Volume 4, Number 1, APRIL 2020.
ISSN 2459-9484
http://seemedj.mefos.unios.hr

SEEMEDJ SOUTHEASTERN EUROPEAN MEDICAL JOURNAL

IVAN HEIL
POGLED NA OSJEČKU PERIFERIJU, 1939.

IZ FUNDUSA MUZEJA LIKOVNIH UMJETNOSTI OSIJEK, KUSTOS VALENTINA RADOŠ.

Josip Juraj Strossmayer University of Osijek
Faculty of Medicine

Southeastern European Medical Journal (SEEMEDJ)

Published by

University Josip Juraj Strossmayer Osijek

Faculty of Medicine Osijek

Published online:

http://seemedj.mefos.unios.hr

ISSN 2459-9484

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Invited review

Stigmatization of Patients With Viral Infections and Mass Psychogenic Illness

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Abstract

Background: The stigmatization of viral patients is primarily a negative attitude and a common opinion about people suffering from various infectious diseases of the viral etiology and their consequences. The belief and the attitude that individuals are not socially acceptable because potentially spreading contagion for the outcome has negative discrimination in our society. Often such persons are excommunicated, which extends through all the social layers and ages.

Methods: The PubMed, ScienceDirect, and SpringerLink databases were used for the research. Keywords stigma, viral infection, infected patient, discrimination, isolation were entered to identify papers dealing with a viral infection, and stigmatization.

Results: After screening available databases in the last five years according to the selected keywords, the PubMed database yielded nine articles, the ScienceDirect identified initially 87 articles, SpringerLink identified 42 articles. Viral infection and stigmatization are of interest to numerous scientists.

Conclusion: The availability of information should create empathy and ensure openness to diversity. Following the available literature, we understand that the biggest problem today is the social exclusion of people due to their viral illness, but equally the self-isolation of those infected due to the fear of being rejected and the misunderstanding of people from the environment.

(Šantić A, Šantić K, Radoja I, Jelinčić I, Degmečić* D. Stigmatization of Patients With Viral Infections and Mass Psychogenic Illness. SEEMEDJ 2020; 4(1); 1-13)

Received: Feb 29, 2020; revised version accepted: Apr 2, 2020; published: Apr 27, 2020

KEYWORDS: stigma, viral infection, infected patient, discrimination, isolation

Introduction

By observing the etymological side, the stigmatization in its widest sense originates from the word stigma (Greek Stigmatos), which in ancient Greece referred to a sign made on the body of morally bad and less valuable persons, created as a consequence of stamping or indentation. Often this was related to uncivilized persons who speak different languages, barbarians, Savages, but also members of the same people, slaves, criminals, traitors, and others from the lowest layers of society, which was then socially acceptable to mark. The main function of such a marking principle was the exclusion of selected persons from society and their social degradation. The aim of such degradation was not to direct the physical punishment of the individual but to punish the person by losing their freedom outside the bars, i.e., in the surrounding world. We notice that it is a much more complex concept, closely related to several prejudices, discrimination, and is very often accompanied by the primarily imposed and subsequently internalized by the self-stigmatization. The advancement of civilization has changed social layers, but stigmatization has always been one step ahead of time. Although the physical marking of persons as a method of humiliation may not be used nowadays, stigmatization is still present in our society (1-7).

One of the most famous sociologists in recent times who dealt with the notion of stigmatization, Erving Goffman, described the term as "an attribute that makes a man socially different from others in the social category and lowers him to the status of infected or reduced," and stigmatized person as "the individual who disqualifies from full social acceptance." Besides, he proposed to divide the stigmatization into three categories: physical differences (visible deficiencies, physical injuries, mutilations, tattoos), recognizable character deficiencies (mental and mental state, addiction, alcoholism, socially unacceptable behavior) and tribal stigmatization (inborn characteristics such as race, nation, religion (6, 8, 9).

There are two forms of stigma that share three common characteristics: stereotypes, prejudice, and discrimination, as outlined in Table 1.

Table 1. Comparing and contrasting the definitions of public stigma and self-stigma

Comparing and contrasting the definitions of public stigma and self-stigma	
Public stigma	
Stereotype	A negative belief about a group (e.g., dangerousness, incompetence, character weakness)
Prejudice	Agreement with belief and/or negative emotional reaction (e.g., anger, fear)
Discrimination	Behavior response to prejudice (e.g., avoidance, withhold employment and housing opportunities, withhold help)
Self-stigma	
Stereotype	A negative belief about the self (e.g., character weakness, incompetence)
Prejudice	Agreement with belief, negative emotional reaction (e.g., low self-esteem, low self-efficacy)
Discrimination	Behavior response to prejudice (e.g., fails to pursue work and housing opportunities)

The first is the Public stigma, which implies the opinion of the majority of the population towards a marginalized group, while the second self-stigma implies a negative opinion of a member of such a marginalized group (10-13). During our past, many diseases have played a very significant role in their devastating influence on society, and consequently, their negative psychological impact has grown. Examples are numerous. Starting with the plague, probably the first described pandemic of all time, which in history has repeatedly

decimated humankind, and it is related to tens of millions of victims. Its most notable outbreaks were 430 BC, 165, 250, 541, 1350, and 1665. In the 11th century, leprosy was first described as a separate disease and its whole dark side (14). The beginning of the 19th century marks cholera, and at the end of it, we can see the first significant pandemics of a viral disease, influenza (15). First, those Russian from 1889., then Spain from 1918., and in the end, those Asian from 1957. year. History then records a couple more significant outbreaks of the causative agent in the later years of the 20th and early 21st centuries (16). The modern age marks the exponential development of science, medicine, and the discovery of antibiotic therapy, so the previously mentioned threats of bacterial etiology, such as plague and leprosy, have become a minor adversary. However, the man did not become immune to disease outbreaks, and since 1981 a new topic has emerged in the professional literature, as well as in daily informative discussions, HIV - AIDS (17). Another disease that causes the virus and is followed by a series of more common occurrences of other viral pandemics. Fortunately, they are no longer counted in the millions but in the hundreds and thousands, but they are discussed daily (18). It is necessary to take into account the dose of globalism, the ease of availability of new information, but also, more generally, better information for the population.

Looking empirically and with great caution, it is not surprising that the human race created an aversion to diseases and their causes. As beings at the top of the food chain, they remained an important link which, from the beginning, represented our most important natural enemy. Therefore, it is not surprising that infectious patients carried a great social stigma. Perhaps as pity, but more likely out of fear for their own lives, often infected persons are excommunicated, which extends through all the social layers and ages (16-18).

Methods

Literature research took place in February 2020. Databases used in the search were PubMed, ScienceDirect and SpringerLink. Keywords stigma, viral infection, infected patient, discrimination, isolation were entered to identify papers dealing with a viral infection, and stigmatization. In the last five years, the PubMed database yielded nine articles. Another 75 articles were found after using the option "similar results" with the first paper, and 40 articles were found for the second paper.

Inclusion Criteria

All articles containing the terms stigma, discrimination, virus infection, social exclusion, cohort, prospective in the title or abstract were included in the review. Exclusion criteria were if a paper included animal research, a person under 19 years. Six articles regarding studies ultimately met the criteria. The final results obtained after applying filters are shown in Table 2.

Table 2. PubMed search methodology and results

Filters	Paper 1	Paper 2
From 2015 until 2020	11	28
Similar results	78	93
English language	77	89
Sex female and male	61	75
Age: Persons 19 – 80+	58	74
Review of abstracts and title of the articles based on including criteria	5	27

The same keywords were used to search the ScienceDirect database, and 87 articles were identified initially, which include articles published from 2015 to 2020. The number of articles was further reduced to 30 by using the option "type of article –research" and to 10 by using the option "publication title." They publish in the journals Journal of the Association of Nurses in AIDS Care, International Journal of Drug Policy, Social Science & Medicine, Applied Nursing Research, HIV & AIDS Review, Journal of Substance Abuse Treatment, Journal of Theoretical Biology, Nurse Education Today, Journal of Adolescence and Sexual & Reproductive Healthcare. After reading the titles and abstracts, three articles were selected.

The keywords mentioned above were used for searching the SpringerLink database, where 45 documents were identified, which include documents published from 2015 to 2020. After applying the filter article, the initial number was reduced to 42 and then to 36 after using the filter "Medicine & Public Health." Further restriction of results accomplishes using the options "Public Health and English," which led to 14 articles. Titles and abstracts were reviewed, and two articles were selected.

Discussion

The stigmatization of HIV patients

AIDS is a disturbed condition of the human immune system, a disease caused by HIV infection. It affects populations worldwide, is not limited to specific subgroups or regions, and attacks without warning. It estimates that around 37.9 million people are currently infected worldwide, of which as many as 1.7 million children are under the age of 15.

Table 3. New HIV infections by region, 2017–2018; Source: UNAIDS/WHO estimates; WHO HIV update July 2019 (Available on: https://www.who.int/hiv/data/en/; accessed on February 2020)

New HIV infections by region, 2017–2018				
WHO region	**Number of new HIV infections 2017**	**Number of new HIV infections 2018**	**New HIV infections all ages (per 1000 uninfected population) 2017**	**New HIV infections all ages (per 1000 uninfected population) 2018**
Africa	1 100 000 [830 000-1 500 000]	1 100 000 [800 000-1 500 000]	1.15	01.07.20
Americas	160 000 [120 000-200 000]	160 000 [120 000-200 000]	0.16	0.16
South-East Asia	170 000 [120 000-210 000]	170 000 [110 000-200 000]	0.09	0.09
Europe	170 000 [150 000-180 000]	170 000 [150 000-190 000]	0.19	0.19
Eastern Mediterranean	39 000 [24 000-64 000]	41 000 [26 000-68 000]	0.06	0.07
Western Pacific	120 000 [110 000-130 000]	120 000 [110 000-140 000]	0.06	0.06
Global	1 800 000 [1 400 000-2 300 000]	1 700 000 [1 400 000-2 300 000]	0.24	0.24

There is no specific therapy, and due to its characteristic structure and inability to retain antigenicity in living attenuated or dead organisms, the vaccine has not been discovered to date. Taking into account the above facts, as well as the devastating and deadly consequences it carries, the fear of HIV infection is reasonably justified. However, knowing the methods of spreading the disease and the relatively successful prevention mechanisms, the stigmatization of the sick is often exaggerated and non-meaningful. There should also be considered clear data on the constant decline in the number of sufferers, with a fall of as much as 38% globally from 2001 to 2003, accompanied by a significant reduction in the number of deaths caused by AIDS (19). In 2018, about 770,000 people died from the effects of AIDS, compared to 1.2 million in 2010, and half a million more in 2004 (Table 3) (20).

The stigmatization of HBV and HCV patients

Unlike human immunodeficiency viruses, hepatitis viruses constitute a heterogeneous group of microorganisms that specifically attack liver tissue, causing acute and chronic diseases. Hepatitis A and E viruses appear sporadically in the world; we call them diseases of "dirty hands," and the result of infection with these agents is mostly an acute disease that usually spontaneously passes through a period of 1 to 4 weeks (21, 22). Hepatitis D is characteristic in that it never occurs independently as a cause of disease, but always accompanies an earlier infection with HBV as a superinfection, i.e., it causes chronic co-infection of HDV and HBV (23). For our research, however, we will pay special attention to those types of viruses that cause a majority of chronic infections and which pose a special challenge for physicians, and the health system a certain socioeconomic problem. This category includes hepatitis B and hepatitis C viruses, which have a long incubation period, after which they cause mild or no symptoms, and once transmitted to chronic disease in some patients can cause cirrhosis or hepatocellular carcinoma (24). Their occurrence is not geographically specific, they are present in all parts of the world, and they can affect members of all social layers. It currently estimates that 250-290 million people are infected with HBV worldwide, and approximately 887,000 people die annually from the infection (26.)

Specific therapy does not exist; treatment is symptomatic and aims to prevent the development of liver cirrhosis and to prevent the occurrence of hepatocellular carcinoma. However, the HBV vaccine exists, and in combination with improved awareness and good preventive measures, it estimates that by 2030, the global HBV threat should reduce to the lowest possible level, which is also the World Health Organization's plan (26). Unfortunately, despite such a good prognosis, the stigmatization of HBV patients persists. By contrast, the number of people infected with HCV in the world estimates to be four times lower, around 71 million, and the death rate is only half the yearly rate than that of HBV, approximately 400,000. There is certainly the fact that in most cases, HCV is transmitted in the vast majority of cases only by blood, and for HBV, there is data of transfer through bodily fluids. Besides, a specific vaccine against HCV is still not available, so it is more difficult to prevent the disease itself if direct exposure occurs (27-30). In addition to the health complications affecting the ill, an additional burden creates by stigmatization and discrimination (31, 32). The basis of stigmatization and, in this case, carries a high level of anxiety and excessive fear of transmission of infection, which consequently leads to social and economic discrimination and financial burdens for the individual and the economy as a whole. The basis of the prevalence of discrimination against these viral infections would also be education and accurately inform the entire world population (33).

The stigmatization of Ebola patients

Ebola virus disease is a rare disease of Ebola virus infection. The risk of Ebola virus infection is extremely small unless you have been in

direct contact with the bodily fluids of people suffering from Ebola, i.e., living or dead animals (34,35). Uncertainty among populations is given by the fact that it is possible to transfer infection by contact with bodily fluids or unprotected sex with patients who have completely recovered from the Ebola virus disease. In particular, special warnings issues to persons traveling to countries where there is a risk of infection with Ebola. The stigmatization of Ebola patients is mentioned in several scientific articles (36-38).

Talking about the discrimination and stigmatization of Ebola-infected in posts about survivors, up to 64% of articles in 2015 were explicitly stigmatized related to survivors (39). Trough many articles, the main cause of any form of stigmatization and/or discrimination has been called fear of illness (40). What cited as the basic problem for survivors is dealing with the consequences, social and economic outcomes (loss of friends, loss of workplace), and experiencing psychological stress. Often, these issues compound by the unreliability of the health system, inconclusive Knowledge about treatments, and ways of transmitting infection with a lack of feedback and the effectiveness of destigmatizing measures (41, 42). The current epidemic mostly affects the Democratic Republic of Congo with Liberia, Guinea, and Sierra Leone, with a survival rate of 53% (Table 4)

Table 4. Chronology of previous Ebola virus disease outbreaks. Latest numbers as of February 23, 2020. Source: Ministry of Health, Democratic Republic of the Congo (Available on: https://www.who.int/emergencies/diseases/ebola/drc-2019; accessed in February 2020)

Chronology of previous Ebola virus disease outbreaks.

Latest numbers as of February 23, 2020.

Year	Country	EVD	Cases	Deaths	Case fatality
2018-2020	**The Democratic Republic of the Congo**	Zaire	3444 – ongoing	2264	65,74%
2018	**The Democratic Republic of the Congo**	Zaire	54	33	61,00%
2017	**Democratic Republic of the Congo**	Zaire	8	4	50,00%
2015	**Italy**	Zaire	1	0	0,00%
2014	**Spain**	Zaire	1	0	0,00%
2014	**UK**	Zaire	1	0	0,00%
2014	**USA**	Zaire	4	1	25,00%
2014	**Senegal**	Zaire	1	0	0,00%
2014	**Mali**	Zaire	8	6	75,00%
2014	**Nigeria**	Zaire	20	8	40,00%
2014-2016	**Sierra Leone**	Zaire	14124*	3956*	28,00%
2014-2016	**Liberia**	Zaire	10675*	4809*	45,00%
2014-2016	**Guinea**	Zaire	3811*	2543*	67,00%

(43, 44). Persons who are treated as survivors, cured of Ebola, experience social isolation by people from their environment, and experience various forms of violence that, apart from psychic, also involve the extent of physical violence (45, 46).

. The similarity of stigmatizing attitudes towards people suffering from HIV and Ebola infection are numerous, from discriminatory racial attitudes, sexual orientation with irrational fears (47, 48). The fundamental difference in the stigmatization of the infections mentioned above is that the mortality of the Ebola virus infection is higher compared to the affected population, it occurs within a shorter period, and therefore, the level of anxiety is even higher (49).

The stigmatization of patients with respiratory infections

Two-thirds of all infections are inflammatory diseases of the respiratory system. The most common causes of respiratory infections are viruses, accounting for 85% of all acute respiratory inflammation (50). Due to the lack of availability of effective antiviral drugs, treatment generally comes down to symptomatic. The basic mechanism of transmission is by drop-through, more frequently occurring in the colder months with symptoms such as fever, cough, shortness of breath, muscle pain, fatigue, and general weakness (51). Although most often, it is a common cold caused by Rhinovirussians, more emphasis places on the flu virus due to the severe clinical images and possible mortality. Complications of influenza in older age, cause the so-called. "Excess mortality." For this reason, influenza in older age should be recognized as a serious disease, especially for those who already have impaired health.

Influenza viruses are constantly changing, with new strains appearing regularly. If you've had influenza previously, your body has already made antibodies to fight that particular strain of the virus. If future influenza viruses are similar to those you've encountered before, either by having the disease or by getting vaccinated, those antibodies may prevent infection or lessen its severity. But antibodies against flu viruses you've encountered in the past can't protect you from new influenza strains that can be very different immunologically from what you had before. There are three types of flu viruses: A, B, and C. Type A and B cause the annual influenza epidemics that have up to 20% of the population sniffling, aching, coughing, and running high fevers. Type C also causes flu; however, type C flu symptoms are much less severe (52, 53).

The flu is linked to between 3,000 and 49,000 deaths and 200,000 hospitalizations each year in the United States. The seasonal flu vaccine was created to try to avert these epidemics. Type A flu virus is constantly changing and is generally responsible for the large flu epidemics. Type B flu may cause a less severe reaction than type A flu virus, but occasionally, type B flu can still be extremely harmful. Influenza type B viruses are not classified by subtype and do not cause pandemics. Type C flu viruses do not cause epidemics. All flu vaccines protect against three influenza viruses: one Influenza A (H3N2) virus, one influenza A (H1N1) virus, and one Influenza B virus. The avian influenza virus causes bird flu. Birds can be infected by influenza A viruses and all of its subtypes. Birds are not capable of carrying either type B or C influenza viruses (54, 55). The flu season in the Republic of Croatia is expected to start at the end of November and ends in late March with roughly an affected population of 75 000 people (Figure 1 and 2). Since it is a serious illness, vaccination is highly recommended.

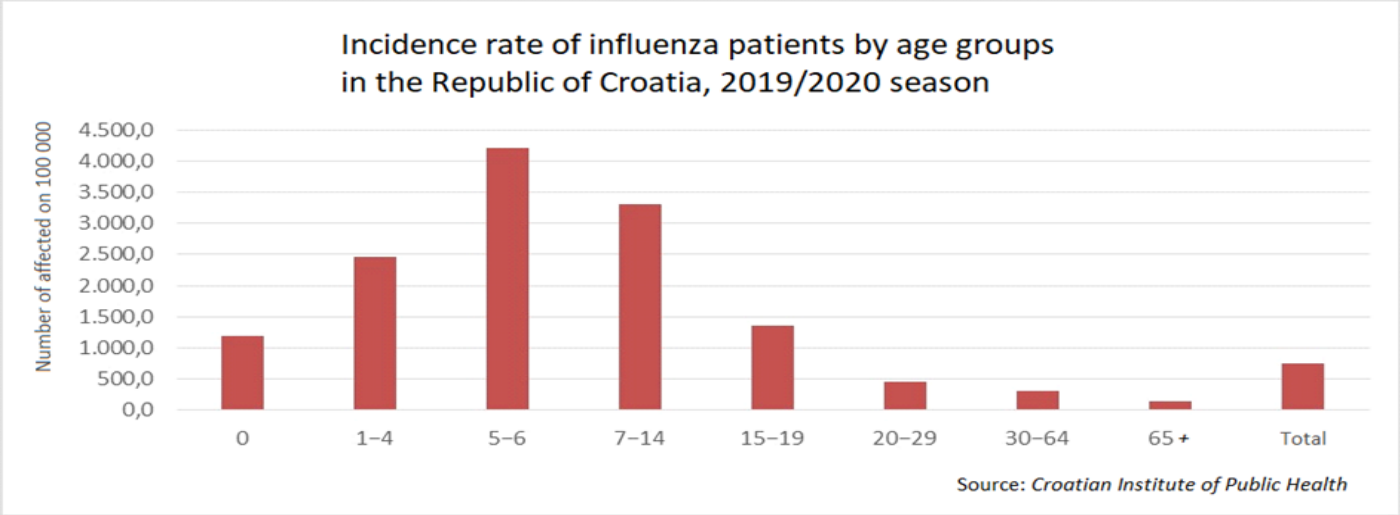

Figure 1. The incidence rate of influenza affected patients by age groups in the Republic of Croatia on 100 000 inhabitants in season 2019./2020. Available on: Croatian Institute of Public Health, https://www.hzjz.hr/; accessed in February 2020

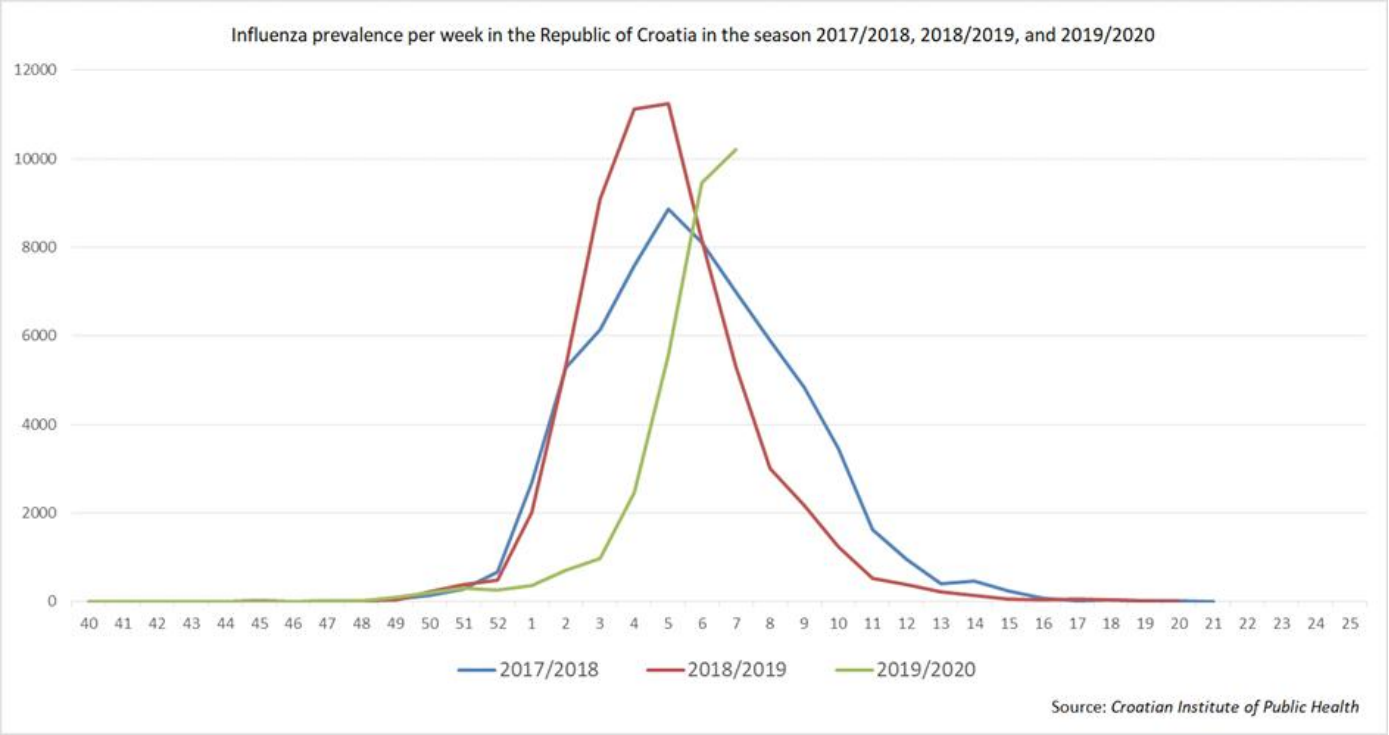

Figure 2. Influenza prevalence per week in the Republic of Croatia in the season 2017/2018, 2018/2019, and 2019/2020. Available on: Croatian Institute of Public Health, https://www.hzjz.hr/; accessed in February 2020

Speaking of respiratory viral infections, we must not forget a microorganism that has occupied the public of the whole world and has become a media and infectious phenomenon in 2019. and in 2020. year, the COVID-19 – Coronavirus disease 2019. Otherwise, it belongs to a group of viruses spread among humans as well as other mammals and birds. After infection, it can affect the respiratory, gastrointestinal, liver, and neurological systems, with consistent symptomatology. Six known types of the virus affect humans, and in general, all manifest by

general infectious symptoms resembling influenza above, and without precision diagnostic technique, it is not possible to distinguish them by the clinical picture. The COVID-19 causes milder respiratory symptoms in most cases. However, there are clear indications of complications and reported deaths (Figure 3) (56).

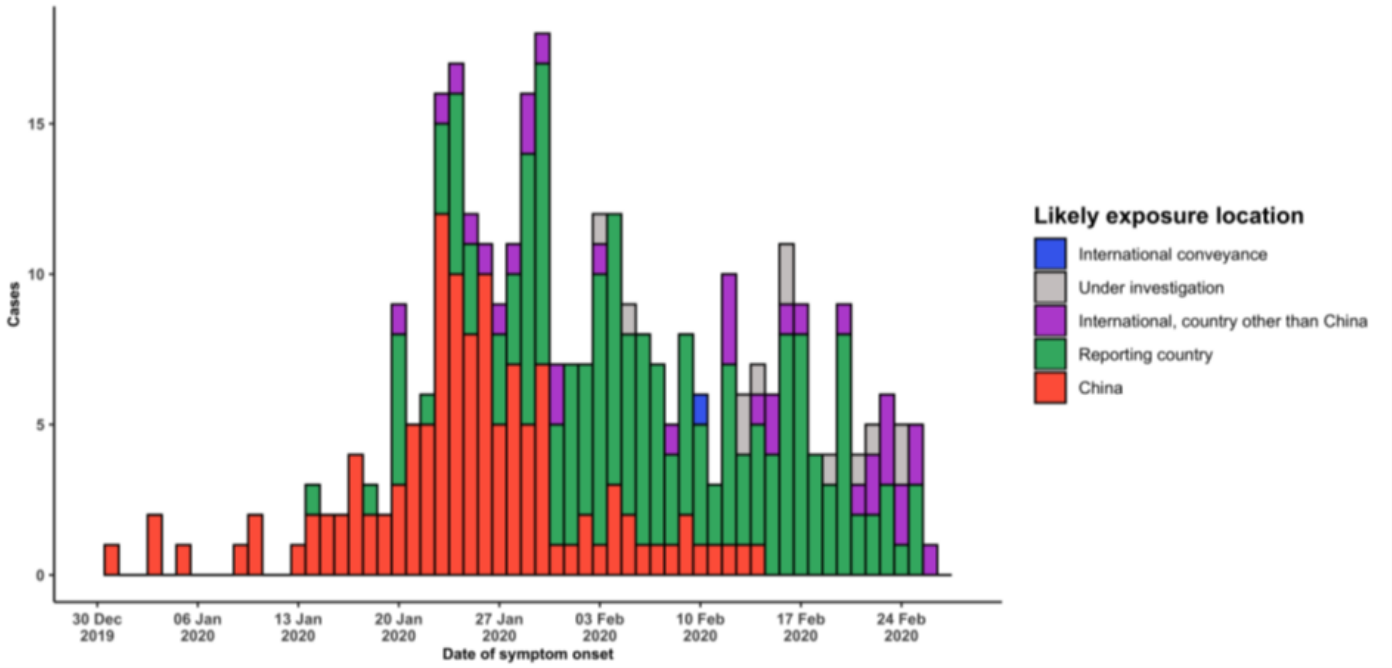

Figure 3. Epidemic curve of COVID-19 cases (n=338) identified outside of China by date of onset of symptoms and likely exposure location, February 27, 2020. Available on: https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200227-sitrep-38-covid-19.pdf?sfvrsn=9f98940c_2; accessed in February 2020

At this time, while the pandemic is still ongoing, the total number of patients is growing worldwide, with a more and more deaths as a result of complications. At the time of writing this paper number of cases in Croatia is growing (18).

