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SEEMEDJ SOUTHEASTERN EUROPEAN MEDICAL JOURNAL

ARPAD MESAROŠ

STROSSMAYER'S PARK IN ĐAKOVO, 1964.

OIL ON CANVAS

CHOSEN BY VALENTINA RADOŠ, MLU, SENIOR CURATOR

Josip Juraj Strossmayer University of Osijek
Faculty of Medicine

ARPAD FRANJO MESAROŠ

(Osijek, 3 April 1904 – Strizivojna, 17 March 1970)

In 1923, he enrolled at the school of painting established by Slavko Tomerlin in Osijek. He also spent some time in the atelier of Prof. Fröhlich in Vienna. He studied at the Royal College of Arts and Crafts in Zagreb for two semesters (1928/1929). In 1929, he returned to Osijek and lived in the village of Musić until 1941. After 1941, he lived in Đakovačka Breznica, in Čajkovci near Vrpolje and in Strizivojna. He primarily painted portraits. After the war, he worked as an art teacher in Vinkovci, Osijek, Đakovo and ultimately in Vrpolje, where he lived until his retirement in 1964. After his retirement, he left for Strizivojna. He exhibited his works in various towns in Slavonia. His first exhibition took place in Osijek (1924), followed by Donji Miholjac (1928), Đakovo (1935, 1947, 1949), Vukovar and Vinkovci (1946 and 1947). Life in rural areas made Mesaroš a painter of personal and solitary art. He established himself as a painter of portraits, landscapes and still lifes. His paintings are distinctively realistic and his portraits are subtly saturated with the psychology of the characters they depict. He also made realistic landscape and still life paintings with strong colourist accents.

References: Švajcer (1974), Švajcer (1980)

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SEEMEDJ SOUTHEASTERN EUROPEAN MEDICAL JOURNAL

Editorial

Dear colleagues,

I am happy to present you with the new issue of Southeastern European Medical Journal (SEEMEDJ 2021; 5(1):1-190). This is the ninth number in the fifth year of journal publication.

This issue brings papers from authors from different countries in south-eastern Europe and worldwide – several topics of general interest. In the first part of the journal one can read articles on epidemiology and public health issues related to infectious diseases. In the era of COVID-19 pandemic it is easy to forget that many other contagious diseases present an enormous burden to health systems. Two articles from West Africa (Ayenigbara et al, and Olofintuyi et al, Nigeria) point towards the necessity to prevent or contain diseases such as Lassa fever and under-five infectious diarrhea, respectively, both diseases having high morbidity and mortality, while is possible to combat both with directed public health care strategies. In Europe, newly emerging issue is minor outbreaks of diseases that have been successfully eliminated and became sporadic due to vaccination programs, such as measles (article by Borocz et al, Hu). Authors pointed out that suspended immunization activities due to the COVID-19 surge might be an ominous precursor to a measles resurgence. Finally, cytomegalovirus (CMV) infection is common opportunistic infections in kidney transplant recipients, and Šisl & Zibar (Cro) provide suggestions for preemptive. CMV prophylaxis and CMV-DNA testing in this group of patients.

COVID-19 pandemic also revealed necessity to further study thrombin homeostasis, since a number of data suggests a crucial role of thrombin in different pathologies accompanied by blood coagulation disorders, in particular diseases causing endothelial dysfunction (Korolova, Ukr).

On the other side, chronic, non-contagious diseases, such as diabetes mellitus, pose challenging questions, as well. Bardak et al (Cro) and Mehovic et al (BiH) investigate diabetic treatments relation to diabetic retinopathy, and quality of life of diabetic patients with cardiovascular complications, respectively. The need to have important psychometric instrument in Croatian language is fulfilled by translation of Clance Impostor Phenomenon Scale from English to Croatian by Čarapina-Zovko et al (Cro). Other published articles are concerned with topics of robotic-assisted rehabilitation of neurological patients (Blazincic et

al, Cro), and with often overlooked condition - underactive bladder syndrome (Radoja, Cro). Kovačević et al (Cro) evaluated immunophenotypes and proliferation index in regard to axillary lymph node invasion in breast cancer in monocentric cross-sectional study.

Importance of telemedicine, that we are aware of even more nowadays, in necessity to keep the physical distance, is discussed in the article by Mihalj et al (Cro); and application of telemedicine in otorhinolaryngology, with historical overview, current status and future perspective is presented,

Two papers (Juzbašič – on oral hygiene in disabled persons and Stanić on dementia and life habits, Cro) are co-edited by senior lecturer Mirna Sabljar, PhD, from the Faculty of Art and Culture of University of Osijek, as contributions from their international meeting on art and disabilities, giving to present issue of SEEMEDJ interdisciplinary perspective. Oral health and dental occlusion in young adults as well is a point of research in article by (Strikić Đula et al, Cro) and body compartmentalization in athletes and non-athletes in article by Seper & Nesic, (Cro). Finally, always intriguing problem of abortion, legislative and practice is a topic of review by Negro et al (It).

It is important to mention the art work at the cover page of this issue. It is a painting of Strossmayer's park in Đakovo, belowed part of the city to all inhabitants, painted by Arpad Franjo Mesaroš, from the Museum of Fine Arts in Osijek (selected by Ms Valentina Radoš, senior curator). Arpad Franjo Mesaroš was a painter of personal and solitary art, renowned in Croatia and born in Slavonija County.

On a behalf of editorial board and my own, I warmly greet our readers and invite them to join us in the endeavor of publishing own scientific work in SEEMEDJ.
Sincerely,

Ines Drenjančević, MD, PhD
Editor-in-Chief
Southeastern European Medical Journal (SEEMEDJ)

Contents

Original article

Imported Infections Versus Herd Immunity Gaps; A Didactic Demonstration of Compartment Models Through the Example of a Minor Measles Outbreak in Hungary

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Abstract

Introduction: In Hungary, where MMR vaccine coverage is 99%, in 2017, a minor measles epidemic started from imported cases due to two major factors – latent susceptible cohorts among the domestic population and the vicinity of measles-endemic countries. Suspended immunization activities due to the COVID-19 surge are an ominous precursor to a measles resurgence. This epidemiological demonstration is aimed at promoting a better public understanding of epidemiological data.

Materials and Methods: Our previous MMR sero-epidemiological measurements (N of total measles cases = 3919, N of mumps cases = 2132, and N of rubella cases = 2132) were analyzed using open-source epidemiological data (ANTSZ) of a small-scale measles epidemic outbreak (2017, Hungary). A simplified SEIR model was applied in the analysis.

Results: In case of measles, due to a cluster-specific inadequacy of IgG levels, the cumulative seropositivity ratios (measles = 89.97%) failed to reach the herd immunity threshold (HIT Measles = 92–95%). Despite the fact that 90% of overall vaccination coverage is just slightly below the HIT, unprotected individuals may pose an elevated epidemiological risk. According to the SEIR model, ≥74% of susceptible individuals are expected to get infected. Estimations based on the input data of a local epidemic may suggest an even lower effective coverage rate (80%) in certain clusters of the population.

Conclusion: Serological survey-based, historical and model-computed results are in agreement. A practical demonstration of epidemiological events of the past and present may promote a higher awareness of infectious diseases. Because of the high R0 value of measles, continuous large-scale monitoring of humoral immunity levels is important.

(Böröcz K, Markovics Á, Csizmadia Z, Najbauer J, Berki T, Németh P. Imported Infections Versus Herd Immunity Gaps; A Didactic Demonstration of Compartment Models Through the Example of a Minor Measles Outbreak in Hungary. SEEMEDJ 2021; 5(1); 1-16)

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KEYWORDS: MMR, vaccine, humoral antibody, epidemics, SEIR model

Introduction

Testing of acquired immunity and effectiveness of vaccination against infectious diseases has been increasingly important in the design of preventive public health strategies. Resurgence in measles cases in the United States and across Europe has occurred, including in individuals vaccinated with two doses of the vaccine (1). According to the Centers for Disease Control and Prevention (CDC), World Health Organization (WHO) and United Nations International Children's Emergency Fund (UNICEF), measles has already been a global issue and now it has been aggravated by disrupted immunization protocols due to the COVID-19 pandemic (2–5). All six WHO regions have reported disrupted immunization activities, with major adverse effects on routine immunization and mass vaccination campaigns (4). According to CDC reports, in 2020, more than 117 million children were at the risk of missing out on measles vaccines as a consequence of the COVID-19 surge (2). Measles immunity gaps resulting from suspended immunization activities are an ominous precursor to a measles resurgence (4). In Ukraine, one of Hungary's neighboring countries that was already endemic for measles, vaccination has been interrupted in many regions (3). Regarding Europe, ECDC surveillance data have indicated an exceptionally high number of measles cases in 2018, 2019 and 2020 in EU/EEA countries.

Vaccination remains one of the safest and most effective interventions available in public health for the primary prevention of infectious diseases, resulting in both direct and indirect immunity in individuals vaccinated (herd immunity) (6–8). Even though a safe and effective two-dose measles/MMR vaccination schedule has been available in Europe since the 1960s, maintaining high vaccine coverage is still difficult, despite the fact that in Hungary, the MMR vaccine is mandatory and consequently the vaccine coverage is estimated to be at 98-99%. According to our previous publications (9,10) and in agreement with the results obtained by our colleagues (11), there are latent immunization gaps in certain age (or immunization) clusters of the Hungarian population, with predominance of the ~35-45-year-old adults. These are individuals who form a significant portion of the active labor force of the country, for instance health care workers (HCWs).

Between January 2017 and May 2019, there were 76 reported measles cases in Hungary (12), 54 of which were reported between 21 February and 22 March 2017 (13). Because of the recent outbreaks worldwide, not only of measles, but also mumps and rubella (MMR) infections, and because of waning of immunity over time after vaccination (14–17), the importance of continuous MMR seroepidemiological screening is evident.

Suboptimal vaccine effectiveness in certain clusters of the population has a negative impact on overall vaccination coverage. Small-scale outbreaks suggest that certain measles vaccines – applied during the early phases of the Hungarian vaccination history – failed to elicit the desired immunological response. The resulting immunization gap(s) raise the concern of potential further outbreaks (9,11). The 2020 COVID-19 outbreak called attention to the importance of mathematical modelling of epidemics (18). Based on a reliable model, the timescale and economic impact of the disease can be predicted and preventive countermeasures can be taken (19). Through the example of the measles epidemic in Makó (2017, southeast Hungary), we demonstrated that, in possession of key epidemiological data (e.g. R0 value, estimated vaccination coverage of a given population, number of infected and recovered individuals and duration of the epidemic), a simple open-source mathematical model can give a good approximation of the course of an infection and may provide better general compliance with protective measures.

Materials and Methods

Experimental work

In this seroepidemiological survey, we combined the data from our previous findings with recent measurements, including anti-

measles, -mumps and -rubella antibody level (IgG) determination. Measurements were performed on the automated Siemens BEP 2000 Advance® platform (Siemens AG, Germany), using our self-developed ELISA assays validated by well-established commercial kits, as previously described (9,10). Indirect immunofluorescent microscopy was used a reference (Euroimmun, Germany).

In case of large-scale seroepidemiological measurements, a serum bank consisting of anonymous patient sera was used (N of total measles cases = 3919, N of mumps cases = 2132, and N of rubella cases = 2132) (Ethical License number 2015/5726). Nationally representative samples included randomly selected clinical residual samples, with the exclusion criteria of neonates, children under the vaccination age and severely immunocompromised patients. Samples were collected from the Department of Laboratory Medicine (University of Pécs, Clinical Centre). Serum samples were from all listed age groups participating in this study and they were categorized based on past changes introduced in measles and MMR immunization schedules. Age group determination was based on the landmarks in the history of measles and MMR vaccination schedules in Hungary (Figure 1). Human sera were stored in the accredited laboratory of the Department of Immunology and Biotechnology (University of Pécs, Medical School, Pécs, Hungary) according to quality assurance criteria (ISO 17025).

Population-level result evaluation and seropositivity ratio assessment was performed in relation to the concept of herd immunity threshold (HIT) values (HIT Measles = 92–95%, HIT Mumps = 85–90%, HIT Rubella = 83–86). The study relies on the full virus antigen repertoire-based indirect ELISA method. Therefore, it must be considered a good surrogate, rather than an absolute correlate marker for immunity – as far as Plotkin's nomenclature is considered normative (20–22). We examined vaccination group-related infection- and vaccine-induced antibody titres using the following software: SPSS, Origin Pro, Excel.

SEIR model example and input data

A small-scale measles outbreak in Hungary in 2017 raised questions about the vaccination coverage rate in the country. Experimental results supported the theory of ineffective vaccines, as previously mentioned (9). In spite of its limitations, it seemed reasonable to set up a SEIR model calculation in order to see whether a few percent decrease in effective vaccination could result in a local epidemic. To demonstrate the disease spread in a well-immunized population where latent immunity gaps may be present, input data were based on the data of the 2017 measles outbreak in Makó, southeast Hungary. The following parameters were used to perform the calculations:

Population (N): The epidemic was linked to the small-town hospital. During that year, 65 physicians were responsible for medical attendance of the estimated 30,000 inhabitants of Makó and the surrounding villages. In our model, a population of N = 400-1,000 people was assumed, including patients, health-care workers and family members.

Number of infected individuals (I): A total of 29 cases were reported.

Incubation time and contagious period: The incubation time for measles ranges from 10 to 12 days on average, an infected person can be contagious even 1-2 days before the first characteristic symptoms are visible, up to 4 days after the rashes appear. In our model, the incubation time (Tinc) was assumed to be 10 days, whereas the contagious period (Tcont) was 6 days. Based on these values, and parameters were determined by equations (5) and (6).

Reproduction rate ranging from 12 to 18 can be found in the literature and both values were tested. The higher value is applicable to communities where no social distancing is present and the ratio of vaccinated or immunized inhabitants is low. In Central Europe, the use of the lower value seems more rational, although this specific epidemic was kept mainly in a hospital, where circumstances promote the spread of the infection. In this case, the start of

the outbreak was defined as the possible first day of the first patient's infection, while the model was set to stop after the recovery of the last infected person. When it comes to large-scale epidemics, a different approach is used. If no new cases are found after a certain period, the outbreak is over. This time period is usually determined by the incubation time, with a calculation method suggested by the WHO.

Based on the ELISA antibody measurements, it can be assumed that only ~90% of the Hungarian population has effective immunization, which is under the theoretical 92-94% of HIT. In the model, 90% of seroprevalence was assumed, but lower values were also tested subsequently. No additional vaccinated (V) compartment was created and immunized individuals were treated as recovered. Vital dynamics was disregarded due to the short period of the epidemic. Death rate was not taken into consideration either, as no fatalities were observed during the Hungarian outbreak. Calculations were performed using Microsoft Excel Visual Basic Application (VBA), but the graphs were plotted in Origin. VBA is a built-in feature of the Microsoft Office Suite with several limitations, but its prevalence and the user-friendly computer language makes it suitable for educational purposes.

Results

Changes and historical data regarding epidemics in the Hungarian measles/MMR vaccination schedule (23–25) have been plotted on a timeline in order to evaluate seroepidemiological data accordingly. Figure 1 shows changes in measles and MMR vaccination schedules in Hungary since the introduction of the vaccine (1969). High age-specific attack rates characterizing major epidemics (1980-81 and 1988-89) along with 93%-99% of vaccine coverage evidence insufficiencies of the early vaccination program.

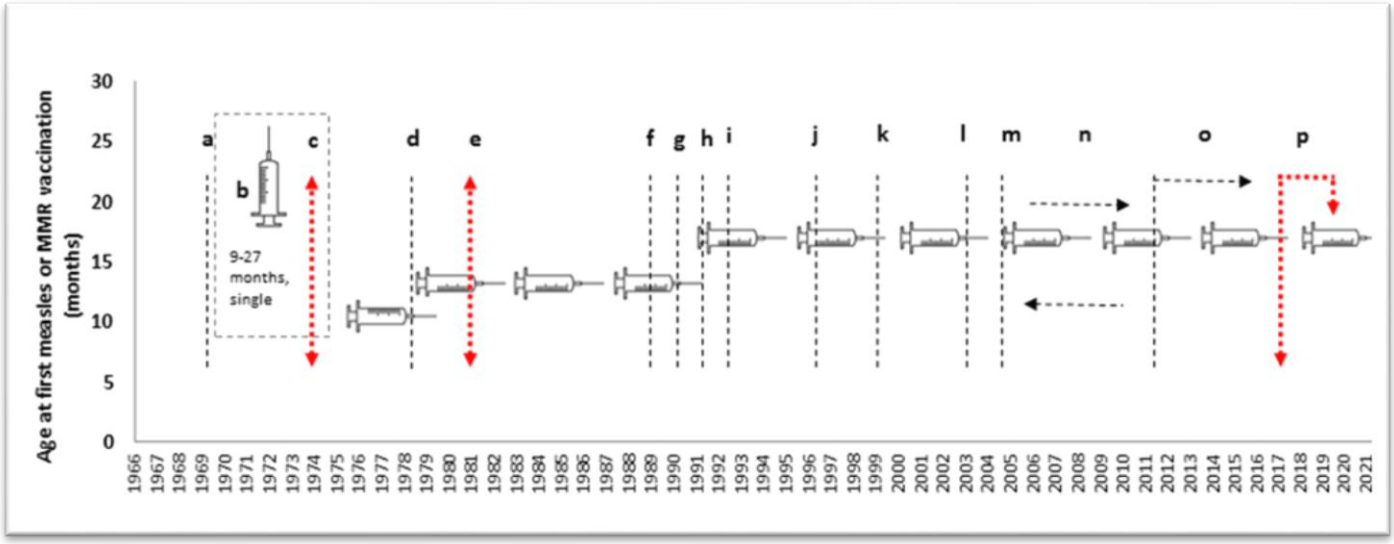
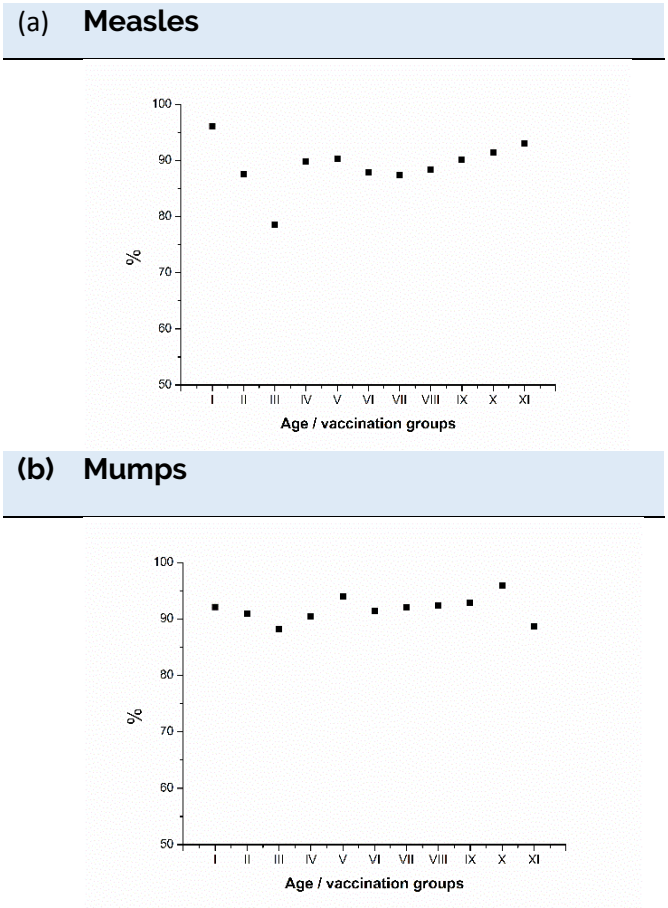


Figure 1. Measles and MMR vaccination schedules in Hungary

(a) Vaccination against measles was introduced in Hungary in 1969. (b) From 1969 to 1974, a single dose of measles vaccine was administered in mass campaigns to persons aged 9-27 months. (c) After vaccination was implemented, the incidence rate decreased until 1973-74, when large epidemics occurred primarily in unvaccinated 6-9-year-olds. (d) The recommended age for vaccination was 10 months until 1978, when it was changed to 14 months. (e) After the 1980-81 epidemic, persons born between 1973 and 1977, who received vaccine when the recommended age was 10 months, were revaccinated. (f) The 1988-89 epidemic mainly affected persons aged 17-21, who had been targeted to receive vaccine during mass campaigns in the first years of the vaccination program in Hungary. After 1989, children were re-vaccinated at the age of 11 with a monovalent measles vaccine in a scheduled manner. Also, in 1989, the rubella vaccine was introduced. (g) In 1990, measles-rubella bivalent vaccines were introduced. (h) The administration of the first vaccine at the age of 14 months lasted from 1978 to 1991. Also, in 1991, the measles-mumps-rubella trivalent vaccine was introduced. (i) In 1992, the administration of the first MMR vaccine was shifted to 15 months of age. (j) In 1996, the MERCK MMR II vaccine (Enders' Edmonston

strain, live attenuated) was introduced. (k) In 1999, measles-mumps-rubella revaccination replaced the monovalent measles vaccine. Also, in 1999, the GSK PLUSERIX vaccine (Measles Schwarz Strain) was introduced. (l) In 2003, the GSK PRIORIX vaccine was introduced. (m) Between 2004 and 2005, the MERCK MMR II vaccine was used. (n) Between 2006 and 2010, the GSK PRIORIX vaccine was in use. (o) Starting from 2011, we have been using a Sanofi-MSD product, MMRvaxPro (Measles virus Enders' Edmonston strain, live, attenuated), for vaccination and revaccination of children. GSK PRIORIX is still on the market, commonly used for vaccination in adulthood. (p) Between January 2017 and December 2019, there were 76 reported measles cases in Hungary (according to ECDC Surveillance reports). (Source of information: MMWR Weekly October 06, 1989 / 38(39); 665-668, International Notes Measles – Hungary, http://www.vacsatc.hu, https://www.ecdc.europa.eu)

Figure 2 shows the age or vaccination group-specific seropositivity and seronegativity ratios for measles, mumps and rubella. The lowest seropositivity ratios in terms of anti-measles antibody titres (IgG) were observed in the groups 'Vaccinated between 1969-1977' (87.56%) and 'Vaccinated between 1978-1987' (78.48%). These results are further confirmed by the abovementioned vaccine insufficiencies of the relative periods, described in Figure 1. Regarding the mumps and rubella seroepidemiological survey, in terms of humoral antibody levels, all vaccination groups satisfied the requirements necessary for the achievement of herd immunity.



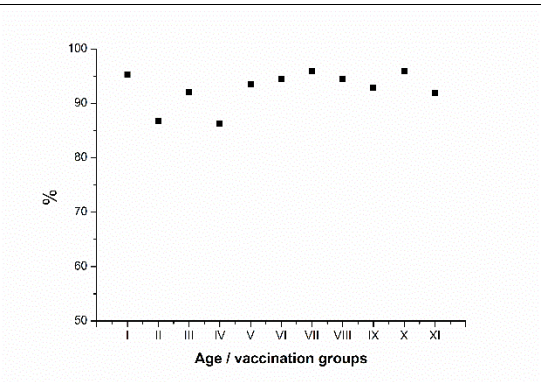


Figure 2. Measles, mumps and rubella seropositivity ratios according to vaccination groups

Age / vaccination groups: (I) Individuals born before 1969. (II) Individuals vaccinated between 1969 and 1977. (III) Individuals vaccinated between 1978 and 1987. (IV) Individuals vaccinated between 1988 and 1990. (V) Individuals vaccinated between 1991 and 1995. (VI) Individuals vaccinated between 1996 and 1998. (VII) Individuals vaccinated between 1999 and 2002. (VIII) Individuals vaccinated in 2003. (IX) Individuals vaccinated between 2004 and 2005. (X) Individuals vaccinated between 2006 and 2010 (XI) Individuals vaccinated after 2011. The lowest seropositivity ratio (78.48%) was observed in the anti-measles antibody titres (IgG) in the group 'Vaccinated between 1978 and 1987'.

In case of measles, mumps and rubella cumulative results, the seropositivity ratios were 89.97%, 91.60% and 92.58%, respectively, as shown in Figure 3. Due to previously detailed

cluster-specific inadequacy of humoral antibody levels, the cumulative anti-measles seropositivity ratios also failed to reach the herd immunity threshold (HIT Measles = 92–95%).

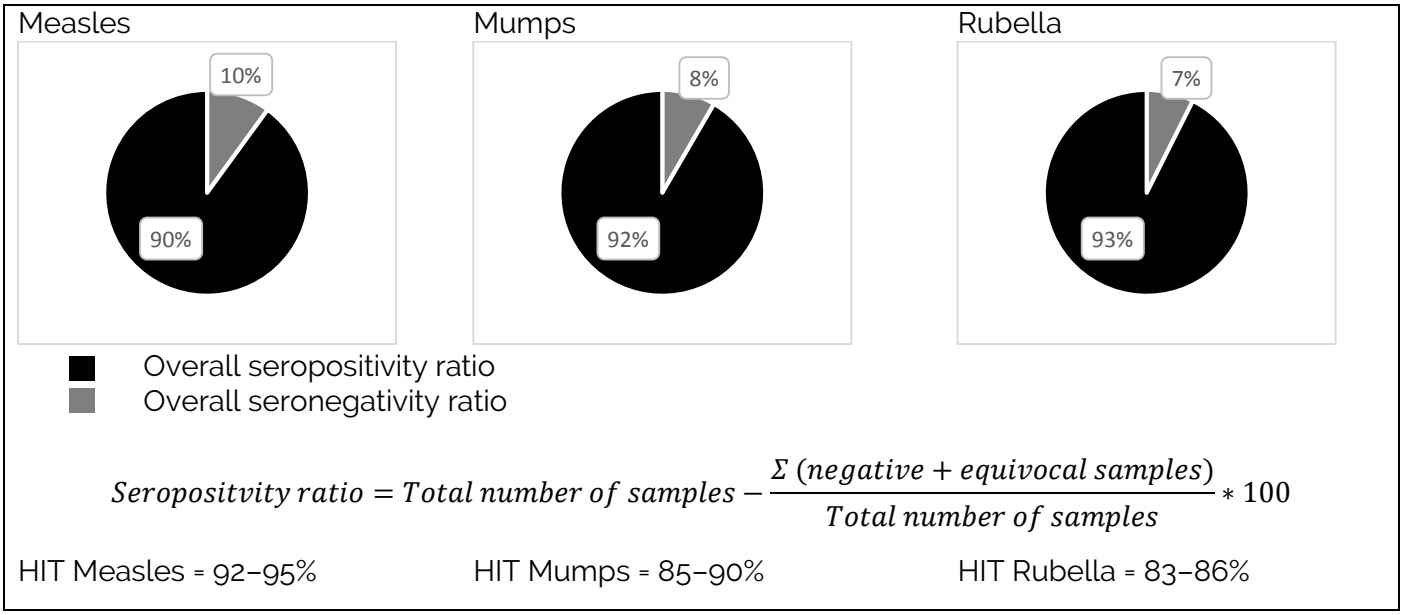


Figure 3. Overall seropositivity and seronegativity ratios

N measles = 3,919; N mumps, rubella = 2,132. In case of measles, mumps and rubella cumulative results, the seropositivity ratios were 89.97%, 91.60% and 92.58%, respectively. The overall ratio of seropositive samples was the lowest in the 'measles' group, where it remained under the threshold value. Seropositivity ratios were calculated as follows:

Using the seronegativity ratio of 89.97% (≈ 90%) obtained by the cumulative data representation of anti-measles (IgG) antibody levels, the model of possible outcomes of a measles outbreak in a hospital as a function of the vaccination coverage rate was investigated. The results of the VBA-based SEIR model of the 2017 epidemic in Hungary are summarized in Table 1. Three parameters – population of the sample, ratio of immunized individuals and reproduction rate of the virus – were set to different values. The effect of these adjustments was investigated and changes in the number of measles cases and timescale of the epidemic were observed.

Table 1. SEIR model results for the 2017 measles epidemic in Makó, Hungary

Population of the sample (N)	Ratio of immunised (%)	Total number of measles cases	Duration of epidemic
		$R_0 = 18$	
1000	90	73	6 months
400	90	29	4 months
400	80	78	3 months
150	80	29	2.5 months
		$R_0 = 12$	
1000	90	2	6 days
400	90	2	6 days
400	80	70	4 months
150	80	26	3 months
Empirical values			
?	90	29	2 months

At R_0=18 and N = 1000, assuming 90% effective vaccination, 100 susceptible individuals can be found in the population. The model estimates a total number of infected persons at 74 and the duration of the epidemic at half a year, which is more than double of the real values. By setting the population at N = 400, 30 infected individuals and 4 months were given by the model. This way the number of infected persons corresponds to the actual clinical data, but the duration is still longer compared to empirical findings.

Timescale of the epidemic can be compressed by increasing the proportion of susceptible people. If the vaccination coverage rate is changed from 90% to 80%, the duration of the epidemic is reduced to 3 months, but the total number of infected individuals becomes higher. Based on this anomaly, it can be presumed that the total number of involved population might be even lower than 400. Unfortunately, the results of the contact tracing procedure were not available for a better approximation.

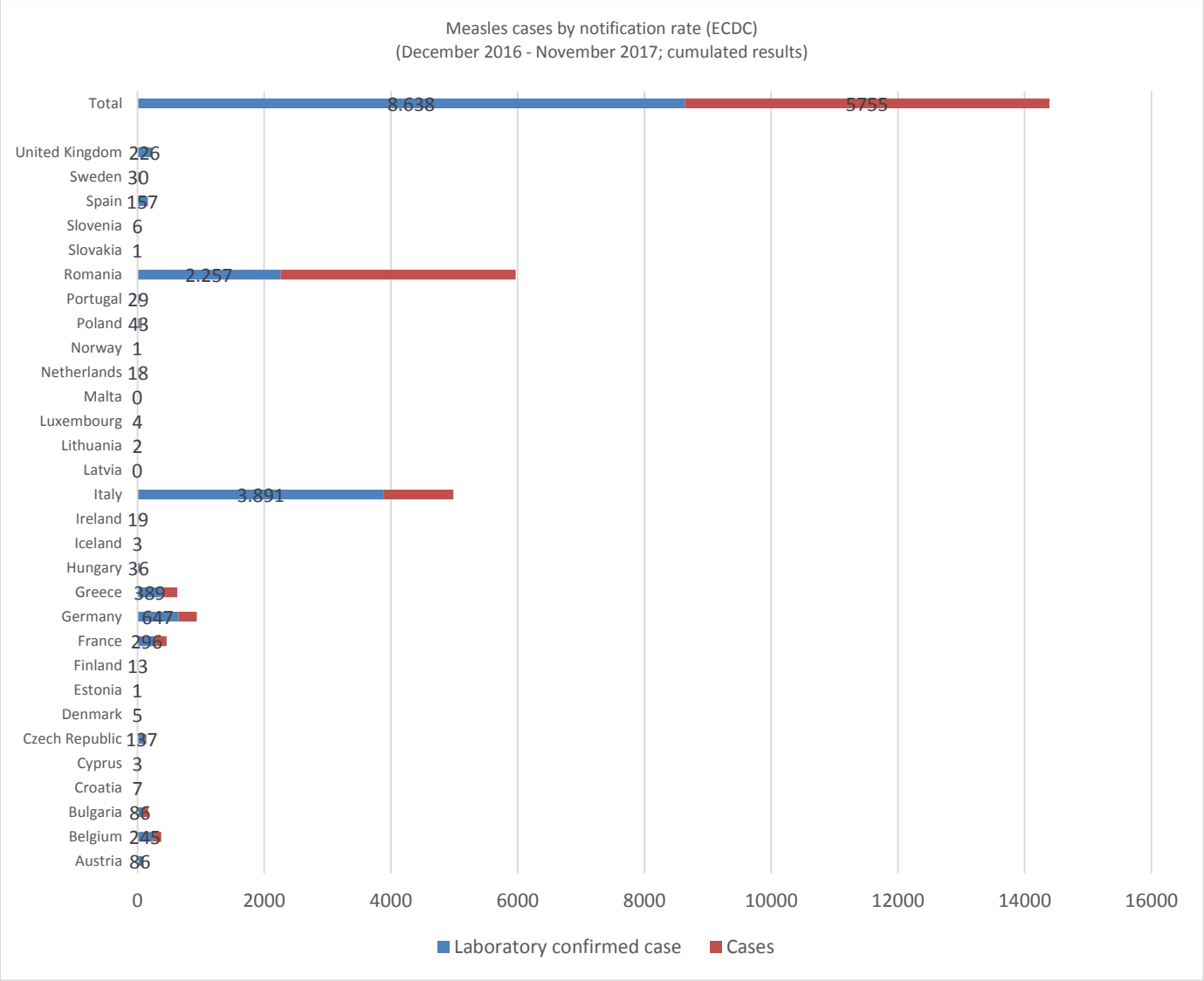
An acceptable correspondence between the model calculations and the clinical data was observed by assuming N = 150 and 80% of vaccination coverage as input parameters.

The results – 30 infections in a two-month period – are close to the official values. For a better comparison, modelling with R_0=12 was also performed. The less contagious the virus, the fewer cases are found. Using this lower reproduction rate, only isolated cases can occur at 90% of vaccination coverage (which is a value that resembles the HIT). By decreasing the vaccination rate, the number of cases increases and the timescale is shortened, similarly to previous test examples.

Discussion

MMR vaccination in Hungary

In Hungary, MMR vaccine is mandatory. A single-dose, live-virus combined measles-mumps-rubella (MMR) vaccine is used to vaccinate infants of ≥15 months of age. A reminder vaccine is given to sixth year primary school students (~11 years of age). PRIORIX (GSK), PRIORIX-TETRA (GSK), ProQuad (MERCK) and the M-M-RVAXPRO (MSD Pharma) vaccines are currently used in Hungary for vaccination of children (at 15 months and 11 years of age) and for adults (62). The vaccines contain live attenuated viruses (26). Regarding insufficient cumulative anti-measles seropositivity levels, we would like to emphasize that potential gaps in the population-level humoral immunity (IgG) are attributable to early vaccination periods and are not a general phenomenon relative to the current immunization practices. The susceptibility of certain cohorts is likely attributable to the thermal instability of the historical Leningrad-16 vaccine, inefficient seroconversion owing to vaccination at a premature age (e.g. 9 months of age) and the questionable efficiency of the inoculum itself (9, 11, 25, 29, 30, 31). The 2017 measles outbreak in Makó was provoked by imported cases. Some of our bordering countries are still endemic for measles (27–30). Supplementary Figure 1 shows the European measles cases in the time period relative to the epidemics in Makó and Szeged. COVID-19 is increasing the risk of measles outbreaks. According to CDC Global Measles Outbreak reports of January 2021, 41 countries may postpone their measles campaigns for 2020 or 2021 due to the COVID-19 pandemic. This increases the risk of bigger outbreaks around the world (31).



Supplementary Figure 1. European measles cases in the time period relative to the epidemics in Makó and Szeged (ecdc.europa.eu)

Between December 2016 and November 2017, numerous measles cases occurred in Europe, most of which were reported by Romania, one of Hungary's neighbouring countries. Source of data: https://www.ecdc.europa.eu/

2017 measles epidemic in Hungary

In 2017, according to the data of the national authorities, a total of 76 persons were infected with measles (corrected to 73 laboratory confirmed cases by ECDC Surveillance reports). The outbreak in the hospital of the small town of Makó involved 29 individuals and lasted from January 2017 to March 2017 (32,33). In order to demonstrate the spread of virus in a well-immunized population, where despite good vaccination coverage, latent immunization gaps (unprotected, seronegative cohorts) are present, we used an open-source epidemiological report of the Hungarian National Public Health and Medical Officer Service (ANTSZ) (17 March 2017): 'At the peak of the Hungarian measles epidemics during the spring of 2017, 52 cases with measles-specific symptoms were reported. Of these, 15 laboratory confirmed cases (National Reference Laboratory for Measles and Rubella, National Public Health Institute, Budapest, Hungary) were registered by 16 March. Of these patients, 12 were health care workers (HCWs) and two were hospitalized patients. One of them was a foreigner, while the

other one was a patient living in the vicinity of a HCW. The epidemic affected two health care institutions, the Hospital of Makó and the clinics of the University of Szeged. The first measles case was imported in mid-February 2017 to the Hospital of Makó. The epidemic affected the hospital staff and their contacts. By 17 March, a measles infection was confirmed in case of a patient who was presumed to be the original importer of the virus, in case of 11 HCWs and in case of one of the HCW's contacts. At the time of this report, additional 11 cases (of which seven HCWs and three patients' contacts) were still under investigation. At the clinics of the University of Szeged, two persons – a HCW and a patient – fell ill with measles. Another 11 persons (six patients and five HCWs) were also suspected at the time of the report. Following the appearance of the abovementioned measles cases, in Csongrád County, a total of 391 people were vaccinated against measles, mumps and rubella (MMR). As the first cases of this period had been revealed, the National Chief Physician ordered strict monitoring and reporting of suspected measles virus infections. Thus, another 15 suspected cases were registered in several other counties. At the time of the report, laboratory testing was still ongoing (12)'.

The second group of imported cases was detected at the end of July 2017 in Nyíregyháza, Szabolcs-Szatmár-Bereg County, Hungary (11). Six unvaccinated Romanian children were hospitalised with clinical symptoms of measles. These cases were later laboratory confirmed (National Reference Laboratory for Measles and Rubella, National Public Health Institute, Budapest, Hungary). The subsequent disease spread among two additional HCWs (also laboratory confirmed) supports the susceptibility of certain clusters in the Hungarian population (11).

Epidemiological model- a didactic representation

In this section, we explain the spreading mechanism of infectious diseases for those who are not familiar with the computational background of modelling. To understand the basics of epidemic models, a simplified mathematical interpretation can be used. The spread of a disease can be described by S-shaped sigmoid mathematical functions, similar to the well-known pH titration curve, or haemoglobin saturation curves. As infectious diseases spread from human to human, the number of susceptible persons is decreasing over time and it influences the propagation of the pathogenic agent. In the beginning of the outbreak, the damping effect of recovered patients is minimal; the curve is very close to exponential and the number of new cases increases rapidly. At a certain time, a kind of equilibrium follows, daily recoveries can balance new infections and the curve reaches its inflection point. Afterwards, in the saturation phase, the epidemic slows down and at the end, no new cases are found and the vast majority of the population has recovered (Figure 4). The curves represented in Figure 4 are a graphic interpretation of a commonly used method for epidemic modelling – the compartment model. In this model, the population is divided into compartments – well-defined categories based on their epidemiological properties. In a compartment, all individuals behave exactly the same, e.g. they are all infected, all vaccinated, all exposed, etc. The simplest among these compartment models is the SIR model, where the letters of the acronym stand for susceptible, infectious and recovered.

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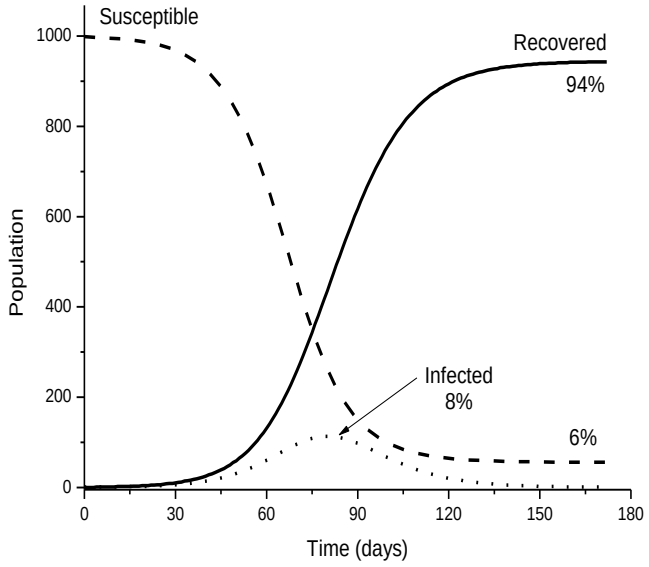

Figure 4. SIR model curves of a hypothetical epidemic

As the disease spreads, the number of susceptible individuals decreases. First they get infected (I), but later on they will progress to the recovered compartment (R). Approximately 6% of the population managed to avoid contact with infected individuals. The peak of infections could be observed almost three months after the first case was recorded, affecting 8% of the population at the same time.

The progression of an individual in this model is easy to follow, each member of the population progresses from susceptible to infectious to recovered.

$$S \overset{\beta}{\Rightarrow} I \overset{\gamma}{\Rightarrow} R \quad (1)$$

Transition between compartments is described by transition rates. Infection rate () represents the probability of transmitting the disease between a susceptible and an infectious person. In other words, the value of shows the number of individuals to whom an infectious person can pass the disease per day (18,39,40) For example, if the infection rate is 0.2, it will take five days on average to infect someone. If we assume that the patient is contagious for 10 days, two new infections are expected in this case.

The overall efficacy of the epidemic can be described by the number of these secondary infections originated from the primary infection, our first patient. This important parameter is the basic reproduction number (R0). Each virus has its own average R0 value – 12-18 for measles and 3.3-5.7 for COVID-19, according to the literature.

The recovery rate (describes the probability of transition into the recovered compartment. For instance, if this rate is 0.1, the contagious period will last for 10 days.

From a mathematical perspective, the transitions can be described by the following differential equations, where S, I and R are the number of individuals in the corresponding compartments, while N is the whole population.

$$\frac{dS}{dt} = -\frac{\beta IS}{N} \quad (2)$$

$$\frac{dI}{dt} = \frac{\beta IS}{N} - \gamma I \quad (3)$$

$$\frac{dR}{dt} = \gamma I \quad (4)$$

Mathematical methods (such as the Runge-Kutta method) are available for solving similar equations, but there is a simpler option. Using the built-in features of Microsoft Excel (or any equivalent spreadsheet application), it is possible to make calculations using an iterative method. Instead of solving the equations, the computer performs calculations that follow the daily changes in different compartments.

For modelling, and R0 have to be defined. Based on the definition of the transition rates, it can be seen that the recovery rate can be determined by the number of contagious days (T_cont).

$$\gamma = \frac{1}{T_{cont}} \quad (5)$$

The basic reproduction number can be given as follows:

$$R_0 = \frac{\beta}{\gamma} \quad (6)$$

Let us assume that in a certain population measles can be transmitted from a single person to 12 others (R_0=12) and they stay contagious for 6 days (T_cont=6). In this case:

$$\gamma = \frac{1}{T_{cont}} = \frac{1}{6} \text{ (7)}$$

$$\beta = R_0\gamma = 2 \text{ (8)}$$

Incubation time plays an important role in the spread of a disease. In a more sophisticated model (SEIR model), this can also be taken into consideration. A new compartment for the exposed part of the population can be generated. The susceptible person first gets exposed and will progress to the infectious state only after a certain time.

$$S \overset{\beta}{\Rightarrow} E \overset{\alpha}{\Rightarrow} I \overset{\gamma}{\Rightarrow} R \text{ (9)}$$

The parameter 'α' is a new transition rate, which can be determined by the incubation time (T_inc) , similarly to γ :

$$\alpha = \frac{1}{T_{inc}}$$

New compartments can be added to the model anytime, such as the compartment M for individuals with maternal immunity or the compartment E for exposed individuals, who are already infected, but not infectious. Based on the characteristics of certain infectious diseases, further models have been developed, such as the SIS, MSIR, SEIR, SEIS, MSEIR and MSEIRS models. The second 'S' in the acronym indicates that after the infection, no permanent immunity can be reached and the individuals step to the S compartment again. In other models, the ratio of hospitalization, the ratio of mild and severe cases and epidemiological interventions can be included, with a more complex mathematical background.

In the examples described above some important parameters are simply disregarded, although it is possible to perform a more detailed computation. Vital dynamics, the natural dynamics of birth and death, can be included by adding two further parameters.

It is necessary to mention that compartment models have their well-known limitations and shortcomings. For instance, all individuals in the population are assumed to have an equal probability of coming in contact with others, although society is inhomogeneous from the perspective of social distancing. Another drawback is that the traditional compartment model cannot handle uncertainty in model parameters. Working with a smaller set of data increases this uncertainty, making predictions unreliable. To overcome this problem, it is usual to calculate the SIR model over a few possible values for each parameter. A more complex solution is to use distribution functions instead of single numbers and if real-time data is available (e.g. we are in the middle of a pandemic), a clinical dataset can be utilized to adjust these parameters (36–38).

Regarding the SEIR model resembling the 2017 measles outbreak in Makó (Figure 4), we would like to note that both the simplified mathematical method and the input data were unreliable. With more sophisticated models, many different parameters can be taken into consideration (37,39). Despite that fact, the calculated values correspond in order of magnitude to the available data on the epidemic and support the experimental results describing the vaccination gap.

Model curves using a lower percentage of the population-level anti-measles protection rate are more fitting. This finding may indicate an even lower percentage of effectively vaccinated population than it was found previously (~90%).

It is concluded that the importance of seroepidemiological surveys is confirmed by the recent outbreaks of measles, mumps and rubella infections in several countries (14,16,17,40–45). Considering the HIT values, suboptimal anti-measles seropositivity ratios were detected in certain clusters of the early vaccination era (78.48% of sufficient anti-measles IgG antibody titres among individuals vaccinated between 1978 and 1987). This finding, which is in accordance with a recent study published by our colleagues (11) and historical literature data (46), suggests the existence of age-specific immunization gaps in the Hungarian population. For mumps and rubella, our preliminary data shows satisfactory immunity levels. Nowadays, in our country, the MMR vaccination coverage is ideal due to the

mandatory administration of safe and modern trivalent vaccines. Nevertheless, dubious immunization practices in some of our neighboring countries, aggravated by the detrimental effect of the COVID-19 pandemic and subsequent suspension of measles vaccination campaigns, may facilitate the occurrence of minor importation-related MeV outbreaks in susceptible cohorts. Using the example of the 2017 measles outbreak in Makó, it has been demonstrated that in possession of key epidemiological parameters (e.g. R0 value, estimated vaccination coverage of a given population, number of infected and recovered individuals, duration, etc.), a simple SEIR model can give a good approximation regarding the course of an infection.

We believe that awareness may significantly reduce the extent of an epidemic (38,47). In the light of current disquieting epidemiological circumstances, we suggest the introduction of open-access mathematical and epidemiological models into modern natural science education of students. Today, online epidemic models are easily available for the public (35,36). Practical introduction to these plain calculation models could help students understand the rationale behind epidemiological data. We believe that a practical demonstration of epidemiological events can promote a better understanding of countermeasures and also allow for an easier adaptation to the current epidemiological regulations.

Limitations of experimental work

The diagnostic ability of our assay was calculated based on the results obtained by well-established kits capable of humoral antibody detection, rather than on neutralizing antibody titres that could serve as an absolute correlate of protection (48–50). It is important to emphasize that immunity to measles is a complex orchestration between the cellular and humoral immunity. For this reason, only antibody-based definitions of vaccine success and failure may be misleading, or at least simplistic and incomplete (51).

Limitations of mathematical modelling

Input data plays a key role in modelling of epidemics. Even when the number of cases is high – like in the 2020 COVID outbreak – the confidence of fitting is poor at the beginning of new cases vs. time graph. The first cases are usually unexpected, quarantine and social distancing protocols are not applied yet and if the disease has a low prevalence in the population, the accuracy of the diagnosis might be low. Besides that, atypical symptoms can be misleading for physicians. Furthermore, statistical values, such as basic reproduction number, incubation and recovery time, depend on other factors, such as social distancing and the health care system.

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Original article

Cytomegalovirus Infection in Kidney Transplant Recipients

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Abstract

Introduction: Present study examined the frequency of CMV infection during follow-up using quantitative nucleic acid amplification testing, the frequency of administration of infection prophylaxis, viremia and infection in kidney transplant recipients who underwent transplantation (TX) at the University Hospital Center Osijek.

Materials and Methods: 107 kidney recipients who underwent transplantation in the period 20 October 2007 – 24 August 2016 were included. Demographic and clinical data, data about pre-transplantation CMV IgG test results of recipients and their donors, data about CMV prophylaxis, viremia, infection, and kidney transplant function were taken from medical records and analyzed.

Results: 92.5% of kidney recipients and 86% of donors were CMV IgG positive before TX. 28% of recipients were CMV-DNA positive at some point after TX, none of whom received a transplant from an IgG negative donor. 89.7% of participants received CMV prophylaxis. Seven participants developed CMV disease, 2 of whom were not administered prophylaxis. Participants were tested for CMV-DNA once a year (median; min 0 max 6). CMV disease was marginally more frequent in those who did not receive valganciclovir prophylaxis (P = 0.066).

Conclusion: It seems wise to enforce the administration of CMV prophylaxis and CMV-DNA testing in accordance with protocol, in order to detect viremia on time and to implement preemptive treatment, aiming at prevention of clinical manifestation of infection and preservation of graft function.

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KEYWORDS: cytomegalovirus, kidney transplantation, CMV-DNA, prophylaxis, valganciclovir

Introduction

Cytomegalovirus (CMV) infection is one of the most common opportunistic infections in kidney transplant recipients. There are two ways of acquiring the infection: reactivation of the virus or de novo infection of an immunocompromised patient. The infection can be asymptomatic, it can jeopardize the function of the graft and/or cause a systemic infection and even death.

CMV infection is defined as virus isolation or detection of viral proteins (antigens) or nucleic acid in any bodily fluid or tissue specimen, regardless of symptoms. CMV disease is defined as evidence of CMV infection with attributable symptoms and can be further categorized as a viral syndrome (i.e. fever, malaise, leukopenia, and/or thrombocytopenia), or as a tissue-invasive ("end organ") disease.

In recent years, administration of CMV infection prophylaxis during the first post-transplantation months has become a standard part of therapy after kidney transplantation (TX). It is also recommended to observe CMV viremia using quantitative nucleic acid amplification testing (QNAT) that detects CMV-DNA (deoxyribonucleic acid). Prophylaxis and diagnostics raise the cost of treatment. However, modern protocols justify this approach with the lower total cost of the treatment, since it is deemed that this approach leads to fewer complications.

The prophylaxis and diagnostics protocol at the University Hospital Center (UHC) Osijek is not strict and has changed in recent years, while the approach to prophylaxis and diagnostics is not completely uniform. The aim of the study was to examine the frequency of CMV infection during follow-up using quantitative nucleic acid amplification testing (QNAT) for CMV-DNA, the frequency of administration of CMV infection prophylaxis, and the frequency of CMV viremia and infection in kidney transplant recipients who received their transplant in UHC Osijek during the period between 20 October 2007 and 24 August 2016.