Mass psychogenic illness

The significant financial and psychological problem of today's time represents mass psychogenic illness. Although attention often does not focus on this issue, it leaves significant consequences for the management of several systems starting from the health system, continuing to the educational and internal control of the functioning of states. It defines as the rapid spread of clinical signs of illness and symptoms among members of certain groups that manifests through disorders of mental state, physical functioning, and the presentation of numerous somatoform complaints that do not have a real organic basis (57). The trigger is sufficient (bad smell, bad taste, unknown sound, suspicious substance) that will convince the individual or the group that it has been exposed to a particular hazard and to experience self-suggestive symptoms of the disease. Specifically, the index case may have an organic problem, but later cases do not necessarily have to be affected by the cause. It is interesting that it affects women more often and manifests in symptoms of "phantom disease" with the onset of anxiety, social isolation, nausea, headache, abdominal pain, fainting, chest pain, weakness, and hyperventilation. Following the available literature, the phenomenon of mass hysteria is

most often associated with persons exposed to different stressors, certain structural characteristics of personality, infrequently socioeconomic status, and individuals who have historically experienced trauma or abuse. The contribution to the development of this phenomenon is media reports with inconsistent and incomplete information filled with sensational concepts, thus losing the primary objective of informing and educating the public about medical information that may consequently harm news consumers by misleading and misinforming (58). As an example of the mass psychogenic illness occurring in a girls' high school at Gopalganj in Bangladesh in April 2013, which resolves with a rapid coordinated response within the affected population (59). An example of this form of mass hysteria can also mirror infections caused by viruses. Fear of the unknown, insufficient information of the population, and the unwillingness of the entire social system can lead to the development of this phenomenon with the subsequent development of stigmatizing attitudes towards the seriously affected (18, 60).

Conclusion

Any stigmatization entails discrimination, especially if the original cause is long-lasting or incurable, which in turn harms the emotional state of the individual, which ultimately leads to self-stigmatization, deepening feelings of rejection, and aggravating the previously disturbed condition of the individual.

In modern society, the attitude towards the diseased characterizing incomparably greater humanity than it was during the history. The quality of treatment, administration more effective, and every day more advanced pharmacotherapy, as well as the more efficient healthcare system, have led to increasing awareness and social acceptance of the patient. The level of awareness of certain diseases is increasing day by day, partly due to excellent information, but also high-quality health education of the population. Patients are no longer treated as the less valuable beings, nor are they physically marked or punished. However, certain stigma in individual clinical branches persists. Perhaps as a consequence of fear or compassion for the sick, it will hardly be eradicated as such for much longer. One stamp from the past may diminish over time, but unfortunately, the future always brings some new ones.

Collective psychogenic illness has been reported in literature since medival times. Everyone has deep personal experience of panic. Epidemic hysteria is a fascinating phenomenon, one that has occured for centuries and is likely to continue to occur. During times of threat, anxious public needs to feel reassured and protected and people look to authority figures to take control and provide that reassurance. Public health agencies with planned, well coordinated, strategic approach will help reduce societal vulnerability to mass hysteria and limit the „contagiousness" of such an event.

Acknowledgement. None.

Disclosure

Funding. No specific funding was received for this study.
Competing interests. None to declare.

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Original article

Influence of Nodal Yield in Individual Neck Dissection Levels on Survival of Patients With Oral and Oropharyngeal Cancer

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Abstract

Introduction: Nodal yield (NY), or the number of collected and analysed lymph nodes in neck dissection, is one of the variables that could supplement the existing TNM classification in order to better stratify patients and their needs for further treatment. The purpose of this paper was to investigate the importance of NY in individual neck dissection levels and its relation to survival.

Materials and methods: A retrospective analysis of medical records of 133 patients regarding primary tumour excision and neck dissection from 2002 to 2013. Seventy-nine patients had a neck dissection divided by levels at the time of surgery and 54 patients had an en bloc resection.

Results: In the group of all patients, there was no correlation between NY and survival. In the group of patients who underwent a selective neck dissection, a NY above the median was an indicator of a better disease-specific survival (5-year DSS < median NY 70.6%, > median NY 95.2%, p = 0.037 log-rank test). The NY of specimens separated by level was significantly higher than the NY of specimens analysed en bloc (median 33 vs 16; p < 0.001, median test). In the group of specimens separated by level, the NY in levels I-II was not associated with survival, but a high NY in levels III-IV in selective neck dissections was an indicator of an improved overall survival (p = 0.05), disease-specific survival (p = 0.022) and disease-free survival (p = 0.05).

Conclusion: High NY in patients with specimens separated by levels could be caused by a more precise pathohistological analysis of a smaller sample. A high NY in levels III-IV can be an indicator of a well-performed selective neck dissection and sufficiently treated regional disease and therefore lead to better survival rates.

(Kvolik A, Butković J, Zubčić V, Popović Z, Leović* D. Influence of Nodal Yield in Individual Neck Dissection Levels on Survival of Patients With Oral and Oropharyngeal Cancer, Comorbidity and Chronic Therapy. SEEMEDJ 2020; 4(1); 14-24)

Received: Oct 15, 2019; revised version accepted: Jan 13, 2020; published: Apr 27, 2020

KEYWORDS: oral cancer, oropharyngeal cancer, neck dissections, lymph node excisions, disease-free survival, Kaplan-Meier estimate

Introduction

Oral and oropharyngeal squamous cell carcinoma is one of the most prevalent malignancies of the head and neck[1,2]. Its treatment has changed significantly in the last decades, after the introduction of novel chemo-/chemoradiotherapeutic protocols, as well as a result of more sparing surgical procedures. It is well-known that oral and oropharyngeal cancer first metastasises in the regional lymph nodes of the neck (Figure 1).

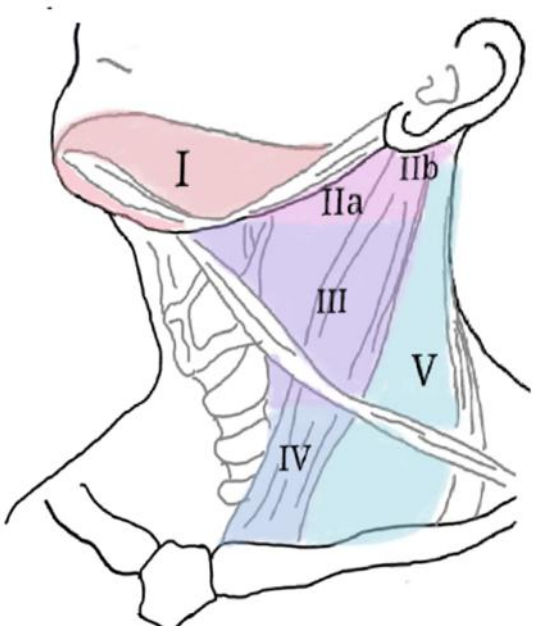

Figure 1. Levels of neck dissection

As metastases in the lower neck levels are quite rare, selective neck procedures have been developed. A sparing approach could provide a good postoperative recovery and minimal function impairment[3], but could also result in understaging of the disease due to the presence of an undetected metastasis or micrometastasis[4]. The question pertaining to the adequacy of the extent of neck dissection, especially when it comes to selective dissection, remains. Nodal yield (NY), or the total number of excised and pathohistologically examined lymph nodes, is a means of quantifying the extent of neck dissection, but it is still unclear whether it is also an independent criterion for survival. The value of nodal yield in clinical practice has been discussed in recent studies[5,6,7], but the results so far have been inconclusive. The aim of this study was to elucidate the connection between nodal yield and survival and the possibility of stratification of high-risk patients based on nodal yield.

Materials and methods

A retrospective study of the influence of nodal yield on the outcome of patients treated surgically due to oral or oropharyngeal cancer was conducted. All patients were treated at the same department of the Clinical Hospital Centre from 1 January 2002 to the end of 2013 and their tumours were classified according to the 2002 TNM staging rules[8]. The study was approved by the Institutional Ethics Committee (number R2-:22512-6/2015 Clinical Hospital Centre Osijek, Ethics Committee).

Patients' medical records and the hospital's electronic database were analysed. General inclusion criteria were the following: pathohistological diagnosis of oral or oropharyngeal squamous cell carcinoma, surgical treatment including a radical or selective neck dissection and follow-up of at least two years or until death. From 229 patients diagnosed with oral or oropharyngeal carcinoma in this period, 133 were included in the study. There were 17 patients whose neck specimens had descriptive pathohistological terms such as "several", "a few" or "conglomerates" of lymph nodes and as such, they were unfit for statistical analysis. Thirty-one patients were treated only with transoral excision without neck dissection due to the early stage of the disease and were not included in the study. The remaining 48 patients were lost to follow-up or refused a proposed therapy. Nodal yield (NY) was defined as the total number of lymph nodes analysed in a neck specimen. If a patient underwent a bilateral dissection, NY was calculated as the sum of lymph nodes on both sides divided by two. Other variables noted and used in the statistical analysis were clinical and pathohistological N status, T status, loco-

regional or distant recurrence and type of dissection. Finally, the study included only the patients with regard to whom the absolute number of lymph nodes was indicated regardless of their quantity. Inclusion criteria were fulfilled by 133 patients on whom 149 neck dissections have been performed. Among them, 118 patients received postoperative radio- and/or chemoradiotherapy, while four patients received preoperative radio- and/or chemoradiotherapy. Due to a poor general condition, medical contraindications, a prolonged postoperative recovery or early stage of the disease, 10 patients did not receive any adjuvant radio-/chemoradiotherapy.

Statistical analysis

All statistical analyses were performed for the all-patients group and subgroups divided by surgical treatment, cN status and pN status. The above-mentioned groups were dichotomised by the median (due to the irregularity of spread of NY values) and cutoff point of 18 lymph nodes according to the recent report of Ebrahimi et al., who found a minimum NY of 18 to be a marker of a well-performed elective selective neck dissection and of a patient's outcome5,9. A separate statistical analysis was additionally made only for the patients (n = 79) whose neck specimens had been divided by levels at the time of surgery. Unfortunately, there was no consistency in the manner of division between various surgeons, e.g. some surgeons divided the upper neck levels (I and II) and lower neck levels (III and IV) as one sample, whilst others separated each level. In order to uniform our data, we presented the results regarding nodal yield in the regions of the upper neck and lower neck, while level V, present only in radical dissections, was analysed separately. We are aware that running multiple statistical analyses on a small data sample increases the probability of a chance finding, which is one of the major limitations of this study. However, it is our opinion that dividing patients into smaller groups was necessary in order to get the data that accurately portrays each specific group of patients.

The statistical analysis was performed using the SPSS 22.0 statistical software (SPSS Inc., Chicago, IL, USA). Two-year and five-year overall survival (OS), disease-free survival (DFS) and disease-specific survival (DSS) were calculated using the Kaplan-Meier survival method (log-rank, Breslow and Tarone-Ware tests). OS was calculated as the time from the treatment to the last follow-up or death, DFS as the time from the treatment to the recurrence of carcinoma, either locoregional or as a metastasis, and DSS as the time from treatment to death due to oral or oropharyngeal SCC. A univariate analysis using the Pearson's chi-square test was made for OS, DFS and DSS. For the data with expected cell frequencies ≤ 5, the Fisher's exact test was performed. Medians were compared with the median test. Level of significance was defined as $p < 0.05$ and all statistical tests that were used in the calculations were two-sided.

Results

From January 2002 to December 2013, 133 patients who were treated for oral or oropharyngeal cancer at the Clinical Hospital Centre Osijek met the inclusion criteria, of whom 122 (91.7%) were male and 11 (8.3%) were female. The mean age was 57.8 ± 8.6 years. The data regarding localisation, stage and treatment of the tumours is shown in Table 1 and Figure 2..

Table 1. Demographical data regarding the study population

		n	%
Tumour site	Oral	89	66.9
	Oropharyngeal	44	33.1
Localisation	Tongue	45	33.8
	Sublingual	25	18.8
	Tonsils	20	15.0
	Tongue base	18	13.5
	Mandibular gingiva	5	3.8
	Uvula	7	5.3
	Retromolar	8	6.0
	Oropharyngeal wall	5	3.8
T	T1	8	6.0
	T2	49	36.8
	T3	42	31.6
	T4	34	25.6
N	N0	54	40.6
	N1	40	30.1
	N2a	10	7.5
	N2b, N2c	25	18.8
	N3	4	3.0
Neck dissection	Selective	38	28.6
	Radical	95	71.4
Chemoradiotherapy	Postoperative	118	88.7
	Preoperative	5	3.8
	No treatment	10	7.5

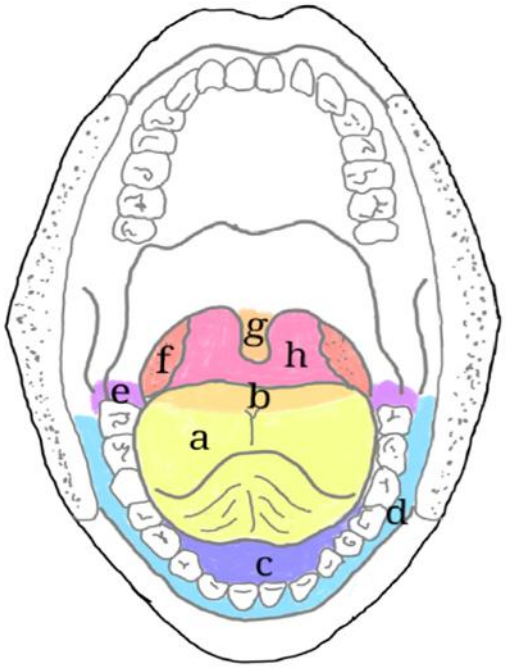

Figure 2. Localisation of the primary tumour. a) tongue b) tongue base c) mouth floor d) mandibular gingiva e) retromolar f) tonsils g) uvula h) oropharyngeal wall

Neck dissection and nodal yield

From 133 patients, 16 had a bilateral neck dissection, yielding a total of 149 dissections in this study. Median nodal yield for the entire study population was 25, with the lowest nodal yield being 4 and the highest being 75. Thirty-eight patients underwent a selective neck dissection, while 95 had a radical neck dissection. Median nodal yield was 25 for radical and 29 for selective neck dissection. There were 54 patients with the cN0 stage and 79 patients with cN+ neck specimens. In the cN0 group, the median nodal yield was 22 lymph nodes, and in the cN+ group, the median NY was 28 lymph nodes, which could have been a result of more sparing neck dissections being performed on patients with the clinically negative neck. We also analysed the patients according to the pathohistological N status. There were 32 patients whose cN and pN status did not match. Forty-six patients had the pN status with a median NY of 29, while 87 patients had the pN+ status with a median NY of 24. Nodal yield for each group can be found in Table 2..

Table 2. Nodal yield in each group or dissection level

Group (n)	Median NY
Overall (133)	25
Selective neck dissection (38)	29
Radical neck dissection (95)	25
cN0 (54)	22
cN+ (79)	28
pN0 (46)	29
pN+ (87)	24
Specimens en bloc (54)	16
Specimens separated by level (79)	33
Level I-II (79)	14
Level III-IV (79)	9
Level V (57)	8

Nodal yield and separation of neck specimens by levels

In 79 patients, the neck specimen was separated by levels at the time of surgery by two experienced surgeons. In this group, the median nodal yield was 33, while the group with en bloc resections had a median nodal yield of 16. The difference was statistically significant ($p < 0.001$, median test). See Table 2.

Survival analysis

Median survival for the entire group of patients was 33 (17-67) months after the surgery. In the first two years after the surgery, overall survival rate was 61.1% for patients with a NY below the median and 63.8% for patients above the median ($p = 0.298$; log-rank test). Disease-specific survival (the Kaplan-Meier curve in Figure 3) showed similar results ($p = 0,103$; log-rank test).

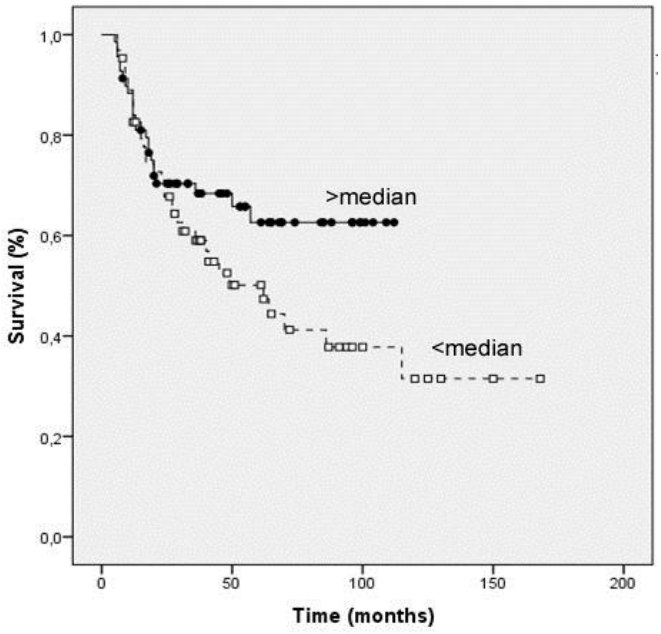

Figure 3. Kaplan-Meier curves of disease-specific survival in the entire study population (N = 133); 2-year survival for NY > median group (n = 69) was 70.3%, and for NY < median group (n = 64) 67.7% (p = 0.103 log-rank test, p = 0.334 Breslow test, p = 0.206 Tarone-Ware test)

For the purposes of survival analysis, we dichotomised all groups according to the nodal yield above and below the median and above and below 18 lymph nodes. There was no difference in survival below and above the cutoff point of 18 lymph nodes in either selective or radical neck dissection.

In the group of selective neck dissections (N = 38), the patients with a NY above the median had a statistically better disease-specific survival as opposed to those with a NY below the median (5-year DSS < median NY 70.6%, > median NY 95.2%, p = 0.037 log-rank test), as seen in Figure 4.

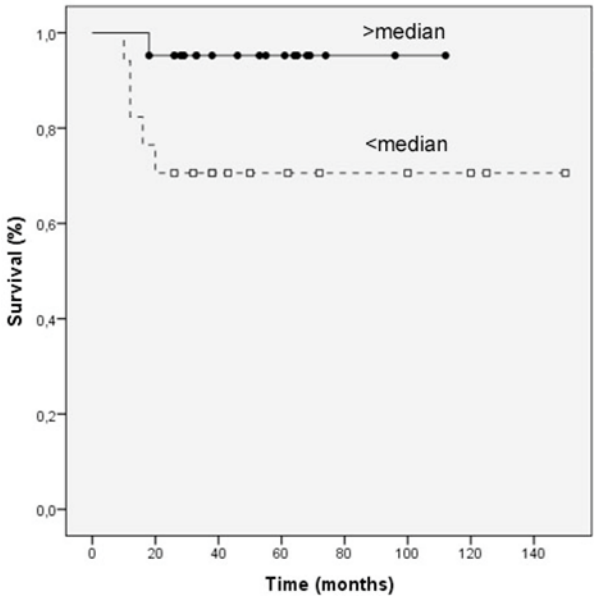

Figure 4. Kaplan-Meier curves of disease specific survival for selective neck dissections; 2-year survival for NY > median group (n = 21) was 95.2%, and for NY < median group (n = 17) 70.6% (p = 0.037, log-rank test, p = 0.035 Breslow test, p = 0.036 Tarone-Ware test)

However, in the group of radical neck dissections (N = 95), the Kaplan-Meier estimates did not confirm a statistical significance of nodal yield as an independent predictor of survival (5-year DSS < median NY 66.6%, > median NY 69.3%, p = 0.459).

We found no statistical difference of survival of patients with a higher and lower NY by dividing the patients into the cN- and cN+ group in any survival study, but a statistical significance was found when we analysed the pN- group. In patients with pathohistologically negative neck specimens, DSS was statistically higher in those patients whose NY was above the median (5-year DSS < median NY 38.4%, > median NY 67.4%, p = 0.045), while DFS was leaning towards a statistical significance (p = 0.057).

NY by neck level and survival

Median nodal yield was 14 lymph nodes in the upper neck (levels I and II) and 9 lymph nodes in the lower neck (levels III and IV). Level V, present only in radical dissections, yielded an average of 8 lymph nodes. We reviewed the upper neck NY separately for cN-, cN+, pN- and pN+ groups, selective dissection, radical dissection and the entire group, but no statistical correlation between survival and NY was found, only a tendency (p = 0.072) for a slightly better disease-specific survival of those with a NY above the median in the cN- group.

However, NY had interesting properties pertaining to the lower neck. In the group of selective dissections (n = 22), a NY above the median was related with a better OS (p = 0.05), DSS (p = 0.022), and DFS (p = 0.05). With regard to the radical neck dissection group (n = 57), findings were quite different. Interestingly, we found worse outcomes in patients with a NY above the median as compared to a NY below the median (OS p = 0.009, DSS p = 0.014, DFS p = 0.007). Kaplan-Meier plots can be seen in Figure 5.

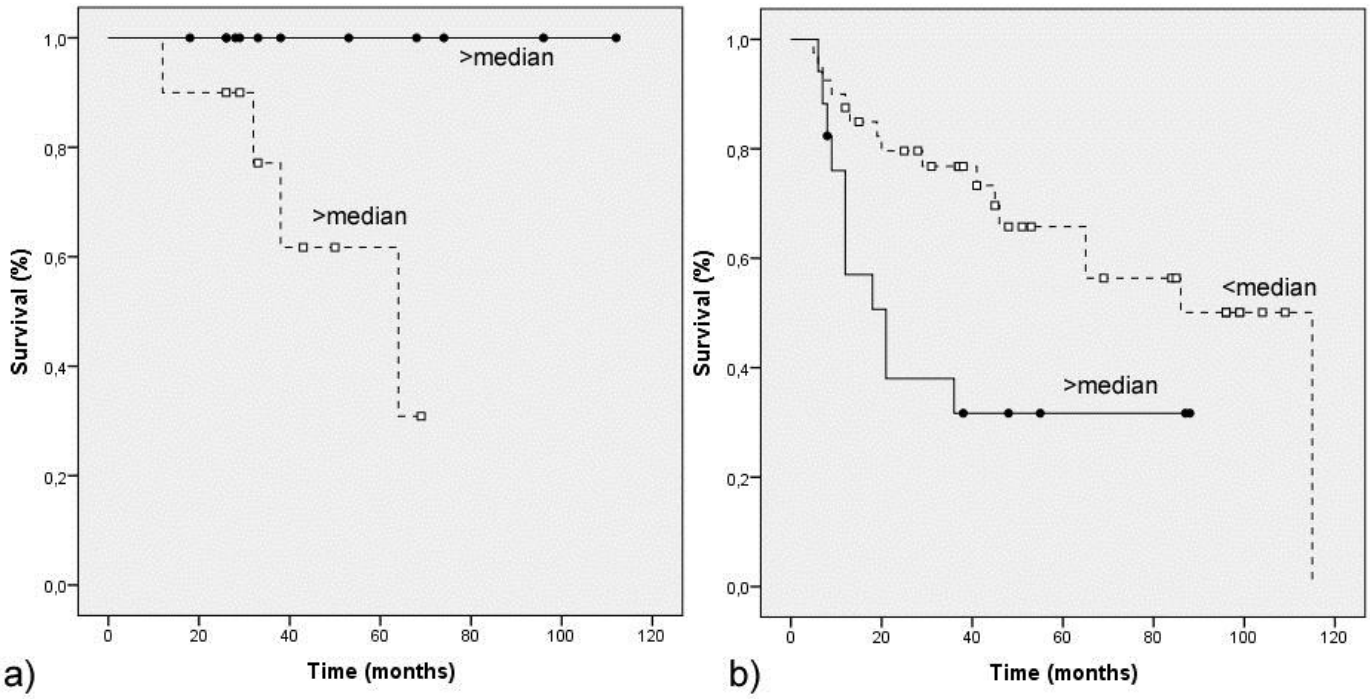

Figure 5. Kaplan-Meier curves of disease-specific survival for NY in lower neck region (3 and 4) in a) selective neck dissections; 2-year survival for > median group (n = 12) was 100%, and for the < median group (n = 10) 30.9% (p = 0.022 log-rank test, p = 0.044 Breslow test, p = 0.029 Tarone-Ware test), and

b) radical neck dissections; 2-year survival for > median group (n = 17) 38.0% and for < median group (n = 40) 56.8% (p = 0.014, log-rank test, p = 0.006 Breslow test, p = 0.007 Tarone-Ware test)

With regard to level V, the results varied, with the lowest nodal yield being 0 and the highest being 27, which was not statistically significant for survival in any group of patients.

Discussion

Even though the gold standard treatment for oral and oropharyngeal carcinoma includes a resection of the primary tumour followed by a neck dissection, the consensus pertaining to the width of the neck dissection has not been reached since its introduction in 1906. Squamous cell carcinoma usually metastasises in the upper neck levels (I, II, III), so the procedures have been shifting to a more sparing and selective neck dissection in order to preserve the function and cause minimal impairment to the patient. Since the introduction of adjuvant chemo- or chemoradiotherapy, surgical treatment options have become even more selective. However, neck dissection is not only vital for treatment, but also for correct staging. Consequently, an over-selective neck dissection could lead to understaging and loco-regional recurrence due to missed lymph nodes in the lower levels or due to micrometastases. The problem of the occult neck disease has been widely studied by Woolgar and Cho et al., who claim that comprehensive neck dissection is an important factor for finding micrometastases in lymph nodes of the neck that cannot be discovered by a routine pathohistological analysis[4,10]. This raises the issue of re-evaluating the margins of a well-performed neck dissection and determining how many levels are enough to ensure the best survival, the least regional recurrence rate and minimal impairment.

Recently, nodal yield has been proposed as a supplement to the standardised TNM classification, as well as a means of quantification of a performed dissection. Even though neck dissection is a standardised procedure, nodal yield in each neck dissection varies dramatically. This could be caused by its

dependence on the three following criteria: width of the dissection, the level of pathologists' scrutiny and individual differences in lymphatic tissue. Patel et al. reported a nodal yield of 2-104 (a mean of 39 ± 23) nodes[11], Ali et al. 7-140 (a mean of 42) nodes[12] and Ebrahimi reported a mean NY of 25.59, 276 and 305 in different studies. The median NY of 25 found in this study does not vary significantly compared to other studies. Nodal yield depends even on the type of surgical technique. Thus, Lörincz et al. [13] proved that a standardised horizontal dissection of the cervical fascia yields more lymph nodes than a caudal-to-cranial dissection. It is noteworthy to add that all our neck dissections have been performed in a standardised horizontal manner and included level I, both for oral and oropharyngeal cancer.

Several studies have tried to analyse NY and its impact on survival, but the results have so far been indecisive. Ebrahimi et al. [9] showed that a nodal yield above 18 is an independent prognostic factor for patients undergoing a selective neck dissection for cN0 oral squamous cell carcinoma. As this study population included patients undergoing both a radical and a selective neck dissection, in all N stages of the disease, we divided the patients into multiple groups in order to homogenise the sample. We found no statistically significant difference in survival of any group of patients with a cut-off point of 18, which could be explained by a higher T and N status at the beginning of the treatment, but also as an inter-institutional difference of overall NY between studies. Lemieux studied cN- patients, divided NY into quartiles and found an improved outcome in two higher quartiles (NY > 22)14. Our study used the median of each group as a cut-off point and showed similar results as Lemieux et al.[14] in the pN- group with a NY above the median having a better disease specific survival as compared to those with a NY below the median. These findings could support the theory that a higher NY increases the likelihood of finding neck metastases and allows for adequate clearance of occult metastases.

Nodal yield is also dependent on the manner of presenting the specimen to the pathologist. In a traditional radical neck dissection, en bloc specimen with non-lymphatic tissue such as the sternocleidomastoid muscle and jugular vein would be presented to the pathologist who was charged with dissecting the specimen by level and analysing it. This procedure was quite imprecise as the tissue of the sample is prone to shrinkage and the identification of level borders was often inaccurate, but this practice became even more imprecise with the introduction of selective neck dissections[15,16]. In selective neck dissection, samples did not have any anatomical structure that could be used for orientation. A correct analysis of each level is an important factor that provides information about the course of future treatment[17]. Dividing specimens by levels at the time of surgery is, therefore, the key to better and accurate staging; it reduces the manipulation of the tumour and tumour spillage. Smaller, more manageable samples could allow the pathologist to find and analyse more lymph nodes and to detect micrometastases if they are present[15,16]. This was confirmed by our data, with a much lower median NY of 19 in en bloc specimens, as compared with a median NY of 35 in specimens divided by levels. However, not all studies have come to the same conclusion; Kerawala[17] et al. found no significant difference between en bloc and divided specimens, while Marres et al.[18] noticed an increase in NY when specimens were examined by a pathological technician as compared to an examination by a pathologist.

To the best of our knowledge, no one has studied the importance of nodal yield in relation with survival on each level. Kerawala et al. [17] reported a median NY in each level as follows: I-3, II-9, III-7, IV-5 and V-9, while Norling et al.[19] studied nodal yield in cadavers as compared to other reports from literature. Both studies showed a notoriously wide range of nodal yield in each level (varying from 0 up to > 20). Our data, therefore, does not vary greatly from other reports. As described in the Materials and methods section, we presented nodal yield separately for the upper neck (levels I and II) and the lower neck (levels III and IV). The data regarding nodal yield in the lower neck levels

was particularly perplexing, especially regarding radical neck dissections having a lower NY than selective neck dissections. This difference might be caused by an imprecise separation, which led to lymph nodes from levels III and IV to end in level V specimens. It remains unclear why radical neck dissection overall had a slightly lower NY than selective dissection, but this could be due to differences in the number of samples (94 radical neck dissections as compared to 38 selective neck dissections).