Materials and Methods

The study included 107 participants, 60 men and 47 women, who underwent kidney TX in UHC Osijek in the period between 20 October 2007 and 24 August 2016. Median age at the time of the TX was 51 (min. 27, max. 71), while at the time of the study it was 57 (min. 32, max. 74). Research methods included collecting data from medical records and statistical analysis. The following data were analyzed: demographic data of the participants (age, sex), clinical features of the participants (primary kidney disease, data about dialysis and TX), data about CMV IgG test results of both recipients and their donors prior to TX, data about CMV prophylaxis, viremia, infection, and about kidney transplant function.

Statistical analysis

Data analysis was conducted using SPSS for Windows (version 16.0, SPSS Inc., Chicago, IL, USA). Nominal data were expressed as absolute and relative frequencies. Numerical data were expressed as mean and standard deviation (SD) in case of normal distribution, and as median and range (min. – max. or interquartile range, IQR) in case of asymmetric distribution. The Kolmogorov-Smirnov test was used to analyze the normality of distribution of the variables. Differences in frequencies were tested using the chi-square test, in numeric variables of normal distribution using Student's t-test and Mann-Whitney U test by asymmetric distribution. Correlations between numeric variables were tested using Spearman's Rho test. Statistical significance was accepted at $P < 0.05$.

Results

For 97 kidney recipients (90.7%) this was the first TX, for 7 of them (6.5%) the second, and for 3 of them (2.8%) the third one. Table 1 shows the demographic data of the participants. Female participants were significantly older both at the time of the study (Mann-Whitney U test, $P = 0.003$) and at the time of the TX (Mann-Whitney U test, $P = 0.001$). By September 2016, 12 (11.2%) participants, 6 men and 6 women, had died, one of whom was CMV IgG negative before the TX.

There was no significant difference in mortality between men and women (Mann-Whitney U test, P = 0.654).

Table 1. Demographic data of patients

Number of patients (%)		**Male** 60 (56%)	**Female** 47 (44%)
Age (median in years)	At the time of the study (IQR)	53 (45–59)	60 (53–65)
	At the time of the TX* (IQR)	50 (42–54)	55 (49–61)

*transplantation

Table 2. CMV* serology data

			N	**%**
CMV IgG prior to TX	Recipient	Positive	99	92.5
		Negative	8	7.5
	Donor**	Positive	92	86
		Negative	14	13.1
Recipient–donor pairs based on CMV serology		R+/D+	84	79.2
		R+/D-	14	13.3
		R-/D+	8	7.5
		R-/D-	0	0

*cytomegalovirus, **data for one donor were unavailable

R – recipient, D – donor

Table 2 shows the CMV serology data. Protocolar administration of CMV prophylaxis was introduced in February 2009. Table 3 shows data related to prophylaxis administration and development of CMV infection and disease. Two out of 3 (66.7%) participants who were not administered prophylaxis and 5 out of 27 (18.5%) of those who were administered prophylaxis developed a clinical manifestation of disease, with no significant (rather, marginal) difference in occurrence (Mann-Whitney U test, P = 0.066). Sixteen (53.4%) positive participants were men, while 14 (46.6%) were women. No significant difference was found in the frequency of CMV-DNA positivity between the sexes (Mann-Whitney U test, P = 0.671).

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Table 3. CMV* prophylaxis and infection data

			N	%
Prophylaxis	Administered		96	89.7
	Not administered		11	10.3
	Duration (mean, in months + SD)		4 ± 2	
CMV infection and disease	Positive for CMV-DNA**	Total	30	28
		Received prophylaxis	27	90
		Did not receive prophylaxis	3	10
		CMV IgG*** positive prior to TX	26	86.7
		CMV IgG negative prior to TX	4	13.3
		R+/D+	26	86.7
		R-/D+	4	13.3
	CMV disease	Total	7	10
		Received prophylaxis	5	71.4
		Did not receive prophylaxis	2	28.6

*cytomegalovirus, **positive for CMV-DNA was defined as having > 1000 copies/mL, ***immunoglobulin G, R – recipient, D – donor

The group in which the recipient was CMV IgG positive and the donor negative was significantly less often CMV-DNA positive at some point after the TX in comparison with the group in which the recipient was CMV IgG negative and the donor positive (Mann-Whitney U test, P = 0.002), and in comparison with the group in which both were CMV IgG positive (Mann-Whitney U test, P = 0.016). There was no significant difference in the frequency of positivity at some point after the TX between the group in which both were positive and the one in which the recipient was CMV IgG negative and the donor positive (Mann-Whitney U test, P = 0.159). No recipient of a CMV IgG negative donor became CMV-DNA positive. There was no pairing in which both the recipient and the donor were CMV IgG negative.

Table 4 shows data related to CMV-DNA testing and follow-up. According to the kidney function criteria for prophylaxis dosing, the participants received the appropriate drug dose. Table 5 shows graft function data. There was no significant difference between the sexes in creatinine concentration in the serum at the start of administration of prophylaxis (Mann-Whitney U test, P = 0.365). The group in which the recipient was CMV IgG positive and the donor negative had significantly lower serum creatinine concentration at the end of prophylaxis administration compared to the group in which both were CMV IgG positive (Mann-Whitney U test, P = 0.009). Women had significantly lower serum creatinine concentration than men at the end of prophylaxis (Mann-Whitney U test, P = 0.002). Seven out of 30 participants (23.3%) who were CMV-DNA positive at some point after the TX developed CMV disease. In 2 of them, it manifested as invasive CMV disease, while in the other 5 it manifested as a viral syndrome with leukopenia, fever, malaise, loss of appetite, diarrhea, and weight loss.

Table 4. CMV-DNA* testing and follow-up data

		N
Number of tests performed	Total	442
	Per patient (median + IQR)	4 (2–6)
	Per patient year	1.03
	Patient years	426
Follow up duration	median + IQR	4 (2–6)
	Min – max	0–9

*cytomegalovirus deoxyribonucleic acid

Table 5. Graft function data

			Value	
	Start of prophylaxis – median (IQR)		35 (19.5–48.5)	
			N	**%**
GFR* (in mL/min/1.73 m²)	Value	> 60	14	14.6
		40–59	26	27.1
		25–39	23	24
		10–24	24	25
		< 10	6	6.3
		Unavailable	3	3.1
				Median (IQR)
Creatinine (µmol/L)	Start of prophylaxis	Men		187 (127–264)
		Women		152 (119–262)
	End of prophylaxis	Men		129 (111–145)
		Women		103 (93–141)
		R+/D+		128 (101–148)
		R+/D-		106 (94–110)
		R-/D+		119 (99–166)

*glomerular filtration rate, R – recipient, D – donor

Discussion

Kidney TX has been performed at the UHC Osijek since 2007, and the availability of CMV-DNA diagnostics dates back to 2009. Before 2007, patients treated at the UHC Osijek dialysis department had their TX at UHC Zagreb, UHC Rijeka or Merkur Clinical Hospital, and CMV diagnostics was performed in Zagreb or Rijeka. This study included only the patients whose TX and diagnostics were performed in Osijek.

CMV is widely present in the population, which has been shown in numerous studies, according to which the prevalence of seropositivity to CMV IgG ranges from 30 to 97% (1, 2). Results of this study agree with those results, finding that 92.5% of recipients and 86% of their donors were CMV IgG positive prior to the TX. This could be considered as a high prevalence.

Since CMV presents a significant risk of morbidity and mortality in the population of persons who have received transplants, the importance of prevention of reactivation or de novo infection has been recognized, whether it is performed by administering prophylaxis or through preemptive treatment (3 – 7, 14). Prevention of CMV infection at the UHC Osijek started in February 2009 with administration of oral valganciclovir, at first during 3 months for CMV IgG positive and 6 months for CMV IgG negative recipients, and since October 2014, a universal 6-month prophylaxis has been in use.

Taking that into consideration, 96 of 107 kidney recipients, or 89.7%, were administered prophylaxis, which is a large share of recipients.

The standard dose of oral valganciclovir in CMV prophylaxis is 900 mg per day. That dose is adjusted for renal function, an indicator of which is creatinine clearance (or calculated GFR). Dose adjustment is extremely important since valganciclovir can cause nephrotoxicity and thus jeopardize the graft function. Some of the side effects of valganciclovir, such as leukopenia, nausea, diarrhea, and elevated serum liver enzymes, overlap with the symptoms of CMV infection and disease, which is why finding the actual cause of such symptoms is of great importance.

Regular testing for CMV-DNA plays an important role, and it can help with early detection of infection and administration of preemptive treatment already at low viral loads, which could lead to prevention of clinical manifestations and better preservation of graft function, since preemptive treatment has been linked to fewer toxic effects on the transplanted kidney in comparison with the prophylaxis (8). CMV infection and disease can, however, appear despite preventive therapy, and the reasons for this are incorrect dosing, discontinuation of preventive therapy, or simply a failure of such therapy (4).

Thirty recipients, or 28% of those who were included in the study, developed CMV infection (and 7 of them had CMV disease), which is a relatively high number if we consider the fact that most of them took prophylaxis. A primary risk factor for the development of CMV viremia and disease is considered to be the serostatus of the recipient–donor pair regarding CMV IgG antibodies; the pairs in which both are positive and in which the recipient is negative and the donor positive are under increased risk for the development of CMV disease (9 – 12). Most of the CMV-DNA positive recipients in this study come from a recipient–donor pair in which both were CMV IgG positive, 26 out of 30 in total, which is 86.7% of all positive recipients. The other 4 CMV-DNA positive participants come from a recipient–donor pair in which the recipient was CMV-DNA negative and the donor positive.

Considering the fact that recipient–donor pairs in which both were CMV IgG positive far outnumbered participants with other serological combinations, it is necessary to mention the share of CMV-DNA positive participants in each group. There were 84 participants belonging to a pair in which both the recipient and the donor were CMV IgG positive, and out of them all, 26 were CMV-DNA positive at some point after the TX, which constitutes 31% of such participants. Seven participants belonged to a recipient–donor pair in which the recipient was CMV IgG negative and the donor positive. Among them, 4 participants became CMV-DNA positive. The remaining 14 participants for whom the serostatus combination is known belonged to a recipient–donor pair in which the recipient was CMV IgG positive and the donor was negative; none of them became CMV-DNA positive. There were no recipient–donor pairs in which both were CMV IgG negative. These findings coincide with previous studies from the pre-prophylaxis era, which studied the natural course of CMV infection in kidney transplant recipients and showed that 56% of kidney transplant recipients from a recipient–donor pair in which the recipient is CMV IgG negative and the donor positive develop CMV disease after TX, as do 20% of those from a pair in which both the recipient and the donor are CMV IgG positive (12).

The participants of this study who received a kidney transplant from a CMV IgG negative donor (all of them were CMV IgG positive in this study), in addition to never becoming CMV-DNA positive after the TX, also had better kidney transplant function compared to those who received their graft from a CMV IgG positive donor. Lack of viremia in such participants was accompanied by better kidney transplant function. There was no significant difference in the occurrence of CMV-DNA positivity between the participants who took prophylaxis and those who did not, but CMV disease was more common in those who did not take it, with marginal significance (P = 0.066). However, with an insufficiently large sample of participants who did not take prophylaxis, the rarity of clinical

manifestations of infections in relatively common viremia could still be interpreted as a consequence of administered prophylaxis and timely detection of viremia with regular testing for CMV-DNA.

Out of 7 participants who had clinical symptoms, 2 developed CMV disease and 5 of them had CMV syndrome. One participant from the pre-prophylaxis era developed early invasive CMV disease and died as a result. One participant developed CMV disease, which manifested in elevated serum liver enzymes and esophagitis. Of the participants who had CMV syndrome, 1 participant had elevated serum liver enzymes, 1 had leukopenia and 1 had weight loss. One participant had diarrhea, fever, and malaise, and 1 had diarrhea and loss of appetite. All of them, except for the deceased one, were successfully treated with antiviral medication.

Previous studies have shown that gastrointestinal symptoms were the most common manifestations of CMV infection and disease (3, 13), which coincides with our findings. Participants were tested for CMV-DNA once a year on average, but the frequency of such testing greatly varied from participant to participant, with some of them not being tested for years during certain periods. Such findings, together with the relatively frequent CMV viremia in the observed population, and the significance which CMV infection has for graft function and overall survival of kidney transplant recipients, lead us to conclude that more work is needed to develop a standardized protocol for prevention of CMV infection in kidney transplant recipients.

The limitations of this study, in the sense of evidence-based medicine, were the epidemiological character (design) and the absence of comparable data in a scenario without prophylaxis, since diagnostics, screening and follow-up of CMV were rare and deficient in the pre-prophylaxis era. Other than that, there remains an important aspect of this problem, which could complement this study in the future. It is the immunosuppressive protocol that was part of the participants' treatment, its dynamics over time, with respect to both the year of TX and the protocols in force during that time, as well as complications and comorbidities other than CMV. Likewise, the promptness of the valganciclovir dose adjustment in relation to kidney function dynamics should be studied, although it appears adequately adjusted at the two studied points of time – at the beginning and at the end of administration of prophylaxis.

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Review article

The Endemicity of Lassa Fever in West Africa; Appropriate Mitigative Measures

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Abstract

Lassa fever is an ancient disease and is endemic in most West African countries. Importantly, Lassa fever is a dangerous and virulent disease because it exerts deleterious effects on many vital organs in the body. Due to its endemic nature and the yearly occurrence of the disease in terms of infection and mortality cases in some West African countries, specifically Nigeria, there is need to reexamine and reemphasize viable prevention alternatives. On this backdrop, this review provides a broad overview of Lassa fever, with the main emphasis on preventive measures. Infection with the Lassa fever virus has severe consequences on health; in this respect, multifaceted preventive measures that ensure and guarantee no contact with multimammate rodents should be adopted. Furthermore, contact with the feces and urine of multimammate rats should be avoided, personal hygiene should always be practiced, environmental sanitation should be ensured and carried out often, the consumption and eating of rats should be discouraged, abolished and ultimately stopped, and food containers should always be kept tight and closed.

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Introduction

Lassa fever develops through the infection by the Lassa virus (LASV) (1, 2). Although the disease was first discovered in the early 1950s, the causative agent was not recognized or known until the late 1960s. The virus that causes the disease belongs to the family of Arenaviridae viruses (3). The history of Lassa fever dates back to the 1950s, when the first case was recorded in Nigeria, Borno State, in the town of Lassa, after which the disease was named Lassa fever in 1969 (3). Presently, the disease is endemic in West African countries because of the yearly cases of infection and death (1, 4-6). Numerical estimates indicate that confirmed cases of Lassa fever range between 350000 and 550000, and over 5000 deaths per year are recorded globally (3).

Hosts of the Lassa virus are infected multimammate rats and rodents, and humans usually become infected with the virus due to contact with urine or feces of infected animals (3). The issue of Lassa fever is a yearly phenomenon in Nigeria, and the surge of infections in humans is usually seen in the dry season between December and April, due to the reproduction cycle of new multimammate rats and rodents in the rainy season (7). The Lassa virus is easily transmitted and highly contagious, and the disease it causes is deleterious because it has severe health consequences for the whole system (3). Once a person is infected with the Lassa virus, the virus weakens organs in the body, consequently resulting in organ malfunctions and depletion of the whole system.

In regard to its public health importance, Lassa fever is a yearly phenomenon in endemic countries because of the rapid multiplication of the virus in the newly infected multimammate rats and rodents, which, once infected, continue to shed the virus in their feces and urine throughout their life (7). Although Lassa fever may not be completely eradicated due to peculiar reasons and lack of existing vaccines against the causative virus, transmission of the Lassa virus could however be diminished and absolutely prevented. Hence, this synopsis presents detailed preventive measures against Lassa fever.

Sources of Information

The review focuses on workable prevention alternatives against Lassa fever. The literature review was purposely selected with keywords on the topic of discourse from PubMed, Google Scholar and SpringerLink databases. All eligible studies included are clinical trials, meta-analyses, randomized controlled trials, systematic and review articles on the topic of discourse. Furthermore, major international and national health agencies' databases were searched to find valuable information for the review.

Biological Description of the Lassa Virus

Lassa viruses are typically enclosed, single-stranded, bisegmented and ambisense RNA in nature (8). Their genome is contained in two RNA segments that code for two proteins each, one in each sense, for a total of four viral proteins. The large segment encodes a small zinc finger protein (Z), which regulates transcription and replication, and the RNA polymerase (L) (9). The small segment encodes the nucleoprotein (NP) and the surface glycoprotein precursor (GP) or the viral spike, which is proteolytically cleaved into the envelope glycoproteins GP1 and GP2 that bind to the alpha-dystroglycan receptor and mediate host cell entry (10).

Lassa fever causes hemorrhagic fever frequently shown by immunosuppression. The Lassa virus replicates very rapidly and demonstrates temporal control in replication. The first replication step is transcription of mRNA copies of the negative- or minus-sense genome. This ensures an adequate supply of viral proteins for subsequent steps of replication, as the NP and L proteins are translated from the mRNA (9, 10). The positive- or plus-sense genome then makes viral complementary RNA (vcRNA) copies of itself. In addition, the RNA copies are a template for producing negative-sense progeny, but mRNA is also synthesized

from it. The mRNA synthesized from vcRNA is translated to make the GP and Z proteins (9, 10). This temporal control allows the spike proteins to be produced last, and therefore delay recognition by the host immune system. Nucleotide studies of the genome have shown that Lassa fever has four lineages: three found in Nigeria and the fourth in Guinea, Liberia, and Sierra Leone (11). The Nigerian strains seem likely to have been ancestral to the others, but further research is needed to substantiate this (11).

The Lassa virus enters the host cell through the cell-surface receptor, the alpha-dystroglycan (alpha-DG), which is a versatile receptor for proteins of the extracellular matrix (10). It shares this receptor with the prototypical Old World arenavirus, the lymphocytic choriomeningitis virus. Receptor recognition depends on specific sugar modification of alpha-dystroglycan by a group of glycosyltransferases known as the LARGE proteins (10). Specific variants of the genes encoding these proteins appear to be under positive selection in West Africa, where Lassa fever is prominent (12). Alpha-dystroglycan is also used as a receptor by viruses of the New World clade Carenaviruses (Oliveros and Latino viruses). In contrast, the New World arenaviruses of clades A and B, which include the important viruses Machupo, Guanarito, Junin, and Sabia, in addition to the non-pathogenic Amapari virus, use the transferrin receptor 1 (12). A small aliphatic amino acid at the GP1 glycoprotein amino acid position 260 is required for high-affinity binding to alpha-DG. Likewise, GP1 amino acid position 259 also appears to be important, since all arenaviruses showing high-affinity alpha-DG binding possess a bulky aromatic amino acid (tyrosine or phenylalanine) at this position (10, 12). Unlike most enveloped viruses, which use clathrin-coated pits for cellular entry and bind to their receptors in a pH dependent fashion, Lassa and lymphocytic choriomeningitis virus instead use an endocytotic pathway independent of clathrin, caveolin, dynamin and actin (12). Once they enter the cell, the viruses are rapidly delivered to endosomes via vesicular trafficking, albeit one that is largely independent of the small GTPases Rab5 and Rab7. On contact with the endosome pH-dependent membrane, fusion occurs and is mediated by the envelope glycoprotein, which at the lower pH of the endosome binds the lysosome protein LAMP1, which results in membrane fusion and escape from the endosome (12).

The lifecycle of the Lassa virus is similar to the Old World arenaviruses. The Lassa virus enters the cell by receptor-mediated endocytosis. The specific endocytotic pathway used is still unknown, but cellular entry is sensitive to cholesterol depletion (13). The receptor used for cell entry is alpha-dystroglycan, a highly conserved and ubiquitously expressed cell surface receptor for extracellular matrix proteins. Dystroglycan, which is later cleaved into alpha-dystroglycan and beta-dystroglycan, is originally expressed in most cells to mature tissues, and it provides a molecular link between the ECM and the actin-based cytoskeleton (13). After the virus enters the cell by alpha-dystroglycan-mediated endocytosis, the low-pH environment triggers pH-dependent membrane fusion and releases the RNP (viral ribonucleoprotein) complex into the cytoplasm. Viral RNA is unpacked, and replication and transcription commence in the cytoplasm (13). As replication starts, both S and L RNA genomes synthesize the antigenomic S and L RNAs, and from the antigenomic RNAs, genomic S and L RNA are synthesized. Both genomic and antigenomic RNAs are needed for transcription and translation. The S RNA encodes GP and NP (viral nucleocapsid protein) proteins, while L RNA encodes Z and L proteins. The L protein usually represents the viral RNA-dependent RNA polymerase (14). When the cell is infected by the virus, L polymerase is associated with the viral RNP and initiates the transcription of the genomic RNA. The 5' and 3' terminal 19 nt viral promoter regions of both RNA segments are necessary for recognition and binding of the viral polymerase. Primary transcription first transcribes mRNAs from the genomic S and L RNAs, which code NP and L proteins, respectively (14). Transcription terminates at the stem-loop (SL) structure within the intergenomic region. Arenaviruses use a cap-snatching

strategy to gain the cap structures from the cellular mRNAs, which is mediated by the endonuclease activity of the L polymerase and the cap-binding activity of NP. Antigenomic RNA transcribes viral genes GPC and Z, encoded in genomic orientation, from S and L segments, respectively. Antigenomic RNA also serves as the template for replication (15). After translation of GPC, it is post-translationally modified in the endoplasmic reticulum. GPC is cleaved into GP1 and GP2 at the later stage of the secretory pathway. Cellular protease SKI-1/S1P is responsible for this cleavage (14). The cleaved glycoproteins are incorporated into the virion envelope with the virus buds and release from the cell membrane (14).

Brief Pathogenesis of Lassa Fever

Lassa fever is mostly caught by humans through exposure to urine or feces of the host rodents, commonly through the contamination of uncovered food items at home (3). Additionally, the spread of the Lassa fever could occur through direct contact between infected humans. People who live in overcrowded areas or environments where the host rodents are abundant, as well as places with a lack of standard hygienic measures, are at a higher risk of infection with the Lassa virus. Furthermore, human-to-human route of Lassa fever transmission has been confirmed, which correlates with infection cases in healthcare workers treating Lassa fever patients. Likewise, family members caring for infected relatives can be infected with Lassa fever through the human-to-human route of transmission (3, 16). Presently, there is no empirical data about viral shielding in human breast milk due to increased and elevated viremia (17).

Symptoms of the disease include a flu-like illness characterized by fever, general weakness, cough, sore throat, headache, and gastrointestinal manifestations. Hemorrhagic manifestations include vascular permeability (15). Upon entry into the body, the Lassa virus infects almost every tissue in the human body. It starts with the mucosa, intestines, lungs and the urinary system, and then progresses to the vascular system (18). The main targets of the virus are antigen-presenting cells, mainly dendritic and endothelial cells (19). Generally, when a pathogen enters a host, the innate defense system recognizes the pathogen-associated molecular patterns (PAMP) and activates an immune response (19). One of the mechanisms detects double-stranded RNA (dsRNA), which is only synthesized by negative-sense viruses (19). In the cytoplasm, dsRNA receptors, such as RIG-I (retinoic acid-inducible gene I) and MDA-5 (melanoma differentiation-associated gene 5), detect dsRNAs and initiate signaling pathways that translocate IRF-3 (interferon regulatory factor 3) and other transcription factors to the nucleus (20). Translocated transcription factors activate the expression of interferons, and these initiate adaptive immunity. NP encoded in the Lassa virus is essential in viral replication and transcription, but it also suppresses the host innate IFN response by inhibiting the translocation of IRF-3 (20). NP of the Lassa virus is reported to have an exonuclease activity to only dsRNAs (20). The NP dsRNA exonuclease activity counteracts IFN responses by digesting the PAMPs, thus allowing the virus to evade host immune responses (21).

Diagnosis of Lassa Fever

Accurate and rapid diagnosis of Lassa fever is especially challenging because of unclear signs and symptoms, different variants of the Lassa virus present in West Africa, and laboratory safety concerns regarding highly virulent pathogens (22). Viral culture remains the “best standard” for Lassa fever diagnosis across the diverse Lassa strains, but requires a clinically nonactionable amount of time and safety level-4 precautions to perform (22). Likewise, nucleic acid-based assays have become the clinical diagnostic standard and may be performed rapidly on inactivated specimens under safety level-2 conditions, but may have false-negative results due to the high variance among viruses (22). Viral antigen assays may provide a rapid diagnosis early on during the illness, but may miss the diagnosis at later stages, once the antigenemia phase has resolved (22). The detection of a new IgM antibody response can

diagnose Lassa fever, but may miss the diagnosis during the early stage of illness, may be falsely negative in severe infections where patients are unable to mount a serological response, and may remain positive for a prolonged period, potentially leading to false-positive results (22). A rise in baseline antibody titers between acute- and convalescent-phase serum or a positive IgM accompanied by the development of a new positive IgG response may be more indicative of acute Lassa fever in regions where it is endemic than a single positive IgM titer (22).

To sum up, an orthogonal diagnostic system employing molecular (PCR-based) and immunological (antibody-based) assays provides the greatest confidence in a diagnostic result for Lassa fever (23). Also, rapid diagnostic tests (RDTs) such as lateral flow immunoassays (LFIs) can be an important addition to the orthogonal system and can significantly reduce infections in ongoing outbreaks and in endemic areas (23). Furthermore, multiplex, magnetic bead-based assays are a significant improvement over traditional enzyme-linked immunosorbent assays (ELISAs) (23).

Clinical Manifestations of Lassa Fever

The period between contact with the Lassa virus and the appearance of signs and symptoms is usually 6 to 20 days (3). There are numerous signs and symptoms of Lassa fever, however, general weakness, migraine and headache, sore throat, muscle pain, chest pain, nausea, vomiting, diarrhea, cough, and abdominal pain are most commonly reported and seen in patients (3, 24). In addition, protein may be seen in the urine of Lassa fever patients, and patients may also experience shock and seizures, while coma could occur in advanced stages of the disease due to the absence of treatment (3, 24). Although other infectious diseases may present with the aforementioned signs and symptoms, it is imperative to visit a hospital or a healthcare clinic for proper diagnosis and early treatment of any diagnosed infection. About 25% of Lassa fever survivors have hearing problems, which are typically reversible (25, 26). Incidences of hair loss during recuperation from Lassa fever have been reported, and death often occurs within a few days in severe cases of the disease without treatment. Clinical presentation of Lassa fever is serious in pregnancy, especially in the last stage of pregnancy, because the disease usually causes miscarriages and stillbirths due to the strong affinity of the Lassa virus for fetal tissue (3). In addition, once a person is infected with the Lassa virus, the virus weakens the organs in the body and causes organ malfunctions and depletion of the entire system (17, 24).

The Epidemiological Trend of Lassa Fever

The Lassa virus was first identified in the late 1960s by a healthcare worker in a healthcare center in Lassa, a town in Nigeria (4). After diagnosis, the contagious Lassa virus was confirmed (27). Over the years, Lassa fever has spread from the shores of Nigeria, and numerous West African countries, such as Burkina Faso, Ghana, Togo, Ivory Coast, Benin, Liberia, Guinea and Mali, are either Lassa fever-endemic or have had cases of infection (28-31, Table 1). The infection rate of Lassa fever is high based on geographic location (i.e. 1.9% in developed countries compared to 56% in developing countries); likewise, Lassa fever caught in hospitals results in more deaths (32, 33). Furthermore, findings from hospital-based serosurveillance related with suspected cases of Lassa virus infection revealed that basic hygiene measures among medical workers resulted in fewer infection cases compared to local inhabitants in villages, where basic hygiene practices are less common (33). The transmission rate of Lassa fever depends on the geographic location, with higher frequencies in developing countries compared to developed countries (34).

Table 1. Summary of West African Nations with Lassa Fever Cases, 1962-2018. Adapted from: (3)

S/N	West African countries with at least one case of Lassa virus infection	Endemic West African countries with Lassa fever	West African countries reporting Lassa fever outbreaks
1	Mali	Nigeria	Nigeria
2	Burkina Faso	Guinea	Guinea
3	Ghana	Liberia	Liberia
4	Togo	Sierra Leone	Sierra Leone
5	Benin		
6	Ivory Coast		

Lassa Fever in Nigeria

As the world battles the current COVID-19 pandemic, Nigeria faces a double battle with two highly contagious viral infections. Many years after it was discovered, Lassa fever is still a major public health problem in Nigeria (35). The aforementioned statement is evidenced by the massive outbreak of the disease in 2018 in the country, where 18 out of the 36 states of the federation were affected by Lassa fever; this was the biggest and the worst case of the disease in history (36, 37). In this respect, the Nigerian health authority declared the disease an emergency (38). From the beginning of 2020 until 26th December, 6732 associated cases of Lassa fever were accounted for in Nigeria, and 1181 samples returned positive from laboratory reports (39)d. Furthermore, from the beginning of 2020 until the end of 2020, 244 deaths occurred in 27 states, and 131 local government areas were affected by Lassa fever across the federation, while the general case fatality rate (CFR) for 2020 was 20.7% (39). Numerical estimates also revealed that Edo, Ondo and Ebonyi states had altogether 75% of the positive cases of Lassa fever in Nigeria for 2020 (39).

Cases of Lassa fever were evident in virtually all age groups; however, the predominant age range of people with confirmed infection for 2020 was 21-30, while the gender ratio for male and female was 1:0.9 (39). Due to the overburdening effects of the coronavirus disease pandemic (COVID-19) on the healthcare system, Lassa fever also exerted additional effects on health practitioners, because many health workers were infected with both diseases mistakenly, with more than two deaths recorded due to Lassa fever in 2020 (7). Lassa fever patients were treated and managed in various hospitals and healthcare centers across the country, secondary contacts were also identified, and follow-ups were done to ensure proper tracking and monitoring of the disease (39). Lassa fever is predominant in Nigeria and the onset of the disease is usually between December and June every year. Imperatively, the seasonal surge in new Lassa fever infection cases and deaths in the whole of Nigeria is a cause for concern and ought to be observed intently by both national and international health authorities (38). In addition, bordering countries, for example Togo and Benin, have had imported cases of Lassa fever from Nigeria in the past; hence, bordering countries should be closely monitored as well (38).

Main Emphasis on the Prevention of Lassa Fever

There are no protective vaccines against Lassa fever as of 2020 (40). Because of the endemic nature of Lassa fever, the disease was listed among future causes of disease outbreaks by the World Health Organization (41, 42). Lassa fever is prevalent in most West African countries and results in the deaths of thousands of people ever year (3). Due to the exponential rise in

mortality and infection cases of Lassa fever in the West Africa subregion, especially Nigeria, it is imperative to advance and reemphasize knowledge about prevention of the disease.

Prevention at Home

The transmission route of the Lassa virus to the secondary host (humans) should be forestalled, hindered and disrupted by limiting and reducing any exposure or contact with the feces and urine of the primary host (rodents) in Lassa fever hotspot areas and geographic locations, because worthwhile and beneficial efforts regarding the prevention of the disease depend on sustainable, adequate and proper personal and community hygiene efforts (43, Figure 1). Furthermore, successful and effective preventive measures against Lassa fever in the home include keeping edible items in closed, tight and clean containers, discarding, disposing of or burning waste and refuse far away from residential areas, basic personal hygiene practices such as washing hands with clean running water and soap before handling any edible items, immediate cleaning and washing of used plates and pots with detergent and clean water, tidy arrangement of kitchen utensils and other materials in clean and well-ventilated cabinets, always keeping kitchen floors clean and tidy, and proper disposal of leftover foods. In addition, prompt caution and preventive measures should be adopted by relatives when caring for persons with Lassa fever (3)..

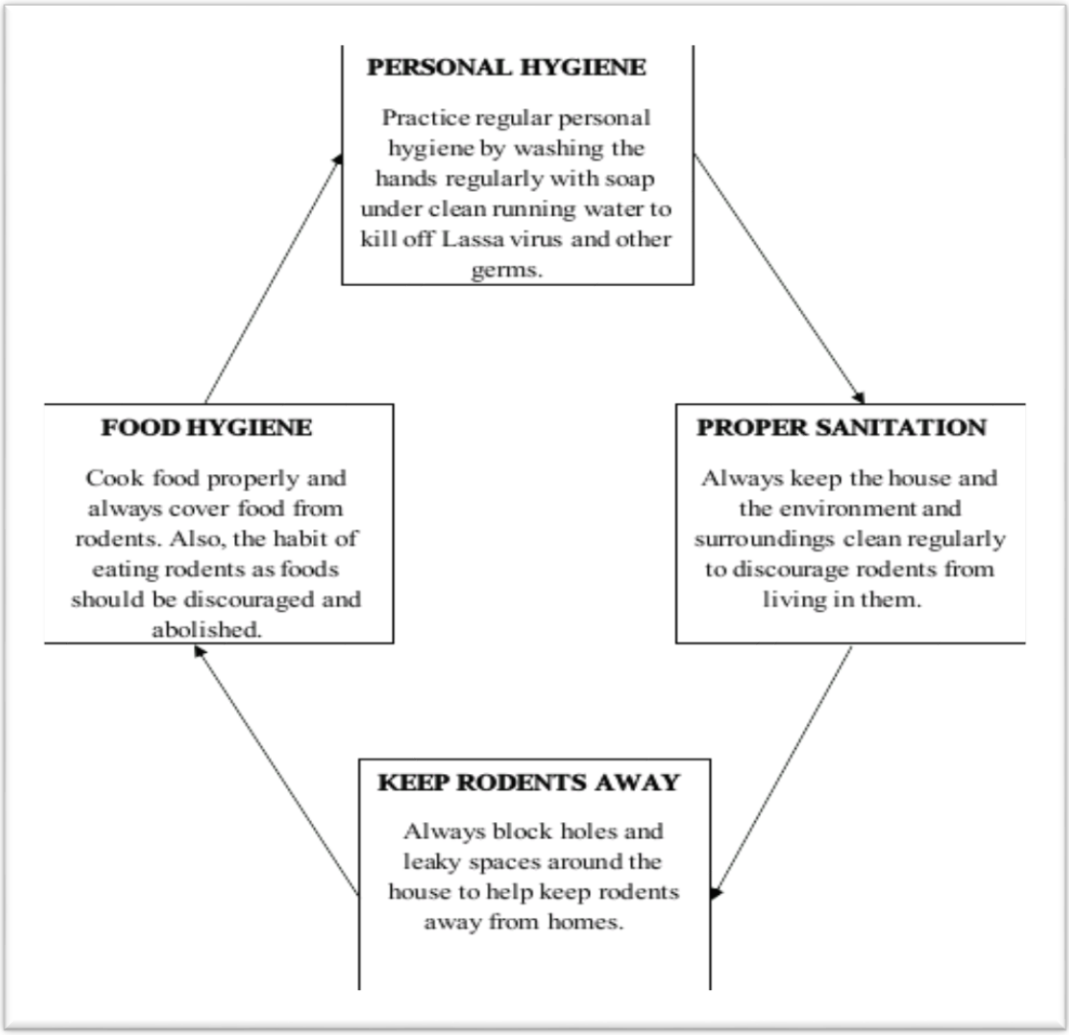

Figure 1. Prevention of Lassa fever at home (self-developed)

Prevention at Hospitals

High safety measures must always be adopted by all healthcare workers in different hospitals, clinics and healthcare centers, even when the diagnosis and cause of infection are unknown. Preventive and control safety measures include essential hand washing with soap, detergent or antiseptic and clean running water, respiratory hygiene and the appropriate use of personal protective equipment (PPE) (3). Furthermore, all rules and guidelines set by the World Health Organization for dealing with cases of highly infectious diseases should be strictly followed by healthcare workers treating Lassa fever patients. In addition, proper checking of personal protective equipment (PPE) should be ensured and the equipment should always be made available to healthcare workers in Lassa fever-prominent areas, such as Nigeria and other neighboring countries. Likewise, laboratory workers are at a higher risk of Lassa virus infection; hence, all Lassa virus samples in the laboratory should be handled with extreme care and caution.

In addition, the infection prevention and control authorities (IPC) should ensure that healthcare centers and laboratories meet the requirements for operation, especially in Lassa fever hotspot areas (44). It is imperative to ascertain that all healthcare centers attending to Lassa fever cases operate with the standard achievable around the world, and routine IPC training should also be made mandatory for all healthcare workers handling cases of Lassa fever (44).

Prevention among Travelers

Migrants moving from countries where Lassa fever is prominent often transfer and import the disease to other locations or regions; this is evident in Togo and Benin, since some of the reported cases in the two countries were imported from Nigeria (38). Strict and continuous border checking and inspection of medical reports of travelers is paramount. Imperatively, all border rules and regulations pertaining to infectious disease checking during border crossing should be strictly enforced by border patrols and healthcare workers stationed at border crossings. Likewise, persons visiting Lassa fever-endemic nations for vacation should visit the respective embassies and consulates in the countries they visit in order to get information on Lassa fever-prone areas in such countries, as well as to get general health information on other infectious diseases prevalent in such countries. Furthermore, febrile migrants who recently returned from Lassa fever-predominant areas should urgently visit a healthcare clinic or hospital in their current location in order to get a proper diagnosis and early treatment (45).

Treatment Options for Lassa Fever

Treatment options for Lassa fever are few and treatments are mainly symptomatic (8). Management of bleeding and hydration is important, particularly in hemorrhagic cases. Likewise, pain management through the use of opiates is prescribed (8). Due to the permeability of blood vessels, pulmonary edema is a concern, and fluid infusion must therefore be carefully monitored (8). An antiviral drug (Ribavirin) offers beneficial effects for Lassa fever patients with poor prognoses, and is usually reserved for patients with higher Herliver enzyme levels (AST value > 150) (8). Even though it is a drug with significant side effects, ribavirin is the drug of choice in many cases of Lassa fever (8, 46).

Recommendations

Based on this study, the following recommendations are made:

a) Contact with multimammate rats, especially their feces and urine, should be avoided.
b) Personal hygiene, such as hand washing with clean running water and antiseptic soap, should be practiced at all times.

c) Environmental sanitation, such as clearing and cleaning of the environment, should be done frequently in order to destroy the habitats of multimammate rats.
d) The barbaric act of eating rats should be discouraged, abolished and ultimately stopped.
e) Containers for food items should be kept tight and closed at all times to prevent rats from accessing them, and any food items with traces of rat feces or urine should be discarded immediately.
f) Healthcare workers, especially nurses, doctors and laboratory scientists, should follow the basic aseptic practices when treating patients infected with Lassa fever.
g) Health education on Lassa fever should be intensified for everyone.
h) The rearing and breading of cats at home should be encouraged. This would help reduce the population of rats in the environment, especially in rural areas.

Conclusions

Lassa virus is highly infectious and could infect any individual, since no one is immune to the disease. Lassa fever is endemic in most West African countries, with yearly cases in terms of infection and mortality in Nigeria. People at severe risk of infection with Lassa fever are individuals living in unhygienic and dirty environments with overcrowded living conditions, because these are the scenarios and situations in numerous Lassa fever-endemic areas. Health consequences caused by the infectious Lassa fever are enormous. In this respect, multifaceted preventive measures that ensure, encourage and guarantee no contact with multimammate rodents and rats should be adopted. Furthermore, there should be a strong synergy and cooperation between governments and border patrol officers of Lassa fever-endemic areas to work out effective ways to minimize the rate of cross-border transmissions of the disease. Likewise, major international health organizations should help and assist with public health experts and personnel, logistic and vital information in curtailing the spread of Lassa fever in developing countries.

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Original article

Maternal Environmental Factors as Predictors of Occurrence of Gastroenteritis among Under-five Children in Akure South Local Government Area, Ondo State

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Abstract

Aim: To examine maternal environmental factors as predictors of the incidence of gastroenteritis among under-five children in Akure South Local Government Area, Ondo State.

Materials and Methods: A descriptive study was carried out in two state-owned hospitals between April and August 2019 using a purposive and convenience sample of 120 mothers of under-five children. Data collected were analyzed using PPMC and regression to test the hypotheses at 0.05 alpha level.

Results: The study shows that three out of four environmental predictors were potent predictors of the incidence of gastroenteritis. They include: quality of water source (β = .387, t = 7.638, P < 0.05), method of sewage disposal (β = .508, t = 9.651, P < 0.05) and hygienic practices (β = .341, t = -6.799, P < 0.05), while area of residence (β = -.048, t = 1.008, P > 0.05) was not a potent predictor. Area of residence, quality of water source, hygienic practices and method of sewage disposal had a significant joint contribution used to predict the incidence of gastroenteritis.

Conclusion: Area of residence, quality of water source, hygienic practices and method of sewage disposal all increase the incidence of gastroenteritis when proper attention is not paid to them. Education about handwashing is necessary for mothers and environmental health workers in collaboration with the Ministry of Health, and the Ministry of Environment should ensure that every house has the proper means to dispose of sewage, especially a septic tank (flush toilet); this will help reduce the disposal of feces in the environment.

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KEYWORDS: gastroenteritis, environment, mothers, infection

Introduction

Children's health is central to the growth and development of any nation's economy, as they are the future leaders. A child's health needs to be addressed immediately, from the day when the mother learns she has conceived. Therefore, it is imperative that the mother provides adequate support to a child's emotional, mental and physical well-being by helping them make healthy choices about hygienic and sanitation practices. Over the years, babies and children below the age of five experience one health challenge or another due to a variety of factors, which may be hereditary, genetic, congenital or environmental in nature, and which often lead to admission into hospitals and an alarming rate of infant mortality.

Gastroenteritis, also known as infectious diarrhea, is the irritation of the gastrointestinal tract – the stomach and the small intestine. It is often accompanied by abdominal pains, diarrhea and vomiting (1). Gastroenteritis can affect people of all age groups (both adults and children can be affected). Persons who are most at risk are individuals with low immunity, especially infants and elderly adults.

According to the World Health Organization (WHO), over 70% of diarrhea-related deaths among children less than 5 years old occur in Africa (2), and Nigeria has one of the highest under-5 mortality rates in the world (328 deaths per 100 000) (3). Recent studies have shown an increase in the prevalence of rotavirus-induced diarrhea in different parts of Nigeria. For instance, a study conducted in Benin City (4) reported a prevalence rate of 19.2%, and one in Sokoto, Northern Nigeria, a high prevalence rate of 25% (5). In Ibadan (6), a prevalence of 18.5% was reported, while Kaduna recorded a prevalence rate of rotavirus-induced gastroenteritis of 32.2% among under-five children (7). Moreover, a study (8= conducted in Akure recorded a high percentage of incidence (31%) in children in the age group between 0 and 12 months. The incidence of gastroenteritis remains a significant burden on children in developing countries due to a range of elements, such as the lack of available healthcare services, lack of safe drinking water, poor sanitation, poor hygiene of both child and caregiver and overcrowding (1).

The transmission of infectious diarrhea (gastroenteritis) can be related to the area of residence. The risk of contracting gastroenteritis can increase as a result of animals living with humans or in close proximity to human dwellings; they can transmit it directly to humans or through contaminated water and overcrowding (9). Any airborne and respiratory infection is likely to spread in overcrowded areas, especially among infants because of their weak immune system.

The quality of drinking water is also linked with the likelihood of suffering from a gastroenteritis infection. The source of water fit for drinking should be considered in regard to its nearness to any toilet facility, in order to avoid water contamination. Several reference works have revealed that children with access to safe drinking water are less likely to suffer from gastroenteritis than those who use water from unsafe sources (10, 11). Likewise, poor handling and storage of drinking water is seen to be significantly associated with an increased risk of infectious diarrhea (12-14).

The method of sewage disposal by the caregiver in a household is quite significant for the incidence of gastroenteritis among under-five children. No access to a hygienic toilet may lead to large amounts of fecal waste being discharged into the environment, especially without adequate treatment, which is likely to have a major impact on infectious disease burden and quality of life (15(. The prevalence of diarrhea is lower among children who live in a house with less dirty sewage than in children who do not (16).

Poor food hygiene practices have been reported to increase the risk of diarrhea episodes among infants when unsterilized feeding utensils and bottle feeders are used (17). The mothers' practice of handwashing before food preparation is associated with a lower risk of diarrhea among children. There is a high risk of

diarrhea among children aged < 2 years if the mother has poor food hygiene practices (18). Some findings (19, 20) also reveal that a combination of environmental factors has an effect on the rapid increase of gastroenteritis. The role of a mother cannot be overemphasized as most activities that help prevent gastroenteritis take place in the home, and the outcome of the illness depends greatly on the initial steps or actions taken by the mothers at home. Due to the high incidence of gastroenteritis in children under five years of age in Akure South Local Government Area, Ondo State, compared to the incidence rate in other parts of Nigeria, this study aimed to investigate the maternal environmental factors as predictors of the incidence of gastroenteritis among under-five children in Akure South Local Government Area of Ondo State.

We hypothesized that: 1) There is no significant relationship between the area of residence, quality of water source, hygienic practices, method of sewage disposal and the incidence of gastroenteritis among under-five children in Akure South Local Government Area of Ondo State; 2) There is no significant relative contribution of maternal environmental factors (area of residence, quality of water source, hygienic practices, method of sewage disposal) on the incidence of gastroenteritis among under-five children in Akure South Local Government Area of Ondo State; and finally, 3) There is no significant joint contribution of maternal environmental factors (area of residence, quality of water source, hygienic practices, method of sewage disposal) to the incidence of gastroenteritis among under-five children in Akure South Local Government Area of Ondo State.

Materials and Methods

A descriptive research design of the correlational type was used for this study. This study was conducted between April and August 2019. The population for this study comprised of mothers of under-five children in Akure South Local Government Area of Ondo State. The sample used for this study included one hundred and twenty (120) mothers of under-five children selected from the Mother and Child Hospital, Akure, and Ondo State General Hospital in Akure South Local Government Area of Ondo State. Purposive sampling technique was used to select two (2) state-owned hospitals in Akure South Local Government Area of Ondo State; both are dedicated to the care of pregnant mothers and children, have a high level of patronage and are located in densely populated areas. Convenience sampling technique was used to select a number of mothers whose children under the age of five have experienced or are currently affected by the infection.

The instrument used for this study was a self-developed questionnaire designed by the researchers in line with the variables under study. The instrument was validated by making a draft copy available for criticism to experts in the field of maternal and health education, which led to subtraction, addition and possible modification of the research instrument. The corrected version of the instrument was used for the collection of data. It was validated by three experts in the field of health education and its reliability was ensured by using Cronbach alpha with the coefficient value of 0.79 obtained. The descriptive statistics of frequency counts was used to present background information of the respondents, while inferential statistics of regression was used to test the research hypotheses at the 0.05 level of significance.

Results

Table 1 shows the relationship between each independent variable (access to clean water, sanitation facilities used, hygienic practices and area of residence) and the dependent variable (incidence of gastroenteritis); there is a significant relationship between the incidence of gastroenteritis and quality of water source ($r = 0.054$, $P < 0.05$), sanitation facilities used ($r = 0.739$, $P < 0.05$) and hygienic practices ($r = 0.461$, $P < 0.05$), but not area of residence ($r = 0.148$, $P > 0.05$). Hygienic practices (mean = 13.81) are the highest contributing factor to reducing the incidence of gastroenteritis among under-five children based on the mean value, followed by

sanitation facilities used (mean = 12.78), while area of residence (9.68) has the lowest mean value.

Table 1. Correlation matrix showing the relationships between study variables.

Variables	Mean	Std. Dev	1	2	3	4	5	p-value
Incidence of gastroenteritis	49.7760	5.70624	1.000					
Access to clean water	11.1083	1.88669	.540	1.000				0.00
Sanitation facilities used	12.7833	2.18160	.739	.347	1.000			0.00
Hygienic practices	13.8083	1.19731	.461	-.069	.274	1.000		0.00
Area of residence	9.6750	1.91044	.148	.001	.078	.178	1.000	0.106

Source: Field survey (2019); Keys: 1 = Incidence of gastroenteritis, 2 = Access to clean water, 3 = Sanitation facilities used, 4 = Hygienic practices, 5 = Area of residence (p is significant if < 0.05)

Table 2 shows the relative contribution of the independent variables (area of residence, access to clean water, hygienic practices and sanitation facilities used) to the prediction of incidence of gastroenteritis. The results showed the following: access to clean water (β = .387), sanitation facilities used (β = .508) and hygienic practices (β = .341), while area of residence (β = .048) is not significant..

Table 2. Relative effect of environmental factors on the prediction of incidence of gastroenteritis

Environmental factors	Unstandardized Coefficients		Standardized Coefficients		
	B	Std. Error	Beta	t	Sig.
(Constant)	-4.012	3.656		-1.097	.275
Quality of water source	1.171	.153	.387	7.638	.000
Method of sewage disposal	1.328	.138	.508	9.651	.000
Hygienic practices	1.623	.239	.341	6.799	.000
Area of residence	.143	.142	.048	1.008	.316

Source: Field survey (2019)

Table 3 reveals a significant joint contribution of the independent variables (area of residence, quality of water source, hygienic practices, method of sewage disposal) to the prediction of incidence of gastroenteritis. The result yielded a coefficient of multiple regressions R = 0.865 and

multiple R-square = 0.748. This suggests that the combination of four factors accounted for 74% (Adj.R2 = .740) variance in the prediction of the incidence of gastroenteritis. The ANOVA result from the regression analysis shows a significant influence of maternal environmental factors on the incidence of gastroenteritis, F (4, 115) = 85.549, P < 0.05.

Table 3. Summary of regression for the joint contributions of environmental factors to the prediction of incidence of gastroenteritis

R = .865

R Square = .748

Adjusted R square = .740

Std. Error = 2.91120

Model		Sum of Squares	Df	Mean Square	F	Sig.
1	Regression	2900.143	4	725.036	85.549	.000[b]
	Residual	974.635	115	8.475		
	Total	3874.778	119			

Source: Field survey (2019)

Discussion

The study revealed that quality of water source has a significant relationship with the incidence of gastroenteritis. The table further reveals that a poor-quality source of water will increase the incidence of gastroenteritis. This may happen if the water used by mothers for their children comes from a bad-quality source, which may be in close proximity to where the toilet is located. Children with access to safe drinking water are almost 20% less likely to suffer from gastroenteritis than those who use water from unsafe sources, such as unprotected dug wells or springs, tanker truck/cart and surface water (11). In the same vein, (10) affirmed that the probability that a child would contract diarrhea increases for households that drink from streams at a significant level of five percent (5%). Thus, households that use streams as their main source of drinking water are 0.032 more likely to have children suffering from diarrhea when compared to those living in households that use piped water as their main source of drinking water.

Hygienic practices have a significant relationship with the incidence of gastroenteritis. The findings of this study revealed that a high percentage of mothers of under-five children always wash their hands before preparing their child's meal as well as after their children defecate or urinate; they also reheat their child's meal. This indicates that a good number of mothers always practice both food and hand washing hygiene. Likewise, the outcomes of this study have shown that there is a positive correlation and a significant relationship between hygienic practices and the incidence of gastroenteritis among mothers of under-five children. The mothers' practice of handwashing before food preparation was associated with a lower risk of diarrhea among children; there is also a significant association between the incidence of gastroenteritis and washing hands with soap after cleaning the infants' perineum (18). Infants whose mothers sometimes or never wash their hands with soap after cleaning the infant's perineum were more likely to have diarrhea than infants whose mothers always wash their hands with soap after cleaning their infant's perineum. That is to say, the practice of handwashing reduces the risk of gastroenteritis.

The type of sanitation facilities used for sewage disposal shows a significant relationship with the

incidence of gastroenteritis in this study. The disposal practices of fecal waste of the youngest children were significantly correlated with the prevalence of gastroenteritis. Children whose mothers reported disposing of the fecal waste of their youngest child in a pit toilet/latrine reported the highest prevalence of diarrhea in comparison to those who throw it into the garbage or rinse it away. Thus, children who lived in a house with less dirty sewage had a significantly lower risk of having gastroenteritis than children who did not (16). This finding is further confirmed by the submission (15) that the proportions of children from households that use a latrine or diaper for stool disposal suffer from diarrhea less when compared to those that do not. The type of sanitation facility used showed that parents and children living in houses with non-flush toilets are twice as likely to suffer from diarrhea compared to houses with a flush toilet. Children living in households with a separate flush toilet are about 50% less likely to suffer from diarrhea than children with access to a pit latrine, dry toilet and toilet shared by other households; they are generally unhygienic and pose a higher risk for the children to get gastroenteritis.

Regarding the relationship between the area of residence (where the mothers live) and the incidence of gastroenteritis, the study has shown that there is no significant relationship. The observation from the results of this study revealed that a significant portion of the respondents reside in a dirty environment, a place surrounded by stagnant water which could be a breeding place for flies, and even that domesticated animals also reside with them. This indicates that the environment where the mothers reside may be a place for infection to be comfortably transmitted. This is in line with the affirmation of (9) that households in rural areas with domesticated animals in close proximity show an increased risk of gastroenteritis. The presence of animals in close proximity to human dwellings adds to the risk of transmission of zoonotic infections to humans directly or through contaminated water.

This study also revealed that when the environmental factors such as hygienic practices, sanitation and quality of water source are combined together, they have a significant relationship with gastroenteritis. This is confirmed from the findings of (19), where in a 2-week study conducted regarding the prevalence of diarrhea, they discovered that it was 23.1%, and that residence, availability of latrine, availability of a handwashing facility, source of water, and waste disposal practices were independently associated with diarrhea. Furthermore, the findings of (20) showed that many factors, such as socio-economic, behavioral like breastfeeding and environmental factors such as water, sanitation and method of waste disposal are linked to gastroenteritis. Environmental factors such as the type of water source, presence of sanitation facilities, solid waste disposal system and floor type in the kitchen are found to be crucial contributors to the high prevalence of diarrhea and gastroenteritis.

Conclusion

Based on the results of this study, it was concluded that: 1) Gastroenteritis is a major health problem that has over the years assumed greater significance in developing countries like Nigeria; 2) Many maternal factors emanating from the environment, such as area of residence, method of sewage disposal, quality of water source and hygienic practices were assessed and most were found to be significant predictors of the incidence of gastroenteritis, except for area of residence; and 3) However, the conglomeration of maternal environmental factors has a significant relationship with the incidence of gastroenteritis.

Recommendations

At this point, it is recommended that environmental health workers in collaboration with the Ministry of Health and the Ministry of Environment ensure that every house has proper means of disposing sewage, especially a septic tank (flush toilet), as this will help reduce the disposal of feces in the environment. Likewise, the government and other well-to-do

people in the society should work hand in hand to provide potable water supply, which the people can easily access, as well as a way to maintain it. Health educators should also train mothers on handwashing hygiene procedures and show them simple ways of how it can be done for them to apply it over time. Mothers should be encouraged to practice handwashing as it prevents infection. Water and sanitation interventions in urban slums should be highly effective in combating the incidence of this disease among children.

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QUESTIONNAIRE ON MATERNAL ENVIRONMENTAL FACTORS AS PREDICTOR OF OCCURRENCE OF GASTROENTERITIS AMONG UNDER-FIVE CHILDREN IN AKURE SOUTH GOVERNMENT AREA, ONDO STATE

Instruction: Please tick (√) the most appropriate column that suits your answer
Background Information
1. Age (in years): (a) 15-20 [ ] (b) 21-25 [ ] (c) 26-30 [ ] (d) 31-35 [ ] (e) 36-40 [ ] (f) 41-45 [ ] (g) 46 and above [ ]

2. Educational status: (a) None [ ] (b) Primary [ ] (c) Secondary [ ] (d) Tertiary [ ]

3. Family size (in person): (a) 1-5 [ ] (b) 6 and above [ ]
4. Monthly income (in Naira): (a) ≤ 15000 [ ] (b) 16000-25000 [ ] (c) 26000-35000 [ ] (d) 36000-45000 [ ] (e) 46000-60000 [ ] (f) 61000-10000 [ ] (g) 101000-150000 (h) 151-250000 [ ] (i) above 250000 [ ]
SECTION B
Instruction: Please tick (√) the most appropriate column to indicate the extent to which you agree or disagree with the statements below.

S/N	Quality of water source	Always	Sometimes	Not at all
1.	Open well			
2.	Bore hole			
3.	River or stream			
4.	Bottled water to drink			
5.	Treated water			
	Method of sewage disposal	**Always**	**Sometimes**	**Not at all**
6.	Septic tank			
7.	Pit toilet			
8.	Open field defecation			
9.	River			
10.	In a bucket			
	Hygienic practices	**Always**	**Sometimes**	**Not at all**
11.	I do not wash my hands before preparing my child's food after feeding my child			
12.	I do wash my child's hands after defecating or urination			
13.	I reheat food before my child eats			
14.	I wash my child's feeding bottle			
15.	I wash my hands before and after I clean up my child's feces			
	Area of residence	**Yes**	**Maybe**	**No**
16.	I live in a crowded environment			
17.	Animals live around my house			
18.	My environment is free from flies			
19.	I reside in a place where water stores			
20.	I live in a clean and tidy environment			

	Occurrence of gastroenteritis infection	**Once**	**Twice**	**More than twice**	**Never**
21.	I have a child who has suffered from this illness before				
22.	It affects all my children when they are between the ages of two and five				
23.	It comes when I stop breastfeeding my child				
24.	It affects my child when taken to day care center				
25.	It starts with my child in the community				
26.	It has affected the child of my neighbor before transfer to my child				
27.	It has affected my child even when I get immunization for the child				
28.	It has affected my child who did not take immunization				
29.	It affects my male child				
30.	It affects my female child				
31.	How many times does your child vomit in one day?				
32.	What is the frequency of diarrhea in one day?				

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Review article

Regulation and Dysregulation of Thrombin Activity

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Abstract

Thrombin is the enzyme of the haemostasis system that stands at the crossroads between the coagulation cascade and coagulation inhibition by protein C, between plasma coagulation factors and cells involved in haemostasis and between haemostasis and the immune system. Allosteric regulation of thrombin and its interaction with various partners in blood plasma and on the surface of the endothelium and platelets provides a wide variety of thrombin functions. The thrombin activity regulation is multifactorial, so a failure of any part of this system leads to serious consequences. An example of this are thrombotic/bleeding complications during endothelial dysfunction, infections, inflammation and uncontrolled treatment.

This review aims to summarize current knowledge about thrombin structure, functions and regulation. Collected data suggest a crucial role of thrombin in different pathologies accompanied by blood coagulation disorders, in particular diseases causing endothelial dysfunction, such as COVID-19.

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KEYWORDS: thrombin, protein C, haemostasis, platelets, endothelium, inflammation, COVID-19

Introduction

Platelet haemostasis and blood coagulation have important and strong interactions, such as positive and negative feedback loops and surface-bound enzyme complexes formation. Thrombin is the resulting enzyme produced by the coagulation cascade. It provides not only clot formation, but also a strong enzymatic feedback activity in procoagulant and anticoagulant reactions and platelet reactivity (1).

The main thrombin functions are: 1) turning soluble fibrinogen to fibrin, that is forming the protein-polymer core of the thrombus; 2) activating platelets; 3) activating clotting factors V, VII, VIII and XI, which enhance thrombin formation; and 4) limiting its own production by binding thrombomodulin and converting protein C (PC) to activated protein C, which subsequently inhibits clotting factors Va and VIIIa.

Thrombin functions extend beyond blood coagulation and include vascular functioning (2) and in particular, thrombin regulates inflammation and regeneration (3). Through PAR-receptors, it stimulates monocytes, T lymphocytes, leukocytes, endothelial cells, fibroblasts, tissue macrophages, neutrophils and mast cells (4-6). This way, thrombin mediates the crosstalk between the coagulation system and the adaptive immune system at the sites of vascular injury (6). Thrombin also enhances proliferation of endothelial cells, epithelium, fibroblasts, smooth muscle and neuromuscular cells and provides an antiviral response (4-6); participates in the development of malignant tumours (stimulates the adhesive and metastatic capacity of tumour cells and activates angiogenesis in the tumour area); regulates the development and apoptosis of nerve cells and neuralgia in the embryonic period and after birth; and controls the survival of the myoblast, the development of the placenta and the embryo as a whole (2-4, 7, 8).

Thrombin is acknowledged as the central enzyme of haemostasis because of the numerous and multi-directional functions it possesses. When it is present in the bloodstream in pathological conditions, it directly triggers intravascular thrombosis and is a marker thereof at the same time. This is why understanding molecular mechanisms of thrombin regulation is important for both laboratory diagnostics and correction of imbalance in the haemostasis system. Thus, the situation with thrombin studies and application is a bit of a paradox. Generation of active thrombin is the main factor of intravascular clotting. However, tests indicating thrombin activity or detecting prothrombin activation products are not used as routine laboratory diagnostic tests. Also, thrombin is the main target of anticoagulant therapy and at the same time, it can provide both pro-coagulant and anticoagulant action. Only a few research groups are working to find ways to switch thrombin activity and direct it to the anticoagulant pathway. This is a promising direction in the search for a fundamentally new treatment of haemostasis disorders.

This article is focused on summarizing current knowledge about the diversification of thrombin functions and substrates selection for the purpose of drawing the attention of medical professionals to the importance of using thrombin-specific tests in routine laboratory diagnostics both with regard to haemostasis disorders and infectious diseases.

Thrombin sites

Thrombin is a multifunctional serine protease of the haemostasis system. It cleaves more than 10 substrates, but remains highly specific to each one of them. Functional plasticity of thrombin is associated with the presence of effector sites (exosites I and II), which together with the active site are involved in the recognition and binding of the substrate; action directionality modulated by Na^+ coordination; and significant conformational mobility and plasticity of the molecule (9, 10). In addition, it should be noted that the multi-directionality of thrombin is ensured by its interaction with different partners, so thrombin is characterized by homotropic

allosteric regulation. In this context, two populations of thrombin can be determined: free and membrane-bound.

The thrombin active site is typical for enzymes of the chymotrypsin family of serine proteases. Thrombin substrates have a positively charged amino acid (usually arginine) on the N-terminus in the cleavage bond (11) (Figure 1).

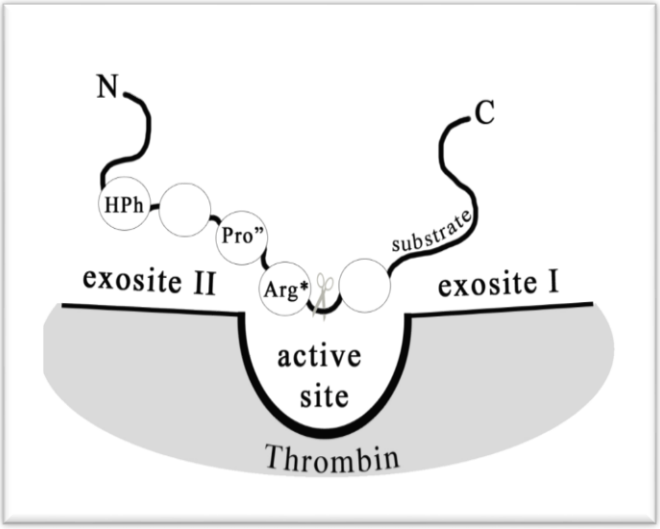

Figure 1. Thrombin substrate recognition scheme (based on [11, 12]).

HPh - hydrophobic amino acid residue

"– Pro or monoamine monocarbon amino acid residue

* - only for heparin cofactor II this position is occupied by Leu

The active site cleft is formed by two loops around the active site, which are longer than such loops in chymotrypsin (12). The 60-loop is hydrophobic and rigid. It forms a cap over an active site and provides interaction with N-terminal hydrophobic substrate residues. The γ -loop is more hydrophilic and flexible in nature. It is adjacent to the active site cleft of thrombin, it can contact substrate residues C-terminal to the scissile bond and can make contacts with the body of the substrate protein. Conformational changes in the thrombin molecule and changes in its catalytic activity result in the binding of exosites I and II of thrombin to the ligands (Table 1). This is confirmed by a functional analysis that demonstrates the relationship between the exosites and the position of the γ -loop of the catalytic centre. The γ -loop can block access to the "gap" of the active site. Active site environments for the individual binary complexes or the ternary thrombin complex are different, such that each binary and ternary thrombin complex could be expected to display unique catalytic properties. At the same time, there is no linkage between exosites I and II structures. Exosite I is formed by Lys36, His71, Arg73, Arg75, Tyr76, Arg77, Lys109 and Lys110; exosite II by Arg93, Lys236, Lys240, Arg101 and Arg233 (11). Exosite I interacts with the C-terminus of the substrate, and exosite II with the N-terminus of the substrate or cofactor

It is suggested that the interaction of ligands with the exosites of thrombin results in the displacement of ions from the exosites. In particular, it is known that the efficiency of interaction with exosite II is strongly dependent on the concentration of NaCl, in contrast to the interaction with exosite I (13, 14). Both exosites require peptides with a high percentage of acidic residues, 50 % for exosite I and 72 % for exosite II, but the hydrophobic content is lower for exosite II. J. Huntington (11) analyzed how thrombin chooses to bind ligands to exosite I or II. The main difference between the exosites is the ratio of negatively charged and hydrophobic amino acids. All exosite I-interacting peptides have a ratio below two and all exosite II-interacting peptides have a ratio above two. Therefore, exosite II is the only true anion-binding site of thrombin and exosite I is actually the "apolar-binding exosite".

Thrombin can interact with ligands by active site only (low-weight substrate), by active site and one of the exosites or by active site and both exosites (thrombomodulin binds simultaneously to thrombin exosite I through its growth factor domains and to exosite II through a chondroitin sulphate moiety). Such interaction variability provides specific recognition of a large range of substrates and their unique rearrangements provide thrombin enzymatic specificity due to

such recognition. The role of exosites in substrate recognition may not be equal. In particular, fibrinogen recognition is dominated by exosite I binding and exosite II plays a secondary role in this process. Eventually, fibrinogen successfully binds to exosite I of active-site-blocked thrombin (15).

Table 1. Thrombin ligands and characteristics of interaction

Ligand	Bound site	Kinetic parameters without Cofactor	Cofactor	Kinetic parameters with Cofactor
Fibrin [87]	exosite I	Kd= 0.52 $*10^{-6}$M (higher affinity site) Kd= 180$*10^{-6}$M (lower affinity site)	-	-
Fibrinogen [88]	exosite I exosite II active site	k_d= 7$*10^{-6}$ M kcat/Km = 13.9 $*10^{6}M^{-1}s^{-1}$ (for FpA release) kcat/Km = 4.0 $*10^{6}M^{-1}s^{-1}$ (for FpB release)	-	-
factor V [89]	exosite I exosite II active site	kcat/Km = 6.3 $*10^{6}M^{-1}s^{-1}$	-	-
factor VIII [90]	exosite I exosite II active site	Km = 12.6 $*10^{-9}$M	-	-
factor XIII [59]	active site	kcat/Km = 1.4$*10^{5}M^{-1}s^{-1}$	Fibrin (in exosite I)	kcat/Km = 1.2$*10^{7}M^{-1}s^{-1}$
GRIb [91]	exosite II	Kd = 5$*10^{-7}$-10^{-8} M	-	-
PAR-1 [91, 92]	exosite I active site	kcat/Km = 3$*10^{6}M^{-1}s^{-1}$	GPIb	kcat/Km = 1.5$*10^{7}M^{-1}s^{-1}$ Kd=10^{-9}-10^{-10} M
Protein C [65, 93]	active site	kcat/Km = 5.6$*10^{2}$ $M^{-1}s^{-1}$	TM (in exosite I, exosite II)	kcat/Km = 5.9$*10^{6}$ $M^{-1}s^{-1}$
TAFI [94, 95]	active site	kcat/Km = 0.96$*10^{3}$ $M^{-1}s^{-1}$	TM (in exosite I, exosite II)	kcat/Km = 1.2$*10^{6}$ $M^{-1}s^{-1}$ Kd = 6.6 $*$ 10^{-9}M
Thrombomodulin [24,96]	exosite I, exosite II	Kd = 1 - 4.9 $*$ 10^{-9}M	-	-
Antithrombin III [97]	active site	kcat/Km = 6.8$*10^{3}$ $M^{-1}s^{-1}$	heparin	kcat/Km = 1.2$*10^{8}$ $M^{-1}s^{-1}$
Heparin cofactor II [20]	active site	-	heparin, dermatan sulphate	Kd = 4$*10^{-8}$M (heparin) Kd =1.9 10^{-6}M (dermatan sulphate)
Heparin [20]	exosite II	Kd = 1.1$*10^{-7}$M (low molecular weight) Kd =0.9 10^{-7}M (high molecular weight)	-	-
Dermatan sulphate [20]	exosite II	Kd = 3.6$*10^{-6}$M	-	-

An interesting example of the regulation significance of thrombin interaction by exosites is fibrinogen γ' binding. Fibrinogen γ' is a product of alternative splicing. It has an alternative chain with the final four C-terminal residues replaced with 20 different residues, with a high proportion of negatively charged residues. Fibrinogen γ' has an average plasma concentration from 8 % to 15 %. [16]. Fibrinogen γ' chain carboxyl terminus strongly binds to thrombin exosite II (16). As a result, fibrinogen γ' reduces thrombin inhibition by antithrombin III (AT III) and heparin cofactor II (HC II) and competes for binding to thrombin with factor VIII. On the other hand, binding to γ' fibrinogen reduces thrombin blood circulation. In addition, fibrinogen γ' forms fibrin clots that are resistant to fibrinolysis. So, even a change in the strength of thrombin exosite II binding to fibrinogen translates into a dramatically different way of clot formation, its content, density and strength of the fibrin network.

It should be noted that there is a competition between cofactors for thrombin exosites binding, which has a significant effect on thrombin's enzymatic orientation. Thrombomodulin (TM) and fibrin have been shown to compete for exosite I thrombin binding. However, thrombin has a 1,000 times greater affinity for TM (Kd = 1 nM) compared to fibrin (Kd = 1 μM), so it always prefers to bind to TM [13]. As the epitopes of binding of TM and fibrinogen, fibrin and PAR-1 overlap and the binding of thrombin to TM interferes with its procoagulant functions (2). Hirudin peptides and fibrinogen bind competitively to exosite I (15).

Exosite II binds to the sulphated region (268-282) of Glycoprotein Ib (GpIb) and heparin with an almost equal affinity, but the thrombin prefers to bind to heparin because its concentration in the intact vascular endothelium is much greater than GpIb (13). This balance can be changed by vascular damage, endothelial dysfunction, inflammation or platelet activation. After coagulation, full clotting factor VIII activation requires cleavage at Arg372, a process involving thrombin exosite II. The sulphated region of GpIb binds to thrombin exosite II and is responsible for the inhibition of the Arg372-Ser373 bond cleavage and activation of FVIII (17).

Another regulative site of thrombin is the Na+-binding site. Na+ is octahedrally coordinated with the three water molecules and the three oxygen atoms of the carboxyl groups of thrombin [14, 18]. The effect is exclusively allosteric because the Na+-binding site is distant from the residues of the catalytic triad (10). The binding of Na+ ions dramatically changes the thrombin's substrate specificity. The "fast" Na+-bound form of thrombin actively cleaves fibrinogen, fibrin, clotting factors V and VIII and PAR. The "slow" Na+-free form has a high affinity for protein C. The transition from "slow" to "fast" thrombin results in the formation of the Arg187:Asp222 ion pair, the optimal orientation of Asp189 and Ser195 for substrate binding, and a significant shift of the side chain of Glu192 linked to the rearrangement of the network of water molecules that connect the bound Na+ to Ser195 in the active site. Under physiological conditions, the concentration of Na+ is 140 mM. The Kd for Na+ binding to thrombin is 110 mM, which implies that nearly half of the thrombin molecules generated in vivo from prothrombin are in the Na+-free, "slow" form (18).

It is suggested that the Na+-binding thrombin loop is directly involved in protein C interaction (2, 14). The binding of Na+ ions plays an important role in fibrinopeptide cleavage by thrombin, since the fibrinogen Aα-chain interacts more closely with the thrombin in the transition condition and, by stabilizing the Na+-bound form of thrombin, makes catalysis more effective (18).

Hypernatremia (Na+ plasma concentration > 145 mM) or hyponatremia (Na+ plasma concentration < 135 mM), which are the most common electrolyte disorders, are often associated with thrombosis or bleeding. Even under physiological conditions, the concentration of Na+ in the blood drops drastically close to platelet clot in vivo, proving the importance of Na+ in controlling the participation of thrombin and other enzymes (clotting factor Xa and protein C) in blood coagulation and thrombosis (18).

Thrombin action during coagulation

Dissociation constants and second-order rate constants of thrombin are quite different for different partners (Table 1). The sequence and speed of cleaving of a substrate depend on the dynamic equilibrium between the concentration of free thrombin, thrombin bound to thrombomodulin, thrombin bound to platelets, thrombin in the antithrombin III-thrombin complex, etc. The thrombin affinity to SERPINs (serine protease inhibitors) is not very high, unlike the thrombin affinity to glycosaminoglycans (dissociation constants of 110 nM and 90 nM for low and high molecular weight, respectively (19, 20)). This is why the only thrombin inhibitors – antithrombin III (ATIII) and heparin cofactor II (HC II) – use heparin and dermatan sulphate to achieve strong interaction with thrombin. Glycosaminoglycans accelerate thrombin inhibition by AT III or HC II 20,000- and 70,000-fold, respectively. They decorate proteoglycans in vascular and extravascular spaces and bind to thrombin exosite II by ionic forces. Thrombin inhibition by SERPINs themselves in the absence of heparin is insignificant. At the same time, heparin presence advances thrombin inhibition and provides strong suppression of coagulation in resting vessels.

Blood clotting cascade activation

In the initial stage of the activation of blood coagulation, subnanomolar amounts of thrombin activate factors V and VIII. Both exosites are involved in thrombin recognition of these factors. This is how thrombin promotes the formation of the prothrombinase complex and thus enhances the process of its own formation through positive feedback regulation. As a result, a few thrombin molecules quickly raise thrombin production (13, 21).

The picomolar concentration of thrombin also activates factor XI (22). The thrombin-GpIb complex can activate FXI on the surface of platelets, where thrombin and FXI are colocalized through separate GpIb interactions. Factor Va also was shown as a cofactor in the activation of FXI by thrombin both in a purified system and in blood plasma and requires phospholipid surfaces (23). But it is not clear whether GPIb and FVa work together or compete.

Thrombin-cells interaction

Membrane-bound enzyme complexes are critical for haemostasis (1). Complexes bound to the sub-endothelium or activated platelets provide blood coagulation activation, while complexes formed on the surface of the endothelium serve for coagulation inhibition. The density of the binding sites on different surfaces can direct haemostasis and change the status of the coagulation system. For example, about 1 % of fVII circulating in blood is normally activated, but it does not lead to coagulation or exceed the activation threshold because of the absence of a binding surface.

While platelet-bound thrombin plays a procoagulant role, thrombin that is bound to endothelial cells provides anticoagulant action (24).

Endothelium binding

Thrombin recognizes three partners on the surface of the endothelium. Interaction of thrombin with heparan sulphate and thrombomodulin (TM) on the endothelium membrane is rapid, reversible and occurs with a high affinity (Table 1), whereas its binding to the membrane protein R-30 is slow, irreversible and occurs with a low affinity (25). This is the way to trigger thrombin activity from coagulant to anticoagulant (Figure 2).

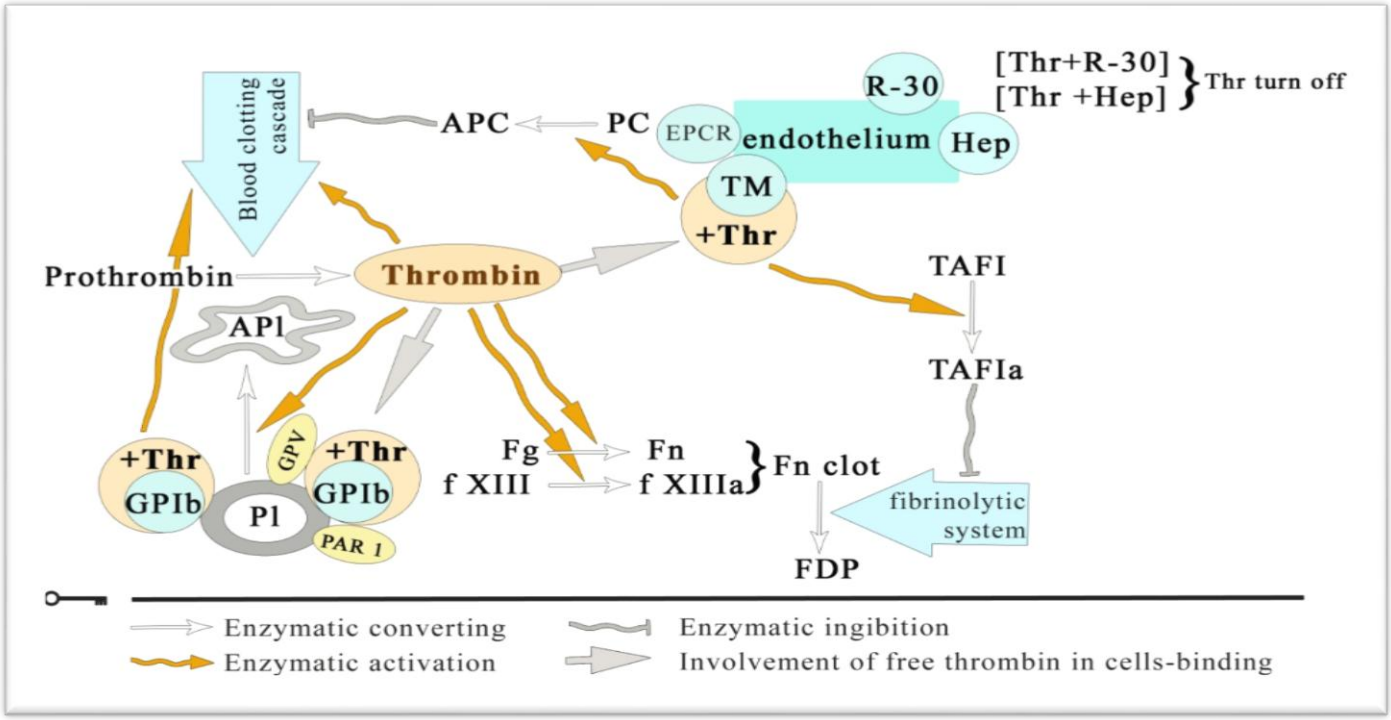

Figure 2. Enzymatic activity points of free thrombin and membrane-bound enzyme

Pl – platelet; APl – activated platelet; Fg – fibrinogen; Fn – fibrin; FDP – fibrin degradation products; TM – thrombomodulin; Hep – heparin; Thr – thrombin; PC – protein C; APC – activated protein C; EPCR – endothelial PC receptor; TAFI - thrombin-activated fibrinolysis inhibitor.© Korolova, 2021

The most striking example of allosteric regulation of thrombin is the change in its enzymatic activity due to its binding to TM. Thrombin recognizes TM by the highly charged regions of exosite I and exosite II. Binding of the chondroitin sulphate moiety enhances the affinity of this interaction. The recognition event also involves conformational changes of the thrombin in the "slow" form, mediated by binding of the EGF-like domains 5-6 to exosite I (24).

Protein C interacts weakly with thrombin due to the placement of polar amino acid residues of protein C near the apolar region of the "slit" of the active site of thrombin. TM interacts with thrombin exosite I by the protein part and with thrombin exosite II by the carbohydrate part. This way, it screens the "unfavourable" amino acid residues of protein C (26). As a result, TM significantly reduces the energy barrier and increases the rate of complex formation [thrombin + protein C] and thrombin activity, in relation to protein C, increases 10,000 times (2, 27). In vivo, the reaction is enhanced approximately tenfold by the endothelial protein C receptor, which binds and localizes PC to the endothelium near TM (Figure 2) (28).

Thrombomodulin is present in an amount of 100,000 copies per endothelial cell. The concentration of free thrombomodulin in large vessels is approximately 0.1-0.2 nM. After interaction with thrombin, its local concentration increases up to 10 nM. It should be noted that in microvessels, the concentration of thrombomodulin reaches 500 nM. This is a prime example of how thrombin-binding sites density can direct haemostasis and provide a significant increase in protein C activity in the microcirculatory system (26). The thrombin-TM complex also activates the carboxypeptidase TAFI (thrombin-activated fibrinolysis inhibitor). Activated TAFI is able to cleave C-terminal lysine residues of fibrin, which typically form fibrinolytic protein binding sites. Thus, the activated TAFI protects the fibrin clot against lysis [29, 30]. This interaction is mediated by thrombomodulin-exosite I interaction, chondroitin sulphate-exosite II interaction and TAFI-active site interaction. Thrombin at high

concentrations binds to the low-affinity R-30 protein on the endothelium surface (25). Thrombin has a low affinity to R-30 and this reaction is slow, but the binding of these proteins is covalent and irreversible. Thrombin R-30 complex serves for thrombin uptake, internalization and degradation.

Platelet binding

Circulating thrombin levels under 100 pM maintain platelets in an inactivated state. Concentration increasing to 1 nM is enough for platelets activation. Thrombin partners on the platelet surface are PARs (protein activated receptors) and GPIb. Platelets provide negatively charged surfaces for coagulation factor assembly. Prothrombinase complex (factor Xa+Va assembled on a phospholipid in the presence of Ca2+) also works on the platelet surface, so thrombin formation is accelerated by acidic phospholipids and platelet activation is upregulated (Figure 2). At low thrombin concentrations, the platelet membrane glycoprotein Ib is involved in the interaction between platelets and thrombin. The binding of thrombin to GPIb is the responsibility of thrombin exosite II on one side and the N-terminal domain and negatively charged GPIb region on the other. Some experimental data (31) suggest that exosite I may also be another site for GPIb interaction. The high-affinity thrombin binding site is located in the α -subunit of the GPIb (268–287 amino acid residues), but only a small fraction of the GPIb exposed on the platelet surface can bind thrombin and most of the receptors specifically bind only to the von Willebrand factor (32, 33). The thrombin-GpIb complex can enhance cleavage of GpV, resulting in hyperactivation of platelets. GPIb/IX/V signalling pathway mediates PI3k/Akt activation and protein phosphorylation (33, 34). It also mediates the increasing intracellular Ca2+ in thrombin-activated platelets. The result of thrombin-GPIb interaction is the stimulation of energy metabolism (glycolysis and oxidative phosphorylation) (34).

GPIb can also be a cofactor in case of factor XI (FXI) and PAR activation by thrombin. Thrombin bound to platelet GpIb via exosite II is brought into proximity to PAR-1 and enhances its activation using exosite I to make contact (11, 13).

Thrombin signalling in platelets depends principally on PARs. Thrombin binds PAR through exosite I and the active site. Thrombin cleavage of PAR is also exosite II-dependent due to the cofactor effect of GPIba, which accelerates the rate of reaction six- to sevenfold (11, 35, 36). Only PAR-1 and PAR-4 are exposed on human platelets and endothelium. PAR-1 is the primary thrombin receptor on platelets, requiring picomolar thrombin concentrations for effective activation, while PAR-4 cleavage is only relevant at high thrombin concentrations (PAR-4 contributes to thrombin-induced platelet aggregation at low thrombin concentrations in the range from ~ 0.4 nM to ~ 0.8 nM (37)). PAR-1 mediates a rapid but transient platelet Ca2+ signalling response to thrombin, whereas PAR-4 mediates a slower, sustained rise, producing the majority of calcium response (38). PAR-4 also plays a more important role in thrombin generation than PAR-1 (39). Both activation of PAR-4 and activation of PAR-1 induce granule release as a feedback mechanism to enhance and stabilize platelet aggregation, with PAR-1 producing reversible aggregation and PAR-4 producing irreversible aggregation (37).

It is probable that the full response of human platelets requires the formation of a pair of receptors PAR-1 and PAR-4 [40]. PAR-4 does not have a sequence complementary to the thrombin exosites. Cleavage of PAR-4 platelets of humans requires a higher concentration of thrombin (~ 100 times) compared to the splitting of PAR-1. Activation of the PAR by thrombin begins with binding and cleavage (Arg41-Ser42) of the N-terminal region of the receptor, which results in the exposure of a new N-terminal region. This amino-terminus serves as a ligand for surfactants, intramolecularly binding to the extracellular portion of the receptor and inducing an intracellular signal (4, 41, 42).

Thrombin activation is associated with G proteins by PAR activation. PARs signalling

mediates the activation of phospholipase C isoform β, PI3- and RhoA/Rho kinases (43-46). PARs also mediate increases in intracellular calcium in thrombin-activated platelets. This leads to changes in cytoskeletal actin and platelet surface exposure of fibrinogen-binding integrins IIbIIIa and activates further signalling and platelet aggregation.

The prothrombinase complex converts prothrombin to thrombin by two pathways. Initial cleavage at Arg320 between the A and B chains generates meizothrombin, which is an active intermediate. The alternative initial cleavage at Arg271 cleaves off the Gla-domain and the two kringles and generates inactive prethrombin-2. Active thrombin is formed as a result of further cleavage of intermediates. Both ways take place in vivo (47–50).

As previously shown, during the first minute of clotting system activation, the in vitro levels of both thrombin and meizothrombin are equal and can reach 0.8 μM (49). Moreover, meizothrombin can bind to the platelet membrane (51), so the local concentration of meizothrombin near the platelet surface may be significantly increased during the initial activation stage. We showed (52) that meizothrombin was able to enhance platelet aggregation induced by ADP, collagen or adrenalin. At the same time, being membrane-bound, meizothrombin is not sensitive to antithrombin III in contradistinction to thrombin (51).

Thrombin can also enhance platelet adhesion through cleavage of ADAMTS13, a proteinase responsible for the von Willebrand factor processing. Since the von Willebrand factor is a key platelet adhesion glycoprotein, by inactivating ADAMTS13, thrombin promotes platelet involvement at the site of injury (53).

Fibrin clot formation

The next step of thrombin activity is fibrinogen cleavage. Thrombin increasing to μM starts the conversion-of-fibrinogen-to-fibrin thrombin activity (Km for fibrinogen cleavage is 7.5 μM). Thrombin cleaves four fibrinogen bonds, cleaving two fibrinopeptides: fibrinopeptide A (fpA: 16-amino acid N-terminal peptide from the Aα chain) and fibrinopeptide B (fpB: 14-amino acid peptide from the Bβ chain) (54).

During cleavage of fpA, thrombin binds directly to the Aα-chain of fibrinogen via exosite I and the active site. The release of fpA is sufficient for the polymerization of both fibrin and this form of monomeric fibrin desA, which spontaneously polymerizes to form protofibrils. When fpB is cleaved, fibrin desAB is formed and lateral association of protofibrils begins. However, the removal of fibrinopeptides B alone does not cause the polymerization of fibrin desB (2, 54, 55). It should be noted that the concentration of thrombin determines the features of the fibrin clot and its resistance to the action of the fibrinolytic system (56).

Thrombin does not react with fibrin through exosite II. This allows the release of fibrin from the active site for clot formation and it is the reason why cleavage of fibrinopeptides is not the main thrombin activity. Thrombin interacts with the N-terminal E region of fibrin to release FpA and FpB, likely disrupting the interaction of the αC terminus with the E-region and exposing knobs A and B in the E-region, which interact with their specific binding pockets in the D-regions of another fibrin molecule, leading to protofibril formation (16).

By converting fibrinogen to fibrin, thrombin provides not only clot material, but also its own cofactor. Being bound to the fibrin E-region, thrombin uses fibrin as a cofactor for cleavage of factor XIII. Factor XIII is bound to the fibrin C-terminal D-region. The fibrin monomers spontaneously polymerize and the E-region of one fibrin molecule is located closely to the D-regions of two other fibrin molecules, moving factor XIII closer to thrombin. Thrombin activates clotting factor XIII by limited proteolysis of its subunit A. Factor XIIIa cross-links neighbouring fibrin molecules by covalent intermolecular bonds and cross-links inhibitors of fibrinolysis to fibrin (13, 57, 58).

The newly generated polymer will provide the cofactor required for 80-fold accelerated factor

XIII activation (59). This ensures that factor XIIIa is generated when it is needed and where it is needed – on the fibrin clot surface.

Interestingly, purified E-regions of fibrin interact with prothrombin, resulting in thrombin-like active site formation in the prothrombin molecule (60, 61). Thus, we can speculate that thrombin-E-region interaction provides not only a thrombin-based approach to factor XIII, but also provides changes in the active site of thrombin and accelerates factor XIII activation.

Role of thrombin in pathology

Historically, the procoagulant function of thrombin (the conversion of fibrinogen to fibrin) has been investigated earlier and more extensively. Based on the aforementioned facts, we can conclude that thrombin has a much wider range of action. Thrombin has a number of functions that can be activated depending on its concentration, localization and accessibility of partner molecules. So, the diversity of thrombin action provides regulation of haemostasis in general and its local concentration influences the thrombus microenvironment and architecture (62). Due to a great variety of protein partners, thrombin is a connector between plasma and platelet coagulation, between coagulation and anticoagulation, between haemostasis and the immune system and between endothelium pathology and coagulation disorders.

Nowadays, thrombin remains a major target of antithrombotic and anticoagulant therapies in cardiovascular medicine. Heparins and direct thrombin inhibitors are currently used in the treatment of acute thrombotic complications, but a strategy that inhibits thrombin at the active site reduces not only procoagulant and prothrombotic functions, but also shuts down activity toward the anticoagulant protein C.

Many researchers have been trying to convert thrombin into a potent and safe anticoagulant for in vivo applications. A new strategy aims at modulating thrombin function, rather than inhibiting it (10, 63). Some authors propose that mutant thrombin be used as a safe physiological anticoagulant (64) or apply a fusion protein, where thrombin and the TM domain are connected through a peptide linker (65).

The shift of balance in pro- and anticoagulant action of thrombin becomes crucial for the development of haemostatic pathologies during inflammation and endothelial dysfunction. It should be emphasized that only the native intact endothelium provides the anticoagulant function of thrombin. So, any endothelium dysfunctions lead to triggering thrombin activity toward coagulation, which appears as thrombotic complications during some diseases.

High levels of angiotensin II cause arterial hypertension by a complex vascular inflammatory pathway that requires leukocyte recruitment and reactive oxygen species production and is followed by vascular dysfunction. The resulting vascular inflammation and dysfunction are mediated by the activation of thrombin-driven FXI feedback, independent of factor XII. FXI receptor GPIb on platelets is required for this thrombin feedback activation [66]. Inhibition of this feedback loop with an antisense molecule against factor XI reduced both vascular pathology and hypertension. At the same time, there are doubts about the importance of feedback activation of factor XI (67).

During bacterial and viral infections, there is an interplay between blood coagulation, immune cells and platelets to restrict the dissemination of pathogens within the body. Endothelial disturbance switches over coagulation and induces thrombotic complications, excessive inflammation and tissue damage. With regard to potential clinical significance, it is possible that interference with the PAR1 pathway by direct thrombin inhibitors or PAR1 inhibitors may increase the risk and severity of viral infection (6).

Thrombin activity leads to endothelial cell activation in Klebsiella infection (bacterial pneumonia). Extrinsic pathway generated thrombin mediates fibrin polymerization and platelet-neutrophil interactions essential for protective immune responses in at least Klebsiella pneumonia–derived sepsis [68]. TF is induced in the lung after an H1N1 IAV infection in

mice, which led to thrombin-induced hyperactivation of coagulation (6). At the same time, activated protein C seems to be involved in coagulation regulation during an H1N1 IAV infection. HIV infection is associated with increased [thrombin-antithrombin III]. Thrombin mediates the crosstalk between the coagulation system and the adaptive immune system at the sites of vascular injury through increased T-cell motility and production of proinflammatory cytokines during an HIV infection (6, 69).

An infection that strongly affects coagulation is the coronavirus disease 2019 (COVID-19). It induces an immune response within the endothelium in blood vessels in several organs (70, 71). Studies of COVID-19 patients demonstrate the presence of fibrin thrombi within distended small vessels and capillaries and extensive extracellular fibrin deposition (72). Disseminated intravascular coagulation (DIC) has been reported to develop in 70 % of patients who succumb to the infection. D-dimer levels were increased far out of proportion to any abnormalities in the prothrombin time (PT/INR), activated partial thromboplastin time (APPT), fibrinogen level, or platelet count; these findings are uncharacteristic of DIC as currently understood.

Hanny Al-Samkari et al. stated that thrombosis is primarily associated with inflammatory markers, rather than coagulation parameters during a COVID-19 infection (73). Marco Ranucci et al. also demonstrated that coagulation is triggered by the release of IL-6 and other cytokines and the consequent release of tissue factor (74).

Thrombin in laboratory diagnostics

In recent times, the most widely used laboratory tests that indicate the imbalance of haemostasis have been the D-dimer test and thromboelastography. D-dimer is the fibrin degradation product appearing in the bloodstream as a result of stabilized fibrin cleavage by fibrinolysis. So, this parameter mainly indicates the existence of stabilized fibrin in the bloodstream and also the balance between coagulation and fibrinolysis (73, 75-77).

The thromboelastography characterizes the clotting process in whole blood, providing information on clot formation and lysis overall (78). The thromboelastography testing has expanded to include managing extracorporeal membrane oxygenation therapy, assessing bleeding and assessing hypercoagulable conditions. In addition, thromboelastography platelet mapping has been utilized to monitor antiplatelet therapy (78).

In particular, in patients with COVID-19, elevated circulating D-dimer levels are associated with mortality (79, 80). But the increasing of D-dimer levels occurs in both thrombosis and bleeding complications, so this parameter cannot be used as a thrombosis marker (81). Management of the thrombotic risk associated with COVID-19 is complicated by heparin treatment (82) and one of the approaches is modification of the thrombin generation assays conditions by adding heparinase as a heparin neutralizing agent.

Markers that directly indicate the appearance of active thrombin in the bloodstream are prothrombin fragment 1+2 (F1+2), prethrombin-1 and soluble fibrin. Unfortunately, they are not incorporated in the routine laboratory practice.

F1+2 is formed during the activation of prothrombin by prothrombinase complex (47), which is why it indicates prothrombinase activity. The appearance of this prothrombin derivative indicates the prothrombin activation to thrombin, so it is a direct method of thrombin detection. The nature of F1+2 makes it a more informative marker of procoagulant changes than clotting tests. Marco Ranucci et al. were able to show the direct appearance of thrombin by measuring F1+2 in the blood of patients with COVID-19 (74). Moreover, the F1+2 level was significantly reduced, whereas it increased in non-survivors.

Another prothrombin derivative is Prethrombin-1, which appears as a result of prothrombin autolysis by thrombin (9). It is present in the bloodstream in case of intensive production of active non-inhibited thrombin, so it is a result of a strong activation of blood coagulation. A high

level of prethrombin-1 evidences the danger of intravascular clot formation (73, 83).

On the other hand, thrombin activity is a useful predictor of the bleeding risk (73). Thrombin acts at the very end of the coagulation cascade and the decrease of its activity is associated with a high risk of bleeding. Several studies indicated the correlation between impaired thrombin generation and the severity of the disease (84). The tendency to bleeding was observed when thrombin generation fell below 20 % of the normal range (73). Similarly, a correlation between the generation of thrombin and the clinical bleeding phenotype in patients with deficiencies in blood coagulation factors FII, FV, FVII, FX and FIX has been observed.

In the same way, as prethrombin-1 is a product of thrombin action on prothrombin, the soluble fibrin appears in the bloodstream as a result of the action of small amounts of thrombin on fibrinogen. Accumulating in small concentrations that are insufficient for clotting, fibrin desA forms oligomers and macromolecular complexes with fibrinogen, circulating in the bloodstream as non-cross-linked soluble fibrin monomeric complexes. Being the direct result of thrombin action, its parameter clearly indicates the activation of blood coagulation even before intravascular clotting (76, 77, 85, 86).

Thus, the level of thrombin as a diagnostic marker has not been neglected during infectious diseases, including COVID-19, and tests that can indicate it directly or indirectly are potentially useful in the prediction of haemostatic abnormalities pertaining to these diseases.

Conclusion

The presence of exosites, active site and Na+ site in the thrombin molecule allows thrombin to recognize a large number of substrates specifically. Thrombin substrates are important for platelet and fibrin clots formation, for activation and inhibition of the blood clotting cascade, for realization of the endothelial anticoagulant function and for the interaction of haemostasis and the immune system. Therefore, thrombin provides wide-scope and multidirectional action in haemostasis. On the other hand, imbalances of various links of haemostasis are reflected in thrombin activity and focus.

This is the reason why a significant number of antithrombotic drugs are aimed at inhibiting thrombin activity. At the same time, inhibition of the general and not procoagulant thrombin activity is not the right approach from the point of view of the current understanding of haemostasis.

Determination of thrombin activity, prothrombin concentration or concentration of prothrombin activation products are not traditional laboratory methods in hemostaseology, despite the fact that thrombin activity is one of the main factors of thrombotic disorders. In our opinion, characterization of the functional state of prothrombin/thrombin should be a required component of everyday laboratory practices.

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Author contribution. Single author article

Original article

Association Between Diverse Diabetic Treatments and Duration of Diabetes Mellitus According to Progression of Diabetic Retinopathy: Experience From a Small Regional Hospital

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Abstract

Introduction: Research objectives of present study were to examine sex and age-related specifics of diabetic retinopathy according to the therapy approach and duration of diabetes mellitus. The study also aimed to determine the association between the presence of diabetic retinopathy and diabetes duration as a prognostic factor of retinopathy progression in such patients.

Materials and Methods: The study was designed as a retrospective study and included 289 patients with diabetic retinopathy, who were treated at the Department of Ophthalmology of the General Hospital "Dr. Josip Benčević" in Slavonski Brod during the period from 2019 to 2020.

Results: 176 patients were treated with oral antidiabetic drugs (OAD), while 113 patients were insulin-dependent. The median age of patients treated with OAD was 77 years. Diabetic retinopathy was present in 35 (19.9%) patients, of whom 33 (18.8%) had non-proliferative diabetic retinopathy, while 2 patients (1.1%) had proliferative diabetic retinopathy. The median age of the insulin-dependent patients was 79 years. Diabetic retinopathy was present in 54 patients (47.8%), non-proliferative diabetic retinopathy was diagnosed in 51 patients (45.1%), while proliferative diabetic retinopathy was diagnosed in only 3 (2.7%) patients. There was a significant difference between the presence of diabetic retinopathy and diabetes duration ($P<0.001$), as well as between the therapy approach and diabetes duration ($\alpha<0.001$).

Conclusion: Various hypotheses have been proposed to explain the worsening of diabetic retinopathy, and we assume that the therapy approach, duration of diabetes and HbA1c have a significant role in retinopathy progression. Hereby, we emphasize that, although there have been significant advances, there is still a pressing need for a better understanding of a new therapeutic modality, new tools for identifying high-risk patients and continued monitoring in order to intervene effectively before vision loss occurs. Further research is needed to identify and implement the best practices to increase diabetic eye screening rates in the long term.

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Introduction

Diabetic retinopathy (DR) is one of the most frequent complications of diabetes mellitus and it remains a leading cause of vision loss globally. Its aetiology and pathology have been extensively studied for half a century, but unfortunately, there are few therapeutic options and prevention of progression is still the ultimate goal. It affects an estimated 126.6 million people worldwide and it is expected to increase rapidly in the future (1).

Even though many studies, such as The Diabetes Control and Complications Trial (DCCT) and The United Kingdom Prospective Diabetes Study (UKPDS), have confirmed a strong relationship between chronic hyperglycaemia and the development and progression of diabetic retinopathy, there is a lack of understanding of the underlying mechanism leading to the development of microvascular damage (2, 3). According to the WHO, diabetic retinopathy is described as a major cause of 5% of vision loss in the developed world and its prevalence is expected to double by 2030. As stated by the American Diabetes Association, 21% of patients with diabetes mellitus have diabetic retinopathy at the moment of first diagnosis of diabetes, and more than 60% of patients develop DR within 20 years after the diagnosis (4). Microvascular damage slowly accumulates in the retinal blood vessels, leading to retinal ischemia, higher retinal permeability, neovascularization and macular oedema, finally resulting in complete vision loss (5-7). The risk of development and progression of diabetic retinopathy is closely associated with the type and duration of diabetes, blood sugar levels, blood pressure levels, proteinuria and possibly hyperlipidaemia (8). Recent studies suggest that apolipoproteins, inflammatory factors and genetic risk factors could also play a role in the development and progression of diabetic retinopathy (9). An ideal model of screening tools for diabetic retinopathy is based on an annual examination of visual acuity and the eye fundus in all diabetic patients. Adults with type 2 diabetes should undergo an eye screening test at the time of diabetes diagnosis. Annual eye exams are recommended, but if there is no evidence of diabetic retinopathy, eye screening every two years thereafter may be considered (10).