Skip metastases, or metastases present only in the lower neck levels without involvement of levels I and II, remain a controversial topic and one of the arguments for a radical surgical treatment. While Feng et al.[20] report a rate of 1.1% for skip metastasis in level III and 3.2% in level IV, they concluded that supraomohyoid dissection does provide adequate care for patients with oral SCC. Khafif et al.[21] also argued that dissection of level IV is necessary only when there is intraoperative suspicion of metastatic involvement in levels II and III. Dias et al.[22] reported only 2% of skip metastasis, but 24.1% of occult neck metastases. It is, therefore, very important to correctly weigh the need to perform a selective or radical neck dissection. In this study, nodal yield in the upper neck showed no statistical significance pertaining to survival. This could be caused by easy surgical access and relatively uniform clearance of this level. A high nodal yield in the lower neck, however, proved to be an important predictor of outcome for node-negative and node-positive neck specimens treated by selective neck dissections. These findings are in consistence with other studies in which the positivity of the lower neck regions was found to be a predictor of a worse outcome[23] and could mean that by dissecting level IV, skip metastases, as well as occult metastases, have been cleared. The group of patients undergoing radical neck dissections showed quite an opposite result, where patients with a higher NY had a statistically worse outcome. Patients undergoing a radical neck dissection belonged to a group of a higher T and N stage. Therefore, it is logical that higher NY dissections would reveal more positive lymph nodes, which negatively affects the outcome. It is rather difficult to compare selective and radical neck dissections because of various other factors that affect a patient's prognosis, such as a more advanced disease, higher risk of perioperative complications and locoregional or distant failure, which are not directly linked with nodal yield. It is also important to evaluate the role of all healthcare providers who participated in the treatment of our patients, such as pathologists, whose impact on NY could not be assessed in detail in this study. However, the number of patients in whom neck specimens were divided into levels and who could partake in the statistical analysis was too small to make any definitive conclusions, but the conclusions that could be made raise some interesting questions and deserve further investigations in a larger cohort study.

Conclusion

Our data showed improved outcomes of well-treated, high NY patients as compared with those with a lower NY. This could indicate the need to review the strategies of surgical approach to the neck metastasis, which has recently shifted towards a more selective approach. A better outcome in patients with a higher NY in the lower neck, even in the pN-group, could mean a higher probability of clearance of occult metastases, which have been linked to a worse outcome[10]. Even so, our data on the importance of NY in the lower neck must be treated with caution because of a small sample, but it could indicate a trend that should be studied in more detail in a larger study.

Acknowledgement. None.

Disclosure. This research did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sectors. This study was conceived as the final diploma work of the first author (AK) and was presented at graduation. Preliminary results were presented at the 12th Congress of Croatian Society of Maxillofacial, Plastic and

Reconstructive Surgery of the Head and Neck; 2-4 June 2016, Osijek, Croatia

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Original article

Survival of Multiple Myeloma Patients Undergoing Dialysis or Plasma Exchange - A Single Centre's 25-Year Experience

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Abstract

Aim: Multiple myeloma (MM) patients might require haemodialysis (HD) and/or plasma exchange (PE) in cases of acute kidney injury (AKI) and/or chronic kidney disease (CKD) and/or other indications. The study analysed the survival of MM patients who required HD and/or PE.

Subjects and methods: All 144 patients treated for MM at the University Hospital Centre Osijek between 1994 and 2018 (of whom 47.9 % were men) were included in the study. Data were collected from medical records. MedCalc Statistical Software version 17.8.2 was used for the statistical analysis, with significance set at α = 0.05.

Results: Forty-three of 144 MM patients (29.9 %) were treated with HD and/or PE. Male patients required HD or PE more often than female patients (62.8 % vs 37.2 %, P = 0.02). Patients who did not require HD or PE were significantly older at the time of their death than the patients treated with HD or PE [75 (interquartile range, IQR, 72 – 77) vs 72 (IQR 66 – 75) years; P = 0.009, Mann-Whitney test]. Among all patients who required acute or chronic HD, PE or a combination of the treatments, the longest life span was found in 17 patients who were treated with chronic HD (median 12 months, IQR 8 – 58).

Conclusion: Kidney failure requiring HD or PE in MM was associated with a significantly shorter life span in comparison with other MM patients. Chronic HD patients had the longest survival among patients who required acute or chronic HD, PE or a combination of the treatments. In general, MM patients in need for HD and/or PE had poor survival.

(Smajić* P, Schönberger E, Periša V, Sinčić Petričević J, Zibar L, Kralik K. Survival of Multiple Myeloma Patients Undergoing Dialysis or Plasma Exchange - A Single Centre's 25-Year Experience. SEEMEDJ 2020; 4(1); 25-31)

Received: Feb 19, 2020; revised version accepted: Mar 18, 2020; published: Apr 27, 2020

KEYWORDS: multiple myeloma, kidney failure, haemodialysis, plasma exchange, survival

Introduction

Multiple myeloma (MM) represents malignant plasma cell proliferation. Tumours, their products and the reactions of the host that they produce lead to a number of dysfunctional organ conditions and symptoms, such as bone-related pain or fractures, kidney failure, susceptibility to infections, anaemia, hypercalcemia and sometimes clotting irregularities, neurological symptoms and symptoms of hyperviscosity (1).

Kidney failure occurs in nearly 25 % of the patients, while in more than half of MM patients, there is some kidney pathology. Hypercalcemia, deposition of amyloid in glomeruli, hyperuricemia, recurrent infections and kidney infiltration with tumour cells contribute to kidney disorders (1). In most cases, kidney damage is associated with immunoglobulin (Ig) and it includes the following conditions: light chain cast nephropathy (LCCN), also known as myeloma kidney, monoclonal immunoglobulin deposit disease and light chain amyloidosis (2). An increased quantity of light chains overloads tubular cells, which causes tubular damage, resulting in direct toxic effects of the light chain or indirect release of cell lysosomal enzymes. It can also cause Fanconi syndrome by the formation of crystalline inclusions in the proximal tubules, resulting in a high loss of glucose and amino acids as well as irregularities of acidification and urea concentration (1, 2). Plasma exchange (PE) has been suggested as a treatment option for LCCN in order to prevent kidney failure. Some retrospective studies have shown that PE can prevent acute kidney injury (AKI), which can verge into chronic kidney disease (CKD) and the need for dialysis (3), while other studies have considered PE as a treatment that does not have any effect on overall survival or need for haemodialysis (2, 4). Other treatment options for patients with MM and renal failure include bortezomib-based therapies or the use of high cut-off dialysis filters to remove free light chains (5). The administration of peritoneal dialysis and kidney transplant in MM patients with kidney failure are controversial treatment procedures because of their low rate of survival and poor tolerance of therapy (6). At the University Hospital Centre Osijek, a proportion of MM patients were treated with acute or chronic HD, PE or a combination of the treatments. The focus of the study was to analyse the outcome in MM patients who required HD or PE.

Subjects and methods

The retrospective study included all 144 patients treated at the University Hospital Centre Osijek for MM during the period between 1994 and 2018. Available patients' data were collected from medical records at the Department of Hematology, the Department of Nephrology and from the register of deaths at the Clinical Department of Pathology and Forensic Medicine of the University Hospital Centre Osijek. The study was conducted between June 2017 and February 2018. MM diagnosis was confirmed if the bone marrow analysis showed plasmacytosis (> 30 %) or if plasmacytosis > 10 % was accompanied by monoclonal paraprotein in serum and/or urine and osteolytic lesions or light chains in serum were present. Criteria for the start of HD treatment were AKI or clinical and laboratory signs of CKD that required substitution of renal function. PE was performed for hyperviscosity to remove the light chains.

Statistical methods

Category data were represented by absolute and relative frequencies. The numerical data were described as median, minimum, maximum and interquartile range (IQR). Differences in nominal variables were tested by Fisher's exact test. Differences in numeric variables, due to deviations from normal distribution, were tested by Mann-Whitney's U Test. All P values were double-sided. The level of significance was set at $\alpha = 0.05$. MedCalc Statistical Software version 17.8.2 was used for the statistical analysis (MedCalc Software bvba, Ostend, Belgium; http://www.medcalc.org; 2017).

Results

The study included 144 MM patients, 43 (29.9 %) of whom required HD or PE. Male patients needed HD or PE significantly more often (27 of 69, 62.8 %) than female patients (16 of 75, 37.2 %), P = 0.02 (Fisher's exact test).

Up to the time of the study, 95 MM patients died (66 %), and at a significantly higher rate if a patient needed HD or PE (34 of 43, 79.1 %) than those who were not candidates for the treatments (61 of 101, 60.4 %), P = 0.03 (Fisher's exact test) (Table 1).

Table 1. Characteristics of patients (N = 144) with multiple myeloma (MM) with regard to haemodialysis (HD) or plasma exchange (PE) treatment and overall survival at the time of study

	MM patients who required HD or PE treatment (n = 43, 29.9 %)	Number of (%) patients MM patients who did not required HD or PE treatment (n = 101, 70.1 %)	Total	*P**
Gender				
Male	27 (62.8)	42 (41.6)	69 (47.9)	**0.02**
Female	16 (37.2)	59 (58.4)	75 (52.1)	
Overall survival				
Alive	9 (20.9)	40 (39.6)	49 (34)	**0.03**
Deceased	34 (79.1)	61 (60.4)	95 (66)	
Total	43 (100)	101 (100)	144 (100)	

*Fisher's exact test

Among 43 MM patients who required HD or PE treatment, 12 (28 %) were treated only with acute HD, 11 (26 %) were treated only with chronic HD and 8 (19 %) only with PE. Seven (16 %) MM patients were treated with both acute HD and PE, 1 (2 %) MM patient was treated with both chronic HD and PE and 1 (2 %) with both acute and chronic HD. Finally, there were 3 (7 %) MM patients treated with acute HD, chronic HD and PE (Table 2)..

Table 2. Distribution of patients according to the type of treatment - acute or chronic haemodialysis (AHD or CHD) or plasma exchange (PE) (N = 43)

Treatment	n	%
AHD	12	28
CHD	11	26
PE	8	19
AHD+PE	7	16
CHD+PE	1	2
AHD+CHD	1	2
AHD+CHD+PE	3	7
Total	43	100

Among 43 (29.9 %) MM patients who required HD or PE treatment, a total of 16 (37.2 %) patients were treated with chronic HD, including those treated with acute HD or PE in combination with chronic HD. Their median survival time was 11 months (IQR 8 – 58). A total of 19 (44.2 %) MM patients were treated with acute HD, including those treated with chronic HD or PE in combination with acute HD and their median survival time was 2 months (IQR 1 – 5). Eight (18.6 %) MM patients were treated only with PE and their median survival time was 1 month (IQR 0 – 20). There was a significant difference in survival time among the examined groups (P = 0.04) (Figure 1)..

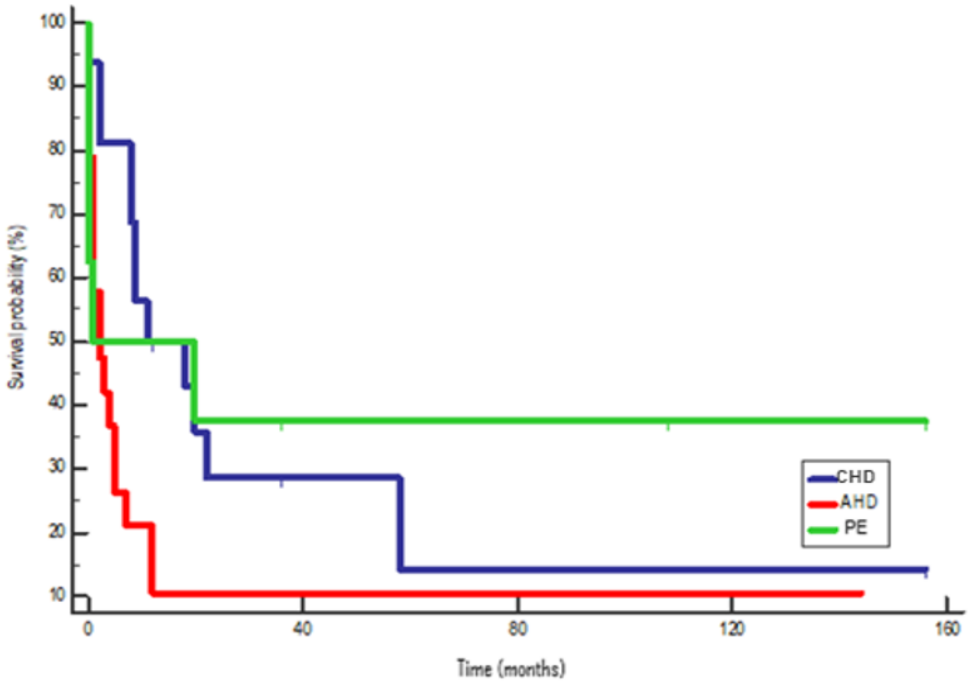

Figure 1. Kaplan- Meier plot of survival time (months) between the groups of patients who required haemodialysis (HD) or plasma exchange (PE) treatment. CHD includes multiple myeloma (MM) patients treated with chronic HD, including those treated with acute HD or PE in combination with chronic HD. Their median survival time was 11 months (IQR 8 – 58). AHD includes MM patients who were treated only with acute HD and those treated with a combination of acute HD and PE. Their median survival time was 2 months (IQR 1 – 5). PE encompasses MM patients who were treated only with PE and their median survival time was 1 month (IQR 0 – 20). There was a significant difference in survival time among the examined groups (P = 0.04).

Survival time was calculated from the start of the treatment until the patient's death.

A total of 35 (81.4 %) MM patients were treated with HD, either only HD or in combination with PE, and their median survival time was 7 months (IQR 2 – 12). There were 8 (18.6 %) MM patients treated only with PE and their median survival time was 1 month (IQR 0 – 20). There was no significant difference in survival time between those who were treated with HD (including patients treated only with HD or with a combination of HD and PE) and those treated only with PE (P = 0.39) (Figure 2)..

P

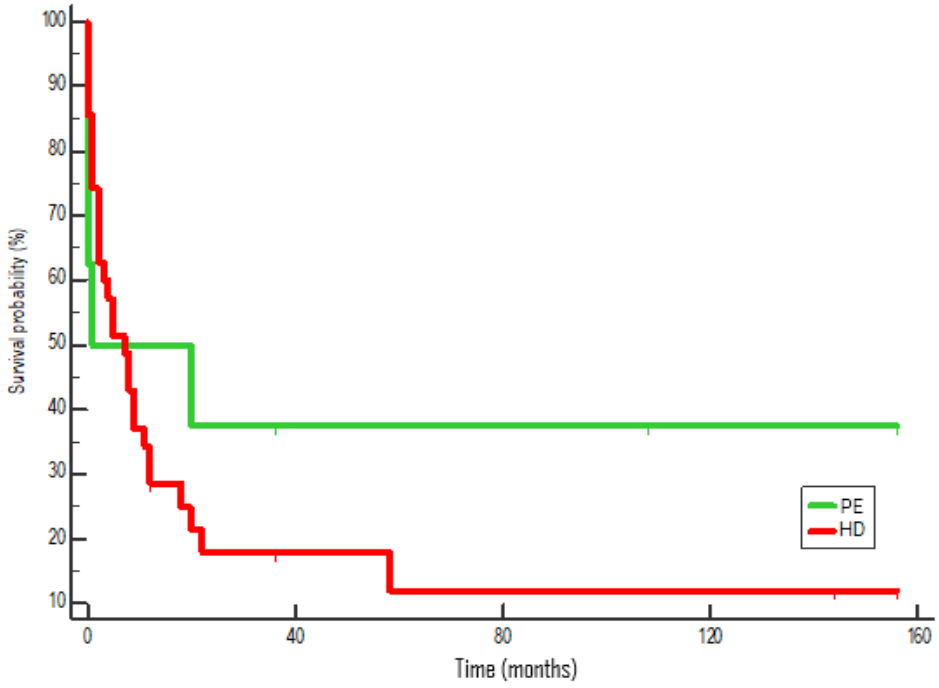

Figure 2. Kaplan-Meier plot of survival (months) between the groups of patients who were treated only with plasma exchange (PE) and those treated with haemodialysis (HD) (includes patients treated only with HD or with a combination of HD and PE). The median survival time of multiple myeloma (MM) patients treated with PE was 1 month (IQR 0 – 20). The median survival time of the other group was 7 months (IQR 2 – 12). There was no significant difference in survival time between these two groups (P = 0.39).

Discussion

Impairment of kidney function in MM patients requiring dialysis or PE was associated with a shorter life span than in MM patients without such treatments. Patients who had no need for treatment with HD or PE were older at the time of death than the patients who needed such treatment, as we had assumed. The need for dialysis or PE was accompanied by a poor outcome in terms of life span in general. At the moment of our research, the outcome of the treatment was negative (death of the patient) in 95 (66 %) cases; significantly, 34 (79.1 %) of these patients needed PE and/or HD.

CKD often appears in MM patients. Despite aggressive treatment, 65 % of MM patients with kidney damage will reach the end-stage renal disease within three months of their MM diagnosis (7). Studies have shown that mortality and morbidity are more common among patients who need renal replacement therapy (RRT) than among those with regular kidney function (8). These findings correspond with our results.

Some research has demonstrated that patients with MM and impairment of kidney function can be treated either with HD or PE and that these procedures are equally effective (9). On the other hand, one retrospective study suggested that PE might be helpful in preventing the initiation or continuation of dialysis in patients with rapidly progressive renal failure which occurred due to MM (10). More recent studies have suggested that PE in combination with chemotherapy, especially bortezomib, leads to renal improvement (11, 12). In this research, among the patients who needed HD or PE, the 17 patients who were treated with chronic HD lived the longest. Studies conducted at different centres found contrasting results. Therefore, in the future, research on this issue should be conducted on a larger number of subjects to get the most accurate results.

Our research was a retrospective epidemiologic study. It should be noted that there were some limitations of this study due to data scarcity for the group without HD or PE treatment, which can account for some of the differences between our research and studies conducted by other researchers. There were missing facts about the time when the diagnoses of MM were confirmed, stage and type of MM, presence of kidney failure in those without non-conservative treatment, presence of other complications and methods of pharmacological treatment. However, the results of our research are important in analysing the outcomes of treatment at one centre and they show the negative association of kidney failure as well as other methods of treatment with non-conservative procedures on lifespan and death outcome. It is also important to emphasize that there has been no similar research published by other centres.

Conclusion

Our study has shown that the requirement of chronic HD is related to a better outcome than that of acute HD or PE when it comes to length of life.

Acknowledgement. None.

Disclosure

Funding. No specific funding was received for this study.

Competing interests. None to declare.

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Original article

Levels of LDL Cholesterol, Triglyceride and Urate in Patients With Type 2 Diabetes Mellitus

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Abstract

Aim: The study aimed to examine LDL cholesterol, triglyceride and urate levels in patients of both sexes with type 2 diabetes mellitus (DM2T) in family medicine offices and to examine whether there is a difference in these parameters between obese patients and patients with normal body weight with DM2T, and between patients with and without manifest cardiovascular diseases.

Participants and methods: The study was organized as a cross-sectional study. It included 136 participants of both sexes diagnosed with DM2T, who were divided into groups of patients with or without adiposity and into groups of patients with or without experience of cardiovascular event. General and demographic data were collected, as well as data on experiencing cardiovascular events and levels of LDL cholesterol, triglycerides and urates.

Results: The average LDL cholesterol level was 2.93 mmol/L, the average triglyceride level was 1.65 mmol/L and the average urate level was 326.36 μmol/L. Only 12.5% of participants reached target LDL cholesterol levels, while levels of triglycerides and urates were within recommended limits. 24.3% of participants had experienced cardiovascular events and 39.7% of participants were obese. There was no significant difference in levels of LDL cholesterol, triglycerides and urates in participants who had experienced a cardiovascular event and those who had not. There was a significantly higher concentration of triglycerides in obese patients than in patients with normal body weight ($p = 0.005$).

Conclusion: In addition to regulation of glycaemia in patients with DM2T, statin doses should be increased in order to reach the target levels of LDL cholesterol. When it comes to obese patients, education courses on physical activity and diet should be conducted more often and, if necessary, fibrates should be included in therapy in order to reduce additional cardiovascular risks.

(Šojat* D, Pirić M, Klarić M, Šapina M, Popović Z, Bačun T. Levels of LDL Cholesterol, Triglyceride and Urate in Patients With Type 2 Diabetes Mellitus. SEEMEDJ 2020; 4(1); 32-39)

Received: Mar 1, 2020; revised version accepted: Apr 2, 2020; published: Apr 27, 2020

KEYWORDS: type 2 diabetes, LDL cholesterol, triglycerides, urates

Introduction

Diabetes mellitus is a chronic and progressive disease. Nowadays it is one of the most important public health problems and for many years it has been among the top ten causes of death in the Republic of Croatia (1). The International Diabetes Federation estimates that more than 420 million people worldwide suffer from diabetes, and it is predicted that in the next 25 years, this number will reach 630 million (2). The number has increased every year in the Republic of Croatia and persons diagnosed with diabetes, currently counting over 260,000 people, are considered to be only half of the actual total number of patients (3). Despite the good-quality and affordable healthcare in the Republic of Croatia, diabetes is often detected or treatment starts in an advanced stage, which affects both the quality and the cost of diabetes treatment. Croatian Health Insurance Fund data for 2016 showed that the total cost of treating diabetes in the Republic of Croatia amounted to HRK 4.6 billion, accounting for almost 20% of the total Croatian Health Insurance Fund budget (4).

Pathophysiology of diabetes is complex and it includes the interaction of genetic and environmental factors (1). Modern, sedentary lifestyle and excess energy intake undoubtedly lead to a state of overweight and obesity, where, due to the increase of visceral fat tissue, insulin resistance occurs; this is, in addition to beta-cell dysfunction and impaired insulin secretion, a major pathophysiological disorder for development of diabetes, along with many other conditions, such as increased gluconeogenesis, increased glucagon secretion in pancreatic α-cells, decreased incretin effect, increased lipolysis, increased renal glucose reabsorption and neurotransmitter disorder (1, 5). For this reason, educating patients on physical activity and diet planning plays an important role in the long-term regulation of diabetes (6).

It is estimated that 75% of diabetic patients suffer from at least one other associated disease. Cardiovascular diseases occur 15 years earlier in such patients, being the leading cause of mortality (7). Diabetic patients have a higher prevalence of coronary heart disease, a more severe degree of coronary ischemia and 2 to 3 times more frequent myocardial infarction with a worse prognosis (8). Factors identified for the development of atherosclerosis, which we can affect, are hyperlipoproteinemia, hyperglycaemia, hypertension, hyperhomocysteinemia, smoking and obesity. It has been shown that by controlling these factors, cardiovascular mortality is significantly reduced, even in the case of patients with type 2 diabetes (9, 10).

According to the cardiovascular risks table referred to in the 2019 guidelines, patients with type 2 diabetes belong to the group of moderate cardiovascular risk if they are under 50 years of age and the disease itself has been present for less than 10 years without additional risk factors. In case of type 2 diabetes lasting for 10 years or longer with the presence of a risk factor or type 2 diabetes without damage to the target organs, cardiovascular risk was assessed as high. Diabetic patients who suffered damage to target organs (e.g., proteinuria or severe chronic kidney disease with glomerular filtration rate – eGFR < 30 mL/min/1.73 m2, retinopathy or neuropathy) or had at least 3 risk factors belong to the group of patients with very high cardiovascular risk.

The target LDL cholesterol level for the group of patients with very high cardiovascular risk is < 1.4 mmol/L and a 50% or greater decrease of the initial level, while target LDL cholesterol levels at the time when this research was conducted, according to the 2016 guidelines, amounted to < 1.8 mmol/L or a 50% or greater decrease of the initial level of LDL cholesterol (11). Despite the available therapy, diabetic patients are still at additional cardiovascular risk regarding the rapid development of atherosclerosis. Although the prevalence of increased levels of LDL cholesterol is lower each day, the prevalence of high levels of triglycerides and low levels of HDL cholesterol has doubled in recent years, which may be connected to the increasing number of obese people (metabolic syndrome). Therefore, body mass regulation and pharmacotherapy for

lowering triglyceride levels and increasing HDL cholesterol levels play an important role (12).

The objectives of this research were to determine the average concentrations of LDL cholesterol, triglycerides and urates in patients of both sexes with type 2 diabetes in family medicine offices of the Healthcare Centre Osijek and to examine whether there is a difference in these parameters between obese patients and patients with normal body weight, as well as between patients with and without manifest cardiovascular disease. The research also examined whether obese diabetic patients and diabetic patients with manifest cardiovascular disease would reach target levels of LDL cholesterol, triglycerides and urates.

Participants and methods

The research was organized as cross-sectional, with data collected at a defined time, and it was conducted in two family medicine offices in the Healthcare Centre Osijek. Before the research, participants were provided with detailed information on the planned research and they read and signed the informed consent document. Participants were over 18 years of age, of both sexes, diagnosed with type 2 diabetes. A total of 136 participants were included in the research, of which 55 were men and 81 were women, with an average age of 69.33 (SD = 10.87). The above-mentioned selection of participants and the size of the sample ensured the representativeness of the sample and objective results. Participants were anonymised, with each participant being assigned a unique code, i.e. a number. For the purpose of the research, the participants were further divided into groups of patients who have experienced a cardiovascular event and those who have not, as well as into groups of obese patients and patients with normal body weight. The research was approved by the Ethics Committee of the Healthcare Centre Osijek and the Ethics Committee of the Faculty of Medicine Osijek of the Josip Juraj Strossmayer University of Osijek.

When visiting the family medicine office, after the participants had been informed of the contents of the informed consent document and after they had signed it, which was a prerequisite for participating in the research, the following data were collected: demographic data (sex, age), data on duration of diabetes, data on body height and weight, and whether the occurrence of a cardiovascular event (acute myocardial infarction, angina pectoris, stroke, transient ischemic attack) was documented in the available medical records. Data on the existence of diabetes-related complications (diabetic retinopathy, nephropathy and macrovascular complications) were also collected from the medical records. Data on concentrations of LDL cholesterol, triglycerides, urates, plasma glucose and HbA1c were collected from laboratory findings.

LDL cholesterol, triglyceride and urate concentrations were measured using the Beckman Coulter Olympus AU680 chemistry analyser in accordance with the manufacturer's instructions. Triglyceride concentration was measured photometrically, using glycerol-phosphate-oxidase (GPO-PAP), LDL cholesterol concentration was measured using a homogeneous enzymatic assay, and urate concentration was determined by photometry using the UV uricase method. Concentrations of glycated haemoglobin were determined using the turbidimetric immunoinhibition method, whereby the sample for analysis was whole blood with EDTA as an anticoagulant.

Statistical analysis

Categorical data were presented as absolute and relative frequencies. Numerical data were described as the arithmetic mean and standard deviation in case of normal distribution and as the median and interquartile range in other cases. Differences between categorical variables were tested using the $\chi 2$ test and Fisher's exact test, where necessary. Normality of distribution of numerical variables between two independent groups was tested using Student's t-test and Mann-Whitney U test in case of deviation from normal distribution. Correlation between the variables was expressed as Pearson's correlation coefficient in cases where variables follow a normal distribution or Spearman's correlation coefficient in cases where variables do not follow a normal distribution. All P values are two-tailed. The level of statistical significance was set at $\alpha = 0.05$. The MedCalc Statistical

Software version 18.11.3 was used for statistical analysis.

Results

The research included 136 participants, of which 81 were women and 55 were men. The average duration of diabetes in patients was 9 (4-14) years. The mean level of fasting plasma glucose test was 8.43 ± 2.99 mmol/L and the mean level of HbA1C was 7.15% with interquartile range of 6.70-8.30%. 97 (71.3%) participants experienced diabetes-related complications and the most common complication was diabetic retinopathy, with 16.2% of participants experiencing it. 15.4% of participants had an acute myocardial infarction and 12.5% of participants had a stroke or a transient ischemic attack. 14.0% of participants experienced two or more complications. 24.3% of participants were diagnosed with cardiovascular diseases, and 39.7% were obese (BMI > 30 kg/m2). There was no statistically significant difference with regard to adiposity (chi-square test, p = 0.084) and incidence of cardiovascular diseases (chi-square test, p = 0.060) between men and women.

In the highest number of participants, 64%, LDL cholesterol levels were > 2.5 mmol/L and only 12.5% of participants reached the recommended target levels of LDL cholesterol (< 1.8 mmol/L). Mean triglyceride and urate levels were within the recommended levels and no statistically significant difference was found between men and women (Table 1)..

Table 1. Mean levels of LDL cholesterol, urates and triglycerides in patients with type 2 diabetes

	Level
LDL cholesterol (mmol/L)	2.93 ± 1.02
Triglycerides (mmol/L)	1.65 (1.25 – 2.40)
Urates (mmol/L)	326.36 ± 98.55

The difference in levels of LDL cholesterol, triglycerides and urates was also examined between the group of participants who have experienced a cardiovascular event and the group of participants who have not experienced it, and no statistically significant difference was found. Target LDL cholesterol levels were not reached in either group, target triglyceride levels were reached in the group of participants who have not experienced a cardiovascular event, and target urate levels were reached in both groups (Table 2).

Table 2. Mean levels of LDL cholesterol, triglycerides and urates in patients with type 2 diabetes – comparison of the group of participants who have experienced a cardiovascular event and the group of participants who have not

	Experienced CV event (N = 33)	Not experienced CV event (N = 103)	p
LDL cholesterol (mmol/L)	2.88 ± 1,06	2.95 ± 1.01	0.733*
Triglycerides (mmol/L)	1.80 (1.30 – 2.45)	1.60 (1.20 – 2.40)	0.650**
Urates (mmol/L)	332.79 ± 11.02	324.28 ± 9.51	0.668*

*Student's t-test; **Mann-Whitney U test

Finally, our research examined the difference in LDL cholesterol, triglyceride and urate levels in obese patients and patients with normal body weight. The mean triglyceride level was significantly higher in the group of obese patients than in the group of patients with

normal body weight (Mann-Whitney U test; p = 0.005), while the tested difference in LDL cholesterol and urate levels was not significant. Participants with normal body weight did not reach target LDL cholesterol levels, while obese participants did not reach target LDL cholesterol, triglyceride or urate levels (Table 3).

Table 3. Mean levels of LDL cholesterol, triglycerides and urates in patients with type 2 diabetes – comparison of obese participants and participants with normal body weight

	Obese (N = 54)	Normal body weight (N = 82)	p
LDL cholesterol (mmol/L)	3.05 ± 0.92	2.86 ± 1.08	0.325*
Triglycerides (mmol/L)	2.20 (1.48 – 2.75)	1.50 (1.10 – 2.08)	0.005**
Urates (mmol/L)	342.13 ± 13.85	316.48 ± 10.64	0.141*

*Student's t-test; **Mann-Whitney U test

Discussion

One of the biggest challenges in family medicine is regular and in-depth monitoring of a chronic patient's medical condition, especially of patients with type 2 diabetes and patients with multimorbidities, as well as continuous education on the importance of maintaining normal glucose levels, physical activity and diet planning in order to prevent the development of microvascular and macrovascular complications, which increase patient mortality as well as reduce the quality of life.

Factors which often complicate regular monitoring and counselling of chronic patients, including diabetic patients, in family medicine offices are the increasing number of patients visiting out-patient clinics per day and their lack of response due to insufficient levels of education. All of the above shows that providing healthcare for patients with type 2 diabetes is a difficult task and a special challenge, as well as that treatment priorities should be set together with the patients, bearing in mind the professional guidelines, associated diseases, existing therapy and the patients' social status.

Guidelines of the American Diabetes Society (ADA) and the European Association for the Study of Diabetes (EASD) issued in October 2018 recommend HbA1c levels < 7% for most patients, ≤ 6.5% for younger patients and patients without comorbidities, and ≤ 7.5% for older patients with longer duration of disease and comorbidities (13). Mean HbA1c levels in our research were 7.15%, which corresponds to the guidelines' recommendations, given that our participants were older patients with multimorbidities whose average age was 69.33 ± 10.87. In a similar large-scale study conducted in family medicine offices in the Republic of Croatia, HbA1c levels were 7.6% on average, which is slightly above the recommended levels (14).

The most common complication in participants is diabetic retinopathy, which 16.2% of participants had been diagnosed with; this can be compared to the results obtained in Croatian and worldwide studies, with values ranging between 18 and 20% (14,15).

The average urate and triglyceride levels in participants were within the reference values irrespective of sex. Only 12.5% of participants reached target LDL cholesterol levels, and LDL cholesterol levels of 64% of participants were over 2.5 mmol/L, which also corresponds to results obtained in large-scale studies conducted at the level of primary healthcare worldwide, where LDL cholesterol levels of 60 to 65% of participants amounted to 2.6 mmol/L and over (16).
The average triglyceride level was 1.65 (1.25 – 2.40) mmol/L, which is a better result than the one obtained in other studies conducted in family medicine offices in the Republic of

Croatia. Research conducted by Naqvi Syeda et al. in 2017 showed a strong correlation between high triglyceride levels and high HbA1c levels in diabetic patients ($r = 0.278$, p value < 0.0001). Given the results, the use of HbA1c levels as markers for dyslipidaemia, especially for hypertriglyceridemia, has been proposed in order to minimize cardiovascular risks in diabetic patients through timely administration of adequate therapy (14,17).

Average urate levels in our research were also within the reference values, 318.97 ± 11.11 μmol/L for women and 337.44 ± 13.09 μmol/L for men. Urates or uric acid are organic substances produced as a final product of purine metabolism. Hyperuricemia is a condition in which plasma urate levels exceed recommended levels and it is frequently found in persons with congestive heart failure. Given that persons with type 2 diabetes are at increased risk for the development of cardiovascular diseases, and ultimately heart failure, it is considered that correction of hyperuricemia is very important for them. Many studies examined the correlation between increased urate concentration and the development of insulin resistance and diabetes, dyslipidaemia, arterial hypertension and abdominal obesity, but the results were contradictory (18, 19, 20).

The average body mass index of our participants was 29.48 ± 5.64 kg/m2, with 39.7% of participants having a body mass index > 30 kg/m2, which classifies them as obese. Tested differences in obesity between men and women were not significant (chi-square test, $p = 0.084$). In Croatia, approximately 25.3% of men and 34.1% of women are considered obese, which is consistent with the results of our research. Nowadays, obesity is almost the biggest health problem as it has become a pandemic, and in pathophysiological terms it is closely linked to the onset of many chronic diseases, including diabetes and cardiovascular diseases. There are many causes of obesity and the healthcare system's attempt to stop this pandemic has not produced satisfactory results so far. Programs in many countries are focused mainly on therapeutic rather than preventive approach, since prevention requires serious long-term investments with generally uncertain results. Prevention programs should focus on children and young people in order for them to adopt healthy habits and behaviour patterns in a timely manner, which, in the long run, would certainly contribute to reducing obesity in adults, and thus to decreasing its negative effects on population health (21). The research compared LDL cholesterol, triglyceride and urate levels between the groups of obese diabetic patients and diabetic patients with normal body weight. As expected, LDL cholesterol, triglyceride and urate levels were higher in the group of obese patients, but a statistically significant difference between the two groups was only shown with regard to triglyceride levels (Mann-Whitney U test, $p = 0.005$). The group of obese patients did not reach the target levels of any of the examined parameters (LDL cholesterol, triglycerides and urates), while the group of patients with normal body weight reached the target levels of urate and triglycerides, confirming the correlation between adiposity and these parameters.

Cardiovascular diseases are considered to be a major cause of mortality in patients with type 2 diabetes, but also in the general population. Many studies have demonstrated the existence of equal mortality risk caused by coronary diseases in patients with type 2 diabetes who have not experienced a cardiovascular event and patients who are not suffering from type 2 diabetes, but who have experienced a cardiovascular event (22). Diabetic patients who had an acute myocardial infarction are 43% more likely to experience a recurrent cardiovascular event than diabetic patients who have never experienced a cardiovascular event (23). Out of the total number of our participants, 24.3% of them have experienced a cardiovascular event and no statistical difference was found between men and women (chi-square test, $p = 0.060$), which is very likely connected to the high average age of participants, i.e. menopause in women, which makes cardiovascular risk equal for both sexes.

A large prospective Korean study examined the correlation between LDL cholesterol levels and

the onset of cardiovascular events in patients with type 2 diabetes who had not experienced a cardiovascular event. The patients were monitored for 7 years. The average LDL cholesterol levels in participants were 2.94 mmol/L, which is similar to the results obtained in our research. The patients were further divided into groups of patients who were taking statin therapy and those who were not. There was a statistically significant increased risk of cardiovascular event occurrence in the group of patients who were not taking statin therapy, while LDL cholesterol levels were > 3.37 mmol/L and > 1.8 mmol/L in the group of diabetic patients taking statin therapy (24).

Target LDL cholesterol levels in our research were not reached in either the group of patients who have experienced a cardiovascular event or the group who have not experienced it, whereas target triglyceride levels were only reached in the group of patients who have not experienced a cardiovascular event.

Despite the use of statin and antihypertensive therapy in corresponding doses and adequate glucoregulation, diabetic patients are at additional cardiovascular risk due to increased triglyceride levels and decreased HDL cholesterol levels, which is why it is extremely important to regulate HDL cholesterol and plasma triglyceride levels in addition to LDL cholesterol levels, as well as to observe urate levels for possible worsening of the cardiovascular condition that would further contribute to micro- and macrocirculatory damage. Given the poor LDL cholesterol levels shown, it can be concluded that it is of great importance for patients with type 2 diabetes to regularly check their lipid profile and to increase statin doses, if necessary, in order to reach the target LDL cholesterol levels. In cases of triglyceridemia, fibrate therapy should be considered in addition to statin therapy. Nevertheless, the most important thing is to educate patients and administer non-pharmacological therapy for regulating body weight, as well as to encourage physical activity, which will, in the long term, have a positive effect on reducing triglyceride and urate levels, as well as on glucoregulation in patients with type 2 diabetes.

Acknowledgement. None.

Disclosure

Funding. No specific funding was received for this study.
Competing interests. None to declare.

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Original article

Decrease in Environmental Temperature May Trigger the Onset of Acute Aortic Dissection

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Abstract

Aim: The most important risk factors for a Stanford type A acute aortic dissection (AAD) include arterial hypertension and connective tissue disorders, while numerous studies have identified meteorology factors, such as environmental temperature also play an important role. The aim of this study is to explore the relationship between environmental temperature and the frequency of AAD surgically threated over a 12-year period at a Croatian university hospital.

Methods: This is a retrospective, monocenter observational study conducted at the University Hospital Centre Zagreb. The study includes 134 patients who were threated surgically for Stanford type A AAD between January 2001 and December 2012. Temperature categories (low, moderate and high) were based on the calculated monthly average environmental temperature and standard deviation given from official daily environmental temperatures for the respective period.

Results: The results show a higher frequency of AAD in days of low temperature compared to days of moderate temperature or high temperature. The frequency of days with AAD was somewhat higher in moderate than high temperature category, but the difference is not statistically significant. The relative frequency of AAD for low, moderate and high temperature categories were 4.55, 2.96 and 1.93, respectively.

Conclusion: Environmental temperature drop induces stressful adaptive body response, including an additional hemodynamic load and increase in arterial blood pressure, strong enough to trigger the AAD-etiopathogenesis. Furthermore, our findings indicate that body response to environmental heat may differ from a response to reduced environmental temperature, due to observed small number of events in days of high temperature.

(Švagelj* I, Vlahović I, Ogresta D, Belina D, Kovač Z. Decrease in Environmental Temperature May Trigger the Onset of Acute Aortic Dissection. SEEMEDJ 2020; 4(1); 40-48)

Received: Oct 21, 2019; revised version accepted: Mar 9, 2020; published: Apr 27, 2020

KEYWORDS: acute aortic dissection, blood pressure, cold temperature, thermotolerance

Introduction

Among cardiovascular diseases, which are the leading cause of death in the developed countries, acute aortic dissection (AAD) stands out as one of the most lethal. With an in-hospital mortality rate of 22 % for Stanford type A (involving ascending aorta) and about 12 % for Stanford type B (no ascending aorta involvement), AAD represents an emergency case in the cardiovascular surgery (1). At a histological level, AAD is the tearing of the inner surface of the aortic layer known as intima, whereupon blood at high pressure splits or dissects the media to form a false channel or lumen that runs alongside the true lumen. A further re-entrance tear allows blood to circulate through the false lumen (2, 3). Retrograde spreading of this tear results in either the penetration of blood into the pericardium, causing hemopericardium and heart tamponade, or the rupturing of the aortic wall. Both of these scenarios are medical emergencies, as they lead to the rapid death of the patient if timely surgical intervention is not performed (2, 4).

AAD affects men at about twice the rate that it does women and it commonly occurs between 60 and 80 years of age (5). Besides age, other well-known risk factors are an arterial hypertension, an aortic aneurism, congenital diseases of connective tissue like Marfan syndrome or Elhers-Danlos syndrome, various types of vasculitis and a bicuspid aortic valve (6). Furthermore, a number of studies have revealed meteorology factors (e.g., environmental temperature) as potentially risk factors which increase the incidence and mortality of AAD during the cold months of the year (7-11). A number of published studies have compared AAD with the exact data on environmental temperature and interestingly, although all the studies were carried out in a moderate zone of Western Europe, the results of the studies are contradictory. In fact, one group of authors from the United Kingdom (12) showed that climate factors have no impact on the occurrence of AAD, whereas a group of authors from Germany failed to identify any relationship between air temperature and the incidence of AAD. However, the German authors did point out possible association which tended to be significant, explaining it as result of a small number of events (13). On another note, a group of authors from France (9) has reported that AAD has a higher incidence during colder months of year, and a research group in the Netherlands (14) noted that a higher incidence of AAD correlates to a lower air temperature (low minimal daily temperature). A second group from Germany (15) noted that changes in the air temperature and amount of cloudiness are the most representative weather predictors among the studied parameters.

The aim of this study is to explore the relationship between relative environmental temperature and the frequency of Stanford type A acute aortic dissection.

Materials and methods

This is a retrospective, monocenter observational study conducted in one university hospital located in Zagreb (Croatia). The study has been approved by the Ethical Committee of University Hospital Centre Zagreb. Information from hospital records were combined with data from local weather forecast archives. This approach assumes that all person in a specified geographic area experienced the same exposure conditions.

Clinical data

The medical records of the patients consecutively admitted to the Department of Cardiac Surgery at the University Hospital Centre Zagreb in the period between 1 January 2001 and 31 December 2012 were investigated. Inclusion criteria were diagnosis and surgically treatment of the patient with a Stanford Type A AAD. The baseline characteristics of patients (gender and age), diagnosis of arterial hypertension and/or connective tissue disorder, as well as precise date and time of the first AAD symptoms were recorded for each candidate. After searching operating protocols

212 patients were identified as fitting the given criteria, but 35 were excluded due to onset of iatrogenic AAD (2 patients) and incomplete or unclear data (33 patients). Upon consultation with Croatian Meteorological and Hydrological Service (CMHS), the geographic area of the specific climate type was determined, meaning a CfB climate type according to the Köppen classification. It is a temperate oceanic climate (C) without dry season (f) but with warm summers (B) (16). The geographic area of interest is a circular area with a diameter of 195 km (with Zagreb as the center) and includes nine Croatian counties inhabited by approximately 2.1 million inhabitants. Accordingly, another 43 of the remaining 177 patients were excluded given that they reside outside of the determined geographic area at the moment of onset of AAD. Hence, a total of 134 patients with surgically treated Stanford type A AAD were included in the study.

Meteorological data

Exact data on environmental temperatures for the determined area and timeframe were collected from the official weather database provided by CMHS. All average daily temperatures of twelve-years studied period were categorized in one of the three relative temperature categories: low, moderate or high. Relative temperature categories, for each month separately, were determined from the calculated average monthly temperature and standard deviation of these months (e.g. all Januaries, Februaries etc.) during the studied period. Hence, the moderate category of each month is defined as the interval between the value of average monthly temperature plus and value of average monthly temperature minus one standard deviation. At the same way, the high and low categories of each month are defined as one standard deviation higher or lower than the average monthly temperature of these months. Consequently, daily average temperatures of the studied twelve-year period which were between the monthly average temperature and one standard deviation interval in which the average daily temperature belongs to, were placed in the moderate category. Daily average temperatures higher than the average monthly temperature plus one standard deviation for the month they belong to were placed in the high category, whereas average daily temperatures lower than the average monthly temperature minus one standard deviation were placed into low category (Figure 1).

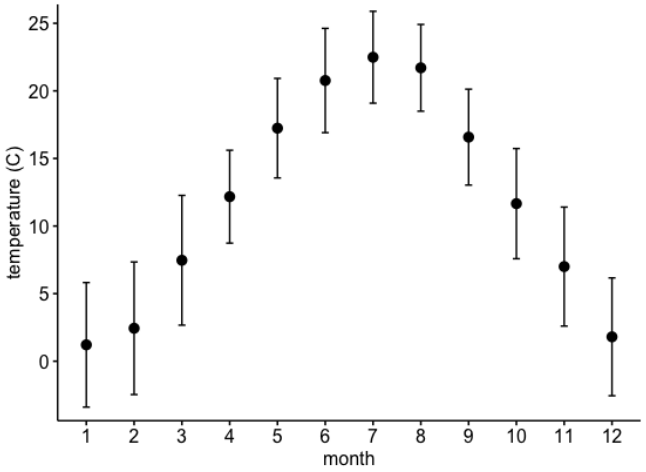

Figure 1. Categorization of average daily temperatures of the studied twelve-year period in respect to the average monthly temperature and standard deviation of each month

The figure shows average monthly temperature (black dots) with one standard deviation interval for each month. Average monthly temperatures plus the respective standard deviation are linked together with a red line and all average monthly temperatures minus the respective standard deviation are linked together with a blue line in order to get the boundaries of relative temperature categories. All average daily temperatures which were fallen above the red line are categorized as a high temperature and those below the blue line as a low temperature. Average daily temperatures which were within one standard deviation interval are categorized as a moderate temperatures.

Furthermore, temperature categories were divided into two groups, one for days when AAD occurred and the other without AAD occurrence. Finally, the same categories from all months were summarized in order to obtain the final table for analysis.

Statistical analysis

Descriptive statistics and statistical analysis were performed using statistical software program R (17). The normality distribution of average monthly temperatures for each month was tested using the Kolmogorov-Smirnov test. Differences in the frequency of days with and without occurrence of AAD between temperature categories was tested using the chi-square test. P-values < 0.05 were considered to be statistically significant.

Results

According to the clinical and geographic criteria given in this study (see the Materials and methods section), a total of 134 patients who were threated surgically for Stanford type A AAD at the Department of Cardiac Surgery, University Hospital Center Zagreb, between January 2001 and December 2012, were included in the data analysis. A descriptive analysis by gender shows that 89 (66.4%) of cases were men and 45 (33.6%) were women. The average age of the patients was 57.8 years with a standard deviation (SD) of 12.8, ranging from 18 to 82 years. Arterial hypertension was noticed in 91 (67.9 %) patients while 25 of them (18.7 %) did not have arterial hypertension, whereas data were missing for 18 patients (13.4%). Furthermore, the Marfan syndrome, one of the most common connective tissue disorder, was detected in one case (0.7 %) (Table 1)..

Table 1. Patient characteristics (SD – standard deviation)

		Patients (n = 134)
Gender	Female	45 (33.6 %)
	Male	89 (66.4 %)
Age (years)	Youngest	18
	Oldest	82
	Mean/SD	57.8/12.8
Hypertension[1]	With	91 (67.9 %)
	Without	25 (18.7 %)
Connective tissue disorder	With[2]	1 (0.7 %)
	Without	133 (99.3 %)

[1]Missing values for 18 patients; [2] Marfan Syndrome

The difference in the frequencies of AAD between the defined relative temperature categories was statistically significant (χ^2 = 8.65, df = 2, p = 0.01). Further analysis shows a higher frequency of AAD in low temperature days compared to moderate temperature days (χ^2 = 4.12, df = 1, p-value < 0.05) or high temperature days (χ^2 = 7.12, df = 1, p < 0.01) (Table 2)..

Table 2. Relative temperature categories including frequencies of days (n) with and without occurrence of AAD (n (%))

	Low	Moderate	High	Total
Days with AAD	33 (0,75)	87 (2)	14 (0,32)	134 (3,1)
Days without AAD	692 (15,8)	2846 (64,9)	711 (16,23)	4249 (96,9)
Total	725 (16,55)	2933 (66,9)	725 (16,55)	4383 (100)

The difference in the frequencies of days with and without AAD between the defined relative temperature categories was statistically significant (χ^2 = 8.65, df = 2, p = 0.01). There is a higher frequency of AAD on low temperature days compared to moderate temperature days (χ^2 = 4.12, df = 1, p-value < 0.05) or high temperature days (χ^2 = 7.12, df = 1, p < 0.01**), while the difference between moderate and high temperature categories is not statistically significant (χ^2 = 1.95, df = 1, p = 0.16)*

Although the frequency of days with AAD is somewhat higher in the moderate than high temperature category, this difference is not statistically significant (χ^2 = 1.95, df = 1, p = 0.16). To get a more comparable and evident relationship, the given results were transformed into a relative frequency of AAD for each temperature category using a particular method. The number of days with AAD in each temperature category were divided by all days (with and without AAD) of the same category and then multiplied by 100. Hence, the relative frequency of AAD for low, moderate and high categories were 4.55, 2.96 and 1.93, respectively (Figure 2)..

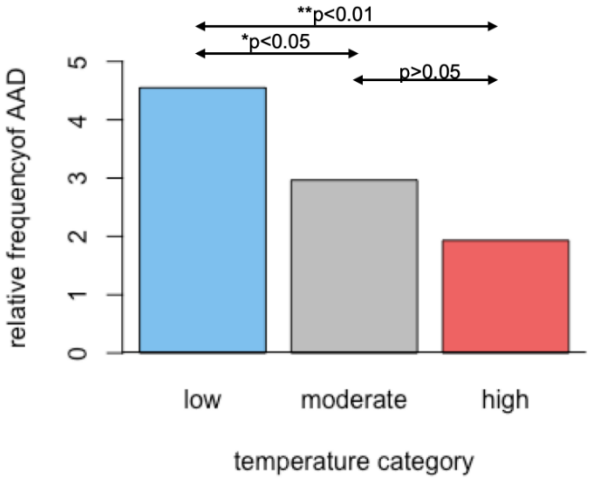

Figure 2. Relative frequency of AAD occurrence between temperature categories

There is a higher relative frequency of AAD occurrence for the low temperature category (4.55) compared to the moderate (2.96) and high (1.93) temperature category, while difference in frequency of AAD between the moderate and high temperature is not statistically significant. The specified P-values between categories correspond with those calculated from Table 2.

Discussion

This study reports a connection between variation of environmental temperature and occurrence of AAD. More than 60 percent of patients included in the present study were men, which correlates to the relevant literature (1, 10). The average age of patients is 58 years with the number of patients aged 70-79 decreasing, which deviates from previously mentioned literature (5). This may be attributed to limitations of the methodology used in the study, meaning patients who died were not included, given that had not undergone surgery and consequently had not been recorded in the surgical operation protocol.

A human physiological, and possible pathological, outcome is strongly connected to numerous internal and external factors and their interplay which is a well-known fact. Hence, arterial hypertension, as one of the most common cardiovascular diseases and a predisposing factor for blood vessel rupture, was also noted within the study and which indicates a more frequent occurrence of AAD in patients with arterial hypertension (68 %), which correlates to the data in the literature (1, 18, 19). The significance of high arterial pressure in the onset of AAD may be attributed to its contribution to damaging the aortic inner layer (intima) which may be the first step in the onset of AAD (2, 3, 20). Due to Laplace's law, a sudden increase in arterial pressure inside the aortic lumen leads to an increase in aortic wall tension, consequently increasing the risk of aortic wall rupture (6). Environmental temperature is an important, but not always perceptible external factor affecting arterial pressure. Hintsala et al. showed that short-term cold exposure increases central aortic blood pressure and cardiac overload, which may contribute to the observed increased winter mortality (21). A similar result is that outdoor temperature and blood pressure are strongly correlated whereby systolic blood pressure decreases with increasing temperature, but only in the elderly population (those over than 80 years of age) as reported by Alperovitch et al. (22). To reduce the well-known influence of a climate type on environmental temperatures, we included 134 patients from only a single climate type (the Cfb type according to the Köppen classification). There has been only one study from France with a similar approach (23). Our results show a higher frequency of AAD for low temperatures regardless of the season and the month(s) of year. On another note, groups of authors from UK (7), former Serbia and Montenegro (8), Korea (10) and Japan (11) have reported that the frequency of AAD is significantly higher in winter and peaking in January. Similar results indicate that cold weather is correlates to a higher incidence of AAD as reported by Verberkmoes et al. from the Netherlands (14). Moreover, Li et al. reported that the onset of Stanford type B AAD was higher in winter than in summer and autumn, and that a low maximal daily temperature is associated with occurrence of the Stanford type B AAD (24), while Xie et al. noted that cold atmospheric temperature and larger daily temperature changes correlated to a higher incidence of AAD (25). Both of these studies were from China.

In our opinion, a relative drop in environmental temperature might be more important risk factor than absolute temperature value, given the highest frequency of AAD when the average daily temperature was categorized as low. This hypothesis was first mentioned in 2005, when Mehta et al. reported that the winter peak for AAD was evident in both cold and temperate climate settings, suggesting that the relative change in temperature, rather than absolute temperature, may be a mechanistic factor (26). Benouaich et al. showed that the incidence of aortic dissection was higher in a winter time than in summer, but also that the days with occurrences of AAD were colder than those without AAD, concluding that a relative change in temperature may be a triggering factor in onset of AAD (9). The results conform with a study by a group of authors from Nantes (France) who arrived to the same conclusion, but using different methodology. They reported that, regardless of the season, a decrease in average daily temperature of more than 5˚C, between days 0-7, significantly increases the risk of acute aortic syndrome at day 0 (23). Finally, a group from China which tried to predict AAD occurrence in respect to environmental temperature changes, concluded that for every 10 ˚C increase, the incidence drops by 0.21 units (27).

An explanation of the possible pathophysiologic mechanism for AAD occurrence regarding temperature changes may be activation of body temperature regulators such as thermogenesis, cytokine response, hemodynamic adaptation and hypermetabolism (6). The human organism retains the heat by decreasing the release of heat (i.e. vasoconstriction) and increasing heat generation (i.e. increased metabolism) what attribute to sympathetic activity increase. (6, 9,

14, 21). Consequently, vasoconstriction and an increased metabolism (including a higher cardiac work load) increases mean arterial pressure, which is inversely proportional to changes in body temperature, and in fact environmental temperature (6, 9, 14, 21).

Edwin et al. reported that the pathogenesis of AAD may result from the interaction of three factors: 1) an existing pathological condition of the aortic media, 2) any agent of intimal injury or tear, and 3) hemodynamic factors that propagate the dissection once it has been initiated (28). Hence, a possible explanation of the connection between a drop in environmental temperature and AAD occurrence is attributed to an increase in arterial pressure leading to a critical level when intimal injury occurs and consequently propagation to AAD caused by the same factor. This value of critical level of arterial pressure is unique for each person individually, while increasing in arterial pressure is generated by the activation of above-mentioned defense (sympathetic) mechanisms. The assumption is that a drop in environmental temperature induces a stressful, adaptive response from the body, including an additional hemodynamic load (and consequently increasing arterial pressure), which is strong enough to trigger and propagate the AAD etiopathogenesis. Also, our findings indicate that body response to an environmental heat may differ from the response to reduction in environmental temperature, given the big difference in AAD occurrence between low and high temperature categories.

These results suggest that a change in environmental temperature does not necessarily lead to a higher frequency of AAD. Instead the temperature must decrease. Moreover, the increase in environmental temperature may have a "protective" effect, given that the lowest frequency of AAD occurs for high temperatures what conforms with results reported by Law et al. (27). However, the difference between the moderate and high temperature category is not significant, and it may be due to the fact that the vast majority of average daily environmental temperatures during the year fall into the category of moderate temperatures (~66%) and as a consequence, AAD as a multifactorial disease, occurs just below these conditions. Consequently, this relationship may be confirmed using a larger number of events. Another limitation of the study should be pointed out. The University Hospital Centre Zagreb is not the only institution where AAD is surgically threated, hence an unknown number of events in the investigated area and period were not included in this study.

Conclusion

Present qualitative retrospective study highlights a dependence between changing of environmental temperatures and AAD occurrence, thus indicating a possible causative correlation, what provides the basis for further thesis research in a future multicenter study.

Acknowledgement. The article is a part of the study which was competed for the rectors award in 2013 at the University of Zagreb Croatia under the mentorship of cardiovascular surgeon Dražen Belina and university professor Zdenko Kovač. Special acknowledgement to Croatian Meteorological and Hydrological Service for providing of weather data.

Disclosure

Funding. No specific funding was received for this study.
Competing interests. None to declare.

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Original article

Employment of Patients After Liver Transplantation

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Abstract

Aim: To determine the prevalence of employment of patients after liver transplantation (TX) and the history of employment, to compare employment with patients' opinions about their ability to work and to establish possible reasons for frequent unemployment.

Methods: Ninety-eight respondents participated in the study. They were the first 98 liver transplant patients who came for a check-up at Merkur University Hospital by the time of the research and agreed to participate in the study. We created and used a questionnaire about the level of education and employment prior to and after the liver TX.