Family medicine physicians have adequate knowledge and awareness of diabetic eye screening guidelines. However, they encounter barriers in ensuring that patients undergo screening due to burdensome and complex tasks they are required to complete during the patient's average 15-20-minute visit to the clinic, as well as due to a lack of access to the patients' eye exam records. Patients should undergo follow-ups by an experienced ophthalmologist using precise eye fundus imaging methods at least once a year. Examination of the eye fundus completed with fluorescein angiography make a gold standard in retinopathy diagnosis and classification (11).

Research objectives of the present study were to examine sex and age-related specifics of diabetic retinopathy according to the therapy approach, duration of diabetes mellitus, as well as accompanying comorbidities. The study also aimed to determine the association between the presence of diabetic retinopathy, diabetes duration and HbA1c as prognostic factors of retinopathy progression in such patients.

Materials and Methods

The retrospective study was conducted on 289 patients treated at the Department of Ophthalmology of the General Hospital "Dr. Josip Benčević" in Slavonski Brod, Croatia during the period from 2019 to 2020. The data were collected from the medical records kept by the Department of Ophthalmology of the General Hospital "Dr. Josip Benčević". The inclusion criteria were the presence of diabetes mellitus and biomicroscope presence of diabetic retinopathy. All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

Statistical methods

Categorical data were presented by absolute frequency and percentage, while numerical data were presented by the median, minimum, maximum and interquartile range. Differences in nominal variables were tested by the Fisher's exact test, while differences in numeric variables were tested by the Mann–Whitney U Test because of deviations from the normal distribution. All P-values were two-sided. The level of significance was set at $\alpha = 0.05$. IBM SPSS Statistics was used for the statistical analysis (IBM Corp. Released 2015. IBM SPSS Statistics for Macintosh, Version 23.0. Armonk, NY: IBM Corp.).

Results

This study was conducted on 289 patients, who were divided into two groups based on the therapy approach. The first group (N=176) was treated with oral antidiabetic drugs (OAD), while the second group of patients (N=113) was insulin-dependent (Table 1 and Table 2).

Table 1. Characteristics of patients (N = 176) treated with oral antidiabetic drugs

	Frequency	Percentage
Gender		
Male	72	40.9%
Female	104	59.1%
Smoking		
Yes	5	2.8%
No	171	97.2%
Alcohol consumption		
Yes	2	1.1%
No	174	98.9%
Hypertension		
Yes	160	90.9%
No	16	9.1%
Diabetic retinopathy		
Yes	35	19.9%
No	141	80.1%
Non-proliferative diabetic retinopathy		
Yes	33	18.8%
No	143	81.3%
Proliferative diabetic retinopathy		
Yes	2	1.1%
No	174	98.9%

Table 2. Characteristics of insulin-dependent patients (N=113)

	Frequency	Percentage
Gender		
Male	55	48.7 %
Female	58	51.3 %
Smoking		
Yes	9	8.0 %
No	104	92.0 %
Alcohol consumption		
Yes	2	1.8 %
No	111	98.2 %
Hypertension		
Yes	101	89.4 %
No	12	10.6 %
Diabetic retinopathy		
Yes	54	47.8 %
No	59	52.2 %
Non-proliferative diabetic retinopathy		
Yes	51	45.1 %
No	62	54.9 %
Proliferative diabetic retinopathy		
Yes	3	2.7 %
No	110	97.3 %

The median age of patients treated with OAD was 77 years (interquartile range of 71-84), with a minimum age of 50 years and a maximum age of 95 years. The median age of the insulin-dependent patients was 79 years (interquartile range of 71-83), with a minimum age of 47 years and a maximum age of 96 years. Out of 176 patients who received OAD, 35 (19.9%) had diabetic retinopathy, while 141 (80.1%) did not. A total of 113 patients were insulin-dependent, of whom 54 (47.8%) had diabetic retinopathy and 59 (52.2%) did not (Table 3). The OAD group included 72 male patients (40.9%) and 104 female patients (59.1%). Five patients (2.8%) were smokers and 171 patients (97.2%) were non-smokers. Frequent alcohol consumption was reported by 2 patients (1.1%), while 174 patients (98.9%) did not consume alcohol frequently. Hypertension was diagnosed in 160 patients (90.9%), while 16 patients (9.1%) did not have hypertension. Diabetic retinopathy was present in 35 patients (19.9%), while 141 patients (80.1%) were not diagnosed with diabetic retinopathy. Out of 176 patients, 33 patients (18.8%) had non-proliferative diabetic retinopathy, while 2 patients (1.1%) had proliferative diabetic retinopathy (Table 1)

Table 3. Characteristics of patients receiving oral antidiabetic drug therapy (OAD) or insulin depending on the presence of diabetic retinopathy

	OAD	Insulin	Total	P*
Patients with diabetic retinopathy	35 (19.9)	54 (47.8)	89 (30.8)	<0.001
Patients without diabetic retinopathy	141 (80.1)	59 (52.2)	200 (69.2)	<0.001
Total	176 (100)	113 (100)	289 (100)	

Fisher's exact test

The insulin-dependent group consisted of 55 men (48.7%) and 58 women (51.3%). Out of 113 patients, 9 (8.0%) were smokers and 104 (92.0%) were non-smokers. Frequent alcohol consumption was reported by 2 patients (1.8%). Hypertension was diagnosed in 101 patients (89.4%), while 12 patients (10.6%) did not have hypertension. Diabetic retinopathy was present in 54 patients (47.8%), while 59 patients (52.2%) were not diagnosed with diabetic retinopathy. Non-proliferative diabetic retinopathy was diagnosed in 51 patients (45.1%), while the proliferative type was diagnosed in only 3 patients (2.7%).

The groups differed based on diabetes duration. Patients receiving OAD had a median duration of diabetes of 7 years (interquartile range of 4-12 years), with a minimum duration of 6 months and a maximum duration of 35 years. A median duration of diabetes in the insulin-dependent patients was 12 years (interquartile range of 7-20 years), with a minimum duration of 3 months and a maximum duration of 55 years. Patients diagnosed with diabetic retinopathy had a median duration of diabetes of 14 years (interquartile range of 8.5-20 years), with a minimum duration of 0 months and a maximum duration of 20 years. The patients without diabetic retinopathy had a median duration of diabetes of 7 years (interquartile range of 4-12 years), with a minimum duration of 6 months and a maximum duration of 55 years (Figure 1).

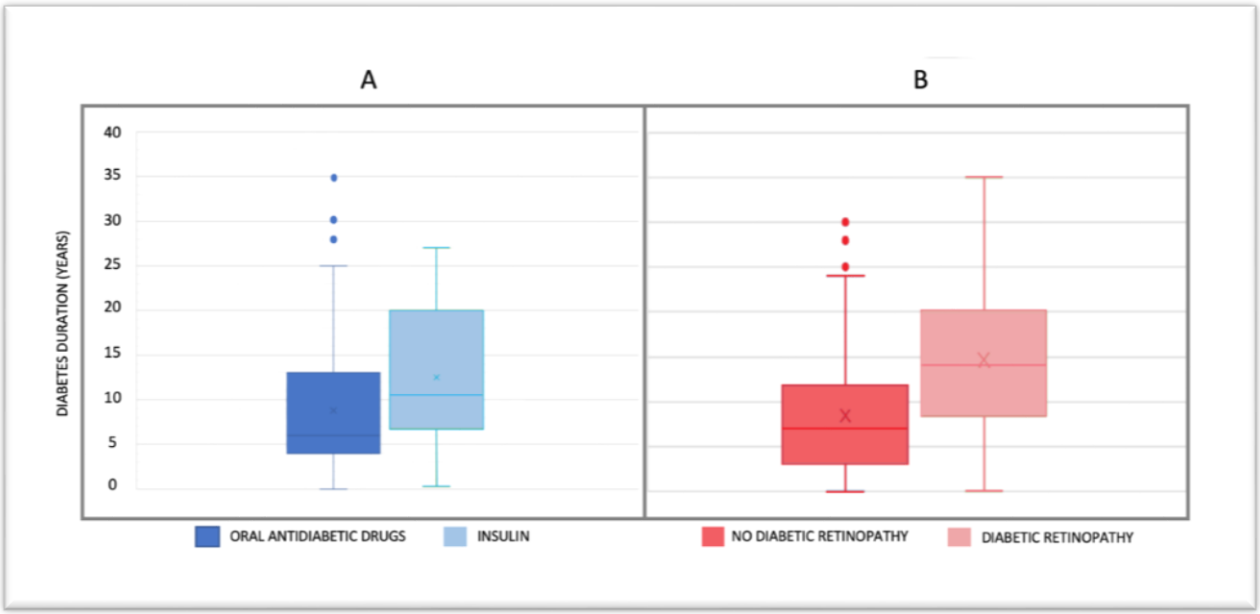

Figure 1. Difference in diabetes duration distribution between patients receiving oral antidiabetic drugs and insulin (A) and between patients with and without diabetic retinopathy (B). p < 0.001 both in Figure 1A and Figure 1B.

Patients diagnosed with diabetic retinopathy had a median duration of diabetes of 14 years (interquartile range of 8.5-20 years), with a minimum duration of 0 months and a maximum duration of 20 years. On the other hand, the patients without diabetic retinopathy had a median duration of diabetes of 7 years (interquartile range of 4-12 years), with a minimum duration of 6 months and a maximum duration of 55 years.

HbA1c concentrations were determined in the patients treated with OAD and insulin. The patients treated with OAD had a significantly lower median concentration of 6.3 mmol/L (interquartile range of 6.1-6.9), with a minimum concentration of 5.5 and a maximum concentration of 7.8 mmol/L. Patients treated with insulin had a median concentration of 7.6 mmol/L (interquartile range of 6.6-8.9), with a minimum HbA1c concentration of 5.5 and a maximum concentration of 12.3 mmol/L (Figure 2).

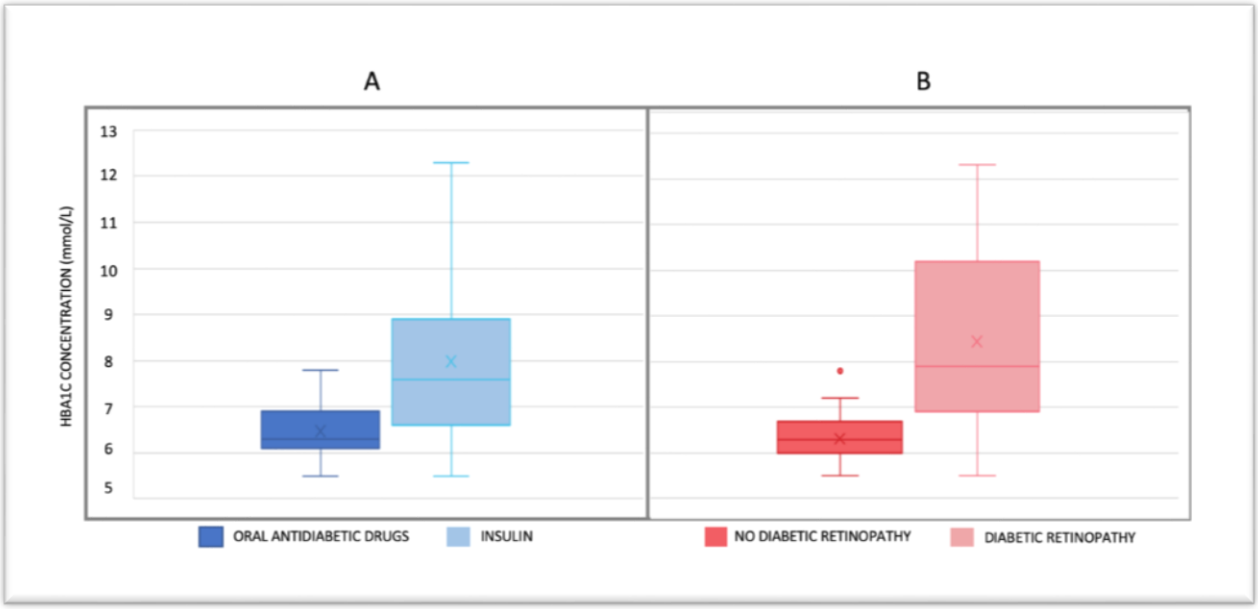

Figure 2. Difference in HbA1c concentration between patients receiving oral antidiabetic drugs and insulin (A) and between patients with and without diabetic retinopathy (B). p < 0.001 both in Figure 2A and Figure 2B.

Discussion

Diabetic eye screening and treatment guidelines are part of the core curriculum for training eye care providers, but the current eye care provider workforce is insufficient to meet the increasing number of diabetic patients. According to epidemiological data, the number of diabetic patients is growing. These patients comprise a large share of eye care provider clinic time, but only one in 20 patients has vision-threatening diabetic eye disease. Our study included 289 patients who suffered from type 2 DM. Patients were divided into two groups according to the therapy approach. There were more patients who used OAD. The majority of the patients using OAD were females who suffered from arterial hypertension, did not consume alcohol and did not smoke. Most of them had no signs of diabetic retinopathy and only two patients had confirmed proliferative diabetic retinopathy (Table 1). In contrast to them, there was a similar number of male and female patients who were insulin-dependent. Most of them suffered from arterial hypertension and half of them had confirmed DR. Only three insulin-dependent patients had proliferative DR. The insulin-dependent patients had more cases of DR confirmed compared to patients who were taking OAD (Table 3). Despite a relatively high prevalence of DR in our study, our results are close to the prevalence of DR reported in the

Region-Specific Information (Europe) and worldwide (Table 3) (12).

Some researchers have pointed out a higher prevalence of DR in patients treated with insulin, suggesting that insulin therapy may be associated with DR and DR severity when compared to the oral antidiabetic drug (OAD) therapy (13). On the other hand, another study, conducted by Gupta et al., has also shown a higher prevalence of DR among insulin users than in patients treated with OAD (52.9% vs 16.3%), discussing how insulin therapy is often started later in the course of the disease, at a stage when glycaemic control is suboptimal for the subject. It was also argued that insulin is simply a marker of disease severity, rather than an independent risk factor for DR, suggesting that starting insulin therapy earlier in the course of the disease might be more beneficial in preventing the development of DR in the longer run (14). In the EURODIAB study, mild forms of non-prolferative diabetic retinopathy (NPDR) were recorded in 25.8%, moderate NPDR in 9.8%, and PDR in 10.6% of insulin-treated patients. The study included 3250 insulin-treated diabetic patients from 13 European diabetes centres, with a mean diabetes duration of 14.7 years. The major factors for vision loss are patient age, diabetes duration, glycosylated haemoglobin and the grade of retinopathy (15, 16).

DM duration is a predictor of diabetic retinopathy (17). Patients with type 1 diabetes develop diabetic retinopathy within five years or less, and only occasionally later, i.e. 27% and 71-90% of patients with diabetes duration of 5-10 and >10 years, respectively. At 20-30 years of diabetes duration, the incidence of diabetic retinopathy increases to 95%. Usually 30-50% of these patients develop proliferative diabetic retinopathy (PDR) (18). In our study, the patients receiving OAD had a median duration of diabetes of 7 years (interquartile range of 4-12 years), while the median duration of diabetes in the insulin-dependent patients was 12 years (interquartile range of 7-20 years). The median age of the patients receiving OAD was 77 years (IQR of 71-84), while the median age of the patients treated with insulin was 79 years (IQR of 71-83). There was a significant difference between the presence of diabetic retinopathy and diabetes duration ($\alpha<0.001$) (Figure 1A). Thus, most of the patients were elderly persons with a comorbidity (e.g. arterial hypertension). Even though the prevalence of DM is relatively high among elderly patients, the incidence of DR and PDR in our study is close to the results of studies conducted in other European countries (12). There are several factors which could explain these observations, some of which are a relatively high quality of life in the last 10 years, newly designed OAD and combinations of OAD, free physical examinations twice or thrice a year and morphological characteristics of the eye structure in elderly persons (posterior vitreous detachment), resulting in slower progression of DR than that observed (19).

Only a few participants in present study consumed alcohol, so alcohol can be excluded as a risk factor and is not directly associated with the presence or progression of diabetic retinopathy (Table 1 and Table 2). Our results are similar to the multicentric study by Lee CC et al., who investigated the association between alcohol consumption and diabetic retinopathy and deterioration of visual acuity in individuals with type 2 diabetes, concluding that alcohol consumption is associated with an increased risk of deterioration of visual acuity, but not with retinopathy in individuals with type 2 diabetes (20). The relationship between cigarette smoking and diabetic retinopathy was examined earlier and data suggest that there is no excess risk of retinopathy in smokers or ex-smokers when contrasted with those who have never smoked. Our study produced similar results due to the fact that a small number of participants consumed cigarettes (Table 1 and Table 2) (21). Landmark multi-centre, randomised controlled trials showed that early identification and proper treatment can prevent the risk of vision loss by 90%, but fewer than 50% of people with diabetes in the USA follow diabetic eye screening guidelines and even lower screening rates (10-20%) have been described (22). Once retinopathy is present, the duration of diabetes appears to be a less important factor than glycaemic control in forecasting progression from earlier to later stages of retinopathy (23). On the other hand, the

link between HbA1c levels and diabetic retinopathy is not conclusive because there are other variables that come into play. In our study, the patients treated with oral diabetic drug therapy had a significantly lower median concentration of HbA1c, without the presence of diabetic retinopathy (Figure 2A and B). Maintaining control of glucose and blood pressure lowers the risk of retinopathy progression and patients should be aware of the importance of maintaining good levels of glycosylated haemoglobin and blood pressure.

Considering a higher prevalence of DM globally, family medicine doctors should improve additional educational programs in diabetic retinopathy screening because multiple workflow and systems-level barriers affect care providers and there is not enough time to follow all diagnostic features in everyday clinical practices (24). The study by Olafsdottir E et al. discusses the benefits of regular screening for diabetes mellitus and diabetic eye disease as the gold standard in preventing diabetic blindness. According to that study, the loss of vision from diabetic retinopathy is uncommon if regular screening is provided and subsequent hospital costs are also lower. The same idea has been confirmed in the study by Bandurska-Stankiewicz E et al., who have confirmed that the incidence of vision loss due to diabetes is significantly lower in the countries which have introduced programs for preventing retinopathy than in those countries which do not have such programs (25, 26). While current evidence indicates that the association between the glucagon-like peptide-1 receptor agonists (GLP-1RA), sodium-glucose cotransporter-2 (SGLT-2) inhibitors and dipeptidyl peptidase-4 (DPP-4i) inhibitors and the risk of DR remains uncertain in patients with T2DM, future studies should focus on such types of drugs, especially on the combinations and prevalence of DR, PDR and NPDR in large-scale, well-designed studies (27).

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Once retinopathy is present, the duration of diabetes appears to be a less important factor than glycaemic control in forecasting progression from earlier to later stages of retinopathy. In our study, there is a lack of information about the type of OAD and DR, so in future studies, we will pay more attention to the association of DR, new OAD and combinations of therapy. In addition, opportunities exist in leveraging team-based care approaches, patient self-management programs and emerging telemedicine imaging technologies.

Conclusion

Our study has confirmed the results of previous studies, namely that the risk of development and progression of diabetic retinopathy is closely associated with the type and duration of diabetes and HbA1c concentration. Also, we emphasise that there is still a pressing need for a better understanding of a new therapeutic modality, new tools for identifying high-risk patients and continued monitoring in order to intervene effectively before vision loss occurs. Further research is needed to identify and implement the best practices to increase diabetic eye screening rates in the long term. There is a lack of additional educational programs in primary health care of diabetic retinopathy screening and a lack of large-scale, well-designed studies of diabetic retinopathy occurrence associated with glucose-lowering drugs.

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Competing interests. None to declare.

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Author contribution. Zvonimir Bosnic and Bozidar Kovacevic were responsible for the conceptualisation and design of the study. Stjepan Kovacevic and Dinko Nizic performed the investigation and collected the data. In addition, they were responsible for data validation. Ana Bardak performed the statistical analysis. Zeljka Vukovic Arar and Bozidar Kovacevic provided participants with the data. Sandra Sekelj and Zeljka Vukovic Arar supervised the study. Zvonimir Bosnic and Ana Bardak wrote the manuscript. Bozidar Kovacevic and Sandra Sekelj reviewed and edited the manuscript. All authors have read and agreed on the published version of the manuscript

Original article

Quality of Life Assessment in Type 2 Diabetes Patients With Cardiovascular and/or Diabetic Complications

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Abstract

Introduction: Type 2 diabetes mellitus is a chronic disease that is causing enormous economic and social costs. It is characterized by many microvascular and macrovascular complications, such as heart attack, stroke, retinopathy, nephropathy, neuropathy, etc. Such complications can cause severe limitations and decrease the quality of life. The objective of this study was to assess the effect of type 2 diabetes mellitus on the quality of life using the EQ-5D-5L questionnaire, taking into account cardiovascular complications (heart attack, hospitalization due to angina pectoris, stroke, hospitalization due to heart insufficiency, transient ischemic attack, coronary revascularisation), complications of diabetes (microalbuminuria, renal failure, retinopathy, and neuropathy), and demographic characteristics (age, gender, body mass index, height, and weight).
Materials and Methods: This cross-sectional study included 484 participants with type 2 diabetes mellitus. Quality of life was estimated by the EuroQol instrument EQ-5D-5L and visual analogue scale (VAS). The following complications related to type 2 diabetes were taken into account: heart attack, hospitalization due to angina pectoris, stroke, hospitalization due to heart insufficiency, transient ischemic attack, coronary revascularization, microalbuminuria, renal insufficiency, retinopathy, and neuropathy.
Results: The mean value of the EQ index was 0.895, with the value of -0.59 as the lowest, and 1.0 as the highest quality of life of the study patients. Multivariate linear regression model showed that heart attack, hospitalization due to unstable angina pectoris, retinopathy, and neuropathy significantly decreased the quality of life of the study participants ($p<0.05$). Spearman's correlation showed that there was a significant correlation between age, height, duration of type 2 diabetes, body mass index, and the EQ index ($p<0.001$).
Conclusion: The results suggest that type 2 diabetes complications, such as heart attack, neuropathy, retinopathy, and hospitalization due to unstable angina pectoris significantly decrease the quality of life of type 2 diabetes mellitus patients (T2DM).

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KEYWORDS: quality of life, diabetic complications, type 2 diabetes mellitus

Introduction

There are two types of methods that can be used for measuring health utilities: direct and indirect. So far, several direct methods have been developed, such as: 1) standard gamble: reflects the assessment of an individual's preference or valuation of their current QoL by quantifying tolerance to risk toward an ultimate bad outcome as a gamble for achieving a perfect state of health (1); 2) time trade-off: individuals are asked to choose between living in their current impaired health state for 10 years or living for a shorter period with perfect health (2); and 3) visual analogue scale: introspective scaling with a rating scale from 0 to 100, where a patient has to choose one number on this scale that correctly reflects his/her quality of life (3). On the other hand, there are many indirect methods which have already been validated, and they can be generic (e.g. the EQ-5D questionnaire) or specific for certain diseases (e.g. FACT-G). Indirect methods do not give immediate values for health utilities; rather, an algorithm must be developed for a transformation of answers to utility values (3).

T2DM is recognized as one of the most common diseases worldwide, which affects every country and different groups of people, regardless of the demographic and socio-economic status (4). Microvascular and macrovascular complications are almost inevitable during the course of the disease. The most common microvascular complications are diabetic nephropathy, neuropathy, and retinopathy, while the most common macrovascular complications are peripheral vascular disease, myocardial infarction, stroke, and congestive heart failure (5). Quality of life assessment in patients with T2DM is important for the development of progressive simulation models used for a long-term assessment of the cost and effectiveness of interventions for the treatment of type 2 diabetes and prevention of accompanying complications. Assessing the utility is the main prerequisite for determining the QALY and it is very important to include participants with a full spectrum of characteristics and health complications (retinopathy, neuropathy, nephropathy, etc.) (6). Zhang et al. conducted a study in the United States of America (USA) on 7327 participants with T2DM. The average EQ index (utility) in this study was 0.80. They concluded that participants without complications of type 2 diabetes, cardiovascular risk factors, and other comorbidities, who were not obese and who had an average income of over USD 40,000, had a better average EQ index, reaching 0.92 (7). Arifin et al. also concluded that T2DM patients with macro/microvascular complications have a lower QoL (0.77-0.79) than those without such complications (0.80). Also, if they have more than two complications, their QoL is even lower (0.74) (8). Pham et al. showed on 214 T2DM patients that those with diabetic retinopathy have a lower QoL (0.92) compared to those who have diabetic heart disease (0.94) or nephropathy (0.93) (9). A study in Japan showed that T2DM patients with more serious microvascular complications have a lower QoL compared to those who have one or no complications. Patients with proliferative retinopathy had a QoL of 0.85, while those with pre-proliferative retinopathy had a better quality of life (0.93). Also, those suffering from stage four nephropathy had a QoL of 0.78, while the QoL of those suffering from stage one nephropathy was as high as as 0.93 (10). There are also other studies showing that patients with complications of type 2 diabetes not only live shorter, but their quality of life is also lower (11,12). However, there is a paucity of studies using the EQ-5D-5L questionnaire for a quality of life assessment in T2DM patients, and their quality estimates vary within a broad range from 0.74 to 0.92 (13-17). EQ-5D-5L questionnaire is a relatively new preference-based instrument with lower ceiling effects and a better sensitivity compared to the EQ-5D-3L. Those two questionnaires are most commonly used in clinical and outcomes research (18).

The aim of this study was to assess the effect of type 2 diabetes mellitus on the quality of life using the EQ-5D-5L questionnaire, taking into account cardiovascular complications (heart attack, hospitalization due to angina pectoris, stroke, hospitalization due to heart insufficiency, transient ischemic attack, and coronary

revascularisation), complications of diabetes (microalbuminuria, renal insufficiency, retinopathy, and neuropathy) and demographic characteristics (age, gender, body mass index, height, weight, smoking status, duration of diabetes, and education level).

Materials and Methods

Study population

This was a cross-sectional study, approved by the Ethics Committee of the Public Institution Health Center of Sarajevo Canton, which included 509 patients from Bosnia and Herzegovina. Between December 2019 and June 2020, 361 patients in total were interviewed in person, while 148 patients were interviewed online. Interviews in person were conducted at several health centers in Sarajevo, such as the Vrazova, Omer Maslić, and Kumrovec Health Center, (urban areas) and Ilijaš Health Centre (rural areas). After reviewing the answers, 484 participants were eligible for the final analysis, while 25 were excluded due to incompleteness. Criteria for inclusion in the study were: diagnosis of T2DM in the patient file, age >18 years, and signed informed consent for participation in the study. Criteria for non-inclusion or exclusion from the study were: persons under the age of 18, not signing the informed consent, pregnancy and/or a diagnosis of a major psychiatric disorder in the patient file.

Questionnaire

In order to collect the patient information, a tripartite questionnaire was used, assessing the demographic/socio-economic characteristics (age, gender, height, weight, education level, duration of type 2 diabetes, and smoking status), quality of life by the EQ-5D-5L questionnaire and by the visual analogue scale (VAS), and cardiovascular and diabetic complications.

The following events were taken into account, reflecting the complications of type 2 diabetes: heart attack, hospitalization because of unstable angina pectoris, stroke, hospitalization because of heart failure, transient ischemic attack, repeated coronary revascularization, microalbuminuria, renal insufficiency, neuropathy, and retinopathy.

EQ-5D-5L questionnaire

The EQ-5D-5L is a generic (19-23) questionnaire validated for the assessment of the quality of life of individuals with various health conditions. It assesses five dimensions: mobility, self-care, usual activities, pain/discomfort, and anxiety/depression. Each dimension is ranked at five levels: no problems – 1, slight problems – 2, moderate problems – 3, severe problems – 4, and extreme problems – 5. After filling in the questionnaire, depending on the patients' answers, the five dimensions are combined in a five-digit number that describes the patient's health state. The five-digit code is further converted to an EQ index using an automatic calculator (24) and the results from our study were checked using the guidelines from a validation study conducted in Poland (25). Since there was no local set of values that had been validated, Poland was selected due to its cultural similarity with Bosnia and Herzegovina. The EQ index ranged between -0.59 (the lowest QoL) and 1 (the highest QoL). The EQ-VAS scale was used together with the EQ-5D-5L questionnaire (26).

A validated Croatian version of EQ-5D-5L was used in our study, since the official language of Bosnia and Herzegovina is the Bosnian/Croatian/Serbian (BHS) language. A laptop/desktop version of the questionnaire was obtained from EuroQol.

Statistical analysis

The results were described by frequencies and proportions for categorical values, and by the arithmetic mean and standard deviation for continuous values (or by the median with interquartile range, if the data were not normally distributed). Normality of data distribution was tested by the Shapiro Wilk and Kolmogorov-Smirnov tests. Nonparametric tests, such as the Mann-Whitney U test, the Kruskal-Wallis test, and non-parametric ANOVA, were used for comparison of the study groups. Impact of health complications, demographic

characteristics, and health state were assessed by a multivariate linear regression model after confirming that the following assumptions were satisfied: linear relationship, independence, homoscedasticity and normality (27). Results with $\alpha \leq 0.05$ or within the 95% confidence interval were considered statistically significant. SPSS for Windows (version 21.0, SPSS Inc, Chicago, Illinois, USA) and Microsoft Excel (version 11.0, Microsoft Corporation, Redmond, WA, USA) were used for statistical analysis.

Results

The final analysis included 484 participants (28.7% men and 71.3% women). Mean duration of T2DM was 8.18 ± 7.08 years and the majority of participants were taking only oral antidiabetic drugs (55.8%) or oral drugs in combination with subcutaneous medication (41.7%). Other demographic characteristics of the study participants are shown in Table 1. More detailed results are shown in Supplement 1.

Table 1. Main demographic/socioeconomic characteristics of study participants (n=484).

Characteristic	Minimum	Maximum	Mean	Median	Std. deviation
Age (years)	20	92	52.68	53	12.11
Body weight (kg)	47	186	89.00	88	18.27
Height (m)	1.45	2.00	1.70	1.69	0.08
Duration of type 2 diabetes (years)	0.02	40	8.18	6.00	7.08
Body Mass Index (BMI) (kg/m²)	18.36	55.54	30.70	30.12	5.68

Characteristic	n (%)
Men	139 (28.7)
Women	345 (71.3)
Education level	
Primary school	17 (3.5)
High school education	275 (56.8)
Associate degree	61 (12.6)
Bachelor's degree	124 (25.6)
Master's/doctor's degree	7 (1.4)
Smoking status	
Yes, active smoker	188 (38.8)
No, never smoked	153 (31.6)
No, stopped smoking	143 (29.5)
Type of therapy	
Oral	270 (55.8)
Oral + subcutaneous	202 (41.7)
Subcutaneous	12 (2.5)

The majority of participants did not have problems with mobility (61%), but 1% of them had extreme problems. Also, most of them did not have problems with self-care (79.5%) and usual activities (62.8%). On the other hand, a high percentage of participants had slight or moderate problems with pain/discomfort and anxiety/depression. More details about the participants' answers are shown in Table 2..

Table 2. Distribution of the EUROQOL 5D-5L answers given by study participants (n=484) according to five dimensions of this questionnaire

	No problems	Slight problems	Moderate problems	Severe problems	Extreme problems
Mobility	295 (61%)	76 (15.7%)	85 (17.6%)	23 (4.8%)	5 (1%)
Self-care	385 (79.5%)	45 (9.3%)	42 (8.7%)	8 (1.7%)	4 (0.8%)
Usual activities	304 (62.8%)	86 (17.8%)	73 (15.1%)	15 (3.1%)	6 (1.2%)
Pain/discomfort	214 (44.2%)	141 (29.1%)	100 (20.7%)	25 (5.2%)	4 (0.8%)
Anxiety/depression	201 (41.5%)	137 (28.3%)	109 (22.5%)	31 (6.4%)	6 (1.2%)

Using the EQ-VAS scale, the participants indicated how they felt at the moment of the interview. The minimum value was 5 and maximum was 100, with 70.40 (SD 20.49) points as the average value. The lowest EQ index was -0.59 and the highest one was 1.0, with 0.895 (SD 0.183) on average (Table 3).

Table 3. Scores on EQ VAS scale and EQ index of study participants (n=484)

	Minimum	Maximum	Mean	Median	Std. deviation	Kolmogorov-Smirnov
EQ VAS scale	5	100	70.40	75	20.49	p = 0.000
EQ INDEX	-0.59	1.00	0.895	0.952	0.183	p = 0.000

Most of the participants did not have any cardiovascular/diabetic complications (46.90%) or had just one (23.97%). The most common complications were neuropathy (31.4%) and retinopathy (28.9%), while microalbuminuria (2.7%) and stroke (3.1%) were the rarest. The most common events occurring due to cardiovascular complications were hospitalization due to unstable angina pectoris (8.3%) or heart attack (8.1%). Renal failure was reported in 9.1% of the cases. More details can be seen in Supplements 2 and 3.

Spearman's correlation showed that there was a significant correlation between age, height, duration of type 2 diabetes and BMI ($p<0.001$) with the EQ index. There was a negative correlation between BMI, age and duration of type 2 diabetes and the EQ index (Table 4).

Table 4. Correlation between demographic characteristics and EQ index of study participants (n=484)

	Spearman's correlation coefficient	p*
Height	0.223	0.000
Body weight	0.019	0.675
BMI	-0.106	0.000
Age	-0.254	0.000
Duration of type 2 diabetes	-0.228	0.000

We analysed whether gender (Mann-Whitney U test) and level of education/smoking status (Kruskal-Wallis test) influenced the EQ index values. The results showed a significant influence of gender (U=19526.500, p=0.001) and level of education (Kruskal-Wallis H 9.949,

p=0.041) on the EQ index. Smoking status did not affect the quality of life (Kruskal-Wallis H 1.082, p=0.582).

Multivariate linear regression was used to determine the effects of study variables on the quality of life. The EQ index was appointed as a dependent variable, and gender, age, level of education, weight, height, BMI, duration of type 2 diabetes, and cardiovascular/diabetic complications were used as independent variables in the analysis. Results showed that four complications – neuropathy, heart attack, stroke, and hospitalization because of unstable angina pectoris – had the greatest impact on the quality of life. These four dependent variables explained 49.3% of model variance (R=0.493, F=34.550, p=4.98*10-25) (Table 5).

Table 5. Effects of certain diabetic complications on the QoL of study participants (n=484): results of multivariate linear regression

Model	B	Sig.	95% confidence interval for B	
			Lower bound	Upper bound
Neuropathy	0.675	0.000	0.610	0.739
Neuropathy	0.115	0.000	0.079	0.150
Heart attack	0.178	0.000	0.119	0.237
Neuropathy	0.103	0.000	0.069	0.138
Heart attack	0.174	0.000	0.117	0.230
Stroke	0.263	0.000	0.176	0.350
Neuropathy	0.097	0.000	0.062	0.944
Heart attack	0.142	0.000	0.080	0.807
Stroke	0.249	0.000	0.162	0.970
Unstable angina	0.081	0.017	0.147	0.776

Discussion

This cross-sectional study conducted in Bosnia and Herzegovina investigated the association between type 2 diabetes, its complications and the QoL using the EQ-5D-5L questionnaire. EQ-5D-5L is an easy-to-understand and validated generic questionnaire for a QoL assessment (28). BMI, age, and duration of T2DM showed a negative correlation with the QoL, meaning that older patients, with a higher BMI and longer duration of illness had a lower QoL. The results also showed that most of the participants did not have problems with mobility or self-care, but they had problems with pain/discomfort and anxiety/depression, which is similar to previous studies. Regarding T2DM patients, Luk et al. reported that more patients had problems with pain/discomfort (24.8%) and anxiety/depression (20.3%) than with other dimensions of the EQ-5D-5L questionnaire (29). In several other studies, it has been shown that pain/discomfort was the most affected domain in patients (30-32). Such results may be due to the fact that the most common T2DM complication was neuropathy, which may cause pain/discomfort. Also, T2DM can change mood and lower self-esteem, leading to anxiety and depression (33). A study by Parik and Patel showed that anxiety and depression have a stronger impact on younger T2DM patients (34). A CODE-2 study on 1,371 T2DM participants showed that anxiety and depression were increased at a younger age and

then decreased with age (35). Auslli et al. suggested that patients with T2DM should be advised carefully about the importance of foot care and exercise. They concluded that physical exercise is a very important determinant of positive clinical outcomes of T2DM and it is associated with better HbA1C levels, a lower BMI, fewer diabetic complications and a higher QoL (36).

Our results also showed that the QoL is dependent on gender, age, and education level, where females, older participants and those with a lower level of education had a lower QoL. Smoking status and weight did not have a significant impact on the QoL. A study in Korea showed that age is an important factor of the QoL of patients with T2DM and that younger participants had a better QoL, most likely because they had T2DM for a shorter period of time and fewer health-related complications (37). Stojanović et al. also showed that lower level of education was highly related to lower QoL. Possible mechanisms include insufficient access to healthcare services, unhealthy habits, poor mental health, and higher frequency of complications (38). A study in Denmark, involving 2419 patients with T2DM, showed that poor socioeconomic status, age, female gender, presence of comorbidity, poor glycemic control and lower level of education were associated with depressive episodes and lower QoL of T2DM participants (39). There have been many other studies showing that these factors are strong determinants of the QoL (37-50).

Most of the participants did not have any diabetic complications (46.90%). Among those who had some of the complications, the most common were neuropathy (31.4%) and retinopathy (28.9%), while microalbuminuria and stroke were the rarest. Data from the American Diabetes Association shows that 75-80% of adults with diagnosed DM will ultimately die from cardiovascular disease due to chronic macrovascular complications (41). Microvascular and macrovascular complications of T2DM put a great burden on every healthcare system, even in developed countries like the USA. However, this is not the only problem. Many studies have shown that patients with T2DM who have one or more diabetic complications also have a significantly lower QoL compared to those who do not have T2DM-related complications (42-46).

In our study, the values of the VAS scale varied from 5 to 100, with 70.40 as the average value. After converting the scores to the EQ index, the lowest value was -0.59 and the highest was 1.0., with 0.895 as the average value. According to Mlitt et al., a negative index is possible when researchers use the EQ-5D-5L questionnaire (40). It suggests that the health state of these participants is deemed to be worse than death. The VAS scale score can vary between different group of participants. A Norwegian study of T2DM patients showed a mean EQ index of 0.85 (51), while an Iranian study reported an even lower average value (0.70) (52).

A multivariate linear regression model based on our data showed that factors such as neuropathy, heart attack, stroke, and hospitalization due to angina pectoris are strong predictors of the QoL, which is a finding congruent with the results of previous studies analysing patients with diabetes type 2. A univariate and multivariate regression analysis by Stojanović et al. also showed that angina pectoris, heart failure, diabetic retinopathy, and diabetic nephropathy are factors that impact the QoL of T2DM patients the most (38). A study in China, which was conducted on 1,275 patients with T1DM, showed that the presence of any of the four major diabetic complications (heart disease, stroke, end-stage renal disease, sight-threatening diabetic retinopathy) have a significant impact on the QoL (48). The United Kingdom Prospective Diabetes Study showed that utilities are significantly reduced in T2DM patients who have serious complications, like end-stage renal disease, blindness, stroke or heart attack (50). There is a plethora of other studies showing that T2DM patients with complications have a decreased QoL compared to T2DM patients without such complications (12,49,51,53,54).

Our study has certain limitations. Firstly, the EQ-5D-5L questionnaire was not previously validated in Bosnia and Herzegovina. Therefore,

the transformation of raw scores to the EQ index had to be based on the results from another population that is as similar as possible to the Bosnian population in terms of culture (the Polish population), which still creates a certain potential for bias. Secondly, the sample used in the study was not ideally balanced in regard to frequency and severity of type 2 diabetes complications, which could have led to an overestimate of the EQ scores.

Conclusions

Our study gave an overview of average EQ index values in the population of diabetes type 2 patients from Bosnia and Herzegovina, and showed a prevailing influence of diabetic complications on the quality of life, which could be used for further pharmacoeconomic analyses. Demographic characteristics, such as the BMI, age and duration of T2DM were negatively correlated with the QoL, meaning that patients with a higher BMI, who are older and have suffered from the illness for a longer period of time, had a lower QoL. Neuropathy, heart attack, stroke, and hospitalization due to unstable angina had the greatest impact on the QoL in T2DM patients.

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SUPPLEMENTS

Supplement 1. EQ Index mean values (n=484)

	Number of patients (n=484)	EQ Index: mean (SD)
Gender		
Male	139	0.894 (0.250)
Female	345	0.895 (0.148)
Age (years)		
18-25	7	0.940 (0.838)
26-35	32	0.926 (0.106)
36-45	101	0.934 (0.100)
46-55	151	0.914 (0.134)
56-65	115	0.888 (0.189)
66+	78	0.803 (0.304)
Education level		
Primary school	17	0.810 (0.181)
High school education	275	0.902 (0.165)
Associate degree	61	0.882 (0.234)
Bachelor's degree	124	0.925 (0.126)
Master's/doctor's degree	7	0.798 (0.216)
BMI (kg/m²)		
18-24.99	95	0.884 (0.257)
25-29.99	134	0.880 (0.216)
30-34.99	161	0.920 (0.115)
>35	94	0.885 (0.128)
Duration of diabetes (years)		
0.01-2.99	121	0.945 (0.077)
3.00-5.99	107	0.888 (0.186)
6.00-8.99	67	0.910 (0.121)
9.00-14.99	119	0.904 (0.107)
> 15	70	0.788 (0.349)
Smoking status		
Yes, active smoker	188	0.897 (0.175)
No, never smoked	153	0.890 (0.164)
No, stopped smoking	143	0.889 (0.214)

Supplement 2. Type 2 diabetes - related complications

Complication	n (%)
Heart attack	
Yes	39 (8.1)
No	445 (91.9)
Hospitalization due to unstable angina pectoris	
Yes	40 (8.3)
No	444 (91.7)
Stroke	
Yes	15 (3.1)
No	469 (96.9)
Hospitalization due to heart failure	
Yes	21 (4.3)
No	463 (95.7)
Transient ischemic attack	
Yes	27 (5.6)
No	457 (94.4)
Coronary revascularisation	
Yes	35 (7.2)
No	449 (92.8)
Microalbuminuria	
Yes	13 (2.7)
No	471 (97.3)
Renal insufficiency	
Yes	44 (9.1)
No	440 (90.9)
Retinopathy	
Yes	140 (28.9)
No	344 (71.1)
Neuropathy	
Yes	152 (31.4)
No	332 (68.6)

Supplement 3. Number of complications discovered in the study population

Number of complications	n (%)
Without complications	227 (46.90)
One complication	116 (23.97)
Two complications	75 (15.50)
Three complications	26 (5.37)
Four complications	26 (5.37)
Five complications	9 (1.86)
Six complications	3 (0.62)
Seven complications	1 (0.21)
Eight complications	1 (0.21)

Author contribution.
Mehović S., Janković S., and Zana S. conceived the study design and participated in data collection. Mehović S. and Janković S. led the data analysis and interpretation. All authors approved the final manuscript.

Original article

Body Components Differences and Their Impact of Phase Angle Values in Athletes and Non-Athletes

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Abstract

Introduction: The aim of this paper was to determine PhA values in athletes and sedentary population. The specific aim was to determine differences between subjects of the same sex and with a different level of physical activity, as well as the factors affecting PhA values.

Materials and Methods: Sixty-six athletes and sedentary students participated in the research. They were divided into four groups according to sex and level of physical activity. Routine BIA at 50 kHz was performed and BMI, PBF, FFM, PMM, TBW, ECW, ICV, ECW/ICE ratio, BMR, BM, PhA and impedance were measured.

Results: Male athletes had higher PhA values (6.85±0.5°) compared to male non-athletes (6.29±0.67°), female athletes (5.61±0.44) and female non-athletes (5.47±0.58°). Statistically significant differences were found in men (PhA p=0.004; ECW/ICE ratio p=0.002), but not in women. The highest positive correlation was found in ICW (ρ +0.71 p≤0.01), while the highest negative correlation was found in impedance (ρ -0.79 p≤0.01). PhA variance was mostly due to PMM (B=+0.44, p=0.002).

Conclusion: Differences found in male athletes and non-athletes may suggest the influence of physical activity, since the variance in PhA values was mostly due to PMM and a positive correlation with ICW.

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KEYWORDS: athletes, students, bioelectrical impedance, body mass

Introduction

During recent years, body composition measurements have been particularly important due to the obesity epidemic and related illnesses, such as cardiovascular diseases and metabolic and endocrine disorders (1). Such measurements can be useful to predict clinical outcomes in children and adults (2) and can be used to characterise physique changes during growth, aging and training. However, they sometimes require expertise regarding the measurement of skinfolds, girths and skeletal breadths (3). Other methods used to asses body composition, such as computed tomography (CT), CT body composition (CTBS), magnetic resonance imaging (MRI), ultrasound (US) or dual-energy X-ray absorptiometry (DEXA), may not always be available or an appropriate choice (1). Cost-efficient, non-invasive and simple assessment of body composition and hydration status remains an important need, which is why bioelectrical impedance (BIA) is widely used (4, 5). BIA is considered superior even to some serum (albumin, transferrin) or anthropometric indicators (weight change, arm muscle circumference, triceps skin-fold thickness) (6, 7).

BIA measures the electrical characteristics of the human body either at 50 kHz or at several frequencies ranging from 1 to 1,000 kHz (5). It is the only method that allows for keeping track of body hydration and cell mass using the phase angle (PhA) and impedance (8). Although the PhA does not indicate body composition, but gives information on tissue capacity, cell size and cell membrane integrity, it correlates with cell mass, nutrition and general health both in children and adults and provides information on tissue resistance, which depends on lean body mass hydration (2). PhA is obtained by measuring the ratio between impedance (R) and reactance (Xc) based on the following equation (6):

$$PhA = Xc/R \times 180/\pi$$

Impedance is defined as a reduction in voltage due to the passage of current through the ionic solution of the body, while reactance is a delay in the passage of current measured as a phase shift. Both measures suggest cell function (9), correlate with body shape and influence the difference in the size and function of the body (3). Also, in adults, it is significant in the prognosis of the outcomes and mortality of haemodialysis, cancer, acquired immunodeficiency syndrome and cirrhosis patients (2).

According to Bosy-Westphal et al. (10), gender and age are the main determinants of the PhA in healthy adults, with men and younger people having higher PhA values (10). As far as body mass is concerned, the PhA increases with an increase in BMI to the value of 40 kg/m^2, after which it shows a negative correlation (10). In addition to age and gender, FFM and height have the strongest influence on PhA values, while the ECW/ICW fluid ratio shows a correlation with the PhA in a clinical environment and obese people (11). Body composition assessment in sports may be useful for estimating total body water (TBW) and FFM, sport performance and effects of a training program (5), whereas higher values of the PhA may be linked to a higher level of physical activity (12). The potential of the PhA and bioimpedance on the whole lies in the fact that it allows for non-invasive tissue monitoring, especially of hydration (1), and it can help with recommendations concerning the volume and intensity of training in sports (13).

The general aim of this paper was to determine PhA values in younger adult athletes and sedentary population. The specific aim was to define which body components the subjects of the same sex, but with a different level of physical activity differ in, as well as which body components relate to the PhA the most. Our hypothesis was that athletes will have higher PhA values than non-athletes. Also, we expected differences between subjects of the same sex, but with a different level of physical activity.

Materials and Methods

The research was performed at the College of Applied Sciences "Lavoslav Ružička" in Vukovar in June 2017, during the competition season. A

total of 66 student volunteers, including active male and female athletes and male and female non-athletes living a sedentary lifestyle, participated in the research. The inclusion criteria for the athletes were the following: 1) age < 35 years, 2) no medical problems according to self-reported information, 3) no smoking or alcohol abuse according to self-reported information, 4) participation in 3-5 training sessions per week and 5) involvement in organised sport activities. The inclusion criteria for the non-athletes were the following: 1) no medical problems reported 2) age < 35 years, 3) no smoking or alcohol abuse reported. Subjects were divided into four groups – male athletes – MA (26 subjects), female athletes – FA (8 subjects), male non-athletes – MNA (22 subjects) and female non-athletes – FNA (10 subjects). The most common men's sports were football and handball, whereas the most common women's sports included handball and volleyball.

The aim of the research and the procedure of BIA measurement was explained to the students. All subjects granted their informed consent for routine BIA and the research was carried out in accordance with the Declaration of Helsinki for medical research involving human subjects. The subjects arrived at 8:00 AM for the BIA exam, on an empty stomach and with an empty bladder. The measurement was performed in a standing position. The students were barefoot, wearing shorts and a T-shirt, with their hands held out and away from the body, feet slightly apart. Prior to the measurement, the participants were advised to sit for 10 minutes (equilibrium period). Earlier on, they had been instructed not to drink alcohol eight hours and not to consume any food four to six hours prior to the measurement. Also, the subjects did not do any physical activity the day before the measurement. Regarding room preparation, the examination was conducted in a lit up, pleasantly air-conditioned room (22 °C). Machine preparation included verification in accordance with the manufacturer's instructions. The measurement was conducted on the TANITA MC-780MA body mass analyser (TANITA Corporation, 1-14-2, Maeno-cho, Itabashi-ku, Tokyo, Japan, 2013).

Height was measured to the nearest 0.1 cm using a stadiometer (Seca, Hamburg, Germany). PhA and impedance were measured using BIA, as well as other body components: BMI, percent body fat (PBF), FFM, TBW, extracellular water (ECW), intracellular water (ICW), extracellular water/intracellular water ratio (ECW/ICW ratio), percent muscle mass (PMM), bone mass (BM) and basal metabolic rate (BMR).

Statistical analysis

Results are presented as mean ± SD. Normal distribution of data was evaluated using the Shapiro–Wilk test. Differences between all four groups were determined by the Kruskal–Wallis H independent samples test. Statistical significance was determined at the $p \leq 0.001$ level. The post hoc Mann–Whitney U test with Bonferroni correction was done to establish the differences between male athletes and non-athletes and female athletes and non-athletes. Statistical significance was set at the $p \leq 0.03$ level. The Spearman's rank correlation coefficient (ρ) was used to determine the correlation between the PhA and other variables. The Stepwise-Backward regression was applied in order to determine which variables influenced the PhA the most. Statistical significance was confirmed at the $p \leq 0.05$ level. Statistical analysis was performed using the IBM SPSS Statistics 23 software (Business Machine Corp, 2015) independently by the authors.

Results

Body composition results for all four groups are presented in Table 1. The highest PhA values were found in male athletes and the lowest in female non-athletes. Female non-athletes had the highest PBF and male athletes the lowest PBF. The PMM and FFM were the highest in male athletes and the lowest among female non-athletes. Impedance was the highest in female non-athletes, and TBW, as well as ICW and ECW, were the highest in male athletes.

Table 1. Values of variables for male and female athletes, male and female non-athletes and for the entire sample

		Age	Height	BMI	PBF	FFM	TBW	ECW	ICW	ECW/ICW ratio	PMM	BM	BMR	Impedance	PhA
MA	$\bar{X}$	20.38	184.34*	23.39*	14.49	67.82*	48.87*	18.91*	29.96*	.63	64.45*	3.37*	8462.00*	558.69	6.85*
	Nr.	26	26	26	26	26	26	26	26	26	26	26	26	26	26
	SD	1.20	7.65	2.70	4.55	8.26	5.48	1.93	3.61	.03	7.87	.38	1094.69	52.35	.50
MNA	$\bar{X}$	20.82	181.22	23.68	16.89	63.96	46.13	18.35	27.78	.66	60.78	3.18	8003.90	586.09	6.29
	Nr.	22	22	22	22	22	22	22	22	22	22	22	22	22	22
	SD	2.36	6.65	3.83	5.96	7.27	4.81	1.75	3.14	.03	6.93	.34	946.15	60.19	.67
	p	0.97	0.08	0.80	0.16	0.16	0.14	0.46	0.052	0.002**	0.16	0.16	0.21	0.17	0.004**
FA	$\bar{X}$	21.63*	172.38	21.33	23.44	48.58	35.03	14.38	20.65	.69	46.11	2.46	6237.38	697.88	5.61
	Nr.	8	8	8	8	8	8	8	8	8	8	8	8	8	8
	SD	3.93	11.12	2.36	3.67	8.46	5.96	2.69	3.29	.02	8.04	.41	981.47	97.81	.44
FNA	$\bar{X}$	20.00	165.70	21.59	25.32*	44.23	31.90	13.25	18.65	.71*	41.99	2.24	5781.50	750.10*	5.47
	Nr.	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	SD	.82	8.25	2.15	3.73	5.98	4.21	1.90	2.36	.03	5.69	.29	698.68	94.04	.58
	p	0.36	0.27	0.90	0.27	0.07	0.07	0.27	0.055	0.32	0.07	0.08	0.17	0.27	0.63
Total	$\bar{X}$	20.62	179.03	22.96	18.02	60.63	43.71	17.32	26.39	.66	57.60	3.02	7633.52	613.70	6.31
	Nr.	66	66	66	66	66	66	66	66	66	66	66	66	66	66
	SD	2.08	10.26	3.11	6.33	11.82	8.29	2.95	5.39	.04	11.26	.59	1417.75	98.39	.77
	p	0.74	0.000†	0.09	0.000†	0.000†	0.000†	0.000†	0.000†	0.000†	0.000†	0.000†	0.000†	0.000†	0.000†

*the highest values; **statistical significance p≤0.03 †statistical significance p≤0.001

(MA – male athletes; FA – female athletes; MNA – male non-athletes; FNA – female non-athletes; $\bar{X}$ – arithmetic mean; Nr. – number; SD – standard deviation; p – statistical significance; BMI – body mass index; PBF – percent body fat; FFM – fat free mass; TBW – total body water; ECW – extracellular water; ICW – intracellular water, ECW/ICW ratio – extracellular water/intracellular water ratio; PMM – percent muscle mass; BM – bone mass; BMR – basal metabolic rate; PhA – phase angle

ECW/ICW ratio was the highest among female non-athletes and the lowest among male athletes. The amount of BM was the highest in male athletes and the lowest in female non-athletes. Male athletes had the highest BMR. Statistically significant differences between the male subjects existed only in the ECW/ICW ratio (p=0.002) and PhA values (p=0.004). As far as the female subjects are concerned, whether athletes or those leading a sedentary lifestyle, there were no statistically significant differences (p>0.03). Regarding the correlation between the PhA and other variables, there was a positive correlation with height (ρ +0.35 p=0.004), BMI (ρ +0.55 p≤0.01), FFM (ρ +0.64 p≤0.01), PMM (ρ +0.64 p≤0.01), TBW (ρ +0.66 p≤0.01), ECW (ρ +0.58 p≤0.01) and ICW (ρ +0.71 p≤0.01), BM (ρ +0.63 p≤0.01) and BMR (ρ +0.64 p≤0.01) and a negative correlation with PBF (ρ -0.35 p=0.004), ECW/ICW ratio (ρ -0.78 p≤0.01) and impedance (ρ -0.79 p≤0.01). Regression analysis showed that PhA values were mostly influenced by PMM (B=+0.44, p=0.002), BMI (B=+0.31, p=0.000) and impedance (B=+0.004, p=0.03), which describes almost 83% of the variance in the values of the PhA (R^2=0.83).

Discussion

PhA is a good prognostic indicator in numerous clinical conditions of patients suffering from HIV, bacterial infection, liver cirrhosis, kidney disease, tuberculosis and cancer, but little is known about PhA values in healthy individuals (6). According to Selberg and Selberg (8), PhA values over 5.4˚ are considered normal, in the range of 4.4˚ to 5.4˚ as borderline and under 4.4˚ as abnormal (8). Lower values usually indicate a cell integrity disorder or even cell death (14). In this research, PhA values of male athletes were 6.85±0.5˚, female athletes 5.61±0.44˚, male non-athletes 6.29±0.67˚ and female non-athletes 5.47±0.58˚. A potential explanation may be that higher PhA values are present in physically active people (15), but in the absence of sport-specific reference values, we can only use general healthy population values as references and hypothesise on the influence of physical activity (16). In their systematic review on bioelectrical impedance phase angle in sports, Di Vincenzo et al. (5) stated that PhA values have been shown to be significantly associated with muscle strength and physical activity and to vary between sexes and with age (5). Since the PhA is considered a simple indicator of muscle mass and is defined by tissue hydration and cell membrane potential (8), and since ECW/ICW ratio is one of the measures that influences PhA variability, changes in PhA values usually correlate with cell size, cell permeability and differences in fluid distribution in various tissue types (11). Lower PhA values are caused by an increase in the ECW/ICW ratio in patients with inflammatory conditions and obese individuals (11). Male athletes in our research had the lowest values of the ECW/ICW ratio and PBF among all four groups and the highest values of FFM and PMM. We also found statistically significant differences in PhA values and the ECW/ICW ratio between male athletes and non-athletes. However, no such differences were found among the female subjects, which may be due to PBF values. According to Gallagher et al. (17), PBF of healthy women aged 20-39 ranges from 21% to 33% and of athletes from 14% to 20% (18). On the other hand, female athletes in this research had PBF values of 23.44±3.67%, while female non-athletes had PBF values of 25.32±3.73%, with a mean difference of 1.88±0.06%, placing them in the same reference category. We found a strong positive correlation between the PhA and ICW, TBW, FFM, PMM, BMR, BM, ECW and BMI, as well as a strong negative correlation with impedance and the ECW/ICW ratio. Differences in the distribution of fluids, an increase in the amount of ECW and a compensatory increase in the ECW/ICW ratio may be leading to a decrease in PhA values (11). Increase in the ECW/ICW ratio leading to a decrease in PhA values may be connected to PBF because adipose tissue influences haemodynamics or fluids (11), although in our research, PBF showed a moderate correlation to the PhA. Higher ICW content in physically active people may be a reflection of physiological cellular adaptations leading to higher PhA values (12). Genton et al. (12) hypothesised that physically active people practice carbohydrate loading to improve performance and

consequently have higher ICW content to store glycogen (12). Silva et al. (19) state that regardless of body composition changes, athletes who increase reactance and resistance reduce ECW, while those who raise PhA increase ICW (19). The correlation with FFM and PMM may be explained by the fact that the PhA is directly related to the amount and function of cell membranes, in this case, muscle cells (6). As for BMI, it explained 31% of the variance in PhA values and according to Bosy-Westphal (10), an increase in PhA values with an increase in BMI only occurs with a BMI of about 40 kg/m^2 and is just a reflection of an increased number of muscle or fat cells (10). BMR also showed a positive correlation with the PhA, which may be linked to greater amounts of FFM in athletes (20). Regarding BM, we found no evidence explaining a moderate positive correlation with the PhA, except that it may be due to the age of the participants. Negative correlation of the PhA with impedance may be due to sex. Men tend to have lower values of impedance than women (15) due to a higher amount of FFM and lower PBF. Our results are only applicable when using a BIA device operating at a single frequency (50 kHz), which can be a limiting factor. For a better estimate of the influence of body components on the PhA, the BIA measurement should be performed in different frequency ranges (5 kHz, 50 kHz, 100 kHz) (15). Another limiting factor is that we had no record of the nutritional status of the subjects. Moreover, regarding the medical condition of the students, we relied on self-reported information. A small sample size may also be a limitation, even though the student athletes played different sports.

In conclusion, PhA values in this research were higher in athletes than in non-athletes and higher in male athletes compared to female athletes. We found significant differences in PhA values and the ECW/ICW ratio between male athletes and non-athletes, but no such differences were found in the female subjects. Variables showing the highest positive correlation with the PhA were ICW, TBW, FFM and PMM, indicating a possible influence of physical activity. Regression analysis in this research showed that the variance in the PhA is mostly influenced by PMM, BMI and impedance.

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Original article

Effects of Robot-assisted Upper Extremity Rehabilitation on Change in Functioning and Disability in Patients With Neurologic Impairment: A Pilot Study

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Abstract

Introduction: The aim is to evaluate the effect of robot-assisted training on the most important aspects of functioning and disability in patients with upper extremity neurologic impairment.
Materials and Methods: A prospective six-week pilot study included robot-assisted training of the upper extremity and conventional neurorehabilitation in 12 participants after a stroke or traumatic brain injury. Outcome measurements were range of motion (ROM), the International Classification of Functioning, Disability and Health (ICF) Core Set for Hand and the Visual Analog Scale (VAS) for pain sensation. A Wilcoxon test was used for the analysis of pre- and post-test differences and Spearman's correlation was used for connecting the data collected.
Results: A statistically significant difference was found for ROM (shoulder abduction/adduction, shoulder flexion/extension, shoulder internal/external rotation and forearm pronation/supination) and a number of ICF categories (Body Function: b280, b710, b715, b730, b760; Activities and Participation: d230, d430, d440, d445, d5). A significant positive correlation of medium intensity (r=0.589) was found between the duration of movement coordination training and the ICF category b760. We did not find a statistically significant difference in pain sensation (VAS) with regard to the direct use of the device. For all analyses, $p<0.05$ and CI was 95%.
Conclusion: Robot-assisted training and conventional neurorehabilitation improved motor and functional recovery. There was a correlation between training a specific goal on the device and one of the ICF Body Function categories.

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Introduction

Advances in medicine are impossible without the combined effort of advances in different technologies. Today, the use of novel technology in medicine is expected. Since 2011, an upper extremity exoskeleton has been commercially accessible for rehabilitation (1). But how can we recognise and subsequently combine the clinical needs of patients with the corresponding devices (2)? Robot-assisted devices for the upper extremity have been created for training and assisting or can combine these two functions (3). According to the World Health Organization, stroke, traumatic brain injury (TBI) and spinal cord injury (SCI) are defined as chronic diseases (4). While various neurological conditions affect different populations and have different pathophysiology, they all damage neural networks and motor system networks in particular. As such, clinical impairment and functional problems of persons with different neurological conditions can often overlap (5) and analysing them together may be of interest (6). Around 70% of patients with stroke (7) and 30% of patients with TBI exhibit upper extremity paresis at rehabilitation admission (8,9). For restoration of upper extremity function in patients with a neurologic deficit, the main therapy strategies target the impaired motor cortex (activation of ipsilateral or inhibition of contralateral) or affect afferent sensory pathways (10). It is well-recognised that an intense training program during rehabilitation for patients in the subacute period (i.e. in the first 6 months) will significantly improve functional outcomes for the upper extremity (10.11). Meta-analyses have demonstrated significant effects on motor control and muscle strength using shoulder/elbow robotics, as well as on motor control using elbow/wrist robotics (12). For persons with neurological deficits in a chronic phase, robot-assisted rehabilitation is more effective than other types of therapies for recovery of upper extremity motor function (13). Together, these results indicate that the use of an exoskeleton device with various possibilities (virtual reality, augmented reality and gamification) lead to general improvement of motoric function in neurorehabilitation (14). But can it lead to an improvement in overall functional status? In the present study, we decided to use the International Classification of Functioning and Disability (ICF) as a measurement tool in light of the notion that limitations of function and disability are not only related to aetiology, but can also be considered as general manifestations in overall health conditions (15). The ICF also has other advantages in its use of a common language (i.e. it is internationally comparable) (16) and has the essential and most relevant categories for personal functioning when using the ICF Core Set information (17). The first aim of this investigation is to assess the efficacy of robot-assisted training on the motoric function of the upper extremity. Secondly, this study aims to examine change in functioning and disability among persons with upper extremity impairment caused by different neurologic aetiology at a clinical level during inpatient rehabilitation. The first expected effect of robot-assisted training is an increase in the range of motion (ROM) of joints in the trained upper extremity and changes in activity and participation among the participants. The second expected effect is a connection between the time spent in training specific goals on the robot-assisted device and selected body domains of the ICF Core Set. Pain sensation in the trained extremity will also be evaluated.

Materials and Methods

This prospective pilot study was conducted at the Special Hospital for Medical Rehabilitation Krapinske Toplice in Croatia.

Prior to the commencement of the study, informed consent was obtained from all participants and the study was conducted according to the Helsinki Declaration. Consent for publication of the study results was obtained from the Ethical Committee of the Special Hospital for Medical Rehabilitation Krapinske Toplice.Twelve participants (3 women and 9 men) aged between 20 and 61 years who underwent acute neurorehabilitation following a traumatic brain injury or stroke met the inclusion

criteria. Inclusion criteria were: aged 18 years and older, paresis of the upper extremity following a traumatic brain injury or stroke, ability to sit (i.e., exhibits trunk control in a sitting position) and a Rancho level of function of 5 or more. Exclusion criteria were: non-acute rehabilitation phase, paresis of the upper extremity of other aetiology, plegia of the upper extremity, inability to sit (i.e., exhibits inadequate trunk control in sitting position), fixed contractures of shoulder, elbow or wrist joints, a Rancho level of function less than 5, apraxia, hemineglect and receptive aphasia. The study was designed as a six-week program. Participants received robot-assisted training during their regular occupational therapy sessions for 30 minutes, 5 days a week. All participants were assessed at the beginning and at the end of the study. Our primary outcome measurements were ROM (for shoulder and elbow joints) and categories of the ICF Core Set - Hand Conditions Brief (for the trained upper extremity). The evaluation of pain sensation in the trained upper extremity was a secondary outcome measurement. For the assessment of ROM, kinematic parameters of the shoulder and elbow joints (i.e. minimal and maximal shoulder abduction/adduction, minimal and maximal shoulder flexion/extension, minimal and maximal inner/outer shoulder rotation, minimal and maximal elbow flexion/extension and minimal and maximal forearm pronation/supination) recorded during training on the robot-assisted device were collected and analysed. Assessment of the selected ICF Core Set was conducted by one investigator following additional education. The ICF Core Set is made up of 23 categories (9 in Body Function, 3 in Body Structures, 8 in Activities and Participation and 3 in Environmental Factors). Every category was assessed using the ICF qualifiers on an ordinal scale ranging from 0 (no impairment) to 4 (complete impairment), with additional possible values of 8 (not specified) and 9 (not applicable). For most categories, assessment was carried out during clinical examination of the participants, while for some categories, information from a patient-report questionnaire was sufficient. Some categories also have additional qualifiers: Body Structures 2 (for nature and location of change), Activities and Participation 2 (for capacity and performance) and Environmental Factors (an). The evaluation of pain sensation was also carried out by one investigator. To assess pain directly related to the use of the device, the Visual Analog Scale (VAS) was conducted immediately before and after the first and last training session on the robot-assisted device. Prior to the beginning of the study, three occupational therapists were trained to use the robot-assisted device. An exoskeleton device, the Armeo®Spring Hocoma, Inc., composed of an upper and lower module for the upper arm and the forearm and a pressure sensitive handgrip for the hand was used (18). Both modules were length-adjustable, which allowed adequate positioning of the exoskeleton device and the arm joints of the participants. In addition, the weight compensation feature of exoskeleton (18) was utilised and individually adjusted for all participants. Using this exoskeleton device, our participants were enabled to train the shoulder and elbow joints, flexo-extension of the wrist and manual grip (19). The repetition of movements of the paretic upper extremity during virtual gaming was conducted within a three-dimensional workspace (18). The goals of robot-assisted therapy were to increase ROM (1D and 2D/3D), to improve movement coordination and to improve grasp function and cognitive training. All participants also underwent conventional neurorehabilitation (i.e. they received therapies individually indicated according to their overall clinical status, which included a multidisciplinary approach if needed)..

Statistical analysis

Statistical evaluation was performed using SPSS, version 26.0 (SPSS Inc., Chicago IL, SAD). In the first analysis, a Wilcoxon signed-rank test was used to evaluate pre- and post-test differences for all data collected (kinematic parameters, ICF categories and VAS). Statistical significance was set at $p<0.05$ and CI was 95%. All quoted p-values are two-tailed. In the second

analysis, we used the Spearman's correlation coefficient to test relationships in the data (duration of training a specific therapy goal on the robot-assisted device and selected ICF categories). A correlation greater than r>0.80 was considered as strongly positive, 0.5<r>0.8 was considered as medium to strongly positive and 0<r<0.5 was considered as weakly positive. A correlation coefficient greater than 0.5, r> 0.5 was considered significant.

Results

All 12 participants (3 women and 9 men) successfully completed the pilot study. Seven participants (58.3%) exhibited neurologic impairment following TBI and 5 participants (41.7%) following stroke. The average age of participants was 39.42 years (standard deviation (SD) 16.94) and ranged between 20 and 61 years. The right arm was treated in half of the participants and all participants exhibited right-hand dominance. The average number of therapy sessions with the robot-assisted device was 25.50 (SD 5.98), where the number of sessions ranged between 14 and 33. The average therapy time using the robot-assisted device per treatment was 13.75 minutes (SD 2.67), ranging from 8.74 to 16.84 minutes. From the total number of therapies using the exoskeleton device, the average time spent performing each exercise was: 5.05 minutes (SD 1.21) for increasing ROM 1D, with a minimal value of 3.16 and a maximal value of 7.43; 6.48 minutes (SD 1.86) for increasing ROM 2D/3D, with a minimal value of 4.26 and a maximal value 10.86; 9.41 minutes (SD 2.86) for movement coordination, with a minimal value of 5.30 and a maximal value of 15.58; 2.88 minutes (SD 1.75) for grasp function, with a minimal value of 0.09 and a maximal value of 6.76; and 2.25 minutes (SD 1.09) for cognitive training (11 participants), with a minimal value of 0.09 and a maximal value of 3.50.

Primary outcomes

Kinematics parameters

A Wilcoxon test showed statistically significant differences between the initial and final measurements for the following kinematics parameters observed: minimal shoulder abduction/adduction, shoulder abduction/adduction range of motion, minimal shoulder flexion/extension, shoulder flexion/extension range of motion, minimal shoulder internal/external rotation, maximal shoulder internal/external rotation, shoulder internal/external rotation range of motion, maximal elbow flexion/extension, minimal forearm pronation/supination and forearm pronation/supination range of motion. CI was 95% (Table 1).

ICF Core Set -Hand Conditions Brief

A Wilcoxon test demonstrated a statistically significant difference between the initial and final assessment using the ICF Core Set for the following categories in Body Function: b280 (sensation of pain), b710 (mobility of joint functions), b715 (stability of joint functions), b730 (muscle power function), b760 (control of voluntary movement function). Similarly, statistically significant differences were found for the following categories in Activities and Participation: d230 (carrying out daily routine), d430 (lifting and carrying objects), d440 (fine hand use), d445 (hand and arm use) and for d5 (self-care). CI was 95% (Table 2).

Table 1. Data of the kinematics parameters (ranges of motion in degrees) for the upper extremity joints presented as test statistics[a]

Variable name	Value before (degrees), $\bar{x}$±SD	Value after (degrees), $\bar{x}$±SD	P-value
Shoulder abd./ ad. min.	-71.14±18.82	-88.12±3.24	**0.009**
Shoulder abd./ad. max.	16.09±13.50	22.61±14.29	0.117
Shoulder abd./ad. range	86.41±26.44	110.73±16.09	**0.028**
Shoulder flex./ext. min.	52.90±3.87	50.22±2.05	**0.019**
Shoulder flex./ext. max.	107.34±17.98	112.34±21.59	0.060
Shoulder flex./ext. range	54.44±18.76	62.12±21.90	0.023
Shoulder int./ext. rotation min.	32.98±18.88	18.16±17.79	**0.019**
Shoulder int./ext. rotation max.	75.36±20.84	106.14±20.14	**0.012**
Shoulder int./ext. rotation range	42.38±18.43	87.98±24.45	**0.003**
Elbow flex./ext. min.	22.72±15.32	18.62±15.81	0.241
Elbow flex./ext. max.	94.77±11.86	102.22±6.17	**0.025**
Elbow flex./ext. range	72.05±21.98	83.60±19.12	0.062
Forearm sup./pron. min.	-23.52±40.66	-48.74±27.39	**0.004**
Forearm sup./pron. max.	55.45±13.19	58.49±9.29	0.285
Forearm sup./pron. range	78.97±37.7	107.24±23.53	**0.012**

a. Wilcoxon signed-rank test p <0.05 was considered significant. Abd./ab.= abduction/adduction; flex./ex.t=flexion/extension; int.=internal, ext.=external, min.=minimal; max.=maximal

Table 2: Data of the assessed ICF Core Set presented as test statisticsa, p<0.05 was considered significant.

Variable name	Value before $\bar{x}$±SD	Value after $\bar{x}$±SD	P-value
b152 (emotional functions)	1.50±0.52	1.42±0.52	0.317
b265(touch function)	0.83±0.94	0.75±0.87	0.317
b270 (sensory functions of sensing surfaces and their texture or quality)	0.17±0.39	0.08±0.29	0.317
b280 (sensation of pain)	1.0±0.95	0.58±0.79	**0.025**
b710 (mobility of joint functions)	1.92±1.24	1.17±1.12	**0.014**
b715 (stability of joint functions)	1.25±1.48	0.75±1.22	**0.034**
b730 (muscle power functions)	2.42±0.80	1.75±0.75	**0.005**
b760 (control of voluntary movement functions)	2.25±0.97	1.50±0.67	**0.014**
b810 (protective functions of the skin)	0.08±0.29	0.00±0	0.317
d230 (carrying out daily routine)	2.67±1.07	1.92±0.90	**0.003**
d430 (lifting and carrying objects)	2.751±1.14	2.17±0.84	**0.008**
d440 (fine hand use)	2.58±0.90	1.92±1.00	**0.011**
d445 (hand and arm use)	2.33±0.65	1.50±0.52	**0.002**
d5 (self –care)	2.42±1.09	1.83±1.12	**0.020**
d7 p (interpersonal interactions and relationships)	1.58±0.90	1.67±0.89	0.317
d7 c (interpersonal interactions and relationships)	1.08±0.79	1.08±0.79	1.000
e1 (products and technology)	2.25±0.75	2.58±1.08	0.194
e3 (support and relationships)	2.75±1.14	2.58±1.96	0.785
e5 (services, system and policies)	1.83±1.12	1.00±1.35	0.655

a. Wilcoxon signed-rank test

Secondary outcomes

Data connectivity

Spearman's correlation coefficient demonstrated a significant positive correlation of medium intensity, between the duration of the movement coordination therapy session using the robot-assisted device and the ICF category b760, indicating a positive relationship between these variables r=0.589 (p-value of 0.044) (Figure 1). No significant correlation between the duration of the therapy session for increasing ROM (1D and 2D/3D) and the ICF category b710 was found (Table 3).

Pain assessment

A Wilcoxon test did not indicate any statistically significant difference in pain sensation for the trained upper extremity, as measured with VAS. CI was 95% (Table 4).

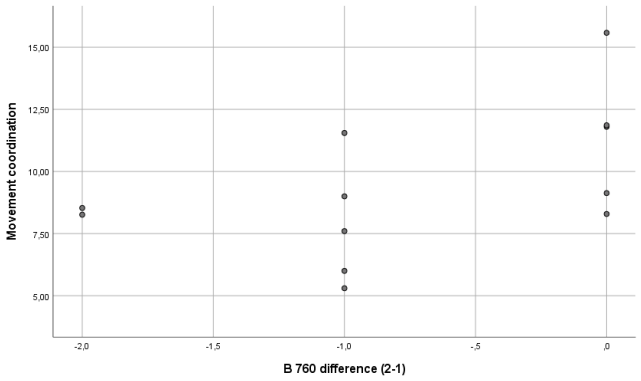

Figure 1: Connection between therapy time for movement coordination and the ICF domain b760) (difference in value of control of voluntary movement functions) as measured by Spearman's correlation coefficient

*Correlation is significant at the 0.05 level (two-tailed).

Table 3. Connection between therapy time, ROM 1D, ROM 2/3D and the ICF domain b710 (mobility of joint functions), as measured by Spearman's correlation coefficient.

Variable name		Therapy time	ROM 1D	ROM 2/3D	b710 (difference)
Therapy time	r	1.0	0.21	0.33	-0.21
ROM 1D	r	0.21	1.0	0.13	-0.35
ROM 2/3D	r	0.33	0.13	1.0	-0.5
B710 (difference)	r	-0.21	-0.35	-0.5	1.0

*Correlation is significant at the 0.05 level (two-tailed). ROM 1D=range of motion, one dimension; ROM2/3D=range of motion, two and three dimension ICF=International Classification of Functioning, Disability and Health.

Table 4. : Test statisticsa for pain sensation data, p<0.05 was considered significant

Variable name	Value before	Value after	P-value
VAS I	13.83±24.53	10.42±13.89	0.385
VAS II	9.17±13.95	15.33±18.36	0.327

a. Wilcoxon signed-rank test

VAS I= sensation of pain immediately before therapy session using the device.

VAS II= sensation of pain immediately after therapy session using the device.

Discussion

This pilot study confirmed the hypothesis that the use of a robot-assisted device in the rehabilitation of patients with paresis of the upper extremity leads to increased ROM in joints of the trained upper extremity. Other studies have also demonstrated motor improvement (ROM, strength or motor control) following robot-assisted training in persons with neurologic impairment (20-23). The added advantage of the robot-assisted device as an assessment tool (24) was used for in this study for measuring ROM in order to attain objective, quantitative data while avoiding the subjectivity of the investigator (25-27). In addition, another feature of the device – the ability to conduct a large number of repetitions – was used for improving ROM. In a study by Lo and colleagues, over 1,000 repetitions per session were achieved during a single hour of robot-assisted therapy (28). Because motor recovery in neurologic impairment is considered to depend not only on CNS damage, but also on the intensity (29) and duration of therapy, the ability to achieve a large number of repetitions in treatment is particularly important. In other words, more intense therapy is positively related to clinical improvements (30). The results of our study demonstrating a significant correlation between the duration of movement coordination therapy using the device and the ICF body category that describes control of voluntary movement are consistent with this evidence. But what happens when participants exercise ROM? The results of a neuroradiology study demonstrated that, when the sensation of movement is induced, the somatosensory, primary and supplementary motor areas of the cortex are activated and different proprioceptive inputs are associated with differently located activation patterns in these cortical areas (31). However, another important question is whether the patient will transfer this acquired motoric knowledge to everyday life? Some investigators have developed and tested new strategies that aim to facilitate the transfer of new motor skills to everyday activities following robot-assisted training (32). In this study, we used the ICF Core Set to understand and measure limitations in functioning and disability among our participants (33, 34) and to examine change following robot-assisted upper extremity training. The results demonstrated significant changes in various categories of Body Function and Activity and Participation. Specifically, participants exhibited significant improvement in motoric function (as measured by the robot-assisted device and the specific ICF Core Set category) and had fewer limitations in functioning during inpatient rehabilitation (as measured by the ICF Core Set categories). A study by Goljar and colleagues demonstrated that the ICF categories have the potential to reveal time-related changes in a patient's functioning (35). The ICF is also considered a useful framework for recognising the possibilities offered by devices in clinical or research settings (15). Our findings are consistent with those of others who have investigated the effects of robot-assisted training on functional recovery. In a study by Colomer and colleagues, significant improvement was found on both function and activity scales for the upper extremities, as measured by the Motricity Index, the Fugl-Meyer Assessment Scale, the Motor Assessment Scale, the Manual Function Test and the Wolf Motor Function Test (36). In another study using the FIM as a measurement tool, Daunoraviciene and colleagues found significant upper extremity improvement among participants who trained with a robotic device, as compared with a control group (37). The update of the Cochrane review by Mehrlotz and colleagues not only demonstrated improved muscle strength and function of the arm, but also improved scores for daily activities following the use of robot-assisted and electromechanical training in rehabilitation for persons after stroke (38). In regard to pain sensation, we did not find a significant difference related to the direct use of the exoskeleton device. In a study by Busching and colleagues, semi-autonomous training was used for patients with severe paresis and no side effects were found with regard to training using the same type of the device (39). It is possible that our participants did not experience changes in pain sensation immediately after the use of the exoskeleton because the degree to which the

upper extremity was unweighted was individually adjusted for all participants with the intention of adequately supporting the paretic extremity and therefore facilitating the number of repetitions (40). However, when pain sensation is considered independently of the direct use of the exoskeleton device, the results of the ICF Core Set demonstrated a significant change in the domain b280 (i.e., pain sensation decreased for participants during the six-week study period). Findings from a double-blind randomised control study conducted by Taveggia and colleagues also demonstrated a decrease in pain sensation over a six-week study period for both a robot-assisted group and a control group (41). In our study, the degree of spasticity was not considered and participants with upper extremity contractures (including those related to spasticity) were not included in the study in accordance with the exclusion criteria. The main limitation of this pilot study is the lack of a control group due to a small number of participants who met the inclusion criteria. As such, the question remains as to the degree of spontaneous recovery that occurred, which is difficult to distinguish from therapy-induced recovery without a control group for comparison. We also did not take into consideration new sensorimotor interactions between the exoskeleton device and the participants (42). Muscle activity and muscle coordination is different in healthy persons (43, 44) when compared to persons with CNS damage (pathological muscle synergies and altered joint coordination) and, because the exoskeleton device has its own mechanical characteristics and there is kinematic incompatibility during the human-exoskeleton interaction (45), currently the intention is to have as little interference as possible between the exoskeleton and the human body. In order to be as ergonomic as possible, new devices aim to be made on principles very similar to functional anatomy (46).

Conclusion

Robot-assisted training and conventional neurorehabilitation improved the motor and functional recovery of patients with upper extremity paresis of various aetiology. We consider the positive connection between the time spent training a specific goal using the robot-assisted device and one of the ICF Body Function categories to be particularly important evidence. Furthermore, improvement in motoric function achieved by affecting afferent sensory pathways might be responsible for improvement in the Activity and Participation category. In order to develop adequate recommendations for the use of robot-assisted devices in accordance with person-specific rehabilitation goals, future research might further investigate the relationship between robot-assisted training and expected clinical improvement. New robot-assisted devices, body-powered robots and their possible advantages (47) or wearable exoskeleton devices that support or replace muscle movement initiation (48) are developed according to new neurophysiological knowledge (49). However, while neurological impairment affects a large number of the population, overall functional recovery of patients with such impairment is limited. As such, further investigation of these available devices, the assessment of their potential for neurologic improvement and any adverse effects is required.

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Review article

Underactive Bladder Syndrome: An Often Overlooked Condition

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Abstract

Urinary bladder outlet obstruction can cause lower urinary tract symptoms in men and women, such as a weak or interrupted urine stream, straining to void, hesitancy, and the feeling of incomplete bladder emptying. These symptoms can also be associated with underactive bladder syndrome. This urological condition has a high prevalence in both genders, especially in older age groups. The most common risk factors that can contribute to the pathogenesis of underactive bladder syndrome are aging, detrusor myopathy, neuroinflammatory, neurodegenerative and other lesions of the central and peripheral nervous system, and diabetes mellitus. Reduced detrusor contractility, or detrusor underactivity, is the most common pathology of underactive bladder syndrome. Detrusor underactivity is a urodynamic definition provided by the International Continence Society. The urodynamic trace is characterised by a low urine flow rate accompanied by low detrusor pressure or brief detrusor muscle contraction. Underactive bladder syndrome is often recognised and treated poorly in clinical practice because the symptoms are not specific. Furthermore, underactive bladder can be asymptomatic or coexist with bladder outlet obstruction and overactive bladder syndrome. The aim of this study was to review recent research concerning the aetiology, classification, diagnostic evaluation, and available treatment methods for patients with symptoms of underactive bladder syndrome. Making an accurate underactive bladder diagnosis is challenging because it is a multifactorial condition with various patterns of manifestation. Consequently, there is still no general agreement and standardisation regarding the most favourable diagnostic and therapeutical approach.

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Introduction

The storage and excretion of urine in women and men depend on the coordinated activity of the neural centres, smooth muscles of the bladder and urethra, and the urethral striated muscle (1). Various urological and neurological diseases can cause voiding dysfunction, which manifests with lower urinary tract symptoms (LUTS), clinical signs, and syndromes (2). Straining to void, a weak urine stream, hesitancy, and other LUTS during the voiding phase of micturition are prevailing symptoms that urologists encounter in an outpatient and clinical setting. These LUTS are more often present in men than in women and are commonly associated with bladder outlet obstruction (BOO) (3). Benign prostatic hyperplasia (BPH) and urethral stricture are the usual suspects in men, while in women, the most common causes of BOO are functional sphincteric obstruction, anti-incontinence surgery, urethral stricture and pelvic organ prolapse (4, 5). These symptoms may be a consequence of detrusor underactivity (DUA) (6). DUA is caused by reduced detrusor contractility, which can be idiopathic or result from various neurological, urological, autoimmune, muscular, and other diseases (7, 8). According to the International Continence Society (ICS) terminology, DUA is a diagnosis based on urodynamic investigations (9). DUA is characterised by a low urine flow rate accompanied by low detrusor pressure, brief detrusor contraction, or absent detrusor contraction (10). The result of this is prolonged micturition, failure to achieve complete bladder emptying in an expected time interval, and a high bladder post-void residual volume (PVR).

Underactive bladder syndrome (UAB) is a contemporary clinical term that describes symptomatic DUA as a cause of voiding dysfunction (11, 12). UAB is defined as a complex of voiding symptoms, such as a weak urine stream, straining to void, and hesitancy (13, 14). Storage and post-micturition symptoms, such as a reduced sensation of bladder filling, terminal dribbling, and a feeling of incomplete bladder emptying may also be present, with or without PVR (15). It can be challenging to distinguish if BOO or UAB is the problem because these conditions have similar symptoms and they can coexist and intertwine, especially in men with BPH (16). UAB is not so popular when compared with overactive bladder syndrome (OAB) and these two syndromes may be present at the same time (17).

Detrusor overactivity (DOA) is another urodynamic diagnosis and OAB represents symptomatic DOA (18). DOA is characterised by involuntary detrusor contractions during the filling phase, which can be spontaneous or provoked during a urodynamic study (9). OAB consists of storage LUTS and it is defined as urinary urgency, with or without urgency incontinence, usually with increased daytime frequency and nocturia, if there is no proven infection or obvious pathology (19). We are better acquainted with the pathophysiology and diagnostic approach of OAB compared to UAB (20). Also, OAB treatment methods are more efficient and safer (21). Storage, voiding and postmicturition LUTS are associated with significant morbidity and mortality of men and women aged 65 and over. For example, nocturia associated with BPH and OAB can lead to a fall-related bone fracture, persistently high PVR associated with UAB can cause bilateral hydronephrosis, chronic cystitis, overflow incontinence, and formation of bladder stones, which can seriously affect the quality of life (QOL) (22-25).

Despite new research initiatives and the latest information about UAB and DUA, there is still no consensus on a diagnostic and therapeutical approach. Furthermore, assessment can be different in men and women. If we could reach a general agreement on diagnosing UAB and DUA, then we could establish the parameters of non-invasive assessment tools, for example, a combination of UAB symptom score questionnaire, ultrasound measurement of detrusor wall thickness, and uroflowmetry result (26). This could help us distinguish patients with UAB and DUA from those with BOO and BPH.

The aim of this review was to describe the clinical and urodynamic aspects of UAB and to emphasise the latest insights in the diagnostic

evaluation and available UAB treatment methods.

Prevalence of underactive bladder syndrome

The prevalence rates vary because UAB is often a poorly recognised condition in clinical practice, frequently mistaken for BOO, and usually not associated with a possible neurological condition. The reason for this is that there is no classification of UAB and a definitive diagnosis can be achieved only by conducting a urodynamic study. According to previous research, UAB has a high prevalence in both genders, especially in older age groups. UAB has the potential of being a serious public health problem in the future because it is expected that the percentage of people aged 65 and over will approach 20% of the total population by 2050 (13, 27). Also, neurodegenerative disorders and other neurologic conditions that can lead to LUTS and UAB increase in prevalence with age (28). The prevalence of UAB in men under the age of 50 ranges from 9% to 28% and increases with age, affecting 48% of men over the age of 70 (6). The prevalence of UAB is approximately 12% in younger women and 45% in older female patients (29). One epidemiological survey, which included a total of 633 participants aged 60 and over, showed that 23% of them had voiding and post-micturition LUTS, with no difference regarding gender or age; however, only 11% of the participants heard about UAB (30). In another original scientific study, urodynamic testing was performed on 1,179 patients with LUTS aged over 65. After the evaluation of the results, it was established that out of 40.2% of men and 13.3% of women who had DUA, 72.6% of the women with DUA had DOA or stress urinary incontinence (SUI) and 46.5% of men with DUA also had DOA or BOO (31).

Aetiology of underactive bladder syndrome

There are many diseases that alone or combined cause bladder innervation disorders and/or damage to the detrusor muscle. This can lead to the inability of the detrusor muscle to generate sufficient intravesical pressure to empty the urinary bladder. Therefore, the aetiology of UAB is multifactorial and the most important aetiological factors are age-related factors, neurological disorders, BOO, and diabetes mellitus (DM) (32). If there is no specific cause, UAB can be classified as idiopathic, which is more common in younger patients (33). Older people are more likely to develop UAB symptoms. The amount of acetylcholine esterase-positive nerves in the bladder decreases with age and a reduced response to bladder filling in the insular cortex occurs (34, 35). Also, the proportion of collagen fibres increases with age and the number of smooth muscle cells and muscarinic receptors decreases (34, 36). Neurological diseases account for the largest share of risk factors for lower urinary tract disorders such as OAB and UAB. Patients could have LUTS but no formally diagnosed neurological condition. LUTS could be an early symptom in the manifestation of an underlying neurological disease, which is referred to as "occult neurology" (37). If we fail to identify an undiagnosed neurological aetiology, we risk poor outcomes for LUTS treatment. OAB symptoms are more common in patients who have had an acute cerebrovascular accident (CVA) and in patients with multiple sclerosis (MS) and Parkinson's disease, but UAB symptoms and high PVR have also been reported (38-40). Urinary retention and DUA can occur in early stages of Guillain-Barré syndrome, but long-term LUTS are not so common.

Traumatic, degenerative and other spinal cord injuries can also cause voiding dysfunction. Symptomatology depends on the spinal cord injury level. Spinal cord injuries below the sacral micturition centre (SMC), which is located in the intermediolateral grey of the sacral cord from S2-S4, are most often partial, resulting in weakened bladder contractility and UAB symptoms. Partial or complete injuries above the SMC and neuroinflammatory and neurodegenerative diseases of the central nervous system often result in the appearance of OAB symptoms. Infectious diseases, such as

neurosyphilis, herpes zoster, herpes simplex, and acquired immunodeficiency syndrome, are also associated with a neurological dysfunction that causes the symptoms of UAB and/or OAB (41-44). Radical prostatectomy, radical hysterectomy, and other pelvic surgical procedures, in which peripheral nerve damage may occur, are examples of an iatrogenic cause of UAB (35). Even OAB and BOO can cause structural changes of the urinary bladder over time, which include a reduction in detrusor blood flow, detrusor muscle deterioration, and proliferation of connective tissue, which can lead to DUA and UAB (45).

DM is one of the most common diseases in general population, which has recently reached epidemic proportions in many countries and is associated with numerous complications and organ damage (46). One of the more common complications of DM is diabetic cystopathy (DC) (47). The prevalence of DC ranges from 20 to 90% (48). As with UAB, we still lack a standardised diagnostic assessment of DC. The symptoms include an impaired sensation of bladder fullness, reduced bladder contractility, increased bladder capacity, and increased PVR. DC can be asymptomatic early in the course of DM (49). DC is a result of sensory and autonomic polyneuropathy, microvascular damage, dysfunction of detrusor smooth muscle cells, and dysfunction of urothelial cells (50). Urothelial cells were considered to be just a barrier between the urinary tract lumen and underlying tissues of the urinary tract wall, but they have a more active role in bladder physiology. These cells form a functional unit that responds to external events by releasing adenosine triphosphatase, nitric oxide, and prostaglandins (51, 52). These modulator agents regulate the activity of afferent nerves and the detrusor smooth muscle and process the information about the urinary tract chemical and physical status (e.g. luminal pressure, urine composition) (53). With all this said, it just goes to show that we still have a lot to learn about the function of the lower urinary tract and surrounding tissues.

Diagnostic evaluation of underactive bladder syndrome

The symptoms of UAB are not specific and they can overlap with other voiding dysfunctions, especially with BOO. The symptoms that may be suggestive of UAB include hesitancy or a delay in passing urine, weak or thin urine stream, interrupted urine flow, post-void dribbling of urine, feeling of having to go again after the stream has stopped, voiding a second or even multiple times after the initial voiding has completed, having to strain to facilitate voiding, and frequent urination day and night in small volumes (54). With history-taking and a physical examination in medical practice, we can only assume that an observed patient has UAB. It is especially important to consider neurological symptoms and diseases. During a physical examination, we palpate the suprapubic region to check the pain and fullness of the bladder. Also, we assess the tone of the anal sphincter, perianal sensation, bulbocavernosus reflex, and plantar reflex, which can be compromised, absent, or significantly diminished (55, 56). Some studies have examined the usefulness of non-invasive methods, such as flow pattern on uroflowmetry, intravesical prostatic protrusion (IPP), PVR volume, and bladder voiding efficiency (BVE) to distinguish between UAB and BOO (15, 57).

Non-invasive methods are very useful for the assessment of patients who cannot go through invasive urodynamic testing. Uroflowmetry is a simple and non-invasive urodynamic test of measuring and recording the voided volume, urinary flow rate, and wave shape throughout micturition (58). It is a screening procedure and its results may be indicative of BOO or UAB. IPP is measured during almost every examination of men with LUTS. It is measured using the transabdominal ultrasound in the sagittal view and defined as the vertical distance from the tip of the protrusion to the base of the prostate (59, 60). Regarding this distance, IPP can be quantified into three grades, which more or less correlate with the symptoms related to BOO: grade 1 (≤ 5 mm), grade 2 (5-10 mm), grade 3 (≥ 10 mm). PVR is measured immediately after

voiding by ultrasound or by inserting a catheter into the bladder through the urethra. Using ultrasound, we measure three dimensions of the bladder: maximal transverse (width), anterior-posterior (height), and longitudinal (length). Then we determine the PVR volume using the Dicuio's formula (volume [mL] = height [cm] × depth [cm] × transverse diameter [cm] × 0.52) (61). To determine BVE, we measure the bladder volume before voiding (BVvoid) using ultrasound and then we measure voided volume (VV) using a measuring cylinder. Using the equation ([VV/BVvoid] × 100), we can express BVE by fraction (%) of the voided volume. A combination of lower IPP (< 8.2 mm) and lower BVE (< 70), sawtooth and interrupted wave shape on uroflowmetry might be useful factors to predict DU and UAB.

Despite all non-invasive methods, pressure-flow study is practically the only method by which we can establish an accurate diagnosis of UAB (62). The parameters observed during this study are peak flow (Qmax) and detrusor pressure at peak flow (PdetQmax) (63). Using these parameters, we can assess the contractility of the bladder by using the bladder contractility index (BCI) formula according to the equation Pdet@Qmax+5Qmax (64). With this formula, we can divide bladder contractility into strong (BCI > 150), normal (BCI 100–150), and weak (BCI < 100). Also, we can exclude or confirm the presence of the obstruction in men using the bladder outlet obstruction index (BOOI) formula according to the equation Pdet@Qmax-2Qmax (65). Based on the results of the BOOI formula, men can be divided into obstructed (BOOI > 40), equivocal (BOOI 20–40), and unobstructed (BOOI < 20). BOOI formula does not apply to women. At the moment, the Blaivas and Groutz nomogram is the most common method used to define BOO in women as a Qmax < 12 mL/sec combined with a Pdet@Qmax of > 20 cm H2O in a pressure-flow study (66). There is a potential future test for UAB that includes urine biomarkers, such as nerve growth factor, and bladder wall biopsy with an electron-microscopic histopathologic ultrastructural assessment of the detrusor (67, 68). The research of these diagnostic methods is still ongoing, so further studies are needed to implement them into clinical practice.

Treatment of underactive bladder syndrome

The treatment of UAB is focused on improving the QOL and preventing complications. If the patients are asymptomatic, we still need to administer the treatment. Also, we must inform patients about possible side effects of each type of treatment. It is necessary to achieve the status of an empty bladder regardless of the aetiology of UAB to avoid chronic urinary retention, bilateral hydronephrosis, recurrent urinary tract infections (UTI), bladder stone formation, and overflow incontinence. Common methods of treatment are timed voiding, double voiding, straining to void, Credé or Valsalva manoeuvre, clean intermittent catheterisation (CIC), pharmacotherapy, and surgical treatment (35, 69, 70).

In patients with vesicoureteral reflux and high intravesical pressure, the Credé and Valsalva manoeuvres are contraindicated. Constipation should be avoided by dietary fibre intake and exercise, which are usually recommended as first-line treatment (71). Prolonged use of laxatives may lead to some adverse effects. CIC must be recommended to patients who have chronic urinary retention with high PVR. If possible, placement of an indwelling urinary catheter and suprapubic cystostomy should be avoided due to complications, such as catheter encrustation, urethral erosion, and increased risk of UTI and bladder cancer (72). We need to explain the benefits of CIC to each patient to enhance compliance. Silicone catheters are most commonly used because they carry a lower risk of allergic reactions. Patients can perform catheterisation on their own or they are catheterised by caregivers. Recommended daily number of catheterisations ranges between 4 and 6 and the amount of urine discharged at every catheterisation should not exceed 400–500 ml. Silicone catheters are most commonly used. CIC is impractical and inconvenient for elderly, visually impaired and mentally handicapped patients, and for patients with

limited manual dexterity. Complications of CIC are urethral injuries (e.g. false passage, strictures), UTI, and haematuria (73).

There are no effective oral drugs for UAB treatment. Contemporary pharmacotherapy of UAB includes the use of α-adrenergic blockers to reduce outlet resistance at the level of the bladder neck and muscarinic agonists or choline esterase inhibitors to increase detrusor contraction (74). These drugs can be used as monotherapy or in combination. α-adrenergic blockers, such as tamsulosin at a dose of 0.4 mg once a day and silodosin at a dose of 8 mg once a day, are in most cases considered for the initial stage of UAB treatment in men (75). α-adrenergic blocker therapy for women with UAB is an off-label regimen in most countries (76). Bethanechol chloride (BC) and pyridostigmine bromide (PB) are the most common orally administered parasympathomimetic agents used in clinical practice (77). The usual adult oral dose for BC ranges from 10 to 50 mg three or four times a day and from 60 to 180 mg for PB. Their effectiveness is limited due to the downregulation of muscarinic receptors and potentially life-threatening side-effects, such as bradycardia and ventricular tachycardia (32). The European Association of Urology (EAU) states that they should not be prescribed for UAB treatment (75). Prostaglandin E2 (PGE2) can increase the inotropic activity in smooth muscles (72). It prevents the release of noradrenaline from sympathetic nerve endings. Intravesical administration of PGE2 can increase detrusor contractions and decrease maximal urethral closure pressure (75). The effectiveness of PGE2 is limited and it is not recommended for routine treatment.

Onabotulinumtoxin A injections into the external urinary sphincter reduce urethral resistance, eliminate the inhibitory effects of urethral afferent nerves on the detrusor and allow easier voiding with the aid of increased abdominal pressure (72). This therapy has not been approved yet and there is no standard dosage. The efficacy of transurethral resection of the prostate and transurethral incision of the bladder neck in patients with BOO and accompanying UAB is questionable. Patients should be informed that they may not benefit from this type of procedure.

Chronic urinary retention associated with UAB often leads to myogenic decompensation because of an overdistended bladder. Reduction cystoplasty decreases the capacity of the bladder and facilitates bladder emptying, but it does not increase bladder contractility (72, 75). This treatment method should be performed only in well-chosen cases and in patients with residual detrusor contractility. Sacral neuromodulation therapy (SNM) is appropriate for well-chosen patients who have not been helped by more conservative treatments, such as drug therapy (78). SNM uses a surgically implanted device to send electrical impulses to the sacral nerves located in the lower back, which modify the function of the detrusor muscle and pelvic floor muscles. It is one of the more expensive methods of treatment and it is performed in specialised high-volume tertiary referral centres. Transurethral intravesical electrical stimulation and use of the stem cells are novel treatment methods with reports that they can improve detrusor contractility and increase weak individual myocyte contractility (79). Neurovascular latissimus dorsi (LD) detrusor myoplasty is suitable for motivated young patients who do not want to undergo CIC. The LD muscle flap is wrapped around the bladder, attached to the pelvic fascia and ligaments, and it is anastomosed to the deep inferior epigastric vessels and lowest motor branches of the intercostal nerve (80). LD detrusor myoplasty can adequately restore bladder emptying on demand (81). Further studies are needed to implement these surgical and novel treatment methods in clinical practice. Regardless of the type of treatment used, patients should have frequent follow-ups with repeated urodynamic examination where necessary.