Results: Before the diagnosis of liver disease, 59.18 % of the patients were employed, while after liver TX, at the time of the research, the employment rate decreased to 8.2 %. During the same time span, the number of retired patients increased from 3.1 % before the diagnosis to 63.3 % after liver TX at the time of the research. The main reasons for unemployment were poor health due to liver disease and employers' unwillingness to hire these patients because of a potential risk of adjustment of working hours. Median follow-up time after liver TX was 3 years (interquartile range 2 – 6).

Conclusion: Many patients with severe liver disease are unemployed. Liver TX did not increase the rate of employment of Croatian patients. Patients should be supported by society in finding appropriate employment.

(Holetić A, Đukić M, Zibar* L. Employment of Patients After Liver Transplantation. SEEMEDJ 2020; 4(1); 49-54)

Received: Feb 13, 2020; revised version accepted: Mar 22, 2020; published: Apr 27, 2020

KEYWORDS: liver transplantation, employment, liver disease, health-related quality of life

Introduction

Liver transplantation (TX) is the standard method of treatment of end-stage liver disease (ESLD), acute liver failure and selected cases of hepatocellular carcinoma. Patients who underwent TX have better survival rates. Due to an improvement in the surgical treatment and modern immunosuppressive medicaments, the 5-year survival rate increased up to 70 % (1,2,3). The term "health-related QoL" (HRQOL, health-related quality of life) has been in use for several decades. This term encompasses the effect of health, i.e. of the illness and of treatment of the illness on the patient's physical, cognitive and social functioning. Ability to work is considered to be a significant indicator of well-being and health status (4,5). Liver TX, as the best method of treatment of ESLD, improves survival, health status, QoL and ability to work (6-8). However, transplant patients frequently face unemployment, sick leave and retirement. We thus presumed that liver TX, although the best method for the treatment, unfortunately does not improve the patients' access to employment. The aims of the study were to determine the prevalence of employment of liver transplant patients, to compare employment with the patients' opinions about their ability to work and to establish possible reasons for frequent unemployment. There are no available published data about the employment of liver transplant patients in Croatia to date.

Patients and methods

The research was conducted at Merkur University Hospital in Zagreb in 2018. Ninety-eight liver transplant patients were selected as the subjects of our study. The first 98 patients who came for a check-up by the time of the research were included in the study. The patients were asked to fill in a questionnaire. The questionnaire was created specifically for our study by the researcher and it included data about employment before the diagnosis of liver disease, before and after liver TX and at the time of the research. We separately compared overall employment and working patients. Overall employment included all employed participants, even those who were on sick leave. Demographic data and data about liver disease were taken from medical records. Seventy (71.4 %) participants were male and 28 (28.6 %) were female. Participants were divided into groups according to age, with an interval of 10 years. Fifteen participants (15.3 %) were born in the period between 1941 and 1950. Most participants, 42 (42.9 %) of them, were born between 1951 and 1960, while 33 (33.7 %) participants were born between 1961 and 1970. The median age of participants was between 58 and 67 years (interquartile range, IQR, 48 – 67). The youngest patient was 26 years old and the oldest one was 67 years old (Figure 1). Median follow-up time (from liver TX to the research) was 3 years (IQR 2 – 6).

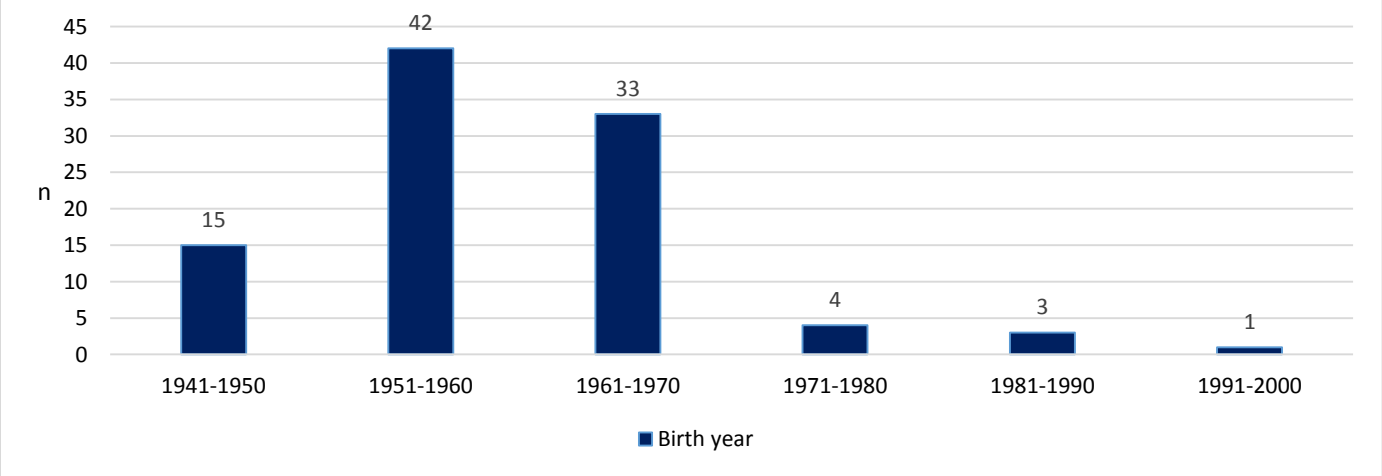

Figure 1. Respondents divided into groups according to age, with an interval of 10 years (N = 98)

Statistical analysis

Data were statistically analysed using SPSS (version 16.0. SPSS Inc., Chicago, IL, USA). Descriptive statistics included the median with IQR for numeric data. Absolute and relative frequencies were used for nominal data. Differences were obtained by the Chi-square test. Statistical significance was accepted if P was < 0.05.

Results

Ninety-eight patients were included in the research. Of 98 participants, 19 completed only primary education or less (19.4 %), 58 finished secondary education (59.2 %), 12 had higher education qualifications (12.2 %) and 9 had a university degree (9.2 %). Most of the participants, 50 of them (51 %), waited for liver TX for more than a year. Twenty-nine participants (29.6 %) waited for liver TX less than six months, while 19 of them (19.4 %) waited between 6 months and a year. All the patients selected for the research underwent liver TX between 2002 and 2017. At the time of the research (in 2018), 4 participants already had two liver TXs. Most of the participants, 21 of them (21.4 %), underwent liver TX in 2017. In 2016, 17 participants underwent liver TX, while 14 of them had liver TX in 2015.

Compared to the period before receiving the diagnosis of severe liver disease, employment among the working patients before liver TX decreased by 37.8 % (59.2 % before the diagnosis, 21.4 % before liver TX). Overall employment decreased by 13.2 % (62.2 % before the diagnosis, 48.9 % thereafter). The number of patients on sick leave (for more than a month) increased by 9 times (3 before the diagnosis, 27 just before TX) (Figure 2)..

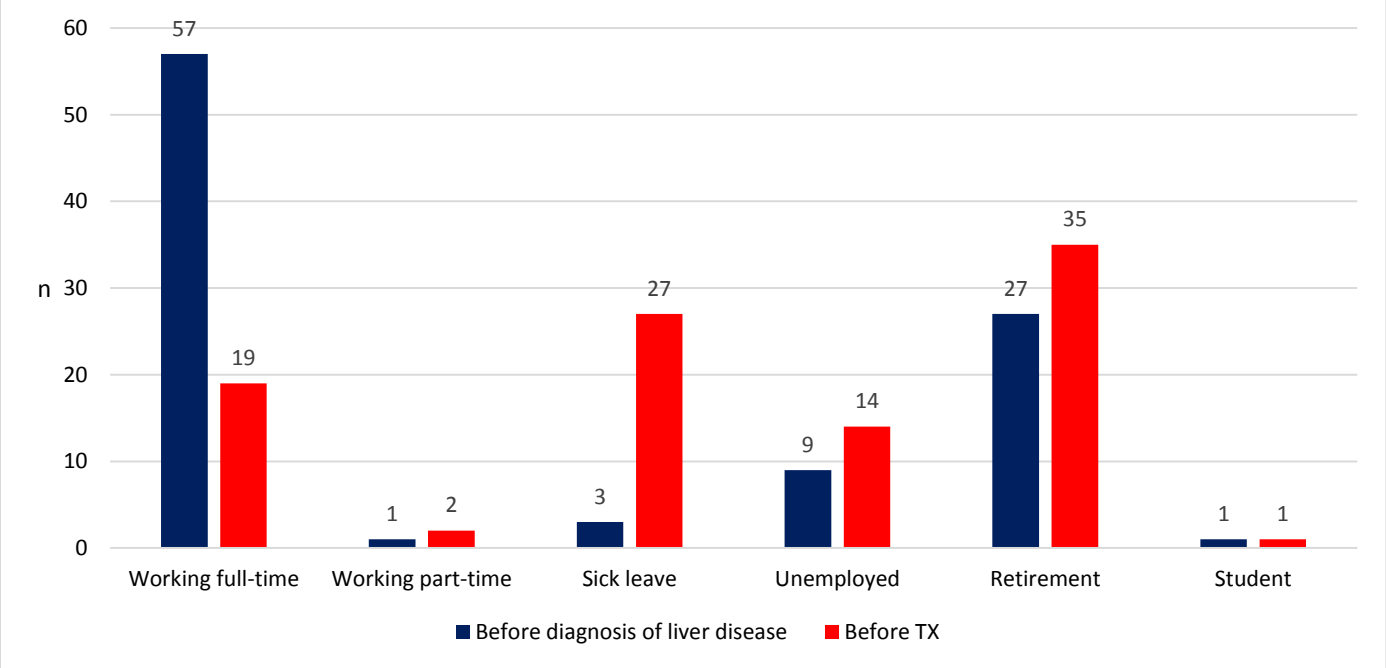

Figure 2. Employment of patients (N = 98) before diagnosis of liver disease and just before liver transplantation (TX)

The number of patients who were working early after TX decreased by 9.2 % compared to the period just before TX (21.4 % just before TX, 12.2 % early after TX). Overall employment decreased by 10.2 % (49 % just before TX, 38.8 % early after TX) (Figure 3).

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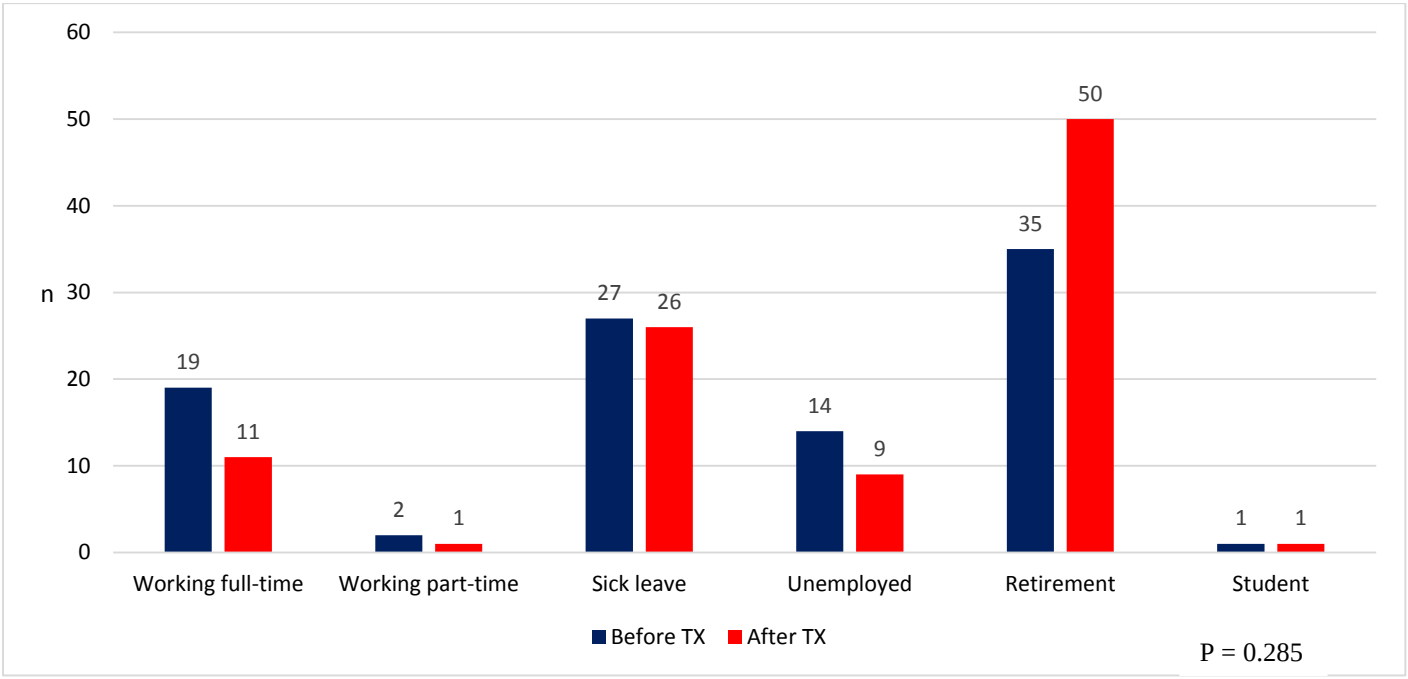

Figure 3. Employment of patients (N = 98) just before and early after liver transplantation (TX)

At the time of the research, the number of employed patients decreased by 4 % compared to the period early after TX (12.2 % early after TX, 8.2 % at the time of the research). The number of patients using sick leave decreased by 7.1 % in the same period (26.5 % early after TX, 19.4 % at the time of research) (Figure 4).

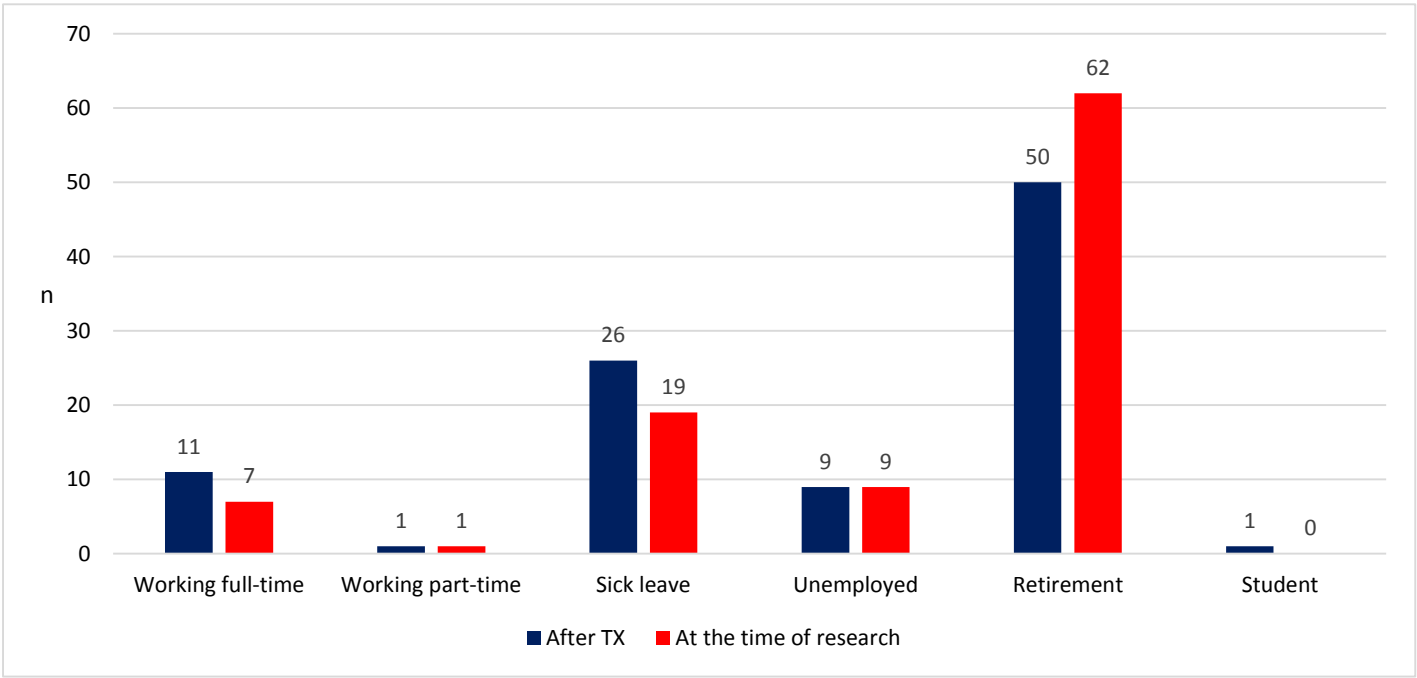

Figure 4. Employment of patients (N = 98) early after liver transplantation (TX) and at the time of the research

At the time of the research, the number of employed patients decreased by 51 % compared to the period before the diagnosis of ESLD (59.2 % before the diagnosis, 8.2 % at the time of research). In the same period, the

number of patients on sick leave increased by 16.3 % (3.1 % before the diagnosis, 19.4 % at the time of the research) and the number of retired patients increased by 60.2 % (3.1 % before the diagnosis, 63.3 % at the time of the research) (Figure 5).

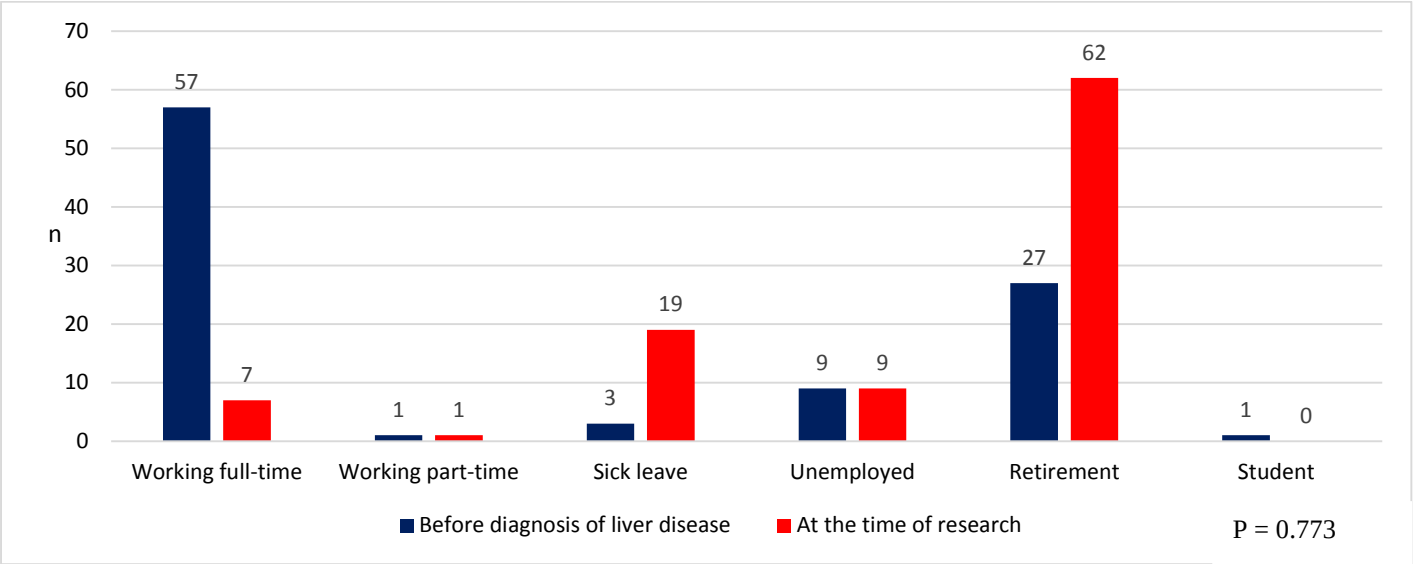

Figure 5. Employment of patients (N = 98) before the diagnosis of liver disease and at the time of the research

At the time of the research, 8 participants were employed (8.2 %), while 34 felt capable of working (34.7 %). Nineteen participants felt capable of working full-time, while 15 felt capable of working part-time (P = 0.773). As the main reason for unemployment, 22 patients (22.5 %) reported poor health due to ESLD, 4 of them (4.1 %) claimed that employers did not want to hire workers who underwent liver TX, 22 of them reported some other reason, while 41 did not give an answer.

Discussion

This is the first research on employment of liver TX patients in Croatia. Just before liver TX, overall employment had already decreased by 13.2 % compared to the period before receiving the diagnosis of severe liver disease. Although patients presumably have a better health status and QoL after liver TX, the number of employed patients early after liver TX decreased by 9.2 % compared to the period just before liver TX. At the time of the research, only 8.2 % of patients were employed. Many researchers described similar results regarding employment after liver TX. In 2018, E. R. Waclawski and P. Noone published a systematic review of the impact of liver TX on employment (9). They included 13 studies published from January 2001 to December 2016. All the studies they found showed either a fall in the employment rate or no effect on the employment rate. Overall employment ranges from 18 to 44 % after liver TX, which was higher than in our research. Employment after liver TX was lower than after other organ TX, such as kidney or heart TX, but higher than after lung TX, as shown in a study which included 281 kidney, heart, liver and lung transplant patients (10). Compared to the research about employment after kidney TX in Croatia, conducted at the Clinical Hospital Centre Osijek in 2017, employment after liver TX at the time of the research was lower than after kidney TX (namely 18.1 %), which corresponded to the results mentioned earlier (11).

What was the reason of unemployment after TX? After TX, patients need to take immunosuppressive medicaments for the rest of their lives. Immunosuppressive therapy has adverse effects, including infections, diabetes mellitus, nephrotoxicity, cardiovascular disease and osteoporosis (12). Moreover, patients have a mental burden because of the disease.

However, our study has shown that 8.2 % of the participants were employed, while 34.7 % of them felt capable of working, which indicates a significant disproportion. The question is what caused that disproportion. As the main reason for unemployment, the majority of respondents reported poor health due to ESLD, while the minority of respondents claimed that employers did not want to hire the patients who underwent TX.

Conclusion

Even though TX improves the patients' health status and survival, it is necessary to ensure that they have a better QoL and allow them to return to their usual activities, including appropriate employment. Social support is needed to improve the chances of employment.

Acknowledgement. None.

Disclosure

Funding. No specific funding was received for this study.
Competing interests. None to declare.

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Review article

Restless Legs Syndrome and Iron

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Abstract

Restless legs syndrome (RLS), also known as Willis-Ekbom disease, is one of the most common neurological disorders that significantly affects quality of life and sleep. It manifests itself in involuntary movements of the lower limbs due to the feeling of discomfort and restlessness that patients feel in their lower limbs.

RLS is of great interest to the experts in various fields of medicine, especially to neurologists, general practitioners, internists and psychiatrists. Numerous clinical conditions and diseases play a role in the pathophysiology of RLS. Some of them are pregnancy, some kidney and stomach diseases, iron deficiency and some disorders of the metabolism.

Moreover, iron is a very important micronutrient in the human body. It is involved in many metabolic processes and, in addition to RLS, it is also associated with other diseases such as hemochromatosis and anemia. This neurological disorder has wide therapeutic choices, which include lifestyle changes, dopaminergic agonists, opioids and iron therapy. Many non-anemic patients with RLS showed reduced levels in brain iron levels compared to healthy control groups in several research. The best course of treatment for this group of patients is iron supplementation.

Oral iron supplementations are the first choice of therapy for patients with low serum ferritin levels. However, when serum ferritin levels are normal or high or when oral iron is not tolerated, intravenous iron is a better choice. There are many intravenous iron formulations, but low molecular weight dextran and ferric carboxymaltose have very efficient effects on the treatment of RLS.

(Pulić * J. Restless Legs Syndrome and Iron. SEEMEDJ 2020; 4(1); 55-62)

Received: Feb 21, 2020; revised version accepted: Mar 18, 2020; published: Apr 27, 2020

KEYWORDS: restless legs syndrome, Willis-Ekbom disease, iron metabolism, serum ferritin levels, treatment

Introduction

Restless legs syndrome (RLS) has been described as a neurological disorder, related to uncontrolled leg movements, and, in much smaller number of patients, it is associated with uncontrolled hand movements. Uncontrolled limb movements are preceded by a sense of discomfort, described by patients as annealing, burning, tickling, etc. It occurs more frequently during inaction. Therefore, it is a common cause of sleep disorders (1-3). In the 17th century, sir Thomas Willis noticed the connection between sleep disorders and lower extremity discomfort among his patients. However, Karl- Axel Ekbom first used the term 'restless legs syndrome' in 1945. That is why this disorder is also known as Willis-Ekbom disease (4).

Unlike some other disorders with similar problems, such as polyneuropathy, the symptoms of RLS decrease with leg movements. In most cases, it is a chronic disorder with worsening symptoms, which vary in intensity and frequency. Therefore, in patients with chronic form of this disorder, symptoms occur at least twice a week if patients do not follow their therapy (1, 5).

RLS has prevalence between 8 and 10% (6) in adult and 2% in children population (7). The study conducted by Manconi et al. has shown that RLS occurs more frequently in women, age 35 and above, than in men of the same age (8).

This disorder can be inherited or be a result of a clinical condition such as kidney diseases (hemodialysis patients) (9), pregnancy (evaluated progesterone and estrogen levels, iron deficiency) (10), anemia, stomach damage, etc. In addition, some substances such as neuroleptic drugs, caffeine, lithium, metoclopramide, antihistamines, dopaminergic agents (1, 11) may increase the risk of developing RLS symptoms (Figure 1).

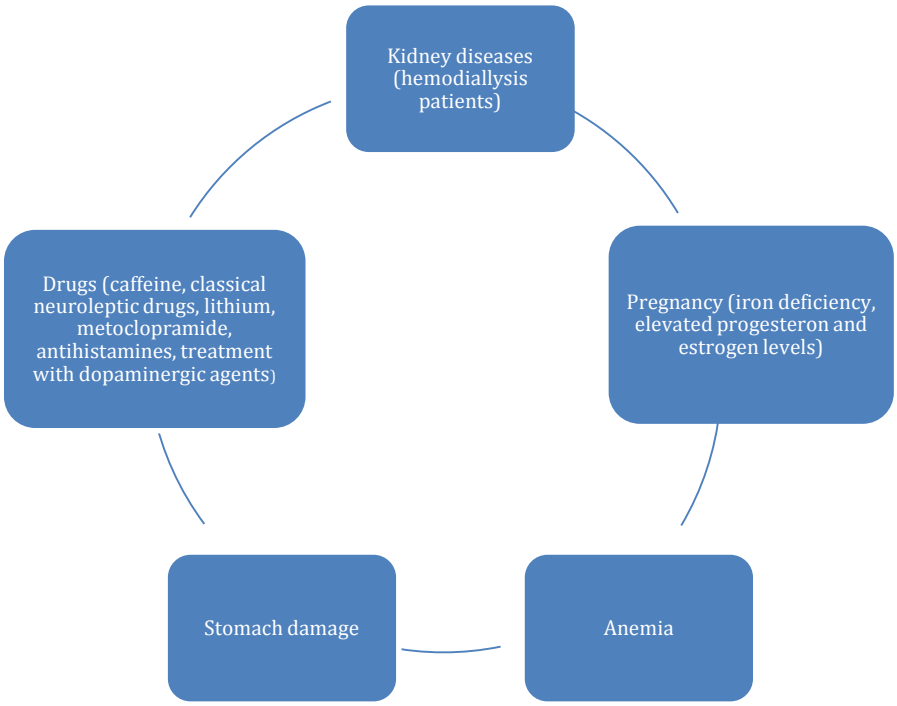

Figure 1. Non-hereditary causes of restless legs syndrome

Summarized findings from studies to date on the most common diseases/clinical conditions/drugs that are associated with restless legs syndrome.

Treatment for RLS depends on its cause. Therefore, the treatment can include dopaminergic agents, lifestyle changes, iron therapy, α -2- δ - ligands, opioids, etc (1).

Compared to the previous European Federation of Neurological Societies (EFNS) guidelines on the management of RLS from 2004, new guidelines from 2012 bring news. Numerous drug studies have been made during this period and new treatments have been examined that could be a potential therapy for RLS. The majority of research deals with dopaminergic agents, considered the first line therapy. The crucial role of iron in pathophysiology of RLS has also been confirmed. Alternative forms of therapy have been investigated; folate, vitamin E, physiotherapy, aerobic training and magnesium. Nevertheless, there is still not enough evidence of their effectiveness (12).

Iron in relation to health and diseases

Iron is one of the most important micronutrient in human body. It has many functions such as a role in metabolic processes; it can cause oxidative stress because it participates in the formation of oxygen radicals (13), and it is a cofactor of numerous enzymes (13, 14).

There are about 4,2 grams of iron in the human body, and much of it is bound to hemoglobin and involved in oxygen transfer (12). About 10% of the iron ingested through food is absorbed in the digestive system, mostly in the duodenum (15).

Ferritin is an intracellular protein that stores iron and plays an important role in regulating iron homeostasis, while transferrin is a glycoprotein that binds iron and transports it into the cells (16). The major regulator of iron homeostasis is hepcidin, 25-amino acid peptide hormone, which is mainly secreted by hepatocytes (17, 18). Its deregulation is linked with excess iron and iron deficiency. Therefore, when level of hepcidin is very high, like in case of inflammation, the absorption of iron is reduced and this can lead to the development of anemia (17-19).