Discussion

ICS conducted a comprehensive review of the terminology pertaining to the lower urinary tract function and dysfunction and recommended the use of the term DUA (9). UAB has not yet been

defined by the ICS. Furthermore, the current definitions of both conditions do not include possible aetiological risk factors. Based on the aetiology, UAB can be classified into four types: neurogenic, myogenic, and idiopathic (29). Neurogenic UAB is associated with changes in the afferent and efferent signals of the micturition reflex (82). Myogenic UAB is associated with modified contraction mechanisms of detrusor muscle cells, which can result in reduced autonomous activity of the bladder (83). UAB can be the combination of myogenic and neurogenic factors or a combination of two or more neurogenic factors. Idiopathic UAB has an unknown cause or mechanism of apparent spontaneous origin (84).

Based on urodynamic findings, UAB can be classified into three types: DU, acontractile detrusor, and OAB/UAB combination (29). Patients with DUA have impaired detrusor contractility and can void incompletely or they have no urine flow during the urodynamic examination. If there are no detrusor contractions during cystometry and a pressure-flow study, then we are talking about an acontractile detrusor. It is an extreme type of DUA, patients have high PVR, and the emptying of the bladder is performed by increased abdominal pressure or CIC. It is important to note that some patients have UAB and OAB symptoms (17). If we suspect this is the case, a urodynamic assessment is mandatory. DOA is recorded during filling cystometry and DUA during a pressure-flow study. Education about the symptoms and clinical signs of the lower urinary system is of utmost importance for all urologists, urogynecologists, and neurourologists. Taking a medical history in an outpatient setting can be complicated at times and for this reason, it is recommended to use questionnaires, frequency-volume charts, and bladder diaries in the evaluation of symptoms. Physicians must also be well acquainted with performing a physical examination, especially of neurological status. Urodynamic testing is very demanding and one must be very familiar with the methodology to adequately interpret the results. Catheter placement and recording of urodynamic traces must be performed according to the ICS standards. There is still no effective pharmacotherapy of UAB. For now, CIC is the only effective therapy for achieving empty bladder status, but unfortunately, not all patients are willing to perform the catheterisation procedure multiple times a day for an extended period of time. All patients should be offered treatment taking into account the cause of UAB and the most distinct symptomatology. CIC is the main treatment method in the management of patients with high PVR and UAB.

Contemporary research has shown that there are drugs which can have a positive effect on DUA, but in most cases, these studies were not randomised clinical trials. Oral parasympathomimetics can improve detrusor contractions and facilitate bladder emptying, but they do not have a long-lasting effect and they have many serious side effects. ICS and EAU guidelines do not recommend or approve the use of parasympathomimetics for UAB. This therapy is contraindicated in patients with coexisting OAB and UAB symptoms because it may worsen bladder emptying, leading to urine retention and UTI. Therefore, we must perform a thorough neurourologic and urodynamic assessment if we suspect that there are multiple voiding dysfunctions in one patient at the same time. α-adrenergic blockers are useful in men with BOO accompanied by PVR, but their use in women with UAB is not supported by the guidelines. SNM and reconstructive surgical methods are expensive and usually performed in experienced high-volume centres. A large number of patients diagnosed with UAB sometimes do not have access to such centres due to distance or cost of treatment. Research is underway on possible new pharmacotherapeutics and new surgical methods of treating UAB.

Conclusion

Regardless of the complexity of the neuromuscular mechanism of controlling the storage of urine and emptying the bladder, we take the process of voiding for granted. When some form of voiding dysfunction occurs, patients experience how much it can affect the

QOL (85). UAB is a relatively new term of voiding dysfunction and was recognised in some aspects years before we knew what it is today. It has a high prevalence in the elderly population (86). Despite new insights, UAB is still shrouded in mystery (6). Many diseases can cause the loss of sensation in the bladder and/or detrusor muscle damage (8). It is a great challenge to determine exactly which disease caused the symptoms of UAB. We must do our best to identify the most likely aetiology of UAB in each patient. Classification of UAB needs to be improved to facilitate diagnostic processing and it should be based on aetiology, urodynamic findings, and symptoms. At this point, we have a great knowledge about the symptoms of UAB and about the urodynamic characteristics of DUA. This can improve through further research on the pathophysiology of UAB. Future developments in the pharmaceutical industry, gene therapy, and biomedical applications are expected to close the gap in treatment. We need to raise awareness among those patients who are highly likely to develop UAB in order to initiate treatment at the right time and avoid complications.

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Author contribution. Single author article

Review article

Implementation of Telemedicine in Otorhinolaryngology

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Abstract

Telemedicine is a term that covers all procedures supported by communication technology, which has the purpose of providing health services at a specific spatial distance. This is an area that is developing rapidly and has found particular application in otorhinolaryngology, given that a large number of surgeries are performed with the help of endoscopes and microscopes. Telemedicine also represents a significant advantage during the coronavirus pandemic, both in terms of treating patients and monitoring them more effectively. For the purpose of preparing this article, research published on Scopus, PubMed, Google Scholar, and Google was reviewed using the keywords "telemedicine" and "otorhinolaryngology". This review article provides a summary and the latest insights in this broad and fast-growing area. The development of telemedicine in Croatia as well as a special review of the application of telemedicine during the coronavirus pandemic is also presented in this article.

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Introduction

Telemedicine is a term that covers all procedures supported by communication technology, which has the purpose of providing health services at a specific spatial distance. The concept of telemedicine includes various activities, such as teleassistance, telemonitoring, teleconsultation, tele-expertise, videoconferencing, and telesurgery (1). These are the most common terms, although standardised terminology is missing due to a very rapid development of telemedicine (2,3). The goal of all these activities is to increase the quality of health care and make health care available to every patient at any place. Telemedicine is a rapidly evolving field that has provided much more efficient access to patients. Telemedicine has been applied in many specialties, including otorhinolaryngology.

For the purpose of preparing this review article on the application of telemedicine in otorhinolaryngology and the latest knowledge in this field, research published on Scopus, PubMed, Google Scholar, and Google was reviewed using the keywords "telemedicine" and "otorhinolaryngology". Abstracts of relevant articles were studied and based on relevant data, full texts of the papers were reviewed. Considering that telemedicine in otorhinolaryngology is heterogeneous, the review article is divided into several parts depending on the field of otorhinolaryngology concerned.

History of telemedicine and its development in Croatia

In the 1960s, NASA sent humans into space as part of the Apollo project. Astronauts should be medically protected in different conditions – this was the beginning of biomedical observation development (4-7). As there was a need for connection and astronauts' mutual education, NASA played a significant role in telemedicine development due to its ability of establishing satellite links. The first complete telemedicine system was installed in 1967, connecting the Boston Airport medical station with the Massachusetts General Hospital. In 1989, NASA conducted the first international telemedicine project, Space Bridge to Armenia, following a strong earthquake in Armenia in 1988 (8).

The first steps in the development of telemedicine in Croatia were made in the 1980s, when an ECG was recorded based on a distant patient's first message at the Clinic for Cardiovascular Diseases in Zagreb. The cardiologist received the imaging record and interpreted it. The technology of the time was not able to transmit mutual, simultaneous change of image and sound. Progress was made after the development of ISDN networks and establishment of the government agency Croatian Academic and Research Network (CARNet) in 1991/1992. In 1993, a national telepathology system with remote hospital centres was established in Zagreb. In 1995, a program was launched to monitor people with diabetes across the country. In the following year, a telecommunication program for cardiac electrostimulation was presented, and in 1998, the national teleradiology and teleneurosurgery programs were in full function. In the same year, the Croatian Academy of Medical Sciences organised a scientific conference on Croatian telemedicine achievements to encourage cooperation between outpatient facilities and higher health system levels. Three years later, central institutions made connections with some islands. During this period, large medical centres developed numerous tele-education programs (9).

Almost every clinical specialty uses telemedicine in a certain way, but some specialties use it to a greater extent than others. Radiologists, for example, use telemedicine extensively. The teleradiology system electronically transmits radiological images and image descriptions. By transferring the image to the radiologist, he can interpret the radiological images very quickly. After NASA, another essential factor in the development of telemedicine was the military.

In the academic year 2009/2010, the Faculty of Medicine in Osijek recognised the importance of knowing the basics of telemedicine, thus

introducing it as a course in the compulsory elective module (Clinical Medicine) as part of the Postgraduate Doctoral Study Programme of Biomedicine and Health. The course consists of 18 lecture classes and 12 seminar classes and over 40 students enrolled in this elective course in the last two generations.

The timeline of the development of telemedicine in Croatia and the world is shown in Figure 1.

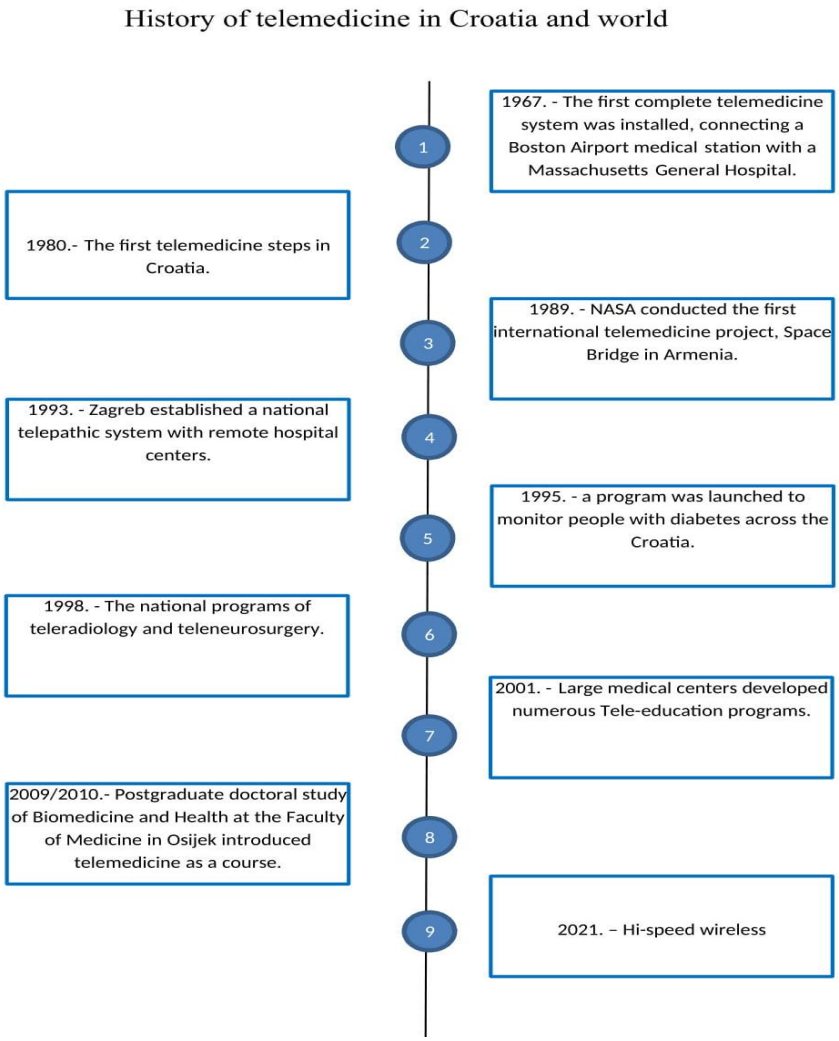

Figure 1. Timeline of telemedicine development in Croatia and the world

Potential of telemedicine development with the development of 5G network

The 5G technology is increasingly mentioned as a driving force of the fourth industrial revolution due to its characteristics and the possibilities it opens up. 5G is as important to humanity as was the invention of the steam engine in the first industrial revolution, the emergence of electricity and oil-powered devices in the second industrial revolution, and the emergence and spread of the Internet in the third industrial revolution. The most important advancements that the 5G technology brings to communication are fast and reliable data transfers, extremely low latency, coverage, and energy efficiency required for such data transfer (10).

The 5G technology improves communication in three main areas using the so-called "5G triangle" consisting of (11): • uRLLC: Ultra-

Reliable Low Latency Communication use cases; • mMTC: Massive Machine Type Communication (IoT) use cases; • eMBB: Enhanced Mobile Broadband – high-speed use cases. The technology called Ultra-Reliable Low Latency Communications (URLLC) brings improvements in the field of healthcare because it is extremely reliable in terms of communication continuity and has a very low response rate, which can be around five milliseconds or less (11).

The latest example of a successful implementation of this technology was given by the Istituto Italiano di Tecnologia in Italy (12), where it was used on the body of a deceased person using robotic technology controlled via a 5G network. The procedure was performed in a manner that the surgeon effectively controlled the surgical robot, forceps, and laser and successfully performed a high-precision laser cordectomy as if he were physically present in the operating room. Surgeries used to be limited due to unreliable and limited networks, which is a problem that the 5G network solves. In this case, it has been proven that a broader implementation of this technology is possible (12).

In addition to surgery, the 5G network will enable fast and reliable transmission of the Internet of Things, which is a network of physical objects consisting of wireless sensors and portable mini-computers that collect data. Such data will be stored in real time due to cloud computing, which provides computing services such as servers, storage, databases, networking, software, analytics, and intelligence (10).

Conditions for the application of telemedicine technology

The biggest advantage of using telemedicine pertains to rural areas and areas of the world where there is a shortage of physicians (13,14). However, certain prerequisites are also required for telemedicine to be used. The application of telemedicine technology primarily depends on the development of telecommunication infrastructure and the relationship between institutions that provide specialist knowledge. At the moment, the most popular approach is based on the "hub-and-spoke" model, in which communications are established between an outpatient department (spoke) and specialist (hub) health care (15). The primary purpose of this model is to have a direct connection between an outpatient department and secondary health care in which there is direct communication between the patient, general practitioner and specialist. Additionally, all sorts of online applications can also provide essential information about the patient and medical data. The fundamental principle is to gather all information we can about a patient in the spoke centre, which is then transferred to a hub centre specialist. This is the point where consultation begins. A primary health care centre must have medical equipment necessary to gather medical information and certain communication equipment, while specialists should only have multimedia equipment at their disposal. Primary health care clinicians should have basic biometric instruments, such as thermometers, pressure gauges, oximeters, and ECG devices. Only with these instruments can we gather important information required for monitoring the patients' condition. Besides biometrics, telemedicine uses all kinds of telemedicine applications that utilise advanced technology to provide static images and videos. Using such interactive technology, we can ensure direct audio-visual communication and transfer static images, such as RTG, CT, or MR scans. It is precisely this technology that enables the classification of telemedicine into tele-education, teleconsultation, telediagnosis, and telemonitoring (15). All of this is important because telemedicine provides telepresence, 3D visualisation and remote control supervision of patients, and better sharing of medical knowledge, which ultimately improves health care for patients.

One of the main limitations of telemedicine in otorhinolaryngology and other medical fields is the need not only for the diagnosis of the disease, but also for treatment (16). Therefore, for some cases when an examination is required, a portable haptic system that transmits a

palpatory impression has been developed, or ultrasound can be used (17,18).

Factors affecting the further development of telemedicine

Telemedicine has proved to be one of the most promising medical branches, with an ongoing increase in distribution worldwide (1). However, it still has not reached its full potential due to many significant reasons. One of the most critical problems regarding telemedicine distribution is the cost of telecommunication infrastructure and technology. Sophisticated telemedicine devices are often over the budget of many hospitals all over the world, especially the ones from developing countries (1).

Secondly, to establish high-quality telemedicine communication with other medical centres worldwide, medical staff should undergo a proper training that will get the best out of the technology that will be used. It is essential to understand the complexity of medical staff involved in telemedicine projects – from physicians, nurses, and technicians to health managers, health administrators, and medical computer scientists. It is easy to conclude that providing these specific groups with training will also take time and funds.

Unfortunately, even when the highest standards in securing top-quality equipment and education for telemedicine are met, one cannot ignore a cultural factor in telemedicine distribution. Many countries do not want to take advantage of modern medicine and are ignorant of creativeness, innovation, and diverse approaches to modern medical issues. If this factor changes over time, there will be significant advancements in modern medicine. Other crucial aspects that most people do not take into account when discussing the factors affecting telemedicine distribution are legal boundaries, which differ from one country to another. For example, some of the United States of America's legal boundaries restrict providing medical services outside of the country (1). Therefore, it would not be legally possible to provide essential medical information to a specific country in Europe that could help in a patient's treatment outside the US. Furthermore, distant telemedicine communication should not result in compromising and disclosing a patient's private information, which is one of the most critical patient's rights.

Because of the numerous advantages that modern telemedicine offers, the highest priority of every country should be to create stable and top-quality medical centres that provide telemedical services and to make them a reference centre. In order to achieve that, every country should have an assessment team, which would be in charge of the evaluation of certain medical centres in order to provide top medical services. Unfortunately, most countries still have infrastructural and organisational limitations in that regard.

Even though there are various problems regarding telemedicine distribution, it is important to emphasise that telemedicine is growing increasingly in the entire world. With high motivation and acceptance of different ideas, telemedicine could be very successful in the future.

Special review of the use of telemedicine during the coronavirus pandemic

COVID-19 disease is a viral infection caused by the coronavirus named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The disease spread quickly across the globe. SARS-CoV-2 transmission occurs via respiratory droplets produced by infected individuals while talking, sneezing, or coughing without covering the mouth and the nose (57). Minimising the virus transmission has become a critical part of patient care during the COVID-19 pandemic. Telemedicine is indeed advantageous in that regard as it prevents person-to-person contact, thus ensuring social distance, and it is therefore recommended by various health agencies for COVID-19 (58). Fortunately, telemedicine is not new to otorhinolaryngologists, so it can be applied in real-time delivery and monitoring of services via video conferencing (synchronous care). On the other hand, there is a store-and-

forward methodology, where test results are stored and reviewed later (asynchronous care). These evaluations have been proven useful for plastic postoperative evaluation, head and neck oncology, facial trauma evaluation, triaging and postoperative visits, allergic or non-allergic rhinitis management, endoscopic sinus surgery, postoperative evaluation, and vocal rehabilitation. The COVID-19 pandemic has propelled telemedicine necessity and utilisation into the mainstays of current otorhinolaryngology practice (59).

Application in audiology

Audiologists have the opportunity to provide services to those who may otherwise have no access to much-needed care, from those in rural areas to patients with limited physical mobility (19). Hearing aid services, such as counselling, fitting, and device testing can be provided through telemedicine. Lack of high-speed Internet connections and limited bandwidth in some rural locations could prevent the delivery of some services (20). Telemedicine in audiology can be applied in real-time delivery and monitoring of services via videoconference. On the other hand, there is a store-and-forward methodology, where test results are stored and reviewed later. Likewise, telemedicine in audiology has been applied in providing education and training to professionals, hearing screening, diagnosis, and intervention (21). Small fitting of hearing aids is possible via the Internet. The technological approach can help with hearing rehabilitation, as patients can ask for help from the comfort of their own homes (22).

Furthermore, teleaudiometry is a reliable, valid, and viable option for hearing tests. Results can be analysed by experts remotely. Moreover, teleaudiometry reduces the costs of hearing screening programs (23). Telemedicine in audiology opens access to vital hearing care.

Telemedicine in Phoniatrics and Phonosurgery

Phoniatrics is a branch of otorhinolaryngology that studies, diagnoses, and deals with voice, speech, language, and swallowing disorders and diseases. The application of telemedicine in phoniatrics and phonosurgery is continuously growing and it can be an essential tool in certain medical situations, such as stuttering or dysphonia (1). The possibility of consulting with colleagues via web conference technology can sometimes be crucial in patient treatment options. According to some reports, around 12% of all speech therapies and assessments in India were performed via telemedicine (1)!

The most important aspect of presenting a phoniatric patient via remote stroboscopy and laryngoscopy is high video and audio recording quality. Higher quality of the footage will provide precise visual and auditory information about particular laryngeal pathology and facilitate physicians' decision about treatment. Furthermore, telemedicine can be essential in voice rehabilitation. It allows for direct consultations with experts across the world on certain phonological issues and provision of optimal healthcare to a patient by eliminating the barriers of time and space.

A fascinating and successful example of telemedicine usage in phoniatrics is the Camperdown Program, a therapeutic approach in the treatment of stuttering (24-27). This therapeutic approach showed no significant difference in curing stuttering telematically in comparison to the in-person approach (28-31). This is only one of many examples highlighting telemedicine as one of the most critical and trustworthy approaches in modern phoniatrics.

Unfortunately, the cost of sophisticated technology and its complexity, which requires trained personnel, are often a limiting factor for a vast number of otorhinolaryngology departments.

However, every medical professional should look forward to dealing with current barriers with a great deal of optimism and motivation to improve every aspect of telemedicine and make it available to every single part of the world.

Application in rhinology

Numerous studies have proven that telemedicine consultation can be applied in rhinology as well. Setzen et al. pointed out that it is possible to triage patients who need to undergo nasal endoscopy by taking a medical history through telemedicine, but this requires guidelines that need to be determined in the future (32). Patients with unilateral sinonasal symptoms and patients with radiologically proven unilateral sinonasal diseases need repeated medical check-ups after nasal endoscopy via telemedicine. Also, during the COVID-19 pandemic, high-risk, immunocompromised patients present a challenge. Telemedicine allows such patients to stay at home during their first examination. In immunocompromised patients, nasal endoscopy should be indicated at the onset of new symptoms, such as severe pain, fever, and other general symptoms (32). When it comes to ENT, patients' condition can be assessed by telemedicine by radiological images (X-ray, CT) and endoscopic images, which can be qualitatively transmitted to distant places. Telemedicine technology has made rapid progress in the last 20 years in terms of the quality of equipment, better internet connectivity, and better image sharing software. In ENT, synchronous telemedicine is also being developed, which has significant advantages over older methods (33,34).

Nasal endoscopy is a high-risk test due to a potential exposure of the examiner to a COVID-19 infection. Also, anaesthetic sprays have aerosol potential, which increases the risk of infection transmission, both by direct contact and indirectly. As numerous studies have shown a correlation and concordance in the evaluation of sinus disorders between CT and nasal endoscopy, CT of the paranasal sinuses represents an interesting alternative to nasal endoscopy (35). Another alternative is intranasal imaging, where, for instance, video files can be sent to a remote device (36). Epistaxis is a common disorder in rhinology, which may be adaptable for a remote evaluation. Telemedicine can help detect trigger risk factors and help cure minor bleeding. A history of uncontrolled bleeding, despite conservative measures provided, requires a prompt live evaluation. In rhinology, telemedicine may also play a role in a postoperative follow-up of patients after a nasal septal surgery and a functional endoscopic sinus surgery (32).

Telemedicine is being developed and is useful in skull base surgery. Telementoring is widely applicable in skull base surgery, in endoscopic endonasal surgery for the surgical treatment of diagnoses such as benign and malignant neoplasms, cerebrospinal fluid leak, and inflammatory diseases. This technology requires two-way real-time video and audio communication using the existing technology. An expert who is not present in the operating room and a surgeon communicate using telemedicine technology and they have robotic technology available to visualise the operating field. Also, with the help of a laser pointer, orientation can be assisted (37).

Application in head and neck oncology

Telemedicine is widely applicable in surgical head and neck oncology. In this area of otorhinolaryngology, the application of telemedicine results in better patient follow-ups, better collaboration among physicians, reduction of unnecessary patient's transfer to specialist facilities, and reduction of patient travel costs (38-41). Kohlert et al. show that 48.6% of consultations in otorhinolaryngology account for head and neck oncological surgery (42). A study from the USA conducted by Prasad et al. describes a virtual examination of patients with head and neck cancer, aiming to set out guidelines for each specific tumour site for the best follow-up possible (43). While extremely useful, this approach can be challenging for both the physician and the patient, mainly because it concerns a new clinical evaluation model for both parties. Head and neck cancer surveillance is often challenging to perform in person, and doing this virtually is even more difficult (38,44).

A multidisciplinary team participates in treating an oncology patient and communication and cooperation between team members are improved using real-time video conferencing (45-49). Also, this form of communication

between multidisciplinary team members has been applied during the COVID-19 virus pandemic due to the inability to gather team members in one place. In addition to real-time video conferencing, communication can be established using various communication platforms. Rimmer et al. stated that for some patients, postoperative telemedicine monitoring is relatively safe and saves time (48). Also, during the COVID-19 epidemic at our institution, psychological counselling was provided to oncology patients by phone, which according to some studies had a positive effect on this type of patients (50,51).

Application in palliative care

Less than a decade ago, the Worldwide Palliative Care Alliance and the World Health Organization concluded that palliative health care represents both a necessity and an essential human right (52). However, palliative care has been suffering from a significant lack of resources for years, primarily in terms of finances. The situation is further aggravated by the ongoing pandemic. Some researchers have noticed that head and neck tumours have shown some similarities with the COVID-19 infection. Regarding both conditions, resources are low, there is a shortage of time, patients' condition can quickly deteriorate, and the family needs consulting about these diagnoses (52). Therefore, the majority of head and neck tumour centres have allowed consultations with patients and their families by phone. The most important goal is to lower the number of unnecessary hospitalisations and consequently reduce the risk of a coronavirus infection for those patients. Another equally important thing is to provide secondary health care through a "hub-spoke" model, in which human empathy and a professional relationship between primary and secondary care physicians should play the key role (52). We live in the age of technology and one should make use of its resources because the definition of palliative care is to provide the best health care possible and to improve the quality of life of patients suffering from a disease in its terminal stage. This is where telemedicine comes in with its potential to improve health care to a degree which every terminal head and neck oncology patient deserves. Therefore, it is necessary to work with telemedicine technology on further development of palliative care, and in otorhinolaryngology, this primarily applies to patients suffering from head and neck cancers (53,54).

Application in sleep medicine

Obstructive sleep apnoea (OSA) is a common disease that leads to severe symptoms and comorbidities. Although general measures are essential, continuous positive airway pressure (CPAP) is the best treatment option (55). However, compliance may be suboptimal and telemedicine may play a role in improving it. To improve OSA management, there is an urgent need to develop new cost-effective strategies, especially those related to OSA treatment, from lifestyle changes to CPAP implementation. Two broad strategies should be applied: 1) appropriate pre-, peri-, and post-titration measures to ensure sufficient continuous positive airway pressure, appropriate training, and appropriate support during follow-up; and 2) use of technological advances, including the optimisation of CPAP devices and the use of telemedicine, specifically focused on the first days or weeks of treatment (55,56). Telemedicine can help with these processes, especially when tailored to each patient.

Traditional methods of diagnosis and treatment of OSA include history, clinical examination, diagnosis, counselling on the condition and treatment of the patient, patient care, and self-treatment of patients with mild or severe obstructive sleep apnoea (56). A multidisciplinary approach is required for an optimal diagnosis and treatment of patients with OSA. The neurologist, otorhinolaryngologist, and head and neck surgeon play the most important role in this multidisciplinary team. The neurologist diagnoses obstructive sleep apnoea by using polysomnography, the golden diagnostic standard. The otorhinolaryngologist tries to locate the site of airway obstruction in a patient by performing a standard clinical

examination, including awake fibre endoscopy and drug-induced sleep endoscopy (DISE). The head and neck surgeon's role is surgical treatment of the detected obstruction and consequent improvement of airflow through the upper respiratory tract. Adhering to the guidelines and best practices of treatment and follow-up of patients, there are also numerous opportunities for improvement with new technologies.

Telemedicine can improve the treatment and follow-up of patients with obstructive sleep apnoea by educating patients more efficiently and reducing the costs of treatment (56). Medical history is essential for a correct diagnosis, and it can be done by phone, video link, or e-mail consultations. Completion of surveys can also take place electronically. Measurements of body weight, BMI, height, and blood pressure can be taken at home, and the results can be sent electronically. Replacing the examination of patients, such as a specialist examination by an otorhinolaryngologist, with video call examinations is challenging. This is because the examination involves methods such as indirect laryngoscopy, fibre endoscopy, or DISE, so in this case, one-on-one examination is essential. Monitoring treatment outcomes and the condition of patients with obstructive sleep apnoea by other specialists can be facilitated by telemedicine because the CPAP device can send information about the patient and therapy given (55). As with other diseases and conditions, OSA telemedicine plays a vital role in the communication between members of a multidisciplinary team, who can share experiences, educate themselves, monitor the patient, and decide on further treatment through videoconferencing.

Conclusion

The development of telemedicine in otorhinolaryngology represents a new approach to diagnostic and therapeutic procedures. This is a rapidly evolving field, which certainly introduces some novelties into everyday practice, improves the quality and availability of medical care in areas where there is a shortage of physicians, and increases the attractiveness of otorhinolaryngology to younger colleagues. It is also necessary to point out the shortcomings of telemedicine technology – primarily expensive equipment and the need for additional staff training. In countries such as Croatia, where there is no shortage of otorhinolaryngologists and resources are limited, telemedicine can be beneficial in situations such as the COVID-19 pandemic and it can also be used for educational purposes.

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Original article

Role of Life Habits as a Construct in Dementia Prevention

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Abstract

Introduction: For over three decades, Europe has been viewed as a continent of the elderly. However, despite the fact that the Republic of Croatia is a part of Europe, it is only nowadays that we have come to face this problem in the form of a large – and rapidly growing – elderly population. Since age is the main risk factor for the onset of dementia, a further increase in the number of patients is expected. Consequently, the need for new insights in the field of constructs that contribute to the prevention of dementia is implied in the context of reducing the number of patients. This research aimed at gaining insight into life habits of individuals using homes for the elderly and infirm, which habits have been shown by previous research as contributory to dementia prevention or mitigation.

Materials and Methods: This research was conducted using an appropriate sample of 443 users of decentralized homes for the elderly and infirm in Osijek-Baranja County.

Results: The obtained research data was divided into four categories: physical activity, cognitive activity, consumption of tobacco products, and alcohol and diet. The results showed that physical and cognitive activities are the least represented in everyday lives of users of homes for the elderly and infirm.

Conclusion: Based on research results, the importance and role of physical activity and maintenance of cognitive skill will be emphasised with an aim of achieving better quality aging, especially in terms of dementia prevention in the elderly.

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Introduction

Dementia describes a group of complex disorders that change and interfere with normal brain activities and result in disorders of various cognitive functions. One of the most common degenerative nervous system diseases from the dementia group is Alzheimer's disease. The disease is characterized by a progressive course, and in individuals older than 65 it is the most frequent cause of dementia (2). Care for the elderly suffering from dementia, given the specific behavioural and psychological symptoms and physical illnesses, requires specific medical interventions and imposes a heavy burden of care. Because of that, it is important to choose appropriate medical institutions that are able to cope with various care needs of individuals suffering from dementia (3). The incidence of Alzheimer's dementia is increasingly gaining the scale of a global health crisis and represents a substantial economic burden on society as a whole (4). Since there are no specialized institutions for taking care of individuals suffering from various forms of dementia in the Republic of Croatia, it is necessary to focus on dementia prevention in order to reduce the number of individuals affected. A significant number of attempts in terms of therapeutic treatment have not been satisfactory, possibly because the treatment starts too late and at advanced stages of the disease, with highly developed pathology. Research is focused on treatment, while prevention is neglected (5). Therefore, an alternative strategy in the fight against dementia can be the prevention of various changeable factors, which also includes adequate control of comorbidities and lifestyle changes with avoidance of harmful habits (6).

Comprehensive clinical and epidemiological evidence points towards a close connection between dementia and heart disease, and due to their increased incidence and coexistence, they demand more attention because they represent a threat to public health (7). Alzheimer's disease is linked to smoking tobacco products since the risk of dementia is increased in the population of active smokers (8). Type 2 diabetes, repeated head traumas, obesity, high blood pressure and social interactions are also changeable risk factors for dementia development. With early detection and measures for prevention of the aforementioned factors, it could potentially be possible to effectively prevent the development of Alzheimer's disease (2). Adequate diet in combination with physical activity also represents a therapeutic potential in terms of prevention and delaying the onset of symptoms (4). Research has confirmed that there are protective factors that contribute to dementia prevention or reduce its severity, for example, a higher level of education, intellectual activity, diet rich in unsaturated fatty acids, physical activity and moderate alcohol consumption (9).

The theoretical and practical findings referred to above are the source from which the purpose of this research emerged. This research aims at getting an insight into life habits of users of homes for the elderly and infirm for which previous research has shown to contribute to dementia prevention or mitigation. Three hypotheses have been set: a) The elderly do not recognize the importance of physical activity as one of the factors contributing to dementia prevention or mitigation; b) The elderly do not recognize the importance of maintaining cognitive activities as one of the factors contributing to dementia prevention or mitigation and c) The elderly do not recognize the importance of avoiding alcohol and tobacco products consumption as factors contributing to dementia prevention or mitigation.

Materials and Methods

The primary purpose and aim of this research is to determine which physical activities, cognitive activities and substances directly affect the life of the elderly, but at the same time contribute to development of dementia. For achieving the aforementioned goal, it was necessary to collect data and carry out the primary research process. The data was collected via an anonymous questionnaire designed by the authors. This research was conducted on an appropriate sample of 443 users of decentralized homes for

the elderly and infirm in Osijek-Baranja County. The questionnaire created by the authors for the purposes of this research was used by interviewing users in person, by interviewers.

Statistical analysis

Analyses of the collected data were performed using the statistical program IBM SPSS Statistics 23 for social sciences. In order to examine the correlation between the variables included in this study, Pearson correlation coefficients were calculated. The respective correlation coefficients, together with their significance, are shown in the results.

Table 1. Socio-demographic characteristics of research participants

	N (%)
Participants' gender	
Female	317 (71.6%)
Male	126 (28.4%)
Participants' age	
Under 50	3 (0.7%)
51-60	8 (1.8%)
61-70	29 (6.5%)
71-80	169 (38.2%)
81-90	207 (46.8%)
91-100	27 (6%)
Qualifications	
No elementary education	86 (19.4%)
Elementary education	151 (34.1%)
Secondary education	151 (34.1%)
Specialist training	40 (9%)
Higher education	14 (3.2%)
Doctorate	1 (0.2%)
Time spent in a home or homes for the elderly and infirm	
Less than 1 year	52 (11.7%)
1-5 years	211 (47.6%)
6-10 years	120 (27.1%)
Over 10 years	60 (13.5%)

Source: authors

Results

Socio-demographic data

This research included 443 participants who are users of homes for the elderly and infirm in Osijek-Baranja County. In terms of gender, 317 (71.6%) were women and 126 (28.4%) were men. Regarding age, 3 participants (0.7%) were under 50 years of age, 8 (1.8%) were between 51 and 60 years, 29 (6.5%) were between 61 and 70 years, 169 (38.2%) were between 71 and 80 years, 207 (46.8%) were between 81 and 90 and 27 (6%) were between 91 and 100 years of age. Regarding qualifications, 86 (19.4%) of the participants have not finished elementary school, 151 (34.1%) have completed their elementary education and the same number (151, i.e., 34.1%) have completed their secondary education, 40 (9%) have finished specialist training, 14 (3.2%) have higher education degrees and only one participant (0.2%) has a doctorate. Since all participants were users of homes for the elderly and infirm, they differed by time spent in homes; 52 (11.7%) participants had lived in a home less than one year, 211 (47.6%) participants had lived in a home between 1 and 5 years, 120 (27.1%) participants had lived in a home between 6 and 10 years, and 60 (13.5%) had lived in a home for over 10 years (Table 1).

Physical activity

Table 2 presents data regarding physical activity of participants. When asked: "How often do you exercise?", as many as 168 participants (37.9 %) answered "never", 160 participants (36.1%) answered "occasionally", 76 participants (17.2%) answered "regularly", and only 39 participants (8.8%) answered "often". When asked: "How often do you take walks in nature?", 74 participants (16.7%) answered "never", 168 participants (37.9%) reported that they do it "occasionally", 117 participants (26.4%) answered "regularly", and 84 participants (19%) answered that they often take walks in nature. Furthermore, as many as 148 (33.4%) of participants never use stairs instead of an elevator, 112 participants (25.3%) occasionally use stairs, 83 participants (18.7%) use stairs regularly, and 100 participants (22.6%) frequently use stairs instead of an elevator...

Table 2. Descriptive data for physical activity parameters

	N (%)
How often do you exercise?	
Never	168 (37.9%)
Occasionally	160 (36.1%)
Regularly	76 (17.2%)
Often	39 (8.8%)
How often do you take walks in nature?	
Never	74 (16.7%)
Occasionally	168 (37.9%)
Regularly	117 (26.4%)
Often	84 (19%)
How often do you use stairs instead of an elevator?	
Never	148 (33.4%)
Occasionally	112 (25.3%)
Regularly	83 (18.7%)
Often	100 (22.6%)

Source: authors

Cognitive activity

Table 3 presents data regarding cognitive activity of participants. Namely, when asked: "How often do you solve crosswords, do puzzles or play memory games?", as many as 258 participants (58.2%) answered "never", 60 participants (13.5%) answered "occasionally", 62 participants (14%) answered "regularly", and 63 participants (14.2%) answered "often". Furthermore, when asked: "How often do you play chess?", 318 participants (71.8%) answered "never", 48 participants (10.8%) answered "occasionally", 34 participants (7.7%) answered "regularly" and 43 (9.7%) answered "often". When asked: "How often do your read books, magazines, newspapers?", 156 participants (35.2%) answered "never", 117 participants (26.4%) answered "occasionally", 82 participants (18.5%) answered "regularly", and 88 (19.9%) answered that they often read books, magazines and newspapers.

Table 3. Descriptive data for cognitive activity parameters

	N (%)
How often do you solve crosswords, do puzzles or play memory games?	
Never	258 (58.2%)
Occasionally	60 (13.5%)
Regularly	62 (14%)
Often	63 (14.2%)
How often do you play chess?	
Never	318 (71.8%)
Occasionally	48 (10.8%)
Regularly	34 (7.7%)
Often	43 (9.7%)
How often do your read books, magazines, newspapers?	
Never	156 (35.2%)
Occasionally	117 (26.4%)
Regularly	82 (18.5%)
Often	88 (19.9%)

Source: authors

Consumption of alcohol and tobacco products

Table 4 presents data that show that 312 participants (70.4%) never consume tobacco products, 30 participants (6.8%) consume them occasionally, 44 participants (9.9%) consume them regularly, and 57 participants (12.9%) consume them often. Furthermore, from Table 4 we see that 295 participants (66.6%) never consume alcohol, 76 participants (17.2%) consume it occasionally, 26 participants (5.9%) consume it regularly, and 46 participants (10.4%) consume it often.

Table 4. Descriptive data for consumption of tobacco products and alcohol parameters

	N (%)
How often do you consume tobacco products?	
Never	312 (70.4%)
Occasionally	30 (6.8%)
Regularly	44 (9.9%)
Often	57 (12.9%)
How often do you consume alcohol?	
Never	295 (66.6%)
Occasionally	76 (17.2%)
Regularly	26 (5.9%)
Often	46 (10.4%)

Source: authors

Diet

Table 5 shows data regarding the participants' diet. Salads, fresh fruit and vegetables are never consumed by 13 participants (2.9%), 90 participants (20.3%) eat them occasionally, 260 participants (58.7%) eat them regularly, and 78 participants (17.6%) often eat salads and fresh fruit and vegetables. Two participants did not provide an answer to this question. Regarding fish, olive oil, nuts and eggs, 43 participants (9.7%) never consume them, 161 participants (36.3%) consume them occasionally, 192 participants (43.3%) consume them regularly, and 47 participants (10.6%) consume them often. When asked: "How often do you consume bakery products, sweets and salty foods?", 54 participants (12.2%) answered "never", 172 participants (38.8%) answered "occasionally", 145 participants (32.7%) answered "regularly", and 72 participants (16.3%) answered they consume these products often..

Table 5. Descriptive data for dietary parameters

	N (%)
How often do you consume tobacco products?	
Never	312 (70.4%)
Occasionally	30 (6.8%)
Regularly	44 (9.9%)
Often	57 (12.9%)
How often do you consume fish, olive oil, nuts, eggs?	
Never	43 (9.7%)
Occasionally	161 (36.3%)
Regularly	192 (43.3%)
Often	47 (10.6%)
How often do you consume bakery products, sweets and salty foods?	
Never	54 (12.2%)
Occasionally	172 (38.8%)
Regularly	145 (32.7%)
Often	72 (16.3%)

Source: authors

Correlation between variables

Table 6 shows significant negative and insignificant correlations between the variables "How often do you walk in nature?" and "Time spent in a home or homes for the elderly and infirm" (r = -0.11; p = 0.027) and variables "How often do you consume tobacco products?" and "Time spent in a home or homes for the elderly and infirm" (r = -0.10; p = 0.029). Other parameters of physical and cognitive activity did not prove to be significantly correlated to the length of stay in a home or homes for the elderly and infirm, nor did alcohol consumption and eating habits.

Table 6. Review of correlations of variables

	Time spent at a home or homes for the elderly and infirm
How often do you exercise?	-0.04
How often do you take walks in	**-0.11***
How often do you solve crossword	-0.04
How often do you play chess?	-0.09
How often do you read books,	-0.06
How often do you consume tobacco	**-0.10***
How often do you consume alcohol?	-0.08
How often do you eat salads, fresh	-0.01
How often do you consume fish, olive	-0.06
How often do you consume bakery	0.05
How often do you climb the stairs	-0.09
How often do you use medications?	0.06

Note: *p<0.05; **p<0.01 (significant correlations are written in bold)

Source: authors

Discussion

The obtained research data was divided into four categories: physical activity, cognitive activity, consumption of tobacco products, and alcohol and diet. The results showed that physical and cognitive activities are the least represented in everyday lives of users of homes for the elderly and infirm, i.e., that the elderly do not recognize the significance of physical and cognitive activities as important factors that contribute to dementia prevention or mitigation, which confirms hypothesis a) and hypothesis b). Positive aspects are that the majority of participants do not consume tobacco products or alcohol, which rejects hypothesis 3, considering that the elderly do recognize the importance of avoiding the consumption of tobacco products and alcohol as important factors that contribute to dementia prevention or mitigation. Furthermore, diet differs when it comes to individual items so the participants in most cases consume fresh fruit and vegetables, but they rarely consume fish, olive oil, nuts and eggs. Furthermore, in showing the correlations between the variables, the results showed that there is a significant, but only slightly significant, correlation between the variables related to the frequency of taking walks in nature and consuming tobacco products with the time spent in a home. Namely, the longer the time spent in a home or homes for the elderly and infirm, the less frequently the users take walks in nature and consume tobacco products.

Previous research has shown that non-pharmacological interventions could have a significant role in prevention, but also in the progression of dementia (10). Research was mostly focused on the treatment of dementia, while prevention was neglected (5), but lately,

due to the increasing number of affected individuals, dementia is becoming a public health problem and a larger number of researchers focus on detecting risk and protective factors in dementia prevention. According to Mimica, we cannot protect ourselves completely against dementia, but if simple recommendations are adopted, the risk of developing dementia can be reduced by one third (11).

In a research conducted by Farina et al. based on six observed studies, it was concluded that physical activity has a positive effect on increase in cognitive functions in the case of dementia (10). Du et al. conducted a research on 869 participants suffering from Alzheimer's disease. Their results also showed positive effects of physical activity on cognitive functions of patients (12). The fact that an active lifestyle is one of the predictors of dementia prevention or mitigation was also confirmed by a research conducted by Rolland et al. (13). They claimed that in the future, prevention of Alzheimer's disease could be based on precisely determined rules regarding management of life habits that include physical activity, cognitive activity and diet (13). Focusing on the principle that physical activity contributes to dementia prevention, Sondell et al. conducted a research in 16 homes for the elderly and infirm in order to observe how exercise programs for the users should be created. Their results showed that group, monitored and individual programs of functional training are the most effective for dementia prevention and that it is very important to motivate users of homes for the elderly and infirm to participate in such programs (14). Furthermore, Rege et al. conducted a study that examined 164 epidemiological, longitudinal, cross-sectional, intervention and randomized controlled studies. Results of this overview confirmed therapeutic potential of physical activity in combination with diet as an important protective factor in dementia prevention (4). According to Kornhuber, a healthy lifestyle with daily activities performed outdoors, Mediterranean diet and reduced consumption of alcohol significantly reduce the risk of dementia, in which context the results showed that cognitively stimulating activities protect even more than physical activities (5). The fact that cognitive engagement, regular physical activity, Mediterranean diet and the consumption of omega-3 fatty acids are important protective factors in dementia prevention was confirmed by a study conducted by Barak and Aizenberg (15). Furthermore, most users come to institutional care when they become dependent on the help and care of others due to their health condition, which reduces their mobility, but research conducted by Močnik et al. (17) shows that even mobile users are not aware of the importance of physical activity and its effects on health in old age and that 71% of respondents do not participate in any physical activity in homes for the elderly and infirm (17). This is why the emphasis should be on motivating and informing users about healthy lifestyles and encouraging lifestyle changes. In addition, literature shows that smoking is significantly linked to an increased risk of Alzheimer's disease. Namely, smoking-related cerebral oxidative stress is a potential mechanism that promotes the pathophysiology of Alzheimer's disease and increases the risk of developing this disease (16). Finally, all conducted studies indicate that dementia prevention is possible. Namely, physical and cognitive activity, Mediterranean diet and reduced consumption of alcohol and tobacco are key protective factors in the fight against this disease.

The results of this research raise the question about the reason why the elderly have life habits shown in the results. Why is it the case that the majority of the elderly do not recognize the importance of physical and cognitive activity as protective factors in dementia prevention? Since all the participants live in homes for the elderly and infirm, the results of this research place a challenge in front of experts employed at those institutions, but also in front of other stakeholders, such as political and public authorities that can contribute to the creation of strategies and programs for dementia prevention. The elderly need to be educated and provided with information on the importance of changing life habits with an aim of dementia prevention. It is also important to note

that, given that the participants were users of homes for the elderly and infirm, where alcohol and tobacco consumption is prohibited, it is possible that this policy influenced the research results and the rejection of hypothesis 3. Namely, the results show that a significant majority of participants never consume alcohol and tobacco products and that the frequency of consumption of tobacco products decreases as time spent at a home increases, which could be the result of obeying house rules of a home they reside in. Furthermore, regarding diet in homes for the elderly and infirm, users receive already prepared meals and cannot choose their menus. Because of that, diet standards in institutions should be harmonized based on the nutrients needed for providing the highest possible level of protective factors for dementia prevention. Cooperation between public authorities and homes for the elderly and infirm with an aim of dementia prevention will give results over a certain period, and we could use a longitudinal research in order to examine the extent to which life habits of the users have changed.

Upon consolidation of author's research and the mentioned previous research, it can be concluded that physical activities, cognitive activities and substances that directly affect the lives of the elderly are imperative in terms of life habits that as a construct have a role in dementia prevention. On the other hand, we can say that the research has brought to light significant segments of risky behaviour of users that are of crucial importance, and as such can contribute to dementia process. Staff communication in the process of everyday interaction with users must be given primacy, through training with a view of improving the users' quality of life. Communication also has a role of transferring information, instructing and activating users; consequently, emphasis has to be on educated staff that plays a daily role in the lives of users of homes for the elderly and infirm. Acknowledging the fact that the elderly have a higher risk of developing some type of dementia, it has been confirmed that dementia prevention is possible and necessary in preserving the quality of life of the elderly. Obtained research results can give a special contribution to future research by the authors, at which occasion initial and new discoveries will be compared, after allowing some time for guidelines to be given to the elderly through direct communication with experts.

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Review article

Translation of the Clance Impostor Phenomenon Scale Into the Croatian Language

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Abstract

Introduction: Clance Impostor Phenomenon Scale (CIPS) is the most common and psychometrically sound instrument used to measure the impostor phenomenon. The aim of this study was to translate and make a cultural adaptation of the CIPS from English into Croatian.

Materials and Methods: The translation process included two independent forward translations, combination of the forward translations into one single translation, back-translation, back-translation review, pre-piloting and drafting of the final translation after several revisions and minor adjustments by a professional reviewer.

Results: We noticed no semantic differences when comparing the original and the back-translated versions of the CIPS. Thus, the final translation was only slightly changed in comparison with the first version.

Conclusions: The version of the CIPS which was translated and culturally adapted into Croatian represents a reliable translation ready to be used in Croatia and Bosnia and Herzegovina.

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Introduction

The psychological term impostor phenomenon (IP) can be identified using a combination of keywords and phrases, including spelling variations and synonyms, such as impostor phenomenon, impostorism, impostor syndrome, impostor, and perceived fraudulence (1). Psychologists P. Clance and S. Imes coined the term in 1978 based on clinical observations and after Clance published her book in 1985, the term attracted increasing attention. Nowadays, the term is widely present due to its strong association with several personal and work-related implications (2–4). Impostorism is not a diagnosable mental illness or condition, yet a pervasive psychological experience of perceived intellectual and professional fraudulence. Impostors doubt their accomplishments and have a persistent fear of being exposed as a fraud (5). Despite adequate evidence of accomplishment, they feel self-doubt and perceive themselves as unworthy of promotion, recognition and reward, convinced that they do not deserve the success (2). Some characteristics indicative of a maladaptive personality style facilitate the vicious cycle of perceived inadequacy. Impostors use increased efforts aimed towards achieving their goal to overcompensate for their frustration, but these well-known perfectionists and workaholics suffer from a considerable amount of pressure, anxiety, fear of failure and life dissatisfaction (3,6).

Impostorism occurs across different cultures and lifetime prevalence is as high as 70% (7–11). Although Clance and Imes first noticed that this pattern was more common in females, subsequent studies yielded inconsistent results, indicating that the impostor experience was equally or even more common among men (3,6,11,12). It should be noted that it does not affect only highly successful people and that anyone could view themselves as impostors if they failed to internalize their success (13). The presence of impostorism has been studied among different occupations, such as college students (9,10,13), academics (11), physician assistants (14), marketing managers (15) and medical students (1,16). A highly competitive environment in the medical field, especially during the training period, probably contributes

to the high prevalence of impostorism. However, this phenomenon, which affects nearly half of females and one-fourth of males among medical students and residents, is still under acknowledged by the healthcare community (17). Initially, it was considered static, but subsequently, its' quite dynamic nature was recognized, and the transition from preclinical to clinical training was described as particularly challenging (9–11). Researchers have identified several factors contributing to the emergence of impostorism, such as perfectionism (2,18–20) and family environment (2,9,21,22). Family dynamics and parenting style can impact the values and behaviours associated with children's success, especially how the child will learn to cope with success and failure (23). General family characteristics were proposed as contributors to the reappearance of the impostor phenomenon: (i) the perception of the impostor that their talents are uncharacteristic compared to family members' talents, (ii) family messages that convey the importance of intellectual ability and that success requires little effort, (iii) disagreement between feedback on the abilities and success of impostors stemming from the family and other sources and (iv) a lack of positive reinforcement or support. The tendency of impostors to reject positive feedback and uphold high standards for self-evaluation while remaining critical of their inability to achieve these standards shows consistency with perfectionism (23). Regarding the IP and personality traits, neuroticism was identified as a strong predictor (23) and there are also positive associations with introversion (5), as opposed to conscientiousness and agreeableness (23).