It is proved that brain and liver contain high levels of iron (13). The highest concentration of brain iron is found in substantia nigra, globus pallidus, red nucleus, putamen and dentate nucleus of the cerebellum (20, 21). In oligodendrocytes, the presence of the transferrin has been confirmed, but a greater amount of brain iron is nevertheless related with ferritin (13, 22). Iron plays a large role in neurotransmitter synthesis and mitochondrial respiration and its status is regulated at the level of the blood- brain barrier (BBB) (13, 23, 24).

It has long been known that iron excess causes hemochromatosis, characterized by skin changes, weakness, loss of sex drive, abdominal pain and symptoms of diabetes; but recent studies have associated brain iron excess with the onset of neurodegenerative diseases such as Parkinson's disease. On the other hand, iron deficiency in the central nervous system (CNS) is associated with irritability, concentration disorder, tiredness and it may play a role in the pathophysiology of RLS (13).

Correlation between restless legs syndrome and iron

The first person who noticed that low serum iron level could be a risk factor for developing restless legs syndrome was Nordlander (25, 26). Ekbom observed iron deficiency among his patients. Although he noticed repeated occurrence of low iron level in RLS, most of his patients suffered from uremia, anemia or were pregnant women (27). In another research, O'Keeffe found low serum iron and ferritin values in patients who complained of RLS symptoms. It was observed that the lower the ferritin level were, the more severe RLS symptoms appeared (28, 29). Iron supplementation has caused improvement in some patients. The potential cause of this is altered management of brain iron in patients with RLS (30, 31).

Two studies have shown that almost 2/5 of the patients suffering from iron deficiency anemia also had symptoms of RLS. Nevertheless, these studies used small groups of participants (32, 33). However, one study, conducted by Allen et al. among the general population, has shown that the percentage of people with symptoms

of RLS is several times higher in the group of participants with iron deficiency anemia than in the general population (34). Abnormalities in the concentrations of ferritin, transferrin in the cerebrospinal fluid (CSF), low CSF ferritin, and high CSF transferrin levels have been noticed (35).

Circadian pattern is characteristic for RLS, with symptoms being dominant at nighttime. Serum iron has a circadian variation, with 30 to 50% drop at night. This can lead to clinically significant drop in brain iron levels with patients with RLS and create the symptoms (36).

Lower iron concentration in substantia nigra and putamen were found in some patients with idiopathic RLS and capillary transport of iron in the brain probably plays a major role in this. In addition, it has been observed that the iron levels in substantia nigra increase with aging (37-39).

There is a small number of studies that have compared the connection between serum hepcidin levels and RLS. However, one of them found higher prohepcidin (inactive form of hepcidin) in putamen and substantia nigra in patients with RLS. This opens up the possibility of discovering new medications, such as hepcidin antagonists, for the treatment of RLS (40-42).

Iron treatment

Iron deficiency anemia is present in more than 1/5 of the patients with RLS (34). Accordingly, oral and intravenous iron supplements are used as therapy for those patients. When serum ferritin level is lower than 75 μg/l, oral iron supplementation is the therapy of choice. While in patients with serum ferritin level higher than 300 μg/l intravenous iron preparations are a better choice (43, 44). O'Keeffe observed among his patients, who had different serum ferritin values, that oral iron supplements had better effect on patients with lower serum ferritin values. However, the problem with this study was that it did not have a control group (25, 29). Furthermore, oral iron supplements have almost the same effect whether taken once a day or divided into two doses. In addition, in both cases these supplementations should be taken with vitamin C in order to improve absorption in the small intestine (44, 45).

According to the American Academy of Sleep Medicine (AASM) guidelines, iron treatment is effective for RLS only in patients who have low ferritin levels. In addition, it is preferred oral over parenteral iron formulations, because parenteral forms are associated with a number of side effects that can endanger patients' life and health (46).

On the other hand, intravenous iron supplementations bypass the intestinal- blood barrier and restriction of iron absorption (47). Intravenous iron forms take precedence over oral iron only in two cases. First is when the patient is severely bleeding and rapidly losing iron, and the second case is when patients have problems with oral iron absorption (44, 48).

There are several intravenous iron formulations available: ferric carboxymaltose, iron sucrose, iron gluconate, low and high molecular weight dextrans (LMW and HMW dextrans), ferumoxytol and iron isomaltose (44). LMW dextran and ferric carboxymaltose have the best clinical evidences for treatment of RLS (1, 44). Infusion of 1000 mg LMW dextran improves the health of RLS patients with early symptoms and significantly increases iron levels in substantia nigra (49). Both oral and intravenous iron preparations have numerous limitations in the treatment of RLS. The most dangerous side effect is related to HMW dextran and it involves anaphylactic shock. On the other hand, ferric carboxymaltose is safe to use because its side effects, such as nausea and headache, are much milder (50).

There are not enough studies on intravenous sucrose, as well as on most intravenous iron preparations. Nevertheless, it is known that intravenous sucrose is not effective for patients that do not have anemia (50). In addition, for iron gluconate, ferumoxytol and iron isomaltose, there are insufficient clinical evidences (44, 51) (Table 1)..

Table 1. Iron preparation limitations in the treatment of restless legs syndrome

Oral ferrous sulfate:	gastrointestinal upset; not effective for patients with serum ferritin level>75 μg/l
Ferric carboxymaltose:	headache nausea
Iron sucrose:	not effective in iron deficiency patients without anemia
High molecular weight dextran:	high incidence of anaphylactic shock
Low molecular weight dextran:	not so effective in patients with symptoms of late onset RLS
Iron isomaltose, ferumoxytol, iron gluconate:	not enough clinical evidences

Note: Summarized findings from studies to date on oral and intravenous iron preparations for the treatment of restless legs syndrome.

Avni et al. have proven in their research that iron preparations are safe and effective for RLS. However, there is still a great need for further research, especially for the research that could determine the exact drug dosages and therapeutic regimen (43)

Conclusion

Numerous studies on the relationship between RLS and iron in the body have provided better insight into the pathophysiology of this disorder and have opened up new possibilities related to therapeutic approaches, such as hepcidin antagonists. Nevertheless, there are still many uncertainties related to iron therapy and a need for further research on that topic. The reason is the fact that many intravenous iron formulations lack sufficient clinical evidences regarding their effect on reducing the symptoms of RLS. Furthermore, many studies have a small or inadequate sample of participants. In addition, for many intravenous iron formulations the exact dosage required for the treatment of RLS has not been determined..

. **Acknowledgement.** None.

Disclosure

Funding. No specific funding was received for this study.
Competing interests. None to declare.

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Review article

Urinary Incontinence: Diagnostic Evaluation and First-Line Treatment

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Abstract

Urinary incontinence is defined as involuntary leakage of any amount of urine that negatively affects the individual's hygienic and social status. It is an important public health problem because it has a high prevalence and incidence. Due to various social prejudices, it is often neglected and unreported. It is also characterized by its chronic appearance and complications such as decreased quality of life, sexual dysfunction and symptoms of anxiety and depression. The financial burden for symptomatic, conservative and surgical treatment of urinary incontinence is high. Both women and men are reluctant to seek medical help, and they most often use coping strategies to deal with urinary incontinence symptoms, which include the use of incontinence aids such as adult diapers and pads.

We conducted a systematic review of high-quality randomized controlled studies and of other review articles to compare circumstances surrounding the diagnostic evaluation and first-line treatment of urinary incontinence in women and men according to International Continence Society guidelines. Because urinary incontinence can occur during any stage of life, diagnostic and therapeutic approach is different in women and men. Social stigmatization created due to urinary incontinence may harm a person's self-confidence and cause many negativities at a social level.

Timely recognition of the type and severity of symptoms of urinary incontinence is necessary to reduce the occurrence of the aforementioned complications.

(Radoja I, Degmečić* D. Urinary Incontinence: Diagnostic Evaluation and First-Line Treatment. SEEMEDJ 2020; 4(1); 63-73)

Received: Feb 29, 2020; revised version accepted: Apr 2, 2020; published: Apr 27, 2020

KEYWORDS: urinary incontinence, cystoscopy, urodynamics, ultrasonography, parasympatholytics

Introduction

The International Continence Society (ICS) has defined new terminology for lower urinary tract symptoms (LUTS), clinical signs and conditions. LUTS is now divided into symptoms occurring during storage of urine within the bladder (incontinence of urine, urgency, nocturia, nocturnal enuresis, overactive bladder syndrome, increased frequency of urination, nocturnal polyuria), symptoms during micturition (dysuria, abnormalities of urine flow) and symptoms after urination (feeling of incomplete bladder emptying, dripping of urine after urination) (1). The most common LUTS occurring in women is urinary incontinence (UI) and the most common LUTS in both sexes is overactive bladder syndrome (OAB). UI is a symptom of involuntary leakage of urine subjectively reported by patients, or a clinical sign of involuntary leakage of urine objectively established by a physician (1). The amount of involuntary leakage of urine considered abnormal has not yet been defined, nor has the impact of UI on the psychological, physical and social functioning of the patient. The new definition specifies that UI is not a disease, but a symptom or clinical sign occurring due to various diseases of the neurological system, lower urinary tract, and muscles of the pelvic floor. Diseases that can cause the onset of UI can be temporary and permanent, neurogenic or non-neurogenic (3). UI can manifest as an occasional involuntary leakage of a small amount urine or as the complete inability to retain urine within the bladder.

Given the complex aetiology of UI and the situations in which it occurs, UI is divided into several types. According to the ICS, the most common types of UI are stress urinary incontinence (SUI), urgency urinary incontinence (UUI) and mixed urinary incontinence (MUI) (1). SUI is defined as involuntary leakage of urine during coughing, sneezing or another physical activity. It is most common in women, but it is also present in some men who underwent prostate cancer surgery. UUI is defined as the observation of involuntary leakage from the urethral orifice associated with the individual reporting of the sensation of a sudden, compelling desire to void. Unlike SUI, this type of incontinence is unpredictable, it can happen at any time and it is independent of physical activity and bladder fullness. MUI is characterized by an involuntary loss of urine associated with urgency and also on effort or physical exertion, including sporting activities, or on sneezing or coughing. Other types of UI include postural UI, nocturnal enuresis, continuous UI, insensible UI and coital UI (4). UI is generally caused by the failure of pelvic floor muscles, the inability of the internal and external sphincter to withstand increased urinary bladder and abdominal pressure occurring during daily activities, or it can be a result of involuntary contractions of the bladder detrusor muscle. In most cases, women and men postpone seeking help from primary care health professionals. Usually, this is because they believe that this is a normal condition occurring due to pregnancy and vaginal delivery in women, due to an enlarged prostate and complications of prostate surgery in men, or due to older age in both women and men. Another reason is because they are not familiar with modern treatment possibilities.

Prevalence and etiology of urinary incontinence

UI is estimated to affect more than 450 million people worldwide and is three times more common in women than in men, regardless of the type of UI (5). In the total adult population of both sexes in the United States of America (USA), the prevalence of UI ranges from 5% to 50%. UI can occur at any age, but the prevalence increases with age and affects approximately 40% of older women and approximately 15% of older men (6). Currently, there are no data on the prevalence and incidence of UI in Croatia, but it is probably similar to those from the USA. According to the latest research, the prevalence of UI in women and men of all ages, ranges from 20 to 50%, and the incidence of UI ranges from 15 to 30% (7). The prevalence of UI increases with age and, therefore, amounts to 38% in patients over 60 years of age (8). The public health impact of UI

is very high because the proportion of older adults in the total population of the USA and other developed Western countries is increasing. According to recent scientific studies, SUI is the most common type of UI, with prevalence ranging from 10 to 39% (9). MUI is the next most common type of UI with a prevalence of 7% to 25% (10). UUI occurs very rarely with a prevalence of 1% to 7% (10). UUI occurs more frequently as part of OAB. OAB is defined as urinary urgency, usually accompanied by increased urination frequency during the day and increased urination frequency during sleep (i.e. nocturia), with or without UUI, and in the absence of a urinary tract infection or another obvious disease of the urinary bladder (e.g. bladder cancer) (4). OAB is often of idiopathic genesis or a consequence of a neurological disease, associated with excessive activity of the bladder detrusor muscle. The prevalence of OAB ranges from 2% to 53% (11). Normal total capacity of an adult bladder is approximately 400 to 500ml of urine. During the day, approximately 1400 ml of urine is produced, depending on the type and volume of the fluids we drink. The frequency of voiding is usually 2 to 3 times a day, and up to 1 time during the night. The normal desire for micturition occurs at a volume of 50% of normal total bladder capacity. Some or most of these physiological functions and parameters are impaired in patients with UI, and are most commonly caused by the following: pelvic floor muscle disorders, bladder mucosa and detrusor muscle disorders, neurological diseases such as cerebrovascular stroke (CVS), multiple sclerosis (MS), dementia and Parkinson's disease (12). UI can also occur in patients with psychological disorders such as schizophrenia, depression, and anxiety, or as a side effect of certain psychiatric medications (13). UI may also be associated with the onset of fecal incontinence, constipation and anorectal pain (14). Neurological signals within multiple micturition centers and neural pathways (e.g. pontine micturition center, periaqueductal gray, pyramidal tracts), which control the response to changes in pressure within the bladder, are often insufficient in older age. This can result in the loss of voluntary and autonomous control of lower urinary tract functions (15). Risk factors for SUI in women are the number of childbirths, injuries to the organs and muscles of the pelvis during childbirth, episiotomies, children with high birth weight and hysterectomies (16).

The occurrence of SUI in men is associated with radical prostatectomy, because of the damage inflicted to the neural structures, the intrinsic urethral sphincter, and muscles and ligaments of the pelvic floor (12). Indications for hysterectomy and prostatectomy can be various benign and malignant diseases of the uterus or prostate. Annually, there are 170,000 new cases of prostate cancer and 60,000 new cases of uterine cancer in the USA (17, 18). In case of a localized prostate cancer, the therapeutic methods of choice are a radical prostatectomy or primary radiotherapy with curative intent. Depending on the surgical method, experience, and skill of the surgeon, the incidence of UI after a radical prostatectomy can range up to 40% (19). In most cases, these men have SUI, but they can also have MUI with a previously unrecognized UUI component (20). Occurrence of UUI or MUI is very common in men and women after they undergo primary radiotherapy for prostate and uterine cancer. This is because the bladder mucosa is damaged by irradiation and becomes sensitive to changes in the volume and chemical composition of urine.

The pathophysiology of SUI includes damaged or weakened internal urethral sphincter of the bladder neck and pelvic floor muscles that support the bladder neck and urethra (16). Pelvic floor muscles are composed of deep and superficial muscle fibers and connective fibers. They participate in a very complex anatomical and physiological relationship that maintains maximum urethral closure pressure in order to prevent leakage of urine. The relaxed bladder neck and pelvic floor muscles allow the bladder to empty. In women, the maximum urethral closure pressure and functional urethral length decrease with age. Mechanisms that cause incontinence in older men are detrusor overactivity and internal sphincter insufficiency. In both sexes, bladder capacity and compliance decrease with age, while

excessive contractions of the bladder detrusor and residual urine simultaneously increase, which may cause UI (21). Increased body mass index (BMI), family history of UI and older age are also associated with the onset of UI (22). As abdominal and bladder pressure increase, the pelvic floor muscles cannot tighten sufficiently enough to hold the urine within the bladder, and, therefore, the intravesical pressure overcomes the maximum urethral closure pressure resulting in urine leakage.

Neurological diseases such as dementia, CVS and MS, and bladder diseases such as chronic cystitis and cystocele, can all cause excessive detrusor contractions with consequent UUI or hypermobility of the bladder neck and urethra with consequent SUI (23). The prevalence of dementia in European countries ranges from 6 to 18% in people over 65, and from 30 to 50% in people over 85 (24, 25). In Croatia, approximately 250 people per 100,000 inhabitants experience CVS annually (26). The prevalence of patients with MS in Croatia is 143 patients per 100,000 inhabitants (27). Some scientific studies predict an increase in the total number of CVS cases by as much as 34% in European countries in the period from 2015 to 2035, and they also predict an increase in the number of CVS survivors (28). The prevalence of Parkinson's disease in the US is 572 patients per 100,000 inhabitants (29). CVS and Parkinson's disease are more common in men, while dementia and MS are more common in women (30).

Diagnostic evaluation of urinary incontinence

In Croatia, family physicians and gynecologists play a leading role in the early identification of UI in primary care. Basic diagnostic methods that can be implemented within primary care include medical history, bladder diary, urinalysis, plain radiography and ultrasonography of the urinary tract with a focus on post-void residual (PVR) urine. After a primary care assessment, women are referred to a urogynecologist and men are referred to a urologist. Diagnostic evaluation of LUTS in urological and urogynaecological outpatient clinics includes detailed specific medical and sexual history, bladder diary, neurologic examination, prostate and genital examination in men, gynecological examination in women, and urethrocystoscopy. The causes of UI are obvious only in uncomplicated cases and a subsequently provided diagnosis is usually based on anamnestic data and a clinical examination. Some cases, however, are very complex and, therefore, a thorough and extensive assessment of UI symptoms is required in order to provide a correct diagnosis and initiate appropriate treatment. During the evaluation, it is useful to perform a urinalysis, a urine culture test and a urethrocystoscopy. Urinalysis and urine culture tests can detect urinary tract infections, hematuria, glycosuria, pyuria, crystalluria, and proteinuria. Urethrocystoscopy is mandatory because it can confirm or exclude diseases such as bladder cancer or urolithiasis. If necessary, it is advisable to refer women and men with UI to a neurourology and urodynamics specialist for further diagnostic evaluation and treatment. Urodynamics is defined as the measurement of physiological parameters relevant to the function of the lower urinary tract (1). Urodynamics in women and men commonly involves uroflowmetry with a full bladder, but without a catheter, measurement of PVR, filling cystometry, a urethral pressure profile and pressure-flow studies (31). In complicated cases, such as post-prostatectomy incontinence and complications of transvaginal mesh surgery, a videourodynamic investigation with simultaneous measurement of urodynamic parameters must be performed, along with a radioscopy of the bladder filled with contrast medium in order to gain insight into the storage and micturition phase.

Sometimes it is difficult to explain the pathophysiology of LUTS only by correlating the patient's symptoms with the results of the urodynamic studies. Additional diagnostic tools may include computed tomography (CT) of the upper and lower urinary tract, and magnetic resonance imaging (MRI) of the brain and spinal cord for purposes of detecting tumors, aneurysms, injuries or other conditions. CT and

MRI can also be used to monitor the activity and progression of the disease that caused the UI.

Treatment of urinary incontinence

Women and men are reluctant to seek professional help and talk about UI problems and related disorders such as sexual dysfunction, anxiety and depression. UI can cause a decrease in social interactions and physical activity, loss of self-esteem, depression, fear of involuntary leakage in society, avoidance of sexual relations and decreased productivity at work. In most cases, patients postpone going to the physician because they are not fully aware of the treatment options for UI, which can be very successful.

As first-line treatment, physicians most commonly use various incontinence aids and strategies to cope with UI problems such as changing their lifestyle. If incontinence aids are not sufficiently effective, patients need to change multiple pieces of the same type of aid or combine different types of aids during a 24-hour period. Because of this, the costs of UI treatment and the financial burden on the healthcare system may be increased and the quality of life (QOL) may be significantly reduced. In older women and men, the problems and costs of UI treatment are increasing. The world's fastest-growing elderly population includes persons aged 85 and over, and it will increase by as much as 12 times by 2025, while the number of people aged 100 and older will increase 15 times, i.e. from the current 210,000 to 3.2 million (32). In Croatia, in 2001, there were 693,540 people older than 65 and 42,553 people older than 85 (33). Depending on the underlying disease causing the UI and depending on the types of UI treatment, there are predictable costs of treatment (e.g. medication, surgery, and hospital stay costs) and unpredictable treatment costs (e.g. treatment of adverse drug effects and possible complications of surgery). Larger share (50-75%) of UI treatment costs is attributed to body care products, laundry detergents, incontinence pads and diapers (34). The total annual cost of UI treatment in the US is very high and in 2000 it was estimated at 19.5 billion dollars (35, 36). The most recent estimate of total annual cost of UI treatment in the USA for all age groups was over 16 billion dollars, which is higher than the annual direct cost of treating breast, ovarian, and cervical cancers altogether (37, 38). According to one study, a person with more severe UI symptoms and a greater impact of UI on QOL spends approximately 900 dollars annually for incontinence aids and body care products (39). Another study found that the daily use of UI aids of one person with symptoms of UI results in an annual cost of 1825 dollars (approximately 150 dollars per month) (39). These differences may stem from the type and effectiveness of incontinence aids, primarily in terms of their power to absorb urine. The cost of treating one patient with UUI or MUI with antimuscarinic drugs alone, without absorbents, ranges from 95 to 290 dollars per month, depending on the type of drug (40). There are no data on the costs of treating UI in Croatia, but we can assume that they are very similar to the previously mentioned data.

In case of contraindications for surgery or pharmacotherapy, a symptomatic treatment that includes only incontinence aids that have a good overall effect can reduce treatment costs and improve the QOL. Depending on the type of UI, the choice of first-line treatment includes conservative methods such as: counselling about lifestyle changes (e.g. weight control, avoiding or reducing intake of alcoholic and other diuretic drinks, reducing fluid intake during the day and before bedtime), bladder training (keeping a bladder diary, delaying the need to urinate, increasing the amount of time between urination), use of incontinence aids (e.g. pads, diapers), pharmacotherapy (e.g. antimuscarinics, mirabegron), pelvic floor muscle exercises, biofeedback, extracorporeal magnetic innervation therapy and functional electrostimulation of pelvic floor muscles. Treatment results of most conservative methods depend on the patient's motivation

and co-operation, except in the case of incontinence aids, such as diapers and pads, where treatment results depend on the absorption capacity and comfort. Treatment of SUI focuses on strengthening the supportive pelvic floor muscles by applying various conservative and surgical methods. The method of choice in UUI treatment is the stabilization of detrusor overactivity by using pharmacotherapy. In the case of MUI, it is necessary to treat the predominant symptomatology component. The most common surgical methods for SUI treatment are the periurethral injection of bulking agents, insertion of pubovaginal slings and colposuspension. If pharmacotherapy proves to be inefficient in treating UUI, either intravesical application of Onabotulinum toxin A or a sacral neuromodulation is indicated. All of the above-mentioned surgical methods include higher costs when compared to conservative methods, and they also include treatment of complications and a number of days spent in the hospital. Treatment of these complications may include the use of incontinence aids. Some patients have absolute and relative contraindications and an increased risk of complications related to conservative and surgical treatments that are otherwise recommended according to the guidelines of the European Association of Urology (EAU) and ICS (41). These include diseases such as a recently experienced heart attack, blood clotting disorders, uncontrolled arterial hypertension, heart rhythm disturbances, the presence of metal implants and heart rhythm electro-stimulators, and glaucoma. These patients can be recommended to use incontinence aids, make lifestyle changes, train their bladder, and do pelvic floor muscle exercises as part of the prescribed therapeutic method in order to help maximize the therapeutic effect. Incontinence aids can also improve the QOL of patients until a proper diagnosis of LUTS is made and treatment initiated. They can also be a permanent solution for patients who have an increased risk of developing complications during the implementation of diagnostic and therapeutic methods related to UI.

Achieving effective and discreet urinary retention with the use of incontinence aids is one of their most important features and it helps improve the users' QOL (42). The most common products used for UI symptoms in men and women are incontinence pads and diapers. They absorb urine after one or more episodes of involuntary urine leakage, depending on their power of absorption. These products protect against leakage, odour, and prevent moisture from getting onto the genital area and skin. Other products that can be used with UI patients include waterproof bed sheets and urinary condoms. Waterproof bed sheets can be washed in the washing machine and reused, but they can also be disposable. They are placed under normal bed sheets and above the mattresses, and they absorb urine. Incontinence pads and diapers have a hydrophobic layer that absorbs urine below the surface, allowing the genital skin to remain dry. They are usually a temporary measure for improving the QOL, as they provide safety and confidence during the day and help maintain a normal social life. Tampons are not recommended for use in women with UI, but they can be useful in situations, such as exercise, as their position can affect bladder neck lifting. This is due to the very close anatomical relationship of the vagina and bladder neck. Menstrual pads should also be avoided when dealing with UI issues. A lot of women use menstrual pads instead of incontinence pads. However, they do not have the same technology and, therefore, these pads remain wet and can cause chronic skin changes, contact dermatitis, and fungal infections. In patients experiencing involuntary leakage of smaller amounts of urine, incontinence pads are more comfortable to wear. In patients experiencing involuntary leakage of moderate to large amounts of urine, which occur several times a day, it is usually necessary to use diapers with a greater absorption capacity, in comparison to that of incontinence pads, or use a combination of both types of supplies. For practical reasons, mostly due to their shape and size, incontinence pads are mostly used by mobile patients who can go to the toilet on their own.

Unlike diapers, pads are more discreet as they are not noticeable under clothing and are easy to put on and remove. In most cases, diapers are used by immobile patients, with or without cognitive impairment, who cannot go to the toilet on their own and who do not need to change supplies frequently. Diapers can absorb greater amounts of urine, but because they need to be fixated with adhesive tapes, they are not practical for removing and repositioning during bladder training or for personal hygiene related reasons. Incontinence pads are more convenient to remove and reuse during bladder training, but they do not have the same absorption power as diapers, meaning that more incontinence pads need to be used during the day.

Discussion

According to EAU guidelines and World Health Organisation Guidelines on Integrated Care for Older People (WHO ICOPE), first-line of treatment for UI symptoms includes lifestyle changes, bladder training, pelvic floor exercises and incontinence aids (43-45). With the help of incontinence aids, it is possible to prevent involuntary leakage on clothing, eliminate the unpleasant odour of urine and reduce the possibility of bacterial infections and chronic dermatitis on the mucous membrane and skin of the genital region. The use of these aids is very important in patients who cannot undergo active treatment by other conservative or surgical methods due to contraindications, and in patients who refuse active treatment. It should be noted that, in most cases, these aids are used in patients who have UUI or MUI with a predominant UUI component due to the different aetiology of these types of UI. Because the onset of SUI symptoms is predictable, various behavioural strategies, such as bladder emptying before a physical activity, can help avoid episodes of involuntary urine leakage, and, consequently, the use of these aids is minimized. UUI is unpredictable because an overactivity of the bladder detrusor can occur at any time and, therefore, implementing similar strategies does not have a positive effect. One of the strategies used by patients with UUI is the mapping of toilets, meaning that they keep a record on the locations of toilets in a city, shopping centres, and public institutions. UUI patients are advised to change their lifestyle, train their bladder and use incontinence aids.

Pharmacotherapy with antimuscarinic and beta-agonist mirabegron is also indicated as first-line therapy in patients with UUI. Some patients may have contraindications for pharmacotherapy. Contraindications to antimuscarinic pharmacotherapy include acute or chronic urinary retention, digestive system diseases (e.g. toxic megacolon), myasthenia gravis, narrow-angle glaucoma, renal failure requiring haemodialysis and severe impairment of liver functions. Contraindications for pharmacotherapy with mirabegron include severe uncontrolled hypertension, defined as systolic blood pressure ≥ 180 mmHg and diastolic blood pressure ≥ 110 mmHg. If there are any contraindications for pharmacotherapy, patients will be provided with incontinence aids. They will also have to change their habits, train their bladder, and implement other behavioural strategies.

The prevalence of neurological diseases and malignant diseases of the genitourinary system is high, and they usually include UI as a consequence. Thanks to modern diagnostic and therapeutic options, the QOL of these patients has greatly improved and their life prolonged. As a result, there are more and more patients with some degree of disability and symptoms of UI. In these patients, a specific approach to treatment and rehabilitation is required in order to maintain their cognitive and motor skills and help them control the UI symptoms. Neurological diseases can cause immobility or impaired mobility, depending on their degree and the level of neurological damage, and this can cause complications such as deep vein thrombosis and decubital ulcers (44). Bladder function in these patients is often neglected, as is the fact that they often have symptoms of UI. It is very important that verticalization and mobility of these patients be restored in a timely manner through daily bladder training. It is also

important to restore their bladder capacity and increase the muscle strength of detrusors, and also to prevent other complications. If patients are immobile and spend most of their 24 hours in a supine or sitting position, the sensory and motor functions of the bladder may deteriorate. When training the bladder, patients are advised to urinate during the day while retaining urine to a certain extent before going to the toilet.

If patients have UI, they must use certain types of aids that will hold the urine until they go to the toilet. Diapers would be the most appropriate choice because they have a greater capacity to absorb urine in comparison to incontinence pads. A negative feature of diapers is that they are not as discreet as pads, because they are larger and noticeable under clothing. This can cause fear of an unpleasant odour of urine, social prejudice, anxiety, depression, decreased QOL and social stigmatization (45). Incontinence pads are more discreet than diapers, but because of their lower absorption capacity, patients usually change up to 7 pads a day (45). This inflicts a huge financial burden on the health system. UI aids of an adequate type and size are required in order to prevent the detection of UI symptoms in social situations. This is achieved by reducing the absorption effect of episodes and the volume of involuntary leakage of urine during 24 hours. In addition, they also help reduce the possibility of complications, such as skin and genital mucosal damage. All patients with UI use some kind of an incontinence aid, regardless of the type and severity of UI symptoms. There are significant differences in design, absorption capabilities and other features between UI aids. There are also significant individual differences in personal attitudes of patients with UI and in their desires for incontinence treatment. Some aids are better designed for men than for women, for example, some aids have better absorption capabilities. Depending on the type and duration of physical and social activities, UI patients usually combine several types of aids and use multiple pieces of aids, especially incontinence pads, over a 24-hourperiod. Reducing the financial burden on the healthcare system could be achieved by providing patients with better-designed UI aids suitable for different circumstances and activities for 24-hour periods. With the help of these conservative first-line treatment methods, patients can maintain an active lifestyle and improve sexual function and their QOL (46, 47). UI is a multidisciplinary problem and all physicians involved in the care of people with UI need to be well informed about the new scientific research data regarding diagnosing and treating of UI, as well as of other LUTS.

Conclusion

With the help of primary prevention measures, women and men should be educated and encouraged to seek help from a family physician, gynaecologist or urologist if they have UI symptoms. After reviewing the patient's medical history, and performing a physical exam, these healthcare professionals can determine the type of UI and begin appropriate treatment, if possible. A three-day bladder diary and various screening questionnaires, such as International Consultation of Incontinence Questionnaire – Short Form and King's Health Questionnaire, are useful in the evaluation of the type and severity of UI symptoms. Sometimes it is very difficult to identify the type of UI and it is, therefore, necessary to refer the patient to a neurourologist for a urodynamic assessment. There are many conservative and surgical treatment methods for UI. In most patients, we can achieve full continence status or a major improvement in alleviating UI symptoms so that a normal lifestyle can be maintained. There are many aids that can be prescribed to patients who have symptoms of UI, such as incontinence pads and diapers, which have different urine absorption properties. UI aids can improve the QOL until proper treatment is initiated, or they can be a permanent solution in patients with contraindications for pharmacological or surgical treatment.

Acknowledgement. None.

Disclosure

Funding. No specific funding was received for this study.

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Competing interests. None to declare.

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Review article

Factors Associated With Sleep Disorders in Patients Undergoing Chronic Hemodialysis Treatment

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Abstract

Introduction: Patients undergoing chronic hemodialysis treatment (HD patients) experience sleep quality deterioration, which is associated with lower quality of life and represents an independent predictor of mortality in HD patients. Recently, the number of research papers aimed at assessing sleep quality in HD patients has been increased, due to the fact that it is such an important factor. Thus, this study aimed to identify the main factors related with low sleep quality in HD patients by reviewing scientific literature.

Materials and methods: A search based on key words was performed in the MEDLINE database and selected scientific papers were analyzed by using the matrix method. The initial search retrieved 472 scientific articles to which certain inclusion and exclusion criteria were applied, combined with a critical analysis, resulting in the selection of a total of 48 papers. Factors related with low sleep quality in HD patients were grouped in three sub-groups, while sociodemographic and clinical characteristics showed significant impact on low sleep quality in HD patients.

Results: It has also been proven that low sleep quality is correlated with biochemical variables such as inflammation factors, low albumin levels and other factors, whereas knowledge concerning factors involved in hemodialysis also contributes to the efforts of medical staff aimed at improving HD patients' quality of sleep.

Conclusion: Etiology of sleep quality deterioration in HD patients is manifold, and significantly affected by psychosocial, biochemical and clinical factors.

(Pojatić Đ, Pezerović D, Mihaljević D, Degmečić* D. Factors Associated With Sleep Disorders in Patients Undergoing Chronic Hemodialysis Treatment. SEEMEDJ 2020; 4(1); 74-86)

Received: Jan 14, 2020; revised version accepted: Feb 17, 2020; published: Apr 27, 2020

KEYWORDS: hemodialysis, depression, sleep disorder

Introduction

End-stage chronic kidney disease (CKD) is characterized by glomerular filtration rate below 15 ml/min, presence of oliguria and electrolyte imbalance. End-stage CKD requires hemodialysis treatment. The hemodialysis procedure can be extremely uncomfortable and painful for patients, who tend to suffer from muscle spasms and drop of arterial blood pressure. In addition, from a long-term perspective, patients with this disease have a lower quality of life due to high incidence of depressive disorder, sleep disorder, secondary alexithymia and other factors related to hemodialysis, comorbidities and general health status of the patient.

Disturbances of sleep quality and other sleep disorders constitute a factor that significantly reduces the quality of life of this group of patients and that is associated with the incidence of depressive disorder, at the same time being an independent predictor of mortality in patients undergoing hemodialysis (HD patients) (1, 2). Prevalence of sleep disorders in HD patients is 41%-83%, depending on the results of the conducted research. In addition, etiology of sleep disorders is manifold and complex (3). Sleep disorders are classified according to their most important features. We thus distinguish the following sleep disorders: insomnia, breathing-related sleep disorders, parasomnia, hypersomnia, disorders of the circadian wake/sleep rhythm and disorders related to movements of extremities (4). Breathing-related sleep disorders, which encompass both obstructive sleep apnea and disorders related to movements of extremities such as restless legs syndrome, are significantly more common in HD patients than in the general population; however, risk factors that contribute to the development of these disorders have not yet been fully elucidated (5, 6). Due to disturbances in endogenous melatonin production cycle and the intermittent character of hemodialysis, HD patients tend to suffer more from disturbances of the circadian wake/sleep rhythm (7). In their studies on sleep disorders in HD patients, majority of researchers decided to focus on sleep quality considering that it is a comprehensive and widely accepted concept and that implies both quantitative and qualitative aspects of sleeping (8). Sleep quality is a concept that encompasses duration of sleep, latency before falling asleep, sleep efficiency and the patient's subjective assessment of whether they feel refreshed after sleep. For this reason, the majority of authors use instruments for assessment of the quality of sleep in their studies (8).

The objective of this paper was to conduct a review of medical literature and determine the impact of socio-demographic, clinical, laboratory- and hemodialysis-related factors on deteriorated quality of sleep in chronic hemodialysis patients.

Methods

The searched scientific database was MEDLINE and the search was performed by using the key words „HD patients" and „sleep quality". Studies that did not contain these key words in their title or in the abstract were excluded from the search. Literature search included original scientific papers, qualitative research and case reports, whereas results of scientific reviews or letters to the editor were not the subject of analysis. Most of the articles were published over the course of last ten years, including some dating back even earlier than that, which were included due to the fact that they provided significant knowledge. This resulted in the analyzed period being the period from 1999 to 2019. Articles that focused on the pediatric population and therapeutic procedures for treatment of sleep disorders unrelated to hemodialysis (except new knowledge about melatonin), as well as articles that were not published in English, were excluded from the analysis, as were articles focused on analyzing the quality of sleep in transplantees and peritoneal dialysis patients.

Results

Database search based on the above-mentioned key words and the analyzed time period retrieved 472 scientific articles. After application of the mentioned elimination criteria and exclusion of scientific reviews, analysis was reduced to 93 articles. Based on a detailed examination of the titles and abstracts of the articles against the objectives of this scientific review, 48 articles were identified as suitable, 17 of which were free-access articles, whereas 31 articles required access authorization. Final analysis included 48 studies published in the period from 1999 to 2019, 35 of which were cross-sectional, 4 were prospective studies, 8 were randomized clinical trials and one was a qualitative study. Results of the studies listed provide insights into the recent discoveries regarding correlation between sleep quality in HD patients and laboratory variables, levels of depression and alexithymia, factors related with hemodialysis, as well as regarding the impact of specific sleep disorders, such as restless legs syndrome, on sleep quality in HD patients (Table 1)..

Table 1. Factors associated with low sleep quality in HD patients

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A) Psychosocial factors	Alexithymia	It implies difficulty distinguishing between feelings and bodily sensations; in HD patients, it predicts low sleep quality independently of depression.
	Depression	Depression reduces sleep quality; prevalence of depression among HD patients is four times higher compared to general population.
B) Sociodemographic factors	Female gender, old age, low physical activity, high body mass index	These factors are related to decreased sleep quality in HD patients independently of depression.
	Poor financial status, unemployment	Poor financial status and unemployment impair sleep quality.
C) Biochemical factors	CRP, IL-6, TNF-alfa, IL-1beta	Sleep disorders in HD patients are associated with high levels of proinflammatory and low levels of anti-inflammatory cytokines.
	Decreased appetite, low serum albumin and creatinine levels	Researchers assess nutritional parameters together with inflammation using the Malnutrition Inflammation Score, which is an independent predictor of low sleep quality in HD patients.
	Secondary hyperparathyroidism, hyperphosphatemia	High PTH, phosphate and calcium levels impair sleep quality in HD patients.
	Loss of circadian rhythm of melatonin secretion	HD patients have a disrupted melatonin rhythm; administration of exogenous melatonin improves sleep quality.
D) Factors related to hemodialysis procedure	Dialysis shift and relative hyperhydration	High relative hyperhydration causes sleep disorders in HD patients; patients treated in the nocturnal shift have better sleep quality.
	Presence of tunneled central venous catheter	These HD patients more often have muscular cramps and uremic pruritus, which causes low sleep quality.

Assessment of sleep disorders in HD patients was performed by using the Pittsburgh Sleep Quality Index (PSQI), Epworth Sleepiness Scale (ESS), the Berlin Questionnaire and the STOP Bang Questionnaire for measuring obstructive sleep apnea, RLS and IRLSSG questionnaires for assessment of restless legs syndrome severity, Insomnia Severity Scale (ISS), CHEQ (Choice Health Experience Questionnaire), Holland Sleep Disorders Questionnaire, Hatoum's Sleep Questionnaire, and Athens Insomnia Scale (AIS).There were two studies that used polysomnography and two that used actigraphy. Three of the studies analyzed different sleep variables by using individual questions, which represents a limitation of those studies.

As far as assessment of depressive symptoms is concerned, researchers used the Beck Depression Inventory (BDI-I and II), the Center for Epidemiologic Studies Depression Scale (CES-D), the Hospital Anxiety and Depression Scale (HAS-D), the Taiwanese Depression Questionnaire (TDQ), and the Patient Health Questionnaire (PHQ-2).

For assessment of alexithymia, the Toronto Alexithymia Scale-20 (TAS-20) was used.

The final result of this integrative scientific literature review was the formation of three sub-topics in this context, one being recent discoveries in the correlation between depression, quality of life and quality of sleep and the other two being laboratory variables and hemodialysis-related variables..

Discussion

Correlation between depression, alexithymia, socio-demographic factors and sleep disorders in HD patients

Alexithymia is a construct, a stable personality trait that implies difficulty distinguishing between feelings and bodily sensations, difficulty describing feelings to other people, constricted imaginal processes and a cognitive style of thinking that is oriented towards external stimuli rather than feelings (9). Prevalence of alexithymia is higher in HD patients than in the general population, as high as 30%, this being a cause of their significantly reduced life quality (1). Alexithymia is a strong predictor of mortality in HD patients, regardless of age, sex or possible depressive disorders. Patients suffering from alexithymia had a 4.29 % higher mortality rate at later stages of the five-year monitoring period (1). The way alexithymia affects sleep disorders has not been sufficiently elucidated; there have only been a few studies dealing with this issue on samples of HD patients and it has long been unclear whether the impact of alexithymia on sleep quality deterioration was indirect or direct. Gennaro et al. conducted a large cross-sectional study on a population of students and reached a conclusion that alexithymia was a confounding factor and that if depression is included in the statistical models, its impact on the quality of sleep disappears (10). Researchers that analyzed the same issue on a sample of HD patients confirmed the impact of alexithymia on poorer quality of sleep, but on a sample of HD patients, this impact was not correlated with depression. In particular, De Santo et al. used a sample of 40 HD patients who underwent parathyroidectomy due to high PTH levels and who were found to suffer from high intensity depression, alexithymia and sleep disorders. Their study showed that the impact of alexithymia on sleep disorders was not related with levels of depression. However, the study was limited by a small number of subjects, which represents a weakness inherent in the study (11). Although research into the correlation of alexithymia and biochemical variables in HD patients is scarce, it has been confirmed that alexithymia, or rather its component in the form of external stimuli-oriented cognitive thinking, represents an independent predictor of low phosphorus levels in HD patients, which are in turn related with poor quality of sleep (12).

Depressive disorder is a mood disorder defined as a feeling of sadness, unhappiness, loss of will and motivation that lasts for more than two weeks. It is one of the most common mental

disorders, whereas sleep disturbance is one of the most prominent symptoms of depressive disorder (13). Based on available discoveries, prevalence of depression in HD patients is almost four times higher than in the general population and amounts to between 43% and 72% (14). Depression has a significant impact on sleep disturbances in patients receiving chronic hemodialysis treatment, it considerably reduces the quality of life and is an independent predictor of patients' mortality (1, 15, 16). Social support has a preventive effect when it comes to depressive disorder and improves the quality of life of HD patients (17). According to the study carried out by Pan et al. , the form of social support that includes analytical appraisal of virtues and positive personality traits of such patients reduces the impact of disturbed sleep quality on life quality and depression in HD patients (17). In their cross-sectional study, Maung et al. found that depressive HD patients tend to suffer more frequently from daytime sleepiness (measured using ESS), lower sleep quality, restless legs syndrome as well as from sleep-related difficulties the night after receiving hemodialysis treatment (15). Latest discoveries point to the importance of additional factors that modify this two-way interaction. According to the results of recent research, sleep disturbances are significantly more frequent in women than in men receiving chronic hemodialysis treatment (18- 20). Only one study found higher incidence of low quality of sleep in the male sex, but the study was conducted on a sample of Malaysian subjects, so it is possible that such result was caused by culturological and racial differences (19). Scientists have also considered the role of depression as a confounding factor in this relationship because it is more frequent in women; however, recent studies have identified female sex to be an independent risk factor when it comes to development of sleep disorders during hemodialysis treatment (14, 21). It is interesting to note that according to the PSQI, the age of HD patients also represents an independent risk factor for sleep disorder. However, Lin et al. found that this influence disappears if monthly pecuniary income of respondents is taken into account. It is possible, as the authors indicated, that the impact of age was dependent on financial status, as financial status drops significantly in the population of retirees, so this is something that should definitely be taken into consideration in future studies (21).

Financial status and unemployment were defined in two studies as the factors that contribute to poor sleep quality in HD patients (23, 24). Tomita et al. found that duration of hemodialysis treatment could also result in poor sleep quality measured by the PSQI questionnaire. Specifically, patients who have been undergoing dialysis for more than five years exhibit more difficulty in functioning during the day as a result of daytime sleepiness, which is an element measured by the PSQI questionnaire, whereas independent analysis shows the correlation between advanced age and sleep disorders, regardless of the levels of patients' depression (24).

Lower physical activity of HD patients has an independent negative effect on the quality of sleep. Namely, using a large sample of 1678 patients undergoing peritoneal dialysis and hemodialysis covered by CDS (Comprehensive Dialysis Study), Anand et al. found that lower physical activity is more frequent in depressive patients, but also that independent of depression, low physical activity functions as a factor that causes poor sleep quality (25). Apart from lower physical activity, studies have shown that BMI (Body Mass Index) is another factor that negatively affects the quality of sleep in HD patients (27, 28).

It is a well-known fact that depression has a seasonal character and that depressive symptoms in patients suffering from depressive disorder as well as depression-related insomnia or hypersomnia tend to become worse during certain seasons. According to the results of Afsar et al., seasonal remission of depression symptoms is not accompanied by an improved quality of sleep. Authors claim that remission of depression symptoms can be perceived only in non-smoking patients and in those with

increased levels of serum albumin during the summer (28).

Many research studies have been conducted on the impact of laboratory variables on sleep disorders and depression in HD patients and most of them concluded that laboratory variables have a stronger impact on depression than on sleep disorders, which is logical since depression is a disorder caused by biochemical imbalance of neurotransmitters, whereas sleeping can be affected by many different socio-economic factors (14). Afsar et al. established that aggravation of chronic kidney disease is accompanied by higher cortisol levels and that these increased cortisol levels in turn cause higher depression frequency. However, this factor does not cause sleep disorders (29). Depression in HD patients is connected with elevated levels of phosphorous, low levels of creatinine and hemoglobin, and low albumin levels, which serve as indicators of their nutritional status (21, 22, 30) .

When discussing disturbances of sleep quality and depression in patients, we must not disregard religious feelings and beliefs, which have been identified, in the context of general population, as factors that help patients to overcome stress even when other anti-stress mechanisms falter. Eslami et al. proved that a high level of spiritual well-being and religious feelings have an independent and positive effect on sleep quality in HD patients (30).

Effect of biochemical variables on sleep disorder in HD patients measured using PSQI

When talking about recent discoveries, one needs to mention the correlation between sleep disorders and inflammatory mediators, which has been confirmed by results of many studies. The factor that all of these studies focus on is the relatively nonspecific but highly sensitive C-reactive protein (CRP), the level of which many cross-sectional studies found to be significantly higher in HD patients suffering from sleep disorders (28, 32). The study conducted by Chiu et al. reveals that the level of pro-inflammatory cytokine IL-1beta, which is responsible for triggering inflammatory responses in the human body, is significantly increased in patients who are undergoing chronic hemodialysis treatment and who are suffering from sleep disorder, and that there is a correlation between the levels of other pro-inflammatory cytokines such as TNF-alpha or IL-6 and deteriorated sleep quality (32). It is possible that elevated pro-inflammatory mediators are the consequence rather than the cause of sleep disorder because, as specified by Chiu et al., sleep deprivation may cause a temporary increase in arterial blood pressure, which then triggers inflammatory response and the synthesis of CRP in the liver cells (32). Results obtained by the group of researchers led by Taraz confirm the pro-inflammatory theory, albeit with reference to lower levels of anti-inflammatory cytokine IL-10 in patients suffering from sleep disorder; however, the importance of this study is limited by the small number of respondents (33). It is possible that lower vitamin D level found in HD patients is the cause of higher levels of pro-inflammatory cytokines TNF, IL-6 and IL-1 as well as lower sleep quality; nevertheless, it is also possible that there is an independent mechanism by which vitamin D affects vitamin D receptors in areas of the brain regulating sleep (34). Conversely, CRP levels represent a more trustworthy proof of the importance of inflammation when it comes to sleep disturbances, because CRP levels are stable during the 24-hour period, whereas cytokine secretion reaches its maximum level according to its specific circadian rhythm (34). The study carried out by Razeghi and Ali included 108 HD patients and revealed that patients who had CRP levels above 3.8 ug/L were more prone to suffer from poor sleep quality according to the Pittsburgh Sleep Quality Index (PSQI), including insomnia, restless leg syndrome (RLS) and obstructive sleep apnea syndrome (35). However, it is necessary to emphasize that several studies did not reveal any kind of correlation between CRP and pro-inflammatory cytokine levels on the one side and poor sleep quality on the other (36).

Another factor that is closely related to inflammatory components and their indirect or direct impact on sleep quality is the nutritional status of patients suffering from end-stage chronic kidney disease. (37). The patient's nutritional status and loss of appetite are closely related to dietary restrictions for HD patients, mental condition, age as well as to the level of inflammatory components. For this reason, some researchers assess these parameters together using Malnutrition Inflammation Score (MIS) (31). MIS and albumin level represent independent predictors of poor sleep quality in HD patients. Research focusing on albumin serum levels obtained the most reliable results, which almost always point to inverse correlation between albumin levels and sleep quality (28, 35, 39). Correlation between low albumin levels on the one side and shorter sleep duration and lower sleep efficiency one the other has also been proven by polysomnography (PSG), which is a gold standard in sleep disorder research (39). Dilek Ongan found that, according to their scale, patients with moderate appetite were exposed to 3.26-fold higher risk of poor sleep quality, whereas patients with bad appetite were exposed to 4.2-fold higher risk of developing sleep disorders (40). An additional confirmation that nutritional status is associated with sleep quality is strong correlation between creatinine levels and sleep quality in the examined groups of patients, considering that the serum creatinine level is a consequence of good appetite. In addition, Dilek Ongan revealed a positive effect that intake of vitamins B1, B6 and B9 has on sleep quality (40). Only a very small number of studies concluded that a patient's nutritional status has no impact on the issue in question (34, 37).

Previous studies found that metabolic acidosis and venous bicarbonate level are associated with higher frequency of obstructive sleep apnea episodes in samples of population not suffering from chronic kidney disease, whereas results of the study conducted on a sample of HD patients confirmed an independent impact of venous bicarbonate and PH levels on poor sleep quality measured by PSQI (38).

Restless legs syndrome (RLS), which is significantly more common in HD patients, is an independent predictor of sleep disturbances, whereas its occurrence is also correlated with higher levels of CRP and white blood cells (41). Patients with RLS have an uncontrollable urge to move their lower extremities due to an uncomfortable feeling in their legs. These symptoms occur during the night when patients are motionless. RLS is not caused by other pathological conditions such as diabetes. The uncomfortable feeling is experienced before the person has moved his or her legs (42). It has been found that homocysteine may potentially be the cause of RLS, considering that the levels of this amino acid are higher in RLS positive patients, provided that the portion of homocysteine bound to serum albumin is taken into account (43).

Loss of circadian rhythm of melatonin secretion is an abnormality detected in HD patients, which clearly has an impact on sleep quality; however, the impact-related mechanisms have not yet been thoroughly examined. Application of melatonin therapy in a randomized controlled clinical trial carried out by Nejad et al. proved that exogenous melatonin administration had a positive impact on sleep quality, sleep efficiency and contributed to lower disturbances during sleep in HD patients with previous sleep disorders (44). It is well-known that application of melatonin during twelve months causes resistance to treatment, lower sleep quality, impaired endogenous melatonin secretion and loss of circadian rhythm of secretion (45). It has also been observed that application of melatonin in a study conducted by Nejad et al. reduced the quantity of erythropoietin required to correct the hemoglobin value, which was also correlated in some studies with poorer quality of sleep in HD patients (46). As early as in 1999, Peterson found, by polysomnography, reduced periodic limb movements and sleep fragmentation as well as total sleep prolongation and improved daytime alertness in a sample of patients suffering from chronic kidney disease whose anemia was successfully corrected by application of short-acting

erythropoietin (47). According to more recent studies, the impact of anemia on sleep quality is questionable. There are many studies that failed to prove the correlation between lower hemoglobin levels and sleep quality, which is most likely the result of the fact that this group of studies did not take into account application of erythropoietin as a confounding factor (18, 32). That such studies are nevertheless carried out is justified by the fact that some researchers have identified anemia as an independent cause of nocturnal sleep fragmentation and daytime sleepiness in HD patients (33, 35).

Secondary hyperparathyroidism is a consequence of end-stage chronic kidney disease because the body uses higher levels of parathyroid hormone (PTH) in order to adapt to the initial hypocalcemia, which is the result of lower vitamin D synthesis and hyperphosphatemia (48). Hyperparathyroidism in end-stage chronic kidney disease is accompanied by high levels of calcium and phosphorous. It seems that PTH is the cause of sleep disorders, depression and alexithymia in patients only when the value of PTH is particularly high and cannot be treated by pharmacological therapy (11). De Santo et al. proved lower PTH levels and ameliorated depression, alexithymia and sleep disorders after parathyroidectomy, after having studied a sample of 40 HD patients (49). Studies that examined PTH levels using a sample of patients receiving pharmacological therapy obtained contradictory results when it comes to the effect of PTH levels on sleep quality. Namely, Nejad et al. identified significantly lower levels of intact PTH in the group of patients with sleep disorders, whereas Taraz et al. identified significantly higher levels (34, 51). Patients suffering from restless legs syndrome, which has already been identified as a predictor of sleep disturbances, also have higher levels of parathyroid hormone (43). PTH has a direct impact on brain tissue. In a study in which magnetic resonance imaging was used, Ma et al. found a decreased N-acetylaspartate to creatine ratio in the thalamus of HD patients, which is significantly associated with the level of parathyroid hormone and patients' PSQI score (51). N-acetylaspartate is the marker of axonal stability in brain tissue and its decreased ratio in comparison with the ratio found in patients not suffering from chronic kidney disease is a sign of axonal injury. Discoveries concerning the impact of ionized phosphorous are unique and the impact of calcium levels on sleep quality deterioration remains unclear. Hyperphosphatemia is a clear factor and predictor of low quality of sleep (20, 35, 37).

Impact of factors related to characteristics of hemodialysis treatment on deteriorated sleep quality

The fact that hemodialysis shift may impact sleep quality and circadian rhythm alteration was examined in several studies, with conflicting and contradictory results. In their study that included 220 respondents, Wang et al. successfully proved that better sleep quality is associated with morning-shift dialysis and with already familiar sleep disturbance factors such as depression and anxiety (52). The authors made a critical remark regarding the results of their study, stating that they did not take into account napping during dialysis as a possible confounder and that nocturnal sleep quality potentially depends on this variable. However, Firoz and Hosseini did not prove either that there is a correlation between hemodialysis shift and deteriorated sleep quality or that sleep quality is affected by change of the dialysis shift (23). Undergoing nocturnal hemodialysis has certain advantages in terms of sleep duration being better in HD patients undergoing hemodialysis in the night shift than in the case of hemodialysis in two daytime shifts. Furthermore, the intensified hemodialysis program, within which hemodialysis is performed six times per week, is associated with better sleep quality. This correlation does not, however, show statistical significance (36).

The quality of hemodialysis treatment is defined by the dose of hemodialysis required by patients with end-stage chronic kidney disease in order to ensure that their mortality and life quality parameters are the same as those of patients not suffering from chronic kidney disease (54). It is clinically defined as urea clearance passing through the dialyzer multiplied by the duration of an individual

hemodialysis session, divided by urea distribution volume in the body (54). Urea distribution volume is total water volume in the body less ultrafiltration volume in an individual hemodialysis session. Recent studies have not succeeded to indicate a correlation between the quality of hemodialysis and deteriorated sleep quality (23, 56). In their study, Unruh et al. divided patients into daily and nocturnal hemodialysis shifts during a one-year period and at the end of this period they found sleep quality to be higher in the nocturnal group, which group also had better hemodialysis quality. However, the results were not statistically significant (54, 57).

One study proved a lower level of disturbances of sleep quality in patients who have a hemodialysis arteriovenous fistula (AVF) in comparison with patients who undergo hemodialysis using tunneled central venous catheter (55). According to the results obtained by Hilda et al., patients who undergo hemodialysis via an arteriovenous fistula are less prone to suffer from muscular cramps and uremic pruritus, which conditions are associated with poor sleep quality (55). Uremic pruritus or itch occurs as a result of higher levels of phosphorus and uremic toxins, and it is also caused by lower hemodialysis quality (2, 56).

During hemodialysis treatment, the level of ultrafiltration or the volume of fluid that is removed from the patient's body represents a special challenge. Bioimpedance is the standard for estimation of dry body weight, which in turn determines the level of ultrafiltration. Studies in which the said method was used in order to study the impact of fluid overload and low ultrafiltration on the quality of sleep revealed predominantly consistent results. Relative hyperhydration in HD patients, which is defined as surplus bodily fluids above 7%, is independently associated with lower sleep quality and daytime sleepiness (57). Abreo et al. found that relative hyperhydration has an impact on lower sleep duration, more frequent night-time waking and more difficulty returning back to sleep (58).

Studies that are focused on different forms of renal replacement therapy usually compare treatment by standard hemodialysis with hemofiltration and hemodiafiltration. As opposed to hemodialysis, in addition to the physical process of diffusion of small molecules, hemofiltration also uses conduction laws for quick flow of dialysate solution, which enables removal of larger harmful molecules (54). Hemodiafiltration is a combination of these two methods. In their cross-sectional study, Hilda et al. found that hemodiafiltration has a positive impact on disturbances of sleep quality only in male patients (55). On the other hand, in their prospective study, Gu et al. found that at the end of a two-year period, treatment using a combination of hemodialysis and hemofiltration, which took up half of the hemodialysis treatment session (HD+HF), had a positive impact on PTH, calcium, phosphorus, CRP and melatonin levels, as well as on sleep quality (59). The study also indicated that HD+HF treatment of patients is associated with an increase in the overall survival in comparison with patients who are treated only by hemodialysis (59).

Conclusion

Previous research studies focusing on the topic of sleep quality in HD patients have consistently arrived at the conclusion that depression has an independent impact on sleep quality in HD patients and that alexithymia has an indirect and significant impact on sleep quality in HD patients. Depression is a factor that is often accompanied by alexithymia and that reduces the quality of life of HD patients and it is an independent predictor of mortality. Results obtained by studies examining the impact of biochemical variables on sleep quality are inconsistent, indicate higher level of correlation with the depressive disorder and fail to provide an answer regarding various confounding factors such as anemia medications, electrolyte imbalance and PTH levels. Future studies should include a prospective approach, they should use more objective methods such as

polysomnography or actigraphy and take into account the impact of medications on the said biochemical variables. The results obtained by studies focused on dialysis and vascular access modalities lead to the conclusion that creation of an AV fistula in HD patients and usage of hemodiafiltration method are justified because of their impact on better sleep and life quality and their contribution to lower frequency of muscle cramps after a hemodialysis session. Given that sleep quality is a factor that is independently associated with life expectancy of HD patients, future studies should focus on identification of factors that reduce sleep quality by using more objective methods, such as PSG, and conducting the study during a longer time period.

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Acknowledgement.

Authors of this article wish to thank Prof Jerko Barbić, PhD, Head of Department of Nephrology of Clinical Hospital Centre Osijek, for his technical assistance in providing the information required in the making of this article.

Disclosure

Funding. No specific funding was received for this study.

Competing interests. None to declare.

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Original article

Cytogenetic Findings in Patients With Intellectual Disability/Mental Retardation and Dysmorphic Features in Eastern Croatia

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Abstract

Introduction: Numerical and structural chromosomal aberrations are some of the most common causes of intellectual disability/mental retardation (ID/MR), especially syndromic, and they represent about 10% of ID/MR that can be detected using cytogenetic methods.

Aim: The aim of this study is to show the results of cytogenetic findings in 340 patients with ID/MR and dysmorphia and/or multiple malformations in Eastern Croatia, examined at the Paediatric Clinic of the Clinical Hospital Centre Osijek and the Medical Genetics Laboratory at the Faculty of Medicine Osijek.

Methods: Cytogenetic analysis of 340 samples from patients with ID/MR and/or dysmorphia was conducted using G-banding with Trypsin/Giemsa (GTG) and fluorescent in situ hybridization (FISH).

Results: A total of 340 patients with ID/MR with dysmorphia and/or multiple malformations were referred for cytogenetic evaluation. The age range of patients was 0-18 years. The analysis included 221 boys (65%) and 119 girls (35%). A chromosomal aberration was found in 24.5% of patients. Numerical aberrations (aneuploidy) were seen in 64 patients (18.8%). The most common type of autosomal aneuploidy was trisomy 21, found in 14.7% of patients. Sex chromosome aneuploidy was detected in 2.6% of patients. Structural abnormalities were found in 6.5% of patients.

Conclusion: The results of our study show that cytogenetic analysis in patients with ID/MR should nowadays be applied when aneuploidies are suspected, since the first-line genetic test for patients with ID/MR, especially non-syndromic, is the Array Comparative Genomic Hybridization (aCGH).

(Tomac* V, Pušeljić S, Wagner J, Kos M, Arambašić N, Damašek M. Cytogenetic Findings in Patients With Intellectual Disability/Mental Retardation and Dysmorphic Features in Eastern Croatia. SEEMEDJ 2020; 4(1); 87-95)

Received: Feb 24, 2020; revised version accepted: Apr 3, 2020; published: Apr 27, 2020

KEYWORDS: intellectual disability/mental retardation, chromosomal aberrations, cytogenetic, children

Introduction

Intellectual disability/mental retardation (ID/MR) is defined as disability characterized by significant limitations in intellectual functioning and adaptive behaviour, covering everyday social and practical skills and starting before the age of 18 (1). It can be syndromic and non-syndromic. The worldwide prevalence of ID/MR is about 2.3% (2). Genetic causes of ID/MR are considered to account for 25-50% of cases (3). Numerical and structural chromosomal anomalies are some of the most common causes of ID/MR, especially syndromic, and they represent about 10% of ID/MR that can be detected with conventional cytogenetic methods (4). Major autosomal and sex chromosome aberrations often cause a number of phenotypic features, such as cardiac anomalies, infertility and growth deficiency (5). In medical genetics, cytogenetic analysis is an important source for evaluation of specific birth defects, genetic disorders, developmental delay and ID/MR (6).

Around 1,000 chromosomal disorders have been reported (7). ID/MR is one of the reasons for the referral of patients and families to genetic counselling. Identification of the causes of syndromic and non-syndromic ID/MR in a patient is very important because of the consequences it has for the prognosis, risk of occurrence in other family members, and prenatal diagnosis. Here we will summarize the result of a cytogenetic study performed on 340 patients with ID/MR and dysmorphia and/or multiple malformations in Eastern Croatia, who were referred to the Paediatric Clinic of the Clinical Hospital Centre Osijek and the Medical Genetics Laboratory at the Faculty of Medicine Osijek.

Materials and Methods

In this retrospective study we included 340 patients (221 boys and 119 girls) examined in the Paediatric Clinic of the Clinical Hospital Centre Osijek and the Medical Genetics Laboratory at the Faculty of Medicine Osijek, who have the diagnosis of ID/MR with dysmorphic features and/or multiple malformations. The evaluation included physical examination and collection of family medical history. The physical examination was focused on dysmorphological and neurological evaluation, congenital malformations, somatometric measurements and behavioural evaluations. In patients with neurological symptoms, such as epilepsy and macro- or microcephaly, neuroimaging was performed for evaluation of brain malformations. A written informed consent document was signed by the child's parent/guardian for cytogenetic testing.

Cytogenetic methods

Peripheral venous blood was collected by qualified medical staff and sent to the Medical Genetics Laboratory of the Faculty of Medicine Osijek. Blood was sampled once by venepuncture and collected into tubes with anticoagulant Na-heparin. Once received, the samples in the laboratory were identified by a unique laboratory number. Cultivation of peripheral blood cells for the purpose of karyotyping was done using a modified Moorhead method from 1960 (8). The chromosomes were analysed using a light and fluorescence microscope (Olympus BX61) and a digital camera (Diagnostic Instruments 0.7x HR070-CMT) coupled with the appropriate software (Cytovision, Applied Imaging), in accordance with European guidelines for constitutional cytogenomic analysis, European Journal of Human Genetics (2019) 27:1–16. For the purpose of the FISH analysis, in accordance with the suspected structural chromosomal anomalies, we used different locus-specific probes, whole-chromosome paint probes, arm-specific probes, centromere probes and subtelomere probes.

Results

A total of 340 patients with ID/MR with dysmorphic features and/or multiple malformations were referred for cytogenic evaluation. The age range of the patients was 0-18 years. We analysed 221 boys (65%) and 119 girls (35%). Out of 340 patients, chromosomal abnormalities were found in 76 patients (24.5%).

Numerical chromosomal aberrations (aneuploidy) were detected in 64 patients (18.8%). The most common type of autosomal aneuploidy was trisomy 21, which was found in 50 patients (14.7%). Sex chromosome aneuploidy was detected in 9 patients (2.6%). The results are listed in Table 1.

Table 1. Numerical chromosome aberrations (N = number)

Syndrome	Karyotype	N
Down syndrome, common type	47,XX,+21	24
	47,XY,+21	24
Down syndrome, translocation type	46,XY,+21,rob(14;21)(q10;q10)dn	1
Down syndrome, mosaicism and translocation type	mos 47,XX,+21,der(21;21)(q10;q10)der(21;21)(p10;p10)[71]/46,XX,der(21;21)(q10;q10)der(21;21)(p21;21)(p10;p10)[29]dn	1
Edwards syndrome	47,XX,+18	2
Patau syndrome	47,XX,+13	2
Klinefelter syndrome	47,XXY	5
Turner syndrome	45,X	2
Triple X syndrome	47,XXX	2
Klinefelter syndrome/Down syndrome	48,XXY,+21	1

Structural chromosomal aberrations were detected in 22 patients (6.5%). Patients' karyotypes and phenotypes are listed in Tables 2.a. and 2.b.

Table 2.a. Structural chromosome aberrations in patients with ID/MR (M – male; F – female)

Patient	Sex	Phenotype	Karyotype
1	F	Large neurocranium, low posterior hairline, broad nasal bridge, low-set ears, synophrys, strabismus, hypertelorism, epicanthal folds, micrognathia, irregular teeth growth, short fingers with clinodactyly. Hyperactivity	46,XX,der(2),t(2;4)(p25.1;q31.3)pat
2	M	Exophthalmos, epicanthal folds, wide nasal bridge, high-arched palate, irregular teeth growth, low posterior hairline, low-set ears, long fingers and wide thumbs	46,XY,der(1)t(10;11;1)(10pter→10p11.2::11q25→11q23::1p34.3→1qter)mat ,der(11)t(1;11)(p34.3;q23)mat,t(18;19)(q23;p13.3)dn
3	F	Microcephaly, short stature, brachycephalic head, microphthalmia, high-arched palate, hypotonia, epilepsy	47,XX,+der(22),t(X;22)(q28;q11.2)mat
4	M	Microcephaly, antimongoloid slant of eyes, microphthalmia, narrow palpebral fissures, bulbous tip of nose, small mouth and cheilognathopalatoschisis, low-set ears, micropenis, hypospadias, small hands, camptodactyly of the third and fourth finger on both hands, syndactyly of the third and fourth toe and hypoplastic second toe on the right foot. Ductus arteriosus	46,XY,del(2)(q31q33)
5	M	Flat occiput, hypertelorism. Tracheoesophageal fistula	46,XY,r(22)dn
6	M	Sharp hair, low posterior hairline, wide nasal bridge, low-set ears, clinodactyly, hirsutism. Atrial septal defect and cryptorchidism	46,XY,der(8)t(4;8)(4pter→4p16.1::8p23.1→8qter)dn

Table 2.b. Microdeletion syndromes (N = number; P = percentage)

Syndrome	Karyotype	FISH	N	P
DiGeorge syndrome	46,XX	46,XX.ish del(22)(q11.2q11.2)(HIRA-)	1	1.1%
	46,XY	46,XY.ish del(22)(q11.2q11.2)(HIRA-)	3	
Prader-Willi syndrome	46,XX	46,XX.ish del(15)(q11.2q11.2)(SNRPN-)	2	
	46,XY	46,XY.ish del(15)(q11.2q11.2)(SNRPN-)	2	1.5%
Prader-Willi syndrome – uniparental disomy (UPD)	46,XY	/	1	
Williams-Beuren syndrome	46,XX	46,XX.ish del (7)(q11.23q11.23)(ELN-)	1	
	46,XY	46,XY.ish del (7)(q11.23q11.23)(ELN-)	1	0.5%
Cri du Chat syndrome	46,XY,del(5)(p15.1)dn	46,XY,del(5)(p15.1).ish del(5)(p15.2)(D5S23-,D5S721-)dn	1	
	46,XY,del(5)(p14.2)dn	46,XY,del(5)(p14.2).ish del(5)(p15.2)(D5S23-,D5S721-)	1	0.5%
Wolf-Hirschhorn syndrome	46,XY,del(4)(p15.3)dn	46,XY,del(4)(p15.3).ish del(4)(p16.3)(WHSCR-)dn	1	
	46,XX,del(4)(p15.32)dn	46,XX,del(4)(p15.32).ish del(4)(p16.3p16.3)(D4S96-, D4Z1+, D4S3360-)	1	0.5%

Patient 1's karyotype is 46,XX,der(2),t(2;4)(p25.1;q31.3)pat. The FISH method (probes Tel2p, CEP2, WHSC1, Tel4q) shows partial monosomy 2p and partial trisomy 4q. The father's karyotype is 46,XY,t(2;4)(p25.1;q31.3). The FISH method (probes Tel2p, CEP2, WHSC1, Tel4q) shows that the father has a balanced reciprocal translocation between chromosomes 2 and 4. The mother has a normal karyotype.

Patient 2's karyotype is 46,XY,der(1)t(10;11;1)(10pter→10p11.2::11q25→11q23::1p34.3→1qter)mat,der(11)t(1;11)(p34.3;q23)mat,t(18;19)(q23;p13.3)dn. The FISH method (probes for centromere – 11, 18, subtelomere – 11qter, 18qter, 19pter, wcp – 1, 10, 11) shows an unbalanced complex chromosomal rearrangement that involves chromosomes 1, 10, 11, 18 and 19. Derivative chromosome 1 in the patient is inherited from the mother. Reciprocal translocation 18;19 is of de novo origin. This is an unbalanced karyotype with 11q25→qter deletion and 10pter→10p11.2 dupllication. The mother's karyotype is 46,XX,der(1)t(10;11;1)(10pter→10p11.2::11q25→11q23::1p34.3→1qter),der(10)t(10;11)(p11.2;q25),der(11)t(1;11)(p34.3;q23),t(13;18)(q14;p11.32)dn. The FISH method (probes for centromere – 1, 10, 11, 18, subtelomere – 10pter, 11qter, 18pter, 18qter, MCB for chromosome 1, 10, 11, 13, 18, 19, ASP – 1p, 11q, 13q, 10p, wcp - 13) shows a balanced complex chromosomal rearrangement that involves chromosomes 1, 10, 11, 13 and 18. The karyotypes of the mother's parents are normal.

The sister's karyotype is 46,XX,t(1;11)(p34.3;q23)?mat,t(13;18)(q14;11.32)mat. The FISH method (probes for centromere 10, subtelomere – 10pter, 18pter, 18qter, MCB for chromosome 1 and 11, wcp – 1, 10, 11, 13, 18) shows a balanced complex chromosomal

rearrangement that involves chromosomes 1, 11, 13 and 18, but not chromosome 10. The karyotype shows two reciprocal translocations 1;11 and 13;18. Since derivative chromosome 1 in the mother includes chromosomes 10;11;1, and in the sister only chromosomes 1;11, it is possible that mother has gonadal mosaicism. The mother and sister have normal phenotypes.

Patient 3's karyotype 47,XX,+der(22),t(X;22)(q28;q11.2)mat shows an extra chromosome. The FISH method (probes LSI N25 – 22q11.2, LSI SHANK3 – 22q13.3, CEP X-DXZ1, Xqter - MS607) shows a derivative chromosome that contains a region from chromosome 22 (22pter→22q11.2) and chromosome X (Xq28→Xqter). The mother's karyotype is 46,XX,t(X;22)(q28;q11.2)mat. She has a balanced reciprocal translocation between chromosomes X and 22, which she inherited from her mother, whose karyotype is 46,XX,t(X;22)(q28;q11.2).

Patient 4's karyotype is 46,XY,del(2)(q31q33). The mother has a normal karyotype, but we do not know the father's karyotype, so we cannot detect the origin of the chromosomal aberration.

Patient 5's karyotype is 46,XY,r(22)dn. The FISH method (chromosome region 22q11.2, 22q12, 22qter) shows deletion of the 22q13 region and de novo formation of ring chromosome 22.

Patient 6's karyotype 46,XY,der(8)t(4;8)(4pter→4p16.1::8p23.1→8qter)dn shows an unbalanced translocation between the short arms of chromosomes 4 and 8. The FISH method (probes WHSC1, CEP4, CEP8) shows segmental trisomy of the chromosomal segment 4pter-p16.1, and deletion of the chromosomal segment 8pter-p23.1. The parents have normal karyotypes.

Discussion

Numerical chromosomal aberrations are common findings that are well-defined clinically, and their prevalence is similar as in the literature. Coco and Penchaszadeh (9) reported on a cytogenetic study conducted on 200 children with ID/MR in Argentina. They found chromosomal aberrations in 21% of patients. Nasiri et al. (10) reported 23.6% of chromosomal aberrations in their study, similar as in ours. Down syndrome is the most common autosomal aneuploidy in our study (14.7%). Our results were consistent with many previous studies (11,12). We found double trisomy in one patient (48,XXY,+21). This is a very rare trisomy with the phenotype of Down syndrome, as features characteristic of the Klinefelter syndrome are not apparent until the post-pubertal stage (13,14,15). There were 0.5% of female patients with the triple X syndrome. This is a very rare syndrome; the phenotype of such women is normal, they are fertile and they have mostly intellectual problems that vary from mild intellectual disabilities, disorders in language development and problems in forming stable interpersonal relationships to severe psychiatric disorders (16). Structural chromosomal aberrations seen in our patients are variable and were found in 6.5% of patients. The study conducted by Celep et al. (17) reported structural chromosomal aberrations in 4.81% of patients with ID/MR and/or multiple congenital malformations. We compared the clinical findings in our patients with similar cases published in the literature.

Patient 1, with partial monosomy 2p and partial trisomy 4q, has facial dysmorphia and moderate ID/MR, but no urogenital and gastrointestinal anomalies or hand anomalies like the patient described in the literature. Clinical phenotypes of 2p;4q are variable because the involved breakpoints vary on a case-by-case basis (18).

In patient 2, 11q25 deletion is related to developmental delay and facial dysmorphia (exophthalmos, epicanthal folds, wide nasal bridge, high-arched palate, irregular teeth growth, low posterior hairline, low-set ears, long fingers and wide thumbs) (19,20). 10p11.2 dupllication is connected with autistic features (21), which are also present. We presume that novel reciprocal translocation 18;19 does not have an impact on our patient's phenotype, since it is related to changes caused by acute lymphoblastic leukaemia (22).

In patient 3, partial monosomy Xq28 could be related to the phenotype listed in Table 2.a., since this region contains the MECP2 gene, which causes the Rett syndrome, severe epilepsy and psychomotor delay (23). Partial trisomy 22q11.23, which is distal from the DiGeorge syndrome region of the long arm of chromosome 22, could be responsible for other features: growth delay, hypotonia, severe psychomotor delay (24,25).

The phenotype of patient 4 (microcephaly, antimongoloid slant of the eyes, microphthalmia, narrow palpebral fissures, bulbous tip of nose, small mouth and cheilognathopalatoschisis, low-set ears, micropenis, hypospadias, small hands, camptodactyly of the third and fourth finger on both hands, syndactyly of the third and fourth toe and hypoplastic second toe on the right foot) is similar to the only published case with del(2)(q31q33) (26).

Karyotype 46,XY,r(22)dn in patient 6 is known as the Phelan-McDermid syndrome, which includes hypotonia, developmental delay, dysmorphic features (long narrow head, pointed chin, ptosis, deep-set eyes, abnormalities of toes and nails) (27,28). Our patient has mild dysmorphia, psychomotor delay and tracheoesophageal fistula which has been successfully corrected, but has no abnormalities of toes and nails or other dysmorphic features typical for the syndrome. The clinical picture could be incomplete, so it would be recommended to perform aCGH.

We have compared the similarity of patient 7's features to other patients carrying only a duplication of the distal part of 4p or a deletion of the distal part of 8p or similar, which include low posterior hairline, hirsutism, wide nasal bridge, low-set ears, clinodactyly, atrial septal defect and cryptorchidism (29,30); similar features can be found in our patient.

Other structural aberrations listed in Table 2.b. have a well-defined phenotype (DiGeorge syndrome, Prader-Willi syndrome (PWS), Cri du Chat syndrome, Wolf-Hirschhorn syndrome, Angelman syndrome, Williams-Beuren syndrome). In our study, the most common syndromes are PWS (1.5%) and DiGeorge syndrome (1.1%). DiGeorge syndrome occurs in 1 in 4.000 people (31) and PWS in 1 in 10.000 to 30.000 people (32). It is interesting that we have a very similar percentage of patients with PWS and DiGeorge syndrome, since the prevalence of DiGeorge syndrome is higher than the prevalence of PWS in the general population (31,32). On the other hand, the small percentage of patients with DiGeorge syndrome in our study could be explained by its variable features. The condition may not be identified in people with mild signs and symptoms, or it may be mistaken for other disorders with overlapping features.

Conclusion

The results of our study show the prevalence of chromosomal aberrations in 24.5% of patients with ID/MR and dysmorphia, which confirms similar findings in other screened groups of numerical anomalies. The frequency of aberrations in patients with ID/MR in our study was 18.8%, and the most common aberration was DS, as seen in other studies. Cytogenetic findings of structural aberrations were 6.5%, which is also similar to other studies. The results of our study show that cytogenetic analysis in patients with ID/MR should nowadays be reserved for suspected aneuploidies, since first-line genetic testing for patients with ID/MR and especially non-syndromic patients is aCGH (33,34).

Abbreviations

MR – mental retardation;
ID – intellectual disability;
GTG – G-banding with Trypsin/Giemsa;
CTG – C-banding with Trypsin/Giemsa;
FISH – fluorescent in situ hybridization;
DS – Down syndrome;
PWS – Prader-Willi syndrome;
UPD – uniparental disomy;
aCGH – Array Comparative Genomic Hybridization.

Funding

This work was supported by the scientific project VIF2015-MEFOS-11 'Aetiology of mental retardation in paediatric population' (project leader Jasenka Wagner) at the Faculty of Medicine, J. J. Strossmayer University of Osijek.

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Original article

He or She, What Will It Be: Old Wives' Tales and Foetal Sex Prediction

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Abstract

Aim: A myriad of myths surround pregnancy, especially regarding the prediction of the sex of the infant. Some of these myths and old wives' tales are, to this day, widespread among expectant parents. The objective of this study was to examine whether common pregnancy-related statements regarding foetal sex prediction vary between mothers of female and male infants.

Methods: The questionnaire-based study was conducted from September 2017 to September 2018 at a well-baby nursery. Participants were mothers of infants (n = 350) admitted to the well-baby nursery with a singleton pregnancy and at > 36 weeks of gestation at birth.

Results: We investigated a number of statements regarding foetal sex prediction. With the exception of one statement, there were no other differences between mothers of male and female infants. Pregnancy with a male foetus is associated with glossier hair during pregnancy. Women with female newborns reported glossier hair during pregnancy in 39.1% of cases, while women with male newborns reported the same in 45.0% of cases (P = 0.04).

Conclusion: Old wives' tales regarding sex prediction of the infant during pregnancy remain myths for a reason, with the possible exception of one statement regarding glossier hair and pregnancy with a male foetus.

(Kardum* D, Kardum Ž, Nađ T, Müller A. He or She, What Will It Be: Old Wives' Tales and Foetal Sex Prediction. SEEMEDJ 2020; 4(1); 96-101)

Received: Feb 22, 2020; revised version accepted: Apr 3, 2020; published: Apr 27, 2020

KEYWORDS: folklore, parents, pregnancy, maternal nausea

Introduction

Since the beginning of recorded history, people have been continuously fascinated with pregnancy. Pregnancy is possibly associated with more myths and folklore tales than any other human condition and this fascination exists even today. A myriad of myths surround pregnancy, especially regarding prediction of the sex of the infant. Since ancient times, several tell-tale signs have been associated with the birth of a male or a female child. Hippocrates states that "women beget females if they have rough spots on the face; those who keep a good complexion beget males" (1). Some of these myths and old wives' tales are, to this day, widespread among expectant parents. The aim of this study was to examine whether common and widespread pregnancy-related statements regarding foetal sex prediction vary between mothers of female and male infants.

Methods

To investigate the popular myths regarding foetal sex prediction in pregnancy, we surveyed 350 mothers of singleton newborn infants with gestational age > 36 weeks, who were admitted to a well-baby nursery at the Department of Gynaecology and Obstetrics at University Hospital Centre Osijek, Croatia from September 2017 to September 2018. The study protocol was approved by the UHC Osijek Ethics Committee.

A questionnaire was administered to mothers of newborn infants and data on eleven common pregnancy myths were collected. In addition, they were asked two questions aimed to determine the link between maternal heartburn intensity and their perception of the infant's hair density, and one question regarding the maternal sentiment about the sex of the infants at the time they found out that they were pregnant.

Statistical anaylsis

Data were described by using descriptive statistical methods. The Chi-squared test was used to analyse the differences between proportions compare samples. All p values are two-sided. Level of significance is set at Alpha = 0.05. Statistical analysis was performed by using the statistical program MedCalc Statistical Software version 18.9 (MedCalc Software bvba, Ostend, Belgium; http://www.medcalc.org; 2018).

Results

A total of 350 women responded to the questionnaire: 179 of them were mothers of female infants and 171 of male infants. The results are shown in Table 1.

Table 1. Common pregnancy myths

Statement	Number (%) according to sex of the infant			P
	Female (n=179)	Male (n=171)	Total	
During the pregnancy, I carried the baby:				
"High"	103 (57.5)	86 (50.3)	189 (54)	0.17
"Low"	76 (42.5)	85 (49.7)	161 (46)	
During the pregnancy, my facial complexion was:				
Poorer than before	39 (21.8)	27 (15.8)	66 (18.9)	0.15
Unchanged or better than before	140 (78.2)	144 (84.2)	284 (81.1)	
During the pregnancy, I had:				
Mild or no morning sickness	124 (69.3)	120 (70.2)	244 (69.7)	0.85
Strong morning sickness	55 (30.7)	51 (29.8)	106 (30.3)	
During the pregnancy, I had:				
Mildly or moderately increased appetite	115 (64.2)	125 (73.1)	240 (68.6)	0.08
Markedly increased appetite	64 (35.8)	46 (26.9)	110 (31.4)	
During the pregnancy, I craved mostly on:				
Sweet foods	87 (48.6)	85 (49.7)	172 (49.1)	0.84
Salty foods	92 (51.4)	86 (50.3)	178 (50.9)	
At the end of the pregnancy, my belly had the shape of:				
A watermelon	92 (51.4)	80 (46.8)	172 (49.1)	0.39
A soccer ball	87 (48.6)	91 (53.2)	178 (50.9)	
During the pregnancy, I had wild mood swings, I was occasionally sad and of lower mood:				
No	83 (46.4)	78 (45.6)	161 (46)	0.89
Yes	96 (53.6)	93 (54.4)	189 (54)	
At the end of the pregnancy, I mostly woke up on my:				
Right side of the body	71 (39.7)	56 (32.7)	127 (36.3)	0.18
Left side of the body	108 (60.3)	115 (67.3)	223 (63.7)	
During the pregnancy, my hair was:				
The same as before the pregnancy	118 (65.9)	94 (55)	212 (60.6)	**0.04**
Glossier than before	61 (34.1)	77 (45)	138 (39.4)	
During the pregnancy, I gained more weight:				
On the central part of my body	104 (58.1)	93 (54.4)	197 (56.3)	0.52
On the hips and backside	75 (41.9)	78 (45.6)	153 (43.7)	
During the pregnancy, my partner:				
Gained weight	63 (35.2)	57 (33.3)	120 (34.3)	0.71
Stayed the same weight	116 (64.8)	114 (66.7)	230 (65.7)	
TOTAL	179 (100)	171 (100)	350 (100)	

*Chi-square test

We found no statistical significance between 10 popular statements regarding sex prediction during pregnancy. The only exception was a myth that states that a pregnancy with a male foetus is associated with glossy hair during pregnancy. In the group of women with female foetuses, 39.1% of them reported glossier hair during pregnancy, while in the group of women with male foetuses, 45.0% of them reported the same. The difference is significant (P = 0.04). With regard to the correlation between hair volume and heartburn intensity, 205 women described that their newborn has "a lot of hair" and 145 women described that their newborn has "scarce or no hair". Mothers of infants with a lot of hair reported severe heartburn in 45.9% of cases and those with "scarce or no hair" reported severe heartburn in 34.5% of cases (P = 0.03). Mothers accurately predicted the sex of the infant at the time they first found out that they were pregnant in 60% of the cases (57.5 % in cases of female pregnancy and 62.6% in cases of male pregnancy).

Discussion

Since the first artistic depictions of the human figure in history, a collection known as Venus figurines, some of which are perceived as representations of pregnant women (2), we have been witnesses of the continuing fascination with pregnancy. This interest in pregnancy has not diminished even today, which is clearly reflected in the fact that a Google search of the term "foetal sex prediction myths" yields more than 14 million search results.

There have been several studies conducted in an attempt to validate some of the folklore tales and myths surrounding pregnancy. Hsu et al (3) found that women with severe hyperemesis gravidarum were more likely to have female foetuses. The authors propose a mechanism of action in which hyperemesis gravidarum is caused by the female foetal effect on hCG concentrations. This finding was first reported by Schoeneck et al (4). They found that the concentration of gonadotropic hormone in the urine of pregnant patients who present symptoms of nausea and vomiting was increased, compared to pregnant patients who are free from these symptoms. Later studies confirmed these findings (5-7).

Another common myth is that severe morning sickness is associated with female sex of the foetus. In our study, we found that severe morning sickness was found in 30.7% of women with female foetuses and in 29.8% of women with male foetuses. The difference was not statistically significant, but some studies have so far reported that women presenting with morning sickness in the first trimester are more likely to give birth to a female infant (8, 9).

It is a common myth that cravings for sweet food are associated with pregnancies with female foetuses. A similar number of pregnant women with both male (49.7%) and female (48.6%) infants craved sweet food. This difference is not significant. The shape of the belly, carrying the baby "low" or "high" and distribution of additional weight gain during pregnancy were not statistically significant in pregnancies with female and male foetuses.

Perry et al (10) investigated some beliefs associated with predicting foetal sex (e.g., whether a woman is carrying her foetus in front or across the hips, prevalence of morning sickness) and concluded that these were ineffective. Ostler et al. (11) investigated the predictive value of three common pregnancy myths (foetal heart rate test, the Chinese calendar test, and the Draino test) and found that these tests had no value in terms of sex prediction.

Among other myths, we investigated a common myth stating that if the partner of an expectant mother gained weight (the so-called "chubby hubby" test), that this is indicative of a pregnancy with a female foetus. Interestingly, 34.3% of male partners