Different definitions of the IP and various measurement scales were made for clinical and research purposes in order to quantify the characteristics of the impostor experience. The Clance Impostor Phenomenon Scale (CIPS), the most common and psychometrically sound instrument (2,24), was developed in 1985. Besides the Harvey Impostor Scale (11), there are also two other separate scales: the Perceived Fraudulence Scale (PFS) (25) and the Leary Impostor Scale (26). It should be noted that researchers defined the construct differently – Harvey, Clance, and Kolligian and Sternberg (3,11,25) suggested multidimensionality of impostorism, while Leary et al. proposed a unidimensional definition (24,26). After years of researching this phenomenon, Clance defined a typical impostor and created the CIPS that accurately measures the intensity an impostor could experience. This most commonly used instrument by researchers and practitioners has been validated in different settings. It contains items which address the fear of failure, attribution of success to luck, error, or charm, the desire to stand out, the feeling of having given others a false impression, the discounting of recognition from others, as well as the fear of evaluation, fear that successes cannot be repeated and the feeling that one is less capable than peers (2,24,27).

This study aimed to translate and culturally adapt the CIPS to the Croatian language, including translation and piloting of the questionnaire for students in Croatia and Bosnia and Hercegovina. The Croatian language is also one of the three official languages in Bosnia and Herzegovina, while the University of Mostar is the only Croatian-speaking university in the country.

Materials and Methods

The CIPS is a 20-item survey in which respondents rate their answers on a Likert scale from 1 to 5, where the numbers have the following meanings: 1 – not at all true, 2 – rarely true, 3 – sometimes true, 4 – often true, or 5 – very true. For each question, respondents are advised to circle the number that best indicates how true the statement is for them and provide the first response that enters their mind, rather than dwelling on and overthinking each statement. The scores for each item add up to produce a total score, and higher scores indicate greater identification with the impostor syndrome (28).

The translation algorithm for the CIPS (used with the authors' permission) was selected according to the previously described methodology, which included two mandatory forward translations

and backward translation by a health professional (29). Forward translations of the instrument were made by translators residing in the country and familiar with the field of health outcomes. A native English bilingual professional translator and a native Croatian translator for the English language forward-translated the CIPS into Croatian. Once these versions were available, we discussed combined translation efforts at a consensus meeting. The statement "I'm often afraid that I may fail at a new assignment or undertaking even though I generally do well at what I attempt" was inadequately translated in the first forward translation, but the second one was more comparable with the original statement. Regarding all other questions, the forward translations were similar to and consistent with the original version. Subsequently, a bilingual native English-speaking medical professional, who was unaware of the original version, back-translated the combined version of the translations into English. The objective of back-translation was to detect errors in meaning and non-equivalence. We found no semantic or stylistic differences between the original and back-translation version of the CIPS. The algorithm of the translation protocol is presented in Figure 1.

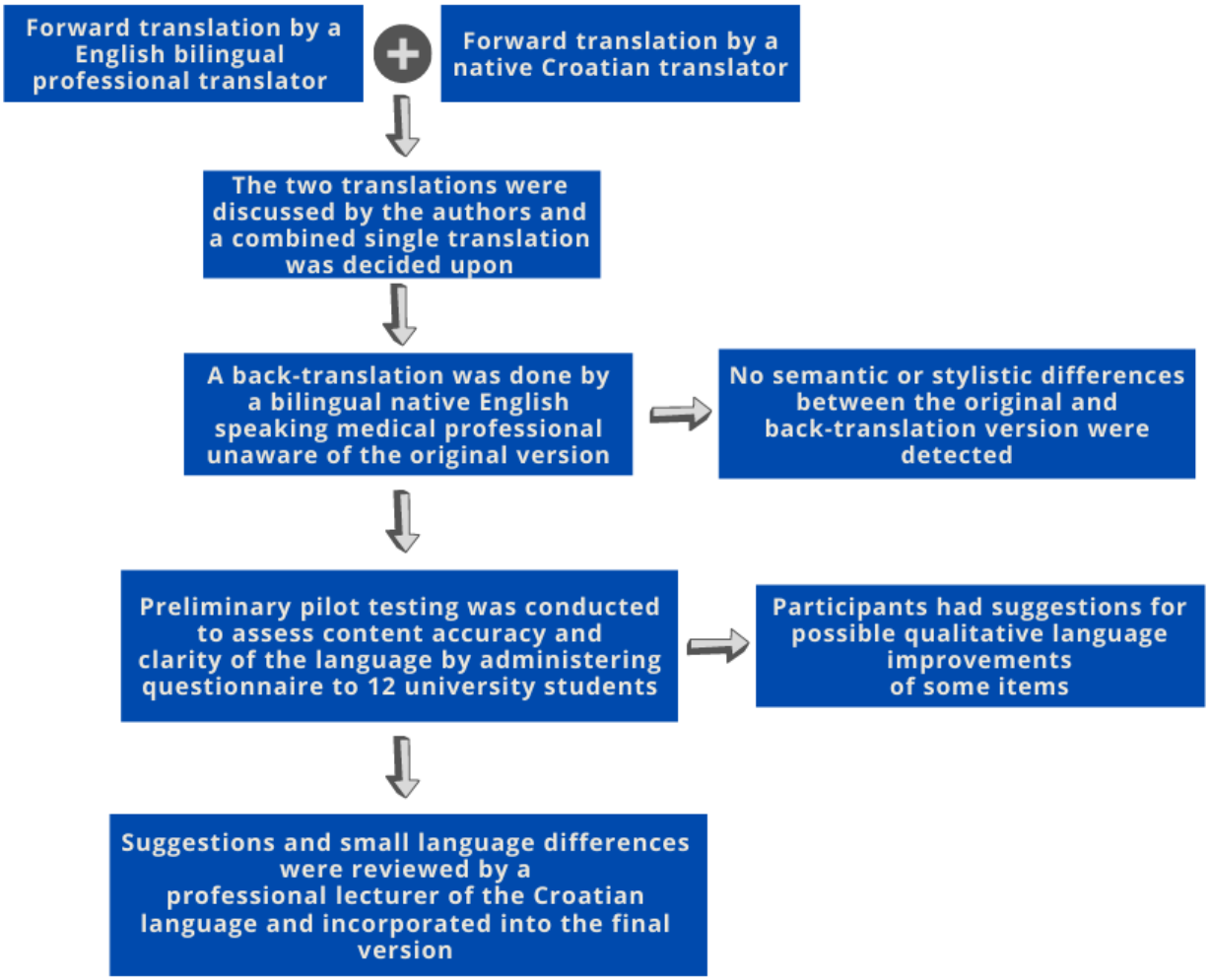

Figure 1. The algorithm of the translation protocol

Preliminary pilot testing was conducted to assess content accuracy and clarity of the language for both countries, Croatia and Bosnia and Herzegovina. The questionnaire was administered to a convenient sample of 12 university students (5 males and 7 females, median age of 21, IQR of 21-35) from the University of Zagreb. During pre-pilot testing,

the interviewer observed that the translations represented the source questionnaire effectively. The participants had suggestions for possible qualitative language improvements regarding some items, which were incorporated into the final version of the questionnaire. Afterwards, the suggestions and small language differences were reviewed by a professional Croatian language reviewer with 20 years of experience and they were incorporated into the Croatian version of the CIPS.

Results

In Table 1, we provided a detailed presentation of the outcome of the translation process, which consists of the original version, two forward translations into Croatian, a combined version of the translations and back-translation into English. The translators provided a translation that is as close to the original as possible and we did not find any significant changes in the meaning or style. When comparing the original English version and the back-translated version, we observed no relevant semantic differences. The only differences between the original and the back-translation were related to using different grammatical forms, which resulted in very similar meanings. These versions are comparable with the original English instrument in terms of content and accuracy, although the Croatian version has been culturally adapted.

Table 1. Steps for the CIPS translation into the Croatian language

Original	Forward translation No. 1	Forward translation No. 2	Combined translation	Backward translation
1. I have often succeeded on a test or task even though I was afraid that I would not do well before I undertook the task.	Često bih uspješno napisao/la test ili izvršio/la zadatak iako sam se bojao/la da ga neću dobro napraviti prije nego sam ga započeo/la.	Često sam bio/la uspješan/na na testu iako sam se bojao/la da neću postići dobar rezultat prije početka rješavanja testa.	Često bih uspješno napisao/la test ili izvršio/la zadatak iako sam se prije početka bojao/la da ga neću dobro napraviti.	I often do well on exams even though beforehand I was afraid of failing.
2. I can give the impression that I'm more competent than I really am.	Mogu odati dojam da sam sposobniji/a nego što zbilja jesam.	Ponekad se činim kompetentnijim/om nego što zapravo jesam.	Mogu odati dojam da sam sposobniji/a nego što zapravo jesam.	I can give the impression that I'm more capable than I truly am.
3. I avoid evaluations if possible and have a dread of others evaluating me.	Izbjegavam procjene ako je moguće i grozim se toga da me drugi procjenjuju.	Ako mogu, izbjegavam evaluacije i strahujem od toga da me drugi procjenjuju na bilo koji način.	Ako mogu, izbjegavam procjene i strahujem od toga da me drugi procjenjuju.	I avoid any comparison and I'm terrified of being judged by others.
4. When people praise me for something I've accomplished, I'm afraid I won't be able to live up to their expectations of me in the future.	Kada me hvale za moja postignuća, bojim se da u budućnosti neću moći ispuniti njihova očekivanja.	Kada drugi pohvale moja postignuća, bojim se da neću ispuniti njihova očekivanja u budućnosti.	Kada me drugi hvale za moja postignuća, bojim se da neću moći ispuniti njihova očekivanja u budućnosti.	When being praised for my achievements, I'm afraid of not being able to meet their expectations in the future.
5. I sometimes think I obtained my present position or gained my present	Ponekad mislim da sam došao/la do sadašnje pozicije ili trenutnog uspjeha	Ponekad mislim da sam trenutnu poziciju i trenutan uspjeh postigao/la	Ponekad mislim da sam postigao/la sadašnju poziciju ili sadašnji uspjeh jer	I sometimes think that I made it to this position or point in life because I was at

success because I happened to be in the right place at the right time or knew the right people.	jer sam bio/la na pravom mjestu u pravo vrijeme ili jer sam poznavao/la prave ljude.	jer sam se našao/la na pravom mjestu u pravo vrijeme ili sam poznavao/la ljude koji su mi to omogućili.	sam bio/la na pravom mjestu u pravo vrijeme ili sam poznavao/la prave ljude.	the right place at the right time or due to knowing the right people.
6. I'm afraid people important to me may find out that I'm not as capable as they think I am.	Strahujem od toga da će ljudi koji su mi važni saznati da nisam sposoban/na koliko oni misle da jesam.	Bojim se da će ljudi koji su mi važni otkriti da nisam sposoban koliko su mislili.	Strahujem od toga da će meni važni ljudi otkriti da nisam sposoban/na koliko oni misle da jesam.	I'm afraid that the people close to me will find out that I'm not as capable as they think I am.
7. I tend to remember the incidents in which I have not done my best more than those times I have done my best.	Sklon/a sam više pamtiti događaje u kojima nisam dao/la sve od sebe nego one kada sam dao/la sve od sebe.	Češće se prisjećam situacija u kojima sam bio/la neuspješan/na nego onih u kojima sam bio/la uspješan/na.	Sklon/a sam češće se prisjetiti događaja u kojima nisam dao/la sve od sebe nego onih kada sam dao/la sve od sebe.	I'm more inclined to remember the times I didn't give my best effort, compared to the times when I did.
8. I rarely do a project or task as well as I'd like to do it.	Rijetko kada projekt ili zadatak napravim onoliko dobro koliko bih volio/ljela.	Rijetko završim projekt ili zadatak zadovoljan/na odrađenim poslom.	Rijetko kada projekt ili zadatak napravim onoliko dobro koliko bih volio/ljela.	Rarely do I accomplish an assignment or project as well as I would of liked.
9. Sometimes I feel or believe that my success in my life or in my job has been the result of some kind of error.	Ponekad se osjećam ili vjerujem da je uspjeh u mom životu ili na poslu posljedica nekog tipa greške.	Ponekad se osjećam ili mislim da je uspjeh u mom životu ili poslu rezultat nekakve pogreške.	Ponekad se osjećam ili vjerujem da je uspjeh u mom životu ili poslu posljedica nekakve pogreške.	Sometimes I feel or believe that success in my life or at work is the result of some type of mistake.
10. It's hard for me to accept compliments or praise about my intelligence or accomplishments.	Teško prihvaćam komplimente ili hvalu na račun moje inteligencije ili uspjeha.	Teško prihvaćam komplimente ili pohvale vezane uz moju inteligenciju ili postignuća.	Teško prihvaćam komplimente ili pohvale na račun moje inteligencije ili postignuća.	It's difficult for me to accept compliments or praise based on my intelligence and success.
11. At times, I feel my success has been due to some kind of luck.	Ponekad se osjećam kao da je za moj uspjeh zaslužna neka vrsta sreće.	S vremena na vrijeme, osjećam se kao da je za moj uspjeh zaslužna sreća.	S vremena na vrijeme, osjećam se kao da je za moj uspjeh zaslužna neka vrsta sreće.	I sometimes feel that my success is due to some sort of luck.
12. I'm disappointed at times in my present accomplishments and think I should have accomplished much more.	Ponekad sam razočaran/a trenutnim uspjesima i mislim da sam trebao/la mnogo više.	Ponekad sam razočaran/a svojim trenutnim postignućima i mislim da sam trebao/la postići puno više.	Ponekad sam razočaran/a svojim trenutnim postignućima i mislim da sam trebao/la postići puno više.	I am sometimes disappointed at the level of my success and feel that I should of done better.
13. Sometimes I'm afraid others will discover how much	Ponekad se bojim da će drugi otkriti koliko znanja i	Ponekad strahujem od toga da će drugi otkriti da mi	Ponekad se bojim da će drugi otkriti koliko mi znanja ili	Sometimes I fear that others will discover how much

knowledge or ability I really lack.	sposobnosti mi zbilja nedostaje.	nedostaje znanja ili sposobnosti.	sposobnosti zbilja nedostaje.	knowledge and ability I'm truly lacking.
14. I'm often afraid that I may fail at a new assignment or undertaking even though I generally do well at what I attempt.	Ponekad se bojim da neću uspjeti u novom zadatku ili poduhvatu iako obično dobro napravim ono u što se upustim.	Često se bojim da ću biti neuspješan/na u novome zadatku ili pothvatu iako sam obično uspješan/na u onome što radim.	Često se bojim da ću biti neuspješan/na u novom zadatku ili poduhvatu iako obično dobro napravim ono u što se upustim.	Often, I fear that I will not succeed in a new task or endeavour even though I usually do well what I embark on.
15. When I've succeeded at something and received recognition for my accomplishments, I have doubts that I can keep repeating that success.	Kada uspijem u nečemu i za to dobijem priznanje, sumnjam u to da ću taj uspjeh moći ponoviti.	Kada uspijem u nečemu i drugi pohvale moja postignuća, bojim se da neću ponovno biti uspješan/na u istom ili sličnom zadatku.	Kada uspijem u nečemu i za to dobijem priznanje, sumnjam da ću taj uspjeh moći ponoviti.	When I get recognition for an achievement, I have doubts that I will be able to repeat that success.
16. If I receive a great deal of praise and recognition for something I've accomplished, I tend to discount the importance of what I've done.	Ako za nešto što sam postigao/la dobijem mnogo hvale i priznanja, sklon/a sam reducirati važnost toga što sam napravio/la.	Ako dobijem puno pohvala i komplimenata za nešto što sam postigao/la, često zanemarim važnost onoga što sam učinio/la.	Ako dobijem puno pohvala i priznanja za nešto što sam postigao/la, sklon/a sam umanjiti važnost onoga što sam učinio/la.	If I receive praise for something I achieved, I tend to reduce the importance of what I did.
17. I often compare my ability to those around me and think they may be more intelligent than I am.	Često uspoređujem svoje sposobnosti s onim ljudima oko sebe i mislim da bi oni mogli biti pametniji od mene.	Često uspoređujem svoje sposobnosti sa sposobnostima drugih i mislim da su inteligentniji od mene.	Često uspoređujem svoje sposobnosti sa sposobnostima drugih oko sebe i mislim da bi oni mogli biti pametniji od mene.	I often compare my abilities with people around me and think that they might be smarter than me.
18. I often worry about not succeeding with a project or examination, even though others around me have considerable confidence that I will do well.	Često se brinem da neću uspjeti s nekim projektom ili ispitom, iako su drugi oko mene snažno uvjereni da ću to dobro napraviti	Često brinem da ću biti neuspješan/na na projektu ili ispitu, iako druge osobe vjeruju da ću biti uspješan/na.	Često se brinem da ću biti neuspješan/na na projektu ili ispitu, iako druge osobe oko mene snažno vjeruju da ću to dobro napraviti.	I often worry about the success on a project or exam even though others around me are strongly convinced that I'll do well.
19. If I'm going to receive a promotion or gain recognition of some kind, I hesitate to tell	Ako trebam dobiti unapređenje ili priznanje neke vrste, oklijevam reći drugima sve dok to nije već ostvareno.	Ako trebam dobiti promaknuće ili nekakvu pohvalu, drugim osobama o tome ne govorim	Ako trebam dobiti promaknuće ili nekakvo priznanje, oklijevam reći drugima dok to nije već ostvareno.	If I need to get a promotion or recognition of some kind, I hesitate to tell others until it

others until it is an accomplished fact.		dok se to napokon ne ostvari.		has already been achieved.
20. I feel bad and discouraged if I'm not "the best" or at least "very special" in situations that involve achievement.	Osjećam se loše i obeshrabreno ako nisam „najbolji/a" ili barem „vrlo poseban/a" u situacijama koje se tiču postignuća.	Osjećam se loše i obeshrabreno ako nisam „najbolji/a" ili barem „vrlo poseban/na" u situacijama u kojima se očekuje nekakvo postignuće.	Osjećam se loše i obeshrabreno ako nisam „najbolji/a" ili barem „vrlo poseban/na" u situacijama u kojima se očekuje nekakvo postignuće.	I feel bad and discouraged if I am not "the best" or at least "very special" in situations concerning success.

The Clance IP Scale translated into the Croatian language and scoring instructions are presented in Appendix 1.

Discussion

The final version of the scale is suitable to assess the impostor phenomenon among medical students in Croatia and Bosnia and Herzegovina. There is extensive variability in the literature associated with the impostor phenomenon, not only regarding synonyms which describe this internal feeling of inadequacy (28). Some reported inconsistencies are most likely attributed to the methodological issues and methodological quality of impostorism validation studies. For our research, we selected Clance's scale, which showed to be a highly sensitive and reliable instrument (1). Validation of the CIP scale is beyond the scope of this research. The sample size for a validation study will be determined when the final version of the questionnaire is administered to a large representative sample of respondents for whom the questionnaire is intended – medical, veterinary and nursing students. The COVID-19 pandemic prolonged the process of obtaining ethical approvals and made the research more demanding in terms of how to distribute the questionnaire to participants. Ideas and suggestions for further research include checking psychometric characteristics and validating the Clance Impostor Phenomenon Scale translated into the Croatian language. The version of the CIPS which was translated into the Croatian language represents a reliable translation ready to be used in Croatia and Bosnia and Herzegovina.

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Appendix 1. The Clance IP Scale translated into the Croatian language and scoring instructions

CLANCE LJESTVICA FENOMENA VARALICE

Molimo zaokružite broj koji najbolje odražava koliko je za vas izjava istinita. Najbolje je da zaokružite prvi odgovor koji vam padne na pamet i ne premišljate se o tome.

1.	**Često bih uspješno napisao/la test ili izvršio/la zadatak iako sam se prije početka bojao/la da ga neću dobro napraviti.**
	1 (nimalo istinito) 2 (rijetko) 3 (ponekad) 4 (često) 5 (posve istinito)
2.	**Mogu odati dojam da sam sposobniji/a nego što doista jesam.**
	1 (nimalo istinito) 2 (rijetko) 3 (ponekad) 4 (često) 5 (posve istinito)
3.	**Izbjegavam procjene ako je moguće i strašim se da me drugi procjenjuju.**
	1 (nimalo istinito) 2 (rijetko) 3 (ponekad) 4 (često) 5 (posve istinito)
4.	**Kada me drugi hvale za moja postignuća, bojim se da u budućnosti neću moći ispuniti njihova očekivanja.**
	1 (nimalo istinito) 2 (rijetko) 3 (ponekad) 4 (često) 5 (posve istinito)
5.	**Ponekad mislim da sam postigao/la sadašnju poziciju ili sadašnji uspjeh jer sam bio/la na pravom mjestu u pravo vrijeme ili sam poznavao/la prave ljude.**
	1 (nimalo istinito) 2 (rijetko) 3 (ponekad) 4 (često) 5 (posve istinito)
6.	**Strahujem od toga da će meni važni ljudi otkriti da nisam sposoban/na koliko oni misle da jesam.**
	1 (nimalo istinito) 2 (rijetko) 3 (ponekad) 4 (često) 5 (posve istinito)
7.	**Sklon/a sam češće se prisjetiti događaja u kojima nisam dao/la sve od sebe nego onih kada sam dao/la sve od sebe.**

1 (nimalo istinito) 2 (rijetko) 3 (ponekad) 4 (često) 5 (posve istinito)

8. Rijetko kada projekt ili zadatak napravim onoliko dobro koliko bih volio/ljela.

1 (nimalo istinito) 2 (rijetko) 3 (ponekad) 4 (često) 5 (posve istinito)

9. Ponekad se osjećam ili vjerujem da je uspjeh u mom životu ili poslu posljedica nekakve pogreške.

1 (nimalo istinito) 2 (rijetko) 3 (ponekad) 4 (često) 5 (posve istinito)

10. Teško prihvaćam komplimente ili pohvale na račun svoje inteligencije ili postignuća.

1 (nimalo istinito) 2 (rijetko) 3 (ponekad) 4 (često) 5 (posve istinito)

11. Ponekad se osjećam kao da je za moj uspjeh zaslužna neka vrsta sreće.

1 (nimalo istinito) 2 (rijetko) 3 (ponekad) 4 (često) 5 (posve istinito)

12. Ponekad sam razočaran/a svojim trenutnim postignućima i mislim da sam trebao/la postići puno više.

1 (nimalo istinito) 2 (rijetko) 3 (ponekad) 4 (često) 5 (posve istinito)

13. Ponekad se bojim da će drugi otkriti koliko mi znanja ili sposobnosti doista nedostaje.

1 (nimalo istinito) 2 (rijetko) 3 (ponekad) 4 (često) 5 (posve istinito)

14. Često se bojim da ću biti neuspješan/na u novom zadatku ili poduhvatu premda obično dobro napravim ono u što se upustim.

1 (nimalo istinito) 2 (rijetko) 3 (ponekad) 4 (često) 5 (posve istinito)

15. Kada uspijem u nečemu i za to dobijem priznanje, sumnjam da ću taj uspjeh moći ponoviti.

1 (nimalo istinito) 2 (rijetko) 3 (ponekad) 4 (često) 5 (posve istinito)

16. Ako za nešto što sam postigao/la dobijem puno pohvala i priznanja, sklon/a sam umanjiti važnost onoga što sam učinio/la.

1 (nimalo istinito) 2 (rijetko) 3 (ponekad) 4 (često) 5 (posve istinito)

17. Često uspoređujem svoje sposobnosti sa sposobnostima drugih oko sebe i mislim da bi oni mogli biti pametniji od mene.

1 (nimalo istinito) 2 (rijetko) 3 (ponekad) 4 (često) 5 (posve istinito)

18. Često se brinem da ću biti neuspješan/na na projektu ili ispitu, premda druge osobe oko mene snažno vjeruju da ću to dobro napraviti.

1 (nimalo istinito)	2 (rijetko)	3 (ponekad)	4 (često)	5 (posve istinito)

19. Ako trebam dobiti promaknuće ili nekakvo priznanje, oklijevam reći drugima sve dok to nije već ostvareno.

1 (nimalo istinito)	2 (rijetko)	3 (ponekad)	4 (često)	5 (posve istinito)

20. Osjećam se loše i obeshrabreno ako nisam „najbolji/a“ ili barem „vrlo poseban/na“ u situacijama u kojima se očekuje nekakvo postignuće.

1 (nimalo istinito)	2 (rijetko)	3 (ponekad)	4 (često)	5 (posve istinito)

Ocjenjivanje testa fenomena varalice

Test fenomena varalice osmišljen je kako bi pomogao pojedincima utvrditi imaju li ili nemaju karakteristike fenomena varalice te, ako imaju, u kojoj mjeri.

Nakon rješavanja testa zbrojite brojeve odgovora na svaku izjavu. Ako je ukupan zbroj 40 ili manje, ispitanik ima malo obilježja fenomena varalice; ako je rezultat između 41 i 60, ispitanik ima umjerena iskustva s fenomenom varalice; rezultat između 61 i 80 znači da se ispitanik često osjeća kao varalica; a rezultat viši od 80 znači da ispitanik često intenzivno osjeća fenomen varalice. Što je rezultat viši, fenomen varalice češće i ozbiljnije utječe na život osobe.

Abbreviations. IP - impostor phenomenon
CIPS - Clance Impostor Phenomenon Scale

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Original article

Differences in the Value of Proliferation Index (Ki67) and Immunophenotypes Between Invasive Breast Cancers With Respect to the Axillary Lymph Node Status

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Abstract

Introduction: Present study aimed to determine the frequency of invasive breast cancer (IBC) immunophenotypes in the General County Hospital Vinkovci, examine a difference between the ages of patients with respect to immunophenotypes and axillary lymph node (ALN) status, and determine differences in the frequency of positive ALNs with respect to immunophenotypes and the proliferation index (Ki67), regardless of the immunophenotype.

Materials and Methods: A monocentre cross-sectional study which included 289 patients diagnosed with invasive breast cancer was conducted in the period from 1 January 2011 to 31 December 2018. The expression of IBC biomarkers was determined by immunohistochemistry.

Results: The most common immunophenotype (41.54 %) was luminal B-like HER2-negative (LumB/HER2-). The mean age was 65.24 (± 12.04), with no age difference with respect to immunophenotypes ($F = 0.64$, $P = 0.43$) or ALN status ($t = 1.59$; $P = 0.11$). A total of 167 patients (58 %) had their ALNs removed, 66 % of which were positive. LumB/HER2- appeared to have significantly more positive ALNs compared to the luminal A-like immunophenotype ($P < 0.01$), while a difference in the size of primary tumours between metastatic breast cancers of these two immunophenotypes has not been detected ($P = 0.17$). ALNs were more likely to be positive in those tumours with Ki67 values higher than 20 % compared to the tumours in which Ki67 was lower than or equal to 20 % ($P < 0.01$).

Conclusions: LumB/HER2- is the most prevalent IBC immunophenotype in patients in our institution and has significantly more positive ALNs compared to the luminal A-like immunophenotype. Also, metastases to ALNs, regardless of the immunophenotype, are more common in patients with Ki67 higher than 20 % than in those with Ki67 lower than or equal to 20 %..

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KEYWORDS: breast cancer; proliferation, immunophenotype, axillary, lymph node

Introduction

Breast cancer is the most common malignancy in women in the European Union (1). Breast cancers are divided into non-invasive (in situ), microinvasive and invasive carcinomas with recognisable subtypes. Non-invasive carcinomas, unlike invasive carcinomas, do not have the ability to invade blood and lymphatic vessels and their tumour cells are limited to ducts. Non-invasive carcinomas include ductal carcinoma in situ (DCIS), intraductal papillary carcinoma, and lobular carcinoma in situ (LCIS) (2). Histologically, invasive carcinomas can be divided into ductal (80%) and lobular (10%), while the rest are special forms of breast cancer (Paget's disease of the nipple, colloid mucinous carcinoma, tubular carcinoma, and solid papillary carcinoma with invasion) (3). According to literature, different types of breast cancers originate from the luminal and basal cells of the terminal duct lobular unit epithelium and they form a heterogeneous group of cancers that cannot be distinguished by histology (4, 8).

When it comes to breast cancer treatment, monitoring and survival rates, molecular subtypes determined by microarrays with more than 2.000 genes take precedence over the standard histological phenotype. This method is not easily attainable and it is quite costly, so it is generally accepted to use the immunophenotypic classification instead, as determined by the immunohistochemical expression of oestrogen receptors (ER), progesterone receptors (PR), proliferation index (Ki67) and amplification of the human epidermal growth factor receptor 2 (HER2) (2, 5). According to the new guidelines (Croatian Association for Cancer Research, 2018), ER and PR are considered weak prognostic and strong predictive factors because hormone receptor-positive cancers have a better survival rate, since they predictively indicate a potential response to hormonal therapy (5-6). ER indicates a response to cancer treatment via hormonal therapy such as tamoxifen, which blocks the growth of oestrogen-stimulated cancers, and a response to aromatase inhibitors that suppress oestrogen production (5). The next immunophenotypic marker, HER2, is associated with a lower survival rate and is also a predictive factor. One of the main reasons for determining the HER2 status is the identification of candidates for targeted anti-HER2 therapy (trastuzumab, lapatinib, pertuzumab, and trastuzumab emtansine) related to HER2-positive breast cancers. The most controversial marker used is Ki67, as it is determined in hot-spot areas, and the result often depends on the quality of tissue fixation, formulation, and the type of antibody used (5). As a rule, a more distinct expression is associated with a lower survival rate, both in general and after the administration of neoadjuvant therapy (2, 5, 7, 8). In addition to being connected with immunophenotypes, the survival rate and treatment also depend on the penetration of malignant cells into blood and lymphatic vessels, tumour necrosis, age of the patient (younger age is a negative prognostic factor), size of the tumour, status of surgical margins and lymph nodes (2, 5).

Studies from different countries show a different prevalence of respective immunophenotypes of breast cancer, which points not only to a number of factors that may play a role in carcinogenesis, but also to possible differences due to non-standardised protocols in pathohistological laboratories. In addition, the diagnosis of the correct immunophenotype is the cornerstone of proper treatment. Therefore, it is necessary to evaluate one's own work, among other things, comparing it with other laboratories in Croatia and other countries in order to detect possible problems in time and improve or at least maintain quality at an acceptable level. Therefore, the objective of this research was to determine the overall incidence of breast cancer and individual immunophenotypes in General County Hospital Vinkovci (CBC), to examine whether there is a difference between the age of women diagnosed with cancer related to immunophenotypes and lymph node status, and to determine the differences in the frequency of positive axillary lymph nodes with respect to immunophenotypes and especially with regard to high Ki67 values, regardless of the immunophenotype.

Materials and Methods

Study Structure and Materials

The paper is based on a monocentre cross-sectional study on historical data (9). The findings of patients diagnosed with breast cancer in the period from 1 January 2011 to 31 December 2018 were collected from the archives of the Department of Pathology and Cytology of the General County Hospital Vinkovci. From a total of 296 patients diagnosed with breast cancer, three were men, who were excluded from further processing. Also, because of the criteria of invasiveness, four female patients diagnosed with carcinoma in situ were excluded. Therefore, the final sample consisted of medical findings of 289 female patients with said diagnosis. The research was approved by the Ethics Committee of the General County Hospital Vinkovci.

Methods

Data of interest in the findings expressed ER, PR, HER2, and KI67 and were obtained using routinely prepared histological samples, formalin fixed paraffin embedded – FFPE, and finally immunohistochemically stained. The preparations were stained with Dako EnVision FLEX kit by treating the sample with Peroxidase Blocking Reagent (for 5 minutes), rinsed with EnVision FLEX wash buffer and treated with the primary antibody called FLEX Monoclonal Rabbit Anti-Human Estrogen receptor α, Clone EP1 (Dako), or other antibodies like Mouse Anti-Human Progesterone Receptor, Clone PgR 636 (Dako), FLEX Monoclonal Rabbit Anti-Human Ki67 Antigen, Clone MIB-1 (Dako) and Monoclonal Rabbit Anti-Human Her 2 Protein (Dako) (for 20 min), rinsed with EnVision FLEX wash buffer and stained with EnVision FLEX Hematoxylin. Finally, the preparations were covered with Sakura Tissue-Tek film in an automatic glass coating device Sakura Tissue-Tek Film (10). Analysis and interpretation of the immunohistochemical staining findings were carried out according to the WHO guidelines (8). The ER and PR findings are considered positive if 1 % or more tumour cells show immunohistochemical nuclear positivity, and negative in case of absence of nuclear positivity or strong nuclear positivity in less than 1 % of total tumour tissue. If tumour cells show absence of membrane positivity or very weak membrane positivity to the HER2 antibody, the HER2 status is indicated with 0 or 1+ and the finding is considered negative. In contrast, if more than 10 % of tumour cells have membrane positivity, the finding is considered positive and marked with 3+. When tumour cells show incomplete membrane positivity in 10 % of tumour tissue or if the positivity is at the borderline of 10 % of the total tumour tissue, the HER2 status is denoted by 2+. In this case, additional in situ hybridisation (FISH/CISH) is required to determine the existence of amplification of the HER2 gene and categorise the tumour as HER2-positive or HER2-negative (8).

According to immunohistochemically determined expression, we distinguish the following five immunophenotypes: luminal A-like, luminal B-like HER2-negative, luminal B-like HER2-positive, HER2-positive and triple-negative breast cancer (Table 1). Luminal A-like immunophenotype has the best survival rate, while a triple-negative immunophenotype has the worst survival rate (2). Luminal B-like HER2-negative breast cancer immunophenotype should exhibit higher Ki67 values than luminal A-like. Both immunophenotypes are HER2-negative and ER-positive, but the luminal B-like HER2-negative immunophenotype has a lower survival rate, so the separation of these two breast cancer immunophenotypes is essential for therapy prescription. According to the recommendations of the St. Gallen Oncology Conferences from 2013 and the 2017 Croatian Guidelines for Quality Assurance in Breast Cancer Screening and Diagnosis, the cut-off value for this factor is 20 % (11-12). The cut-off value is important in clinical practice because it indicates which patients should receive a more intensive therapy (13).

Table 1. Immunophenotypes of breast cancer

Immunophenotype	ER	PR	Her2	Ki67
Luminal A-like	+	+	-	< 20 %
Luminal B-like HER2-negative*	+	- or low*	-	> 20 %*
Luminal B-like HER2-positive	+	+/-	+	+/-
HER2-positive	-	-	+	+/-
Triple-negative	-	-	-	+/-

*At least one of the following criteria is required

After thorough processing, the pathohistological findings indicate tumour size, malignancy, Ki67, hormone receptor status, HER2 status, tumour-to-resection-margin ratio, number of examined and positive axillary lymph nodes, and tumour-to-blood and lymphatic vessel ratio. Finally, after processing these data according to the TNM staging system, the stage of breast cancer is determined indicating the probability of being cured (14).

Statistical methods

The variables were collected in MS Excel and processed in the program R (15). The sample was described using descriptive statistical methods. The Shapiro–Wilk W-test (or, in case of a large number of samples, the Kolmogorov–Smirnov test) was used to examine the distribution of numerical variables. A comparison of categorical variables was carried out using the Pearson's chi-squared test and the Fisher's exact test. ANOVA was used when comparing several groups of numerical variables, given the distribution of data was normal, and an adequate post-hoc test was used in cases of statistically significant results. The parametric Student's t-test was used to compare two groups of numerical variables with normal distribution. In this paper, the level of statistical significance for all tests used for comparisons was defined at $P < 0.05$.

Results

The research included 289 patients with breast cancer. The highest number of patients (45) was in 2018, and the lowest (26) in 2011 ($\chi 2 = 5.09$, $P = 0.65$). A difference was observed in the frequency of occurrence of certain immunophenotypes throughout the research period: the luminal B-like HER2-negative immunophenotype was more common in 2013 than in other years ($\chi 2 = 3.41$, $P = 0.03$), while the luminal B-like HER2-positive immunophenotype was prevalent in 2017 ($\chi 2 = 3.89$, $P < 0.01$) (Figure 1). According to the pathohistological diagnosis, the majority of patients (238) were diagnosed with invasive ductal carcinomas. Invasive lobular breast cancers were present in 30 patients and invasive mucinous carcinomas in 10. The rest of the patients had less common pathohistological types of breast cancer (Table 2).

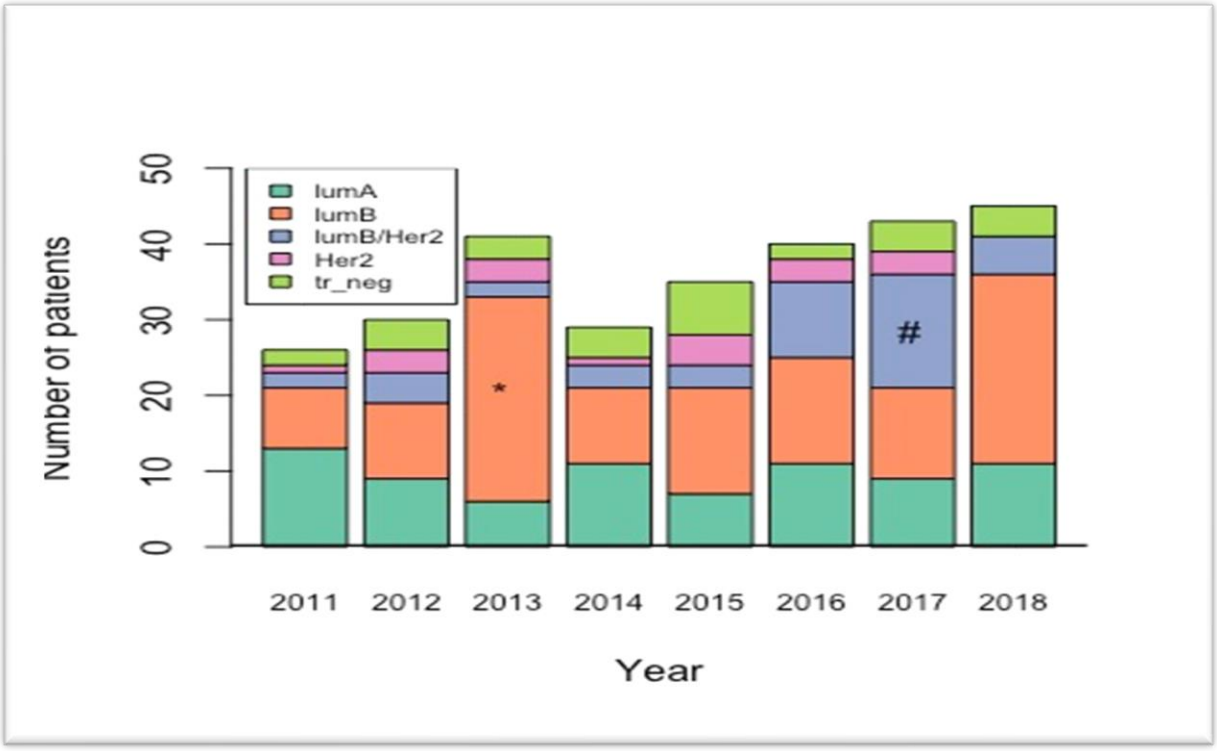

Figure 1. Distribution of patients with breast cancer with regard to immunophenotypes by years during the research period

Luminal B-like HER2-negative immunophenotype was more common in 2013 than in other years (* χ 2 = 3.41, P = 0.03), while the luminal B-like HER2-positive immunophenotype was most common in 2017 (# χ 2 = 3.89, P < 0.01). When it comes to other immunophenotypes, there was no difference in the frequency between the years within the research period (Figure 1). Index: lumA = luminal A-like; lumB = luminal B-like HER2-negative; lumB/Her2 = luminal B-like HER2-positive; Her2 = HER2-positive, tr_neg = triple-negative

Table 2. Pathohistological diagnosis of breast cancer (n (%))

Histological type of breast cancer	Number (%) of patients
Infiltrating duct carcinoma NOS	238 (82.3)
Lobular carcinoma NOS	30 (10.4)
Mucinous adenocarcinoma	10 (3.5)
Solid papillary carcinoma with invasion	5 (1.7)
Metaplastic carcinoma NOS	3 (1.0)
Tubular carcinoma	2 (0.7)
Apocrine adenocarcinoma	1 (0.4)
Total	289 (100)

The mean age of the patients was 65.24 (95 % CI 63.84 – 66.63). The sample follows the normal distribution (d = 0.05; P = 0.4). Regarding the immunophenotype of breast cancer, there was no difference in the age of the diseased patients (F = 0.64, P = 0.43) (Figure 2)..

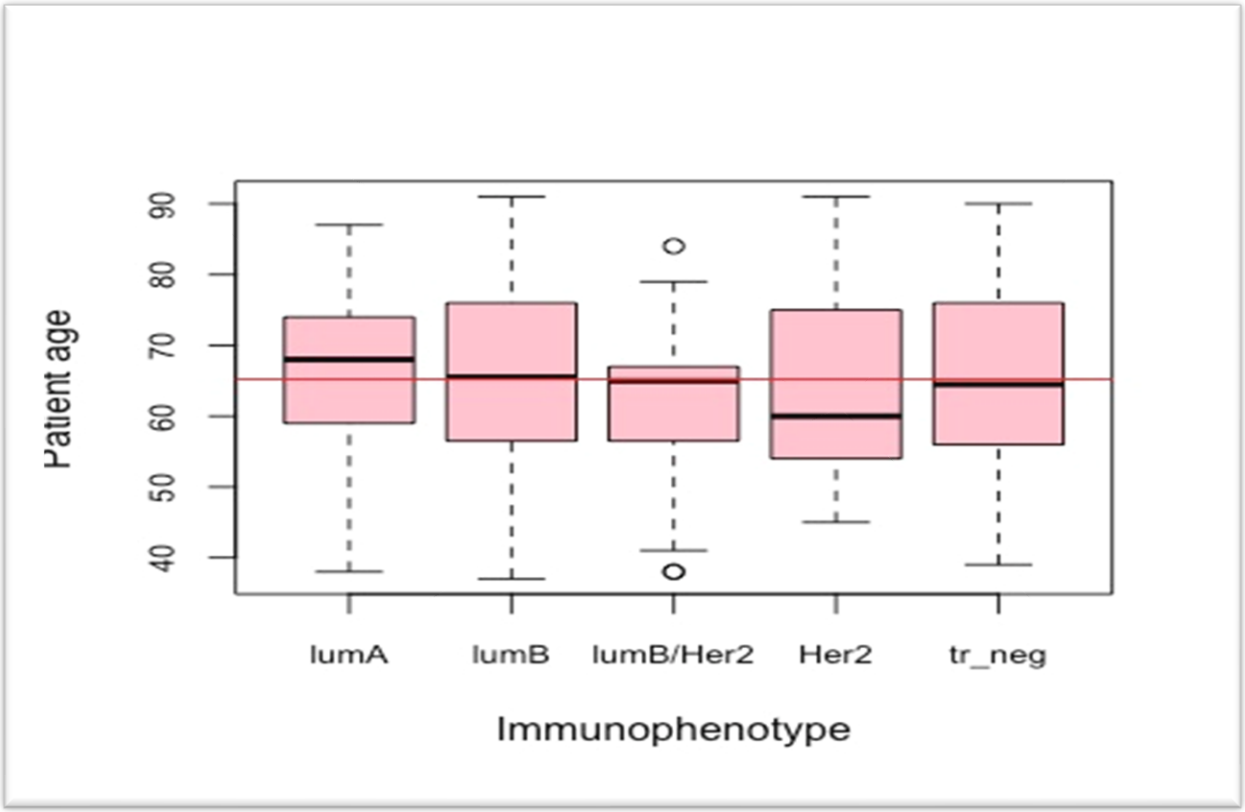

Figure 2. Age of patients with breast cancer with respect to immunophenotypes

The Shapiro-Wilk test helped determine that the age of all patients with respect to the immunophenotypes was distributed according to the normal distribution. There was no difference in the age of patients with regard to the breast cancer immunophenotype (F = 0.47, P = 0.49). The red line indicates the mean age of all patients, which is 65.24 (Figure 2). Index: lumA = luminal A-like; lumB = luminal B-like HER2-negative; lumB/Her2 = luminal B-like HER2-positive; Her2 = HER2-positive; tr_neg = triple-negative.

Axillary resections were performed in our institution on a total of 167 (58 %) patients. Of these 167 patients, 111 (66 %) had positive axillary lymph nodes (proven metastases of cancer tissue), and 56 (34 %) had negative results. The difference between respective immunophenotypes was determined with regard to the presence of positive axillary lymph nodes. The luminal B-like HER2-negative immunophenotype has positive axillary lymph nodes significantly more frequently than the luminal A-like (P < 0.01) (Figure 3). However, no difference in the primary tumour size (relative to pT) was observed between these immunophenotypes with positive axillary lymph nodes (P = 0.17) (Table 3). Furthermore, no age difference was observed between patients with positive and those with negative axillary lymph nodes (t = 1.59; P = 0.11). Patients with a Ki67 value higher than 20 % were more likely to have positive axillary lymph nodes than those with a Ki67 value lower than or equal to 20 %, regardless of the immunophenotype (χ 2 = 9.26, P < 0.01) (Figure 4)..

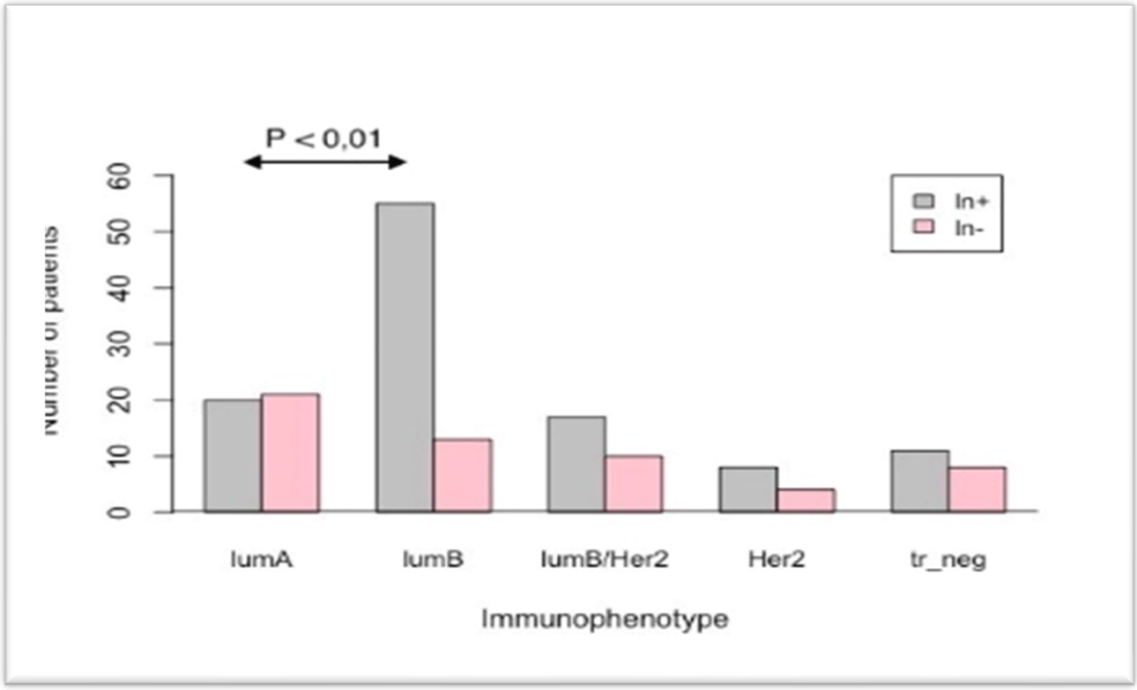

Figure 3. Incidence of positive axillary lymph nodes with respect to immunophenotypes of breast cancer

A difference was observed between the immunophenotypes of breast cancer in the frequency of detected positive axillary lymph nodes (χ 2 = 12.87, P < 0.05), and a post-hoc analysis found that the luminal B-like immunophenotype has positive axillary lymph nodes much more frequently compared to the luminal A-like immunophenotype (P < 0.01) (Figure 3). Index: lumA = luminal A-like; lumB = luminal B-like HER2-negative; lumB/Her2 = luminal B-like HER2-positive; Her2 = HER2 positive; tr_neg = triple-negative; ln+ = positive axillary lymph nodes; ln- = negative axillary lymph nodes.

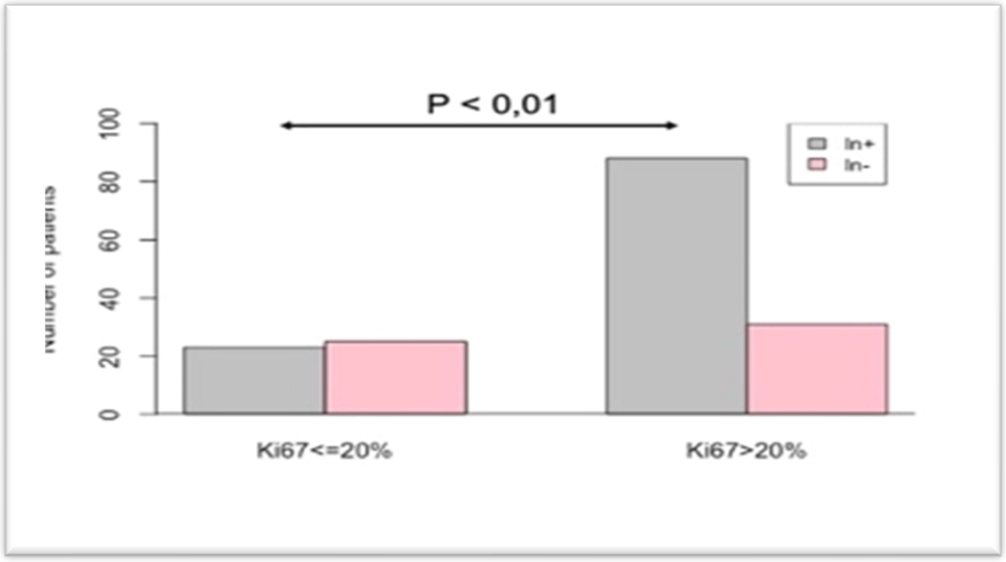

Figure 4. Number of patients with positive and negative axillary lymph nodes depending on the level of the proliferation index (Ki67)

Positive axillary lymph nodes are more common in breast cancer patients with Ki67 higher than 20 % (Ki67 > 20%) compared to those with Ki67 lower than or equal to 20 % (Ki67 ≤ 20 %), regardless of the immunophenotype in question (χ 2 = 9.26, P < 0.01) (Figure 4). Index: ln+ = positive axillary lymph nodes; ln- = negative axillary lymph nodes

Table 3. Primary tumour size in luminal A-like and luminal B-like HER2-negative carcinomas that have positive axillary lymph nodes.

	Primary tumour size				
Immunophenotype	pT1	pT2	pT3	pT4	Total
Lum A	10	7	1	2	20
Lum B/Her2-	17	26	10	2	55
Total	27	33	11	4	75

There was no difference in the size of the primary tumour between the luminal A-like and luminal B-like Her2-negative immunophenotypes of breast cancers with positive axillary lymph nodes (P = 0.17). Index: LumA = luminal A-like; lumB/Her2 = luminal B-like HER2-negative

Discussion

This research has shown that the luminal B-like HER2-negative immunophenotype is the most common immunophenotype of breast cancer in patients who have undergone surgery in our facility. In addition, positive axillary lymph nodes are more common in case of this immunophenotype than the luminal A-like immunophenotype, regardless of the size of the primary tumour. By way of explanation, due to the known correlation between tumour size and metastases of breast cancer to axillary lymph nodes (8), the observed difference in the incidence of metastatic breast cancers regarding these immunophenotypes was further analysed with respect to primary tumour size (pT). Although the difference in the size of the primary tumour subject to examination has not been confirmed, it should be emphasised that the possibility that this could be determined by research on a larger number of samples cannot be ruled out. Also, this paper indicates that metastases to axillary lymph nodes, regardless of the immunophenotype, are more common in patients with breast cancer with a Ki67 value higher than 20 % than in those with a Ki67 value lower than or equal to 20 %. The analysis of 289 patients with breast cancer found that the most common histological type was invasive duct carcinoma (82.3 %), followed by invasive lobular carcinoma (10 %) and invasive mucinous carcinoma (3.5 %). The same order of histological subtypes can be found in studies conducted in Germany (16), Saudi Arabia (17), Pakistan (18) and Nigeria (19), while the invasive ductal type was slightly less common (59 %) in one Italian study, and it was followed by invasive lobular type with 14 % (20). On the other hand, invasive ductal carcinoma is the most common in China (94 %), but it is followed by invasive mucinous carcinoma, with invasive lobular carcinoma in the last place (21).

The mean age of the patients included in this research was 65.24 years, which is similar to the results in clinical hospital centres, general hospitals, clinics and polyclinics all across the Republic of Croatia (22), as well as Serbia (65.59 ± 10.17) (23). A slightly lower mean age (55-57) was detected in studies carried out in Germany (16), Japan (24) and Brazil (25), while the lowest mean age (43-48) was in Sweden (26), Nigeria (19) and Pakistan (18) (Table 3). The significantly lower age of breast cancer patients in Africa is explained by Nigerian authors by the thesis that breast cancer occurs up to 15 years earlier in black people (19).

An almost equal representation of respective immunophenotypes as found in our sample was noted in another research conducted in Croatia (22), and in a country bordering Croatia – Serbia (27), but also in Sweden (26) (Table 4). The luminal A-like immunophenotype is most prevalent in certain European countries (Germany and Italy) (16, 20), Asia (China and Japan) (21, 24), as well as in Saudi Arabia and the United States (17, 28). It is interesting to single out countries such as Pakistan (18), Vietnam (26), and especially Nigeria (19), in which HER2-positive and triple-negative immunophenotypes that are more common in younger people, are more aggressive and have a lower survival rate,

prevail partially or predominantly (29). This is a possible explanation as to why these three countries also have slightly lower age statistics when it comes to patients with breast cancer (Table 4). In addition to the presence of said immunophenotypes, the age of patients is certainly affected by the implementation of prevention programs, which is evident in the example of Sweden, which, despite the distribution of immunophenotypes similar to that in our research, has a much lower median age. In Sweden, the age threshold of women included in the prevention program is 40, i.e. 10 years younger than in the Republic of Croatia, where the threshold is 50 (30, 12). The observed geographical grouping of certain immunophenotypes suggests that, in addition to genetic, a certain significant role in the carcinogenesis of breast cancer is also played by environmental factors, which should certainly be investigated in future research.

When it comes to the frequency of positive axillary lymph nodes, results from our sample made it clear that the luminal B-like immunophenotype metastasises significantly more frequently to axillary lymph nodes compared to the luminal A-like immunophenotype. A group of authors from Serbia obtained similar results (27). Contrary to our result, the authors of studies from Italy (20) and Brazil (25) did not come across significant differences. However, it should be pointed out that a general limitation of these comparisons with other studies lies in the fact that few of them have used a slightly different methodology in the determining of breast cancer immunophenotypes (indicated in Table 4). Minor differences in the methodology of the first group of studies is a consequence of a change in the cut-off value of the proliferation index from 14% to 20%, accepted after the conclusions of the St. Gallen Oncology Conferences in 2013 (11). In our opinion, although these important differences in determining the proliferation index have a huge impact on the type of treatment of each individual patient with breast cancer, they have a minor effect on the total immunophenotype ratio because a small minority of cases could be reclassified into a different immunophenotype if another classification system (the same as in our study) was used. On the other hand, major differences in the methodology presented in the second group of studies are the result of a lack of use of the proliferation index, which can be seen from the absence of the luminal B-like HER2-negative immunophenotype category (Table 4). Therefore, the previously discussed comparison of our results with the results from these studies could be biased.

Regardless of the immunophenotype, in our research, axillary lymph nodes were more frequently positive in those patients with a Ki67 value higher than 20 %. A group of authors from Serbia obtained a similar result, indicating the connection between positive axillary lymph nodes and elevated Ki67, but, in contrast to our study, it had to do with tumours in which Ki67 was higher than 14 % (27). In a research conducted in Turkey, it was found that patients with higher stages of positive lymph nodes (pN2 and pN3) have a higher average level of Ki67 expression than those with lower stages and negative lymph nodes (pN0 and pN1) (31). Contrary to these conclusions, a research in Ethiopia found that there was no difference in the level of the Ki67 proliferation index between breast cancers that have positive and negative axillary lymph nodes, regardless of whether all cancers were divided into three groups according to the Ki67 level (Ki67 < 15 %; 15 % < Ki67 < 30 %; Ki67 > 30 %) or the average level of the proliferation index (13). Since the level of the Ki67 proliferation index amounting to 20 % is the limit that groups certain breast cancers into luminal A-like and luminal B-like immunophenotypes, the result obtained indirectly confirms that the luminal B-like immunophenotype has a lower survival rate than the luminal A-like immunophenotype, which is consistent with the literature (11).

Table 4. Frequency (%) of immunophenotypes in different countries of the world

Country	Sample size	Age (in years)	lumA (%)	lumB (%)	lumB/Her2 (%)	Her2 (%)	tr_neg (%)	Reference
Germany	4102	57	44.7	31.8	6.2	5.0	12.3	(16)#
Japan	363	56.7	30.6	26.2	19.0	11.3	12.9	(24)#
Brazil	269	55.4	23.79	10	34.61	14.50	17.10	(25) #
Serbia	108	61	25.92	47.22	19.44	1.85	5.55	(27) #
Italy	1487	-*	34.09	25.21	11.49	10.15	19.03	(20) #
China	3198	51	65.3	-	19.0	6.5	9.2	(21) §
Saudi Arabia	359	49.8	58.5	-	14.5	12.3	14.8	(17) §
Nigeria	118	46.9	28.8	-	6.7	17.9	46.6	(19)§
Pakistan	285	43.3	21.05	-	48.77	18.4	11.22	(18) §
USA	50 571	-*	72.7	-	10.3	4.6	12.2	(28) §
Vietnam	237	47.7	10.6	33.5	23.0	19.3	13.6	(26)
Sweden	237	51.3	31.6	41.3	8.9	7.6	10.6	(26)
Croatia	1868	62.3	31.32	45.67	11.67	4.50	6.80	(22)
Croatia	289	65.24	26.64	41.54	15.22	6.22	10.38	This research

* The data on patients' age are not expressed as mean or median.

cut-off value of proliferation index is 14%

§ proliferation index was not used

lumA = luminal A-like; lumB = luminal B-like HER2-negative; lumB/Her2 = luminal B-like HER2-positive; Her2 = HER2-positive; tr_neg = triple negative

However, it is important to point out one of the limitations of these comparisons. In clinical pathology, the above-mentioned immunohistochemical marker Ki67 is observed by a microscope, using a semiquantitative method, the interpretation of which is subject to numerous factors, such as the experience of the pathologist, quality of equipment, and quality of sample processing. Therefore, that may be the cause of interlaboratory discrepancies in the interpretation of samples, and therefore lead to different results.

This paper has potentially useful clinical as well as public health implications. Primarily, this study describes the distribution and characteristics of breast cancer, which may be useful in planning, adjusting, and improving treatment options, but also in assessing the risk of disease recurrence and death depending on the immunophenotype of breast cancer. In addition, a slight but

continuous increase in the number of diagnosed cancers during the research period, as well as the data on the age of patients, can serve as criteria in evaluating the preventive program for early detection of breast cancer and in planning further steps in its improvement.

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Review article

Importance of Oral Hygiene and Maintaining Oral Health in Persons With Disabilities

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Abstract

This paper aims to approximate and facilitate the routine of daily oral hygiene for persons with disabilities, as well as to emphasize the importance of educating persons with disabilities and their caregivers about oral health as an essential part of overall health. Desk research of electronic databases was conducted with the aim of writing this paper, using the following keywords: 'oral hygiene', 'dental plaque', 'oral health' and 'persons with disabilities'. Literature research has shown that persons with disabilities have poor oral hygiene, as well as that there is a lack of education among them and their caregivers about the importance of oral health and proper oral hygiene. Poor oral hygiene can affect a person's quality of life due to discomfort during eating, bad breath, poor self-esteem, pain, and disturbed sleep, which is a result of caries or other diseases of the oral cavity. Maintaining oral health is an essential part of overall health.

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Introduction

It is estimated that around one billion people, which is about 15% of the world's population, have some form of disability. The term disability is broad and covers persons with physical, sensory, intellectual, medical, emotional and social disorders, and most commonly a combination of these disorders (1). Persons with disabilities often need extra help to achieve and maintain good health, and oral health is no exception.

Oral hygiene is the process of cleaning the hard and soft tissue of the oral cavity (teeth, gums, and tongue), fixed and removable prosthodontic restorations, and dental appliances. Irregular or poor oral hygiene results in an increased number and variety of bacteria in plaque (2). Dental plaque plays a significant role in the development of the two most common oral diseases, dental caries and periodontal disease.

Most toothaches are caused by caries. At first, caries looks like a painless white stain; at this stage, the process is reversible with good hygiene, and remineralization of the enamel is possible. With the advancement of demineralization, cavitation and caries coloration occur. If left untreated, caries progresses to the pulp and causes inflammation accompanied by severe pain. Inflammation can then spread to the top of the tooth, forming a granuloma, abscess, or cyst (2). Persons with disabilities mostly have genetic predispositions for the development of gingival and periodontal disease, and the medications that they take, in combination with poor oral hygiene, inevitably lead to rapid disease progression, gum recession, and tooth loss (3, 4).

It is important to raise awareness among persons with disabilities and their caregivers about the importance of maintaining oral hygiene and ways to achieve it, with emphasis on the need for regular visits to the dentist, who will teach them about routine and special procedures.

Dental plaque

Dentobacterial plaque is a soft deposit of living and non-living microorganisms in a matrix rich in polysaccharides and glycoproteins, which adheres tightly to the tooth surface and can only be removed by mechanical cleaning. The formation of dentobacterial plaque is a complex process that occurs in several stages that are not restricted. Initially, a pellicle is formed, the first acquired plaque on the teeth. The pellicle is a thin, translucent glycoprotein layer with no cells and bacteria. It is 10 μm thick and forms 20 minutes after the tooth has been cleaned. Once the pellicle has formed, bacteria begin to settle. Bacteria can adhere to the pellicle: through direct contact between the bacterial wall and the pellicle, through fibrous extensions on the walls of the bacteria, through mediation of a fluffy layer of epithelial cells. At this stage, the plaque matrix is still sufficiently permeable, and aerobic conditions prevail (5). Gram-positive streptococci such as S. mutans, S. sanguinus, S. oralis, S. mitis, and Neissera spp. are the first to colonize the tooth surface. The primary colonizers of dentobacterial plaque are either aerobes or facultative anaerobes. Secondary colonizers are mostly gram-negative bacteria such as Actinomyces spp., Fusobacterium, Prevotella intermedia, and Capnocytophaga spp. (6).

From the third to the seventh day, the formation of extracellular polysaccharides dextran, mutan and levan intensifies. They increase the volume of plaque and reduce its permeability. Only small molecules such as sucrose, to which great cariogenic potential is attributed, can now penetrate the plaque. Due to the lack of oxygen, anaerobic conditions occur, in which the final products of decomposition of sucrose are pyruvic acid and lactic acid. The pH values are lowered to critical values of 5.2 to 5.4 (5).

If dental plaque remains undisturbed for about seven days, tertiary colonizers accumulate. These are mostly strict anaerobes that opportunistically exploit the environment provided by other bacteria, including pathogenic bacteria such as Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans, and

spirochetes such as Treponema denticola. When living as a microbial community in dentobacterial plaque, individual bacteria tend to share their virulence properties through gene transfer, particularly antibiotic resistance genes found in plasmids (6). The pathogenic biofilm of dental plaque results not only in dental diseases such as dental caries and periodontal disease, but may also be involved in the development of cardiovascular, respiratory and renal diseases, as well as diseases of other organs (6). It is therefore important to understand the mechanism of occurrence and the possibilities of prevention and control of dentobacterial plaque.

Oral hygiene in persons with disabilities

Persons with disabilities usually have poorer oral health, more extracted teeth, more caries, fewer fillings and treated teeth, gingivitis, a higher rate of edentulism, fewer preventative procedures, and more inadequate dental care compared to the general population (7, 8). As oral diseases primarily have a microbiological origin, it is necessary to establish plaque control, which can be achieved mechanically and chemically (9). The essential means for removing plaque from teeth are mechanical brushing agents and interdental cleaners such as dental floss and interdental brushes (10). Use of soft toothbrushes as well as modified and electric toothbrushes is recommended, especially for persons with reduced motor skills and intellectual abilities. Studies have shown greater plaque removal efficacy in this population when electric toothbrushes are used (11). It is equally important to clean the spaces between the teeth, so it is essential to educate patients, in line with their perceptual and cognitive abilities, about the use of interdental brushes and dental floss. Most of these patients will find it more convenient to use dental floss with a holder (12).

In addition to mechanical cleaning, chemical control of plaque is of great importance. It includes the use of toothpaste, gels, mouthwashes, etc., the composition of which contains active ingredients such as fluorides and chlorhexidine. Various studies have demonstrated the efficacy of using chlorhexidine in persons who are unable to maintain proper oral hygiene mechanically, and it is the first choice for supporting the treatment of gingivitis and periodontal diseases (13). Adequate use of fluoride is recommended from an early age, because of the high risk of caries in this population. Where lower risk is assessed, the recommendation is to use a toothpaste with up to 1000 ppm fluoride for young children, and with around 1500 ppm after the age of 6. In case of high risk, which pertains to most children with disabilities, toothpaste or gel with 1000 to 1500 ppm fluoride is administered immediately when the first teeth appear in the mouth (pea-size). For 12-year-old children at high risk, 2800 ppm of fluoride is recommended, and up to 5000 ppm at the age of 16 (14).

Education of persons with disabilities and their caregivers

Persons with disabilities and their caregivers need to be educated about and conscious of the importance of oral health: how to achieve and maintain it. Despite evidence that this population generally has poor oral health, oral hygiene training programs are not readily available for their caregivers (15). The results of the research show an improvement in the knowledge, skills, and attitudes of caregivers about oral hygiene after completing an educational program (15, 16).

Education about oral hygiene and dental cleaning procedures should be provided from an early age in order for children to develop the habit of maintaining oral hygiene and oral health. To support this, one study conducted among children with disabilities of preschool age showed a significant correlation of good tooth brushing with adaptive skills and practicing oral hygiene. Multiple oral hygiene steps could be performed by children who developed the habit of brushing their teeth before the age of 1 and who regularly brushed their teeth at least twice a day, as opposed to children who started with oral hygiene later and brushed their teeth only occasionally (17). Since one type of education does not suit everyone, such education needs to

be tailored to the specific condition of the individual or group.

Recent research has proved the importance of tailor-made education for achieving significantly better results in regard to understanding oral health and oral hygiene. A study conducted among the deaf and hard of hearing showed that participants with standard education in print form achieved good results compared to their pre-education status. However, a group of participants with tailor-made training, which included a video in sign language, showed statistically significant results when compared to their initial knowledge and skills in oral hygiene (18).

It is crucial to regularly visit the chosen doctor of dental medicine, who will monitor oral health and provide additional oral hygiene instructions depending on the psycho-physical capabilities of the individual patient. For example, a dentist will demonstrate the process of tooth brushing to blind and partially sighted people using the 'hand-to-hand' technique, guiding the patient's hand, alerting them to particular locations in the mouth that have a more significant physiological tendency to accumulate plaque, such as areas of contact between tooth and gums, gritted and rotated teeth, etc. (12). Other methods, also used with the general population in the event of higher anxiety when performing dental procedures, help reduce fear in persons with disabilities. One of them is the so-called 'tell-show-do' method, in which, before any procedure is performed, it is explained to the patient using understandable language, and instruments that will be used are shown. It always begins with a tool or procedure that will cause the least fear in patients (13, 19). The doctor of dental medicine will apply various other methods in communication, preventive and curative action, depending on the patient's medical history and individual needs. Regular visits to the dentist and intervention training decrease dental phobia and aversion to the doctor's office, as well as to necessary preventive and curative treatments (20).

The importance of healthy and balanced nutrition, which is an essential factor in oral health, should undoubtedly be included in the education of persons with disabilities and their caregivers. For example, this could include highlighting the negative role of sugar in the onset of dental disease and pointing out some ingredients in the diet that have a potentially anticarcinogenic effect, such as xylitol (13, 21).

Sedation and general anaesthesia

Most persons with disabilities can be treated routinely in dental practices, with procedures tailored to them. However, some of them cannot cooperate well enough for the necessary dental procedure to be performed (22). Appropriate sedation or general anaesthesia is used on all patients who are unable to cooperate during a visit to the dental office: very young children, patients with motor or cognitive dysfunctions, general population with severe dental phobia; this is likewise used in cases when procedures are extensive and cannot be done under local anaesthesia (23, 24). Today, sedation is most often performed using an oral sedative or inhalation of nitric oxide. These methods can be very effective in relaxing anxious patients, but require a certain dose of cooperation; e.g., for inhalation, the patient must be willing to wear a mask through which gas is inhaled and must breathe through the nose (22, 25).

General anaesthesia involves a reversible loss of consciousness caused by the use of one or more anaesthetics, during which the patient cannot be awakened by painful stimulation. It does not require patient cooperation and its application is considered relatively safe and has been widely described as a useful way of treating patients with a disability (22, 26). General anaesthesia allows for complete oral rehabilitation in the same visit, including tooth extraction, pulp and root canal treatment, placement of necessary tooth fillings, treatment with fixed and removable prosthodontic restorations, scaling and root planing, prophylactic procedures, etc. (27).

Conclusion

Poor oral hygiene and diseases of the teeth and oral cavity limit normal daily functions such as eating, swallowing, chewing, and ultimately result in poorer general health. The impact of oral diseases on systemic diseases and the functioning of the organism in this specific population plays a significant role, even more so than in the general population (13, 28). Therefore, a doctor of dental medicine should be an indispensable part of a multidisciplinary team that looks after the health of persons with disabilities. Society should also contribute to better quality of life and the realization of optimal healthcare by providing high-quality health insurance, more frequent visits to the dentist, and more preventative and curative procedures, which are key to maintaining oral health (14, 29). Oral disease prevention as an integrative part of overall health should be imperative, with emphasis on the mandatory continuous education of persons with disabilities or their caregivers, good oral hygiene, which includes mechanical and chemical plaque control, regular visits to the dentist, and proper nutrition. Good oral health improves the quality of life.

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Original article

Slide in Centric on a Random Sample of Students of the School of Medicine in Split

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Abstract

Introduction: A slide in centric is defined as a slide from centric relation to maximum intercuspation. Understanding contact between natural teeth is important for longevity of the stomatognathic system, diagnosis and therapy planning. The aim of this study was to determine the difference in the length of slide in centric in population according to dental status, sex and previous orthodontic therapy.

Materials and methods: The study was conducted on a sample of 33 students at the University of Split, School of Medicine (dental study).

Results: Slide values do not follow normal or Gaussian distribution according to the Kolmogorov–Smirnov test ($p<0.05$). For that reason, they were represented by the median as a measure of central tendency. The arithmetic mean of a slide in centric is 0.95 mm ± 0.47 mm. A slide in centric was not present in only 10% of the subjects. A slide between 0.5 mm and 1.5 mm to maximum intercuspation was present in 90% of the examinees. There was no statistically significant difference in the length of slide between the subjects who had all teeth and those who had missing teeth 1-4 ($z= 0.507$; $p= 0.612$). There was no significant difference in the length of slide between women and men ($z= 0$, $p=1$). There was no significant difference in the length of slide between the patients who underwent orthodontic therapy and those who did not ($z=0.253$; $p=0.800$).

Conclusion: There is some controversy about slide in centric and its etiological role in the development of temporomandibular disorders. Slide in centric is very significant because it indicates occlusal instability and can eventually lead to temporomandibular dysfunction, which do not have to be of the same aetiology..

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KEYWORDS: dental occlusion, centric; temporomandibular joint dysfunction syndrome

Introduction

Understanding the contact of teeth in certain positions and during the movement of the mandible is important for longevity of the stomatognathic system, diagnosis, planning prosthodontic treatment, and treatment of dysfunctions (1). Occlusion principles are gnathological or of a "freedom in centric" type. In the gnathological type of occlusion, maximum intercuspation is equal to centric relation. Therefore, there are no initial contacts during the closing movement around the kinematic axis. This type of occlusion is called centric occlusion (2). A group of gnathological or organic occlusion ensures that during laterotrusion, contacts occur only on cuspids, while any other contact or slide on any other tooth represents interference (3). Numerous authors, among whom Lauritzen (4), define cuspid guided occlusion as physiological and thus desirable. Cuspid guided occlusion is generally found in young population. The other type of occlusion is "freedom in centric" (4, 5, 6). Posselt was the first author to describe the concept of "freedom in centric" (7). The freedom in centric concept allows a slight initial contact of the antagonists during the closing movement of the mandible, around the kinematic axis, and a slide to the maximum contact between the lower and upper teeth. This slide is considered normal and physiological only if it occurs in the sagittal direction. A slide in centric is defined as movement from the initial contact of the antagonistic teeth in centric occlusion to maximum intercuspation. The slide is approximately 0.5-1 mm. It is acceptable when it occurs in the anterior direction (8). Mann and Pankey use the term "long centric" to describe the case where there is an anterior slide between the retruded contact position and habitual occlusion in a length of approximately 1 mm (9).

Freedom in centric defines the possibility of movement from the initial centric contacts to maximum intercuspation in all orthogonal planes: the horizontal, frontal, and sagittal plane. Over time, the attitude about physiological relations in the temporomandibular joint has been changing. Centric relation and its definition have been evolving for years. There are at least 25 definitions of centric relation (10). Initially, it was the posterior superior position of the condyle in relation to fossa articularis to an anterior superior position. The currently recognised centric relation definition indicates the maxillo-mandibular relation in which the condyle articulates with the thinnest avascular part of the articular disc with the disc-condyle complex in the anterior-superior position against the inclined plane of the articular eminences (11). Therefore, it is also the most distal unstrained physiological relation of the mandible against the maxilla, from which lateral movements are possible.

Despite differences between definitions, centric relation is a repeatable position and it is used as a reference position in prosthetic treatment. Only in 10% of the population does maximum intercuspation coincide with centric relation, which represents a mutually protected occlusion or gnathological occlusion. Regarding the rest of the population, there is a difference between the initial contact in centric relation (retruded cuspal position) and maximum intercuspation. This slide is approximately 0.5-1.5 mm. Changing occlusal surface due to prosthetic rehabilitation, a prosthodontic appliance or a dental filling can cause a premature contact during the closing movement in central relation and consequently to the loss of equilibrium or pathological occlusion (12). Slide in centric of 2 mm is one of the most important occlusal parameters pointing to joint pathology (13, 14) and relates to mandibular instability (15). Some studies have confirmed the influence of slide in centric (in a length of over 2 mm) on joint pathology (16). As far as such slide exists between the position of centric relation and maximal intercuspation, diagnoses given in clinical practice can very often reveal pain in the lateral pterygoid muscle. This muscle pain disorder represents temporomandibular dysfunction and can be easily misunderstood for intracapsular temporomandibular disorders.

The aim of this study was to determine if slide in centric occurs in young healthy population

without any signs of temporomandibular dysfunction according to dental status, sex and previous orthodontic treatment (with or without therapy).

Materials and Methods

A cross-sectional study was conducted as a clinical examination on each subject. It included a random sample consisting of student volunteers of the University of Split, School of Medicine (dental study). All students were informed about the study and 33 students signed an informed consent.

Of the total sample tested, 25 persons were students of Dental Medicine and 8 were students of Medicine. The youngest subject was 20 years old and the oldest subject was 24 years old. In terms of sex, there were 13 men and 20 women participating in the study. The mean age of female subjects was 22.4+/-1.2 and the mean age of male subjects was 22.6+/-1.1 years. The mean age of the entire sample was 22.4+/-1.2 years. From the total sample tested, 21 subjects underwent previous orthodontic treatment, whereas 12 participants did not.

During the clinical examination, Decayed, Missing, and Filled Permanent Teeth (the DMFT index) was defined for each student sample. Slide in centric of every participant was measured using a wax bite record. The participants were positioned in a dental chair in an upright position, with the head resting on a headrest. A warmed, trimmed and softened wax plate was adjusted to the maxillary dental arch. Mouth closing in centric relation was achieved using the Dawson bimanual guiding technique to the point of initial contact between the mandibular teeth and the wax bite plate. Closing under guidance continued until the wax plate was bitten through and the initial contact of the antagonistic teeth was made. After registering the initial contact in centric relation, participants bit the wax plate to the point of maximum intercuspation. After removing the wax plate from the mouth, the length of slide was measured using a caliper. The statistical analyses used included the Kolmogorov–Smirnov test and descriptive analyses.

Results

In this clinical examination, dental status was recorded. Eleven male subjects and 14 female subjects had all teeth, representing 79% of the sample. One male subject and two female subjects were missing one tooth, representing 9% of the sample. One male subject and one female subject (3% of the sample) had two missing teeth. One female subject (3% of the sample) had three missing teeth and two female subjects (6% of the sample) had four missing teeth. Veneer or other dental restorations were not observed in any of the participants. Orthodontic therapy was administered to eight male subjects and 13 female subjects or 64% of the sample. The mean value of slide in centric in the entire sample was 1 mm (ranging from 0 mm to 1.5 mm). Regarding the male subjects, the values of slide in centric measured ranged from 0 to 1.5 mm, with a mean value of 1 mm. The same values were obtained upon examination of 20 female subjects. The slide values do not follow normal or Gaussian distribution according to the Kolmogorov–Smirnov test ($p<0.05$). For that reason, they were represented by the median as a measure of central tendency (min-max). Arithmetic mean of slide in centric was 0.95 mm ± 0.47 mm.

Based on the dental status of the subjects with all teeth, slide in centric of 1 mm was observed in 25 subjects, ranging from 0 to 1.5 mm, using the Mann–Whitney U test 0.612. Slide in centric of 1 mm (0-1.5 mm) was equally observed in both genders using the Mann–Whitney U test value of 1.0. Participants who underwent orthodontic therapy had slightly higher values of slide in centric – 0-1.5 mm – compared to the participants who did not undergo orthodontic therapy, whose values ranged from 0 to 1.4 mm, Mann–Whitney U test 0.800.

Table 1 shows the subjects according to variables in relation to slide in centric (no centric, positive side). Of 25 participants who had all teeth, 21 of them had slide in centric with an initial contact during closure in centric relation, with an equal distribution between male and female subjects. A higher percentage of existing slide in centric was observed in the participants who underwent orthodontic therapy.

Table 1. Subjects according to variables in relation to slide in centric (no slide, positive slide)

		Slide in centric	
		0 (no slide)	Slide
Dental Status	Have all teeth	4	21
	Missing 1-4 teeth	2	6
Sex	Women	4	16
	Men	1	12
Orthodontic therapy	No	1	12
	Yes	4	16

No statistically significant difference in the length of slide in centric between the examined subjects who have all teeth and those who had missing teeth 1-4 (z= 0.507; p= 0.612) was observed in this study. Furthermore, no significant difference in the value of slide in centric between women and men (z= 0; p = 1) was observed in this study. A statistically significant difference in the value of slide in centric between the subjects who underwent and the subjects who did not undergo orthodontic therapy (z = 0.253; p = 0.800) was not observed. Table 3 shows the number of subjects according to the variables studied in relation to slide in centric (no slide, slide in centric present). Slide in centric was not observed in only five subjects.

Discussion and Conclusion

There is some controversy about slide in centric and its etiological role in the development of temporomandibular disorders. In 1918, Harris observed slide in centric of 1 mm or less. Only in 10 % of the population does centric relation coincide with central occlusion, but in 90 % of the population, a slide from retruded contact position to maximum intercuspation occurs. Mandible slide (there is a slide) in an amount of 0.5 mm-1.5 mm. Results of this study comply with the data from the literature because slide in centric was not observed in only five subjects (33). Ramfjord and Ash (17) and Froemden (18) assume that the freedom in centric occlusion increases proportionally with age, based on the degree of tubercle abrasion. Results of this survey do not support Ramfjord's, Ash's and Froemden's opinion. This study was carried out

on a compact and young age group and it indicates a large percentage of slide in centric occurrence. Some researchers state that slide in centric over 2 mm has a significant etiological impact on temporomandibular dysfunctions (p-value of 0.008) (15, 19). Using a sample of 749 patients, Nilner showed that temporomandibular dysfunction correlates with slide in centric (20). Gnathologists confirm that malocclusion contributes to the pathology of the temporomandibular joint by selective grinding after orthodontic treatment (21). In this study, a statistically significant difference in slide in centric between the subjects who have all teeth and the subjects with missing 1-4 teeth ($z = 0.507$; $p = 0.612$) was not found, as shown in Table 1. Those results point out the fact that functional adaptation persists by entrenched neuromuscular adaptation after a partial loss of the teeth. Although a stable relation between the mandible and maxilla in the maximum habitual intercuspation existed before the loss of teeth, it persisted during partial loss of teeth. Initial contact of the remaining teeth during the closing movement initiates the same movements as a jaw with teeth. In this study sample, there was no significant difference in the length of slide in centric between the subjects who underwent orthodontic therapy and those who did not ($z = 0.253$; $p = 0.800$). This was also assumed by Haralur, who confirmed, in his study on a sample of 36 patients (who underwent orthodontic therapy) and a control group (who did not undergo orthodontic therapy), that the length of slide in centric and orthodontic therapy were not correlated (22). There is no significant difference in the length of slide between women and men ($z= 0$, $p=1$). It is obvious that sexual dimorphism was not observed in our study. In some studies, there is no correlation between slide in centric and temporomandibular disorders (23). Slide in centric is highly significant because it indicates a premature contact during the closure of the jaw and possible occlusal instability, which can eventually lead to temporomandibular dysfunction, which do not have to be of the same aetiology. In contrast, Huber and Hall stated in their study that slide does not affect the temporomandibular joint (24). There is no common opinion about slide in centric potential pathology because the central nervous system could diminish potentially damaging forces through neuromuscular control and the compensatory mechanism.

The measurements and applied methodology are basic, useful in everyday practice and dental offices lacking sophisticated equipment. Accuracy is not on a high level, but can provide useful information for practitioners. Such a study should be repeated on a more comprehensive sample in order to identify the differences between each group more easily. By using a greater sample, the result will be more valuable. However, this investigation included only student volunteers, who participated in the study during one semester. Therefore, the sample is relatively small. Nevertheless, the data obtained in this study can provide useful information for practitioners. In everyday work, the difference between the closure of the jaw in centric relation and slide of 1 mm can produce harmful forces on teeth and TMJs as well as cause muscle fatigue and pain. Using a simple and quick method, the practitioner can obtain necessary information regarding the type of occlusion. By doing so, it is possible to avoid interreference during reconstructive procedures.

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Review article

Medical vs Surgical Abortion. Overview of European Legislation and Health Care Practice

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Abstract

Introduction: Abortifacient drugs, such as RU-486 or mifepristone, used in combination with a prostaglandin analogue (misoprostol) for the purpose of achieving medical abortion, have given rise to major legal, ethical and moral quandaries, which legislators all over Europe have striven to overcome by reconciling the reproductive rights of women with those of dissenting medical personnel.

Materials and Methods: We have conducted a comparison between international legislative approaches from the 1970s to 2020 upon the subject of voluntary abortion, with an eye on their applicability as well as other ethical concerns, supported by the analysis of the scientific debate on medical vs surgical abortion.

Results: The unresolved rift between the reproductive will of women and medical professionals' claim to conscientious refusal to treat, i.e., refusal to perform abortions or to prescribe abortifacient medicine, in such overwhelming numbers in Italy and elsewhere, has given rise to the impossibility of many women to terminate their pregnancies as they choose to. As a matter of fact, in 2018, only 64.9% of Italian public hospitals were able to guarantee access to abortion services. Hence, 35% of Italian facilities fail to meet the standards set by Law 194/78.

Conclusion: The authors have aimed to shed light on how medical abortion is to be preferred over a surgical one, and how major European countries have dealt with such an extremely thorny issue that has polarised the public opinion and scientific community members alike.

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Introduction

RU-486 (mifepristone) is an active antiprogesterone and antiglucocorticosteroid agent, generally used in combination with a prostaglandin analogue (misoprostol) in order to bring about a medical abortion during pregnancy. Except for Poland, Ireland, and Malta, where abortion is banned, access to the medication is regulated throughout the European Union, albeit through varying, rather than uniform protocols, as reflected in Table 1 (1, 2). Much like in the EU, in the United States and several Eastern European countries, as well as in India, China, and all countries where abortion is legal, mifepristone combined with misoprostol is the most widespread means to induce an abortion. The World Health Organization itself has deemed the drugs safe and effective (3, 4).

Table 1. Comparison between voluntary termination of pregnancy vs medical abortion in diferent european countries

COUNTRY	Voluntary Termination of Pregnancy	Medical Abortion
Austria	Legal within the first 3 months of pregnancy (20).	Legal and accessible. The regulation for mifepristone allows the drug to be administered only in medical facilities; medical abortion can therefore only be performed in hospitals (21).
Belgium	Legal within the twelfth week of pregnancy.	Available in Belgium and given until up to 49 days of amenorrhea (22).
Bulgaria	Legal within the twelfth week of pregnancy (23).	Abortifacient drugs are not registered, thus illegal.
Croatia	Legal within the tenth week of pregnancy (24).	Medical abortion has been available since 2015. Only recently has the Croatian Agency for Medicinal Products and Medical Devices (HALMED) approved the drug combination used in medical abortion. Yet, their administration is only allowed in hospitals accredited to perform abortions, with professional supervision.
Cyprus	Legal within the tenth week of pregnancy since 2018 (25).	Misoprostol is legally usable for termination of pregnancy.
Czech Republic	Legal within the first 3 months of pregnancy (26).	Legal upon demand since 2013.
Denmark	Legal within the twelfth week of pregnancy since 1973 (27).	Medical abortion is used until end of 8th week. Surgical abortion may be chosen until end of 12th week. Until 8th week, most abortions are medical. Overall, approx. 40% of all the abortions are medical and approx. 60% of all the abortions are surgical (28).

Estonia	Legal within the eleventh week of pregnancy (29).	Mifepristone in combination with misoprostol (Arthrotec) for the purpose of medical abortion was registered in 2003. Medical abortion can be used up to the 63rd day of pregnancy (30).
Finland	Legal within the twelfth week of pregnancy. Up to 20 weeks if there is a risk to physical health of woman or if the woman is younger than 17. Up to 24 weeks in case of major fetal malformation; no limit if there is the woman's life is in danger (31).	Legal and free of charge, on outpatient basis.
France	The ten-week limit was extended to the twelfth week in 2001 (32).	France was the first country to legalize the use of RU-486 as an abortifacient in 1988, allowing its use up to seven weeks of pregnancy under medical supervision. According to a United Nations Population Division estimate, 19% of all French abortions used RU-486 as of 2002. Medical abortion represents almost 50% of all performed abortions. In response to the Covid-19 pandemic, France has extended access to medical abortions to nine weeks of pregnancy (33).
Germany	Legal within the first 3 months of pregnancy; mandatory counseling is required; abortion is also legal later in pregnancy in cases of medical necessity (34).	Legal within 9 weeks (63 days) since last menstruation, requires medical prescription (35).
Greece	Legal within the first 3 months of pregnancy (36).	Mifepristone and misoprostol are registered, available and affordable. However, medical prescription and hospitalization are required (37).
Hungary	Legal during the first twelve weeks of pregnancy (38).	Banned

Ireland	Legal since 2018 (following a constitutional amendment approved by a referendum in May 2018) within the twelfth gestational week and later in cases where the pregnant woman's life or health is at risk, or in the cases of a fatal fetal abnormality.	Abortifacient drugs are illegal (39).
Latvia	Legal during the first twelve weeks of pregnancy (40).	Legal and available since September 2008. Prescription and gynecological assistance are required. Medical abortion can also be carried out in certified in-patient facilities.
Lithuania	Legal during the first twelve weeks of pregnancy (41).	Banned.
Luxembourg	Legal during the first twelve weeks of pregnancy, following two consultations with a medical doctor and a psychologist, and a waiting period of at least three days (42).	Legal within 7 weeks (49 days) of pregnancy.
Malta	Banned under all circumstances. Malta is the only country in the European Union to ban abortion altogether (43).	Banned.
The Netherlands	Legal during the first 24 weeks of pregnancy (i.e., when it is believed that the fetus has develop vital functions enabling it to live outside of the womb) (44).	Legal (45).
Poland	Only legal in cases where the mother's life or health is at risk, in cases of major fetal malformations or pregnancy as a result of rape.	Banned (46).
Portugal	Legal during the first ten weeks of pregnancy.	Legal since 2007 (47).
Romania	Legal during the first fourteen weeks of pregnancy.	Legal with prescription (48).
Slovakia	Legal during the first twelve weeks of pregnancy.	Not available (49).

Slovenia	Legal during the first ten weeks of pregnancy.	Mifepristone available (50).
Spain	Legal within the 14th week of pregnancy, and at later stages in cases of serious risk to the health of the mother or fetal abnormalities.	Legal within the first trimester of pregnancy. Not easily accessible and costly. Surgical abortions account for most termination of pregnancy procedures (51).
Sweden	Legal within the twelfth week of pregnancy (52).	Medical abortion up to 63 days of pregnancy was approved in Sweden in 1992. Medical abortions accounted for 93% of all abortions in 2018 (53).
United Kingdom	Legal within 24 weeks of pregnancy (54).	Mifepristone, approved for use in Britain in 1991, and Misoprostol are legally accessible up to the ninth week (55).

In Italy, the legislation enacted to regulate access to voluntary termination of pregnancy (Law 194/1978, titled "Norme per la tutela sociale della maternità e sull'interruzione volontaria della gravidanza", or "Norms on the Social Protection of Motherhood and the Voluntary Termination of Pregnancy"), has effectively repealed Articles 545 to 555, which used to criminalise the termination of pregnancy in any way or form (5).

As a continuation of this legislative development, following the completion of the experimentation process, RU-486 has been marketed in Italy since 10 December 2009. Nonetheless, it is worth noting that, unlike other European countries, where it was already legal to medically terminate a pregnancy up to 63 days of amenorrhea, Italy has lowered that time limit to 49 gestational days.

The impact that the "procreative revolution" has had on the awareness of the population, however, has pushed the Italian Supreme Court to issue rulings that have helped to overcome several legal and practical hurdles over the years (6, 7).

Materials and Methods

Materials and study design

A large and qualitative review of the literature between the seventies and 2020 has been conducted to analyse the scientific background on medical and surgical abortion. The aim was to clarify the differences among the current laws and guidelines governing voluntary termination of pregnancy in different European countries, with an eye on the Italian situation, where the applicability of these laws is faced with ethical concerns. The study was conducted between 2019 and 2020 at the Sapienza University of Rome, Department of Anatomical, Histological, Forensic and Orthopedic Sciences, in collaboration with the Department of Medical and Surgical Sciences, University of Foggia.

Methods

The authors have examined the main medical databases, e.g. Pubmed, Google Scholar, Scopus and Cochrane Library, as well as legal databases (Lexis, Justia, Kleagle) by applying effective combinations of terms, i.e. surgical abortion; conscientious refusal to treat; mifepristone; medical abortion; voluntary

termination of pregnancy; abortion guidelines; health standards; emergency contraception; conscientious objection; contraception; RU-486; WHO guidelines on medical abortion procedures.

Discussion

The development and availability of new procedures and treatments undoubtedly entail novel ethical quandaries, at least theoretically. The issue of procreative freedom has unfolded along two distinct and irreconcilable lines of reasoning: if, on the one hand, medically assisted procreation has made it possible for women of relatively advanced age to achieve motherhood (8, 9), voluntary termination of pregnancy is in keeping with the woman's will not to become a mother (10). Nowadays, access to abortion services, as codified in Italian statutes, presents considerable difficulties, even more so in cases of unplanned pregnancies, when contraceptive methods fail or when sexual abuse results in pregnancy. In fact, access to emergency contraception, which has positively contributed to lowering abortion rates, may not be easily available in a timely fashion (11-13).

Contraceptive use rising as abortion falls: differences between Italy and European countries

In 2018, more than 64.9% of Italian public hospitals guaranteed access to abortion services. Hence, 35% of Italian facilities fail to meet the standards set by Law 194/78 (11).

One of the most relevant factors that led to such a situation is certainly conscientious refusal to treat by medical personnel, which is codified as a right in Law 194/78, under Article 9. According to said provisions, objectors may opt out of "performing procedures and activities specifically and necessarily aimed at achieving a termination of pregnancy" (14). Conscientious refusal to treat does not however exempt professionals from providing care before and after an abortion procedure or intervening in cases of emergency or imminent danger to the patient's life. After all, the Italian healthcare system is bound to uphold the free exercise of women's right to sexual and reproductive freedom by guaranteeing access to abortion procedures through the services and professionals set in the provisions of Law 194/78, by minimising the detrimental effects of conscientious refusal to treat under such a right, and possibly even ordering transfers of objecting physicians if no one else agrees to perform the procedure.

The practical execution of such measures is undeniably complicated in a country such as Italy, where conscientious objectors account for roughly 70% of health care professionals (15), which is an extremely high share compared to the European average – 10% in the United Kingdom, 7% in France, and none in Sweden (16-21). In most European countries, the law allows surgical abortion upon a woman's request in the first weeks of pregnancy or in an advanced gestational period under certain circumstances (16-51) (Table 1).

France was the first European country to legalise abortion by virtue of Law 75-17 of 17 January 1975 (Law on the Termination of Pregnancy). According to the French law, every pregnant woman has the right to an abortion until the twelfth week of pregnancy. After this time limit, the French law consents to abortion only if the continuation of pregnancy proves to pose a real and serious danger to the woman's health or life (16). In France, RU-486 has been legal since 1988. The pill is administered within the first seven weeks of pregnancy and under medical supervision. In this country, medical abortions represent approximately 50% of all abortions performed. In particular, according to an estimate from the United Nations Population Division, 19% of all abortions in France registered since 2002 have occurred by taking RU-486. Moreover, in response to the COVID-19 pandemic, France has extended access to medical abortion until the ninth week of pregnancy (17).

In contrast, the Republic of Ireland has an extremely restrictive approach to abortion,

unless there are circumstances that put a woman's health and life at risk. In Ireland, the constitution recognises the right to life for unborn children. Should such conditions arise that endanger the health of the woman and/or the child, however, the law does not set time limits for terminating pregnancy, though a surgical abortion is the only possible option (47).

Regarding drugs used for medical abortion, there are only a few European countries where abortifacient drugs are not registered (45), or they are illegal or banned (46-51) (see Table 1). In the European Union, Malta is the only state that has banned both surgical and medical abortion (49). These differences between various European countries, from the north to the south of Europe, have historical, political and religious origins. Medical abortion in Spain, for example, is not easily accessible and it is very expensive. For these reasons, surgical abortions account for the majority of pregnancy termination procedures (44). Conversely, in Sweden, medical abortions accounted for 93% of all abortions in 2018 (19). Any woman who turns to a healthcare professional has the right to thorough and comprehensive consultations on abortion practices (also, and above all, in relation to the clinical condition of a pregnant woman) as well as on the risks and benefits of one method compared to the other. Consultation is advised, but not mandatory for adult women, while it is required for minors. The "contraceptive revolution", or rather the introduction of the abortion pill, in fact, started in the 1960s and 1970s in Western European countries, which were the first to legalise abortion and where, therefore, it is perceived as a fundamental right of all women.

Italy: government legislation and abortion plan

The high degree of sensitivity in Italy towards ethically and religiously contentious issues has most likely played a role in stymieing and delaying scientific progress in terms of access to abortion and medically assisted procreation (MAP) procedures.

Based on Law 194 ("Norms on the Social Protection of Motherhood and the Voluntary Termination of Pregnancy"), women may legally resort to voluntary termination of pregnancy at national public facilities within the first 90 days of gestation, after which pregnancies may only be terminated for therapeutic purposes (5).

Nevertheless, the share of conscientious objectors has grown by 12% over the past 10 years, reaching as much as 90% in regions such as Molise, Trentino-Alto Adige and Basilicata. Significantly, in the whole region of Molise, there is currently only one registered physician who has not expressed a refusal to treat (11).

In 2014, the European Committee of Social Rights of the Council of Europe formally reprimanded three hospitals in the central Marche region, Jesi, Fano and Fermo, where all medical personnel had expressed a refusal to treat. The Committee claimed that such a situation constituted a violation of women's right to health, which is enshrined in the European Social Charter (52).

Another element negatively affects medical abortion: in compliance with the recommendations issued by the High Council of Health, most Italian regions require women seeking abortifacient drugs, such as RU-486, to be hospitalised to terminate a pregnancy (53). Due to that requirement and the organisational and ethical challenges which it engenders, many facilities have mostly opted for surgical abortion instead, to the extent that in 2018, fewer than 25% of Italian women could resort to medical abortion (11).

In December 2015, the Association of Italian Physicians for Contraception and Abortion (AMICA), counting on the support of various organisations and high-profile backers, sent an open letter to the Italian Ministry of Health, in which it asked to make medical abortion procedures less restrictive, on a day hospital basis and, where possible, accessible even in family counselling centres and ambulatory care facilities, for the sake of ensuring health care services availability and adequacy. On 8 August 2020, the Ministry of Health updated the set of guidelines regulating access to the abortifacient drug RU-486, allowing for its administration on an outpatient basis, i.e. with no need for

hospitalisation, and even extending the ultimate time limit for abortion from the seventh to the ninth gestational week. Such a development, based on scientific evidence, undoubtedly constitutes a meaningful step forward for Italy in terms of fully enforcing Law 194/78. Nevertheless, such a consequential decision has occurred within an uneven socio-political context. As a matter of fact, the ministerial decision has been made on the heels of an opinion which was asked from the High Institute of Health, following a regulatory decision made by the regional government of Umbria. That opinion was meant to discourage the tendency on the part of regions to perform medical abortion on a day hospital basis. The Umbria legislative initiative followed the 2010 set of ministerial recommendations (53), after the Italian Medicines Agency (AIFA) had authorised the marketing and distribution of the drug in compliance with European standards, albeit with restrictions in place that do not apply in other countries, such as the three-day hospitalisation requirement and the seven-week gestational limit.

The newly introduced ministerial directive has raised widespread controversy. The Legal Affairs Department of the regional government of Piedmont, in agreement with the Italian Episcopal Conference, has opposed the directive, arguing that it may run counter to Law 194/78 and stressing that outpatient administration of RU-486 could lead to serious complications for women.

Conclusion

It is obviously essential that the new guidelines do not conflict with the original primary purpose of Law 194/78 in terms of providing sound healthcare and psychological consultations for women in order to "try to remove the underlying reasons that induce women to seek an abortion" (5). Furthermore, it is essential to guarantee access to follow-up support activities for those women who ultimately decide to terminate their pregnancies, for the purpose of ensuring the fetus is expelled in a complete and safe fashion in the interest of the patient's well-being.

Furthermore, the WHO itself has been advocating for medical abortion (4) over surgical abortion in light of the many advantages in terms of financial benefits for healthcare providers (as a result of fewer hospitalisations, reduced use of anaesthesia and fewer surgical procedures) and a higher degree of safety, given the lower rates of complications arising from surgical interventions, such as suction aspiration and curettage. Moreover, in cases where medical abortion is not carried out in a timely fashion, resorting to surgical abortion is inevitable, often as an emergency procedure, which increases the risk for patients to contract infections or haemorrhages during surgery (54, 55).

The Italian ministerial directive is ultimately and consistently aimed at guaranteeing and upholding the "right to responsible and conscious procreation" by removing some of the barriers hindering access to termination of pregnancy and extending the period in which such procedures can be accessed, in an effort to discourage illegal abortions and reduce the need for late surgical abortions, often performed under emergency circumstances.

The nationwide application of said ministerial decree, released on 8 August 2020, will make it possible to gather more consistent and reliable data as to the rates of adverse events associated with medical abortion, which will provide scientifically reliable analytical elements for assessing its impact compared to surgical abortion procedures.

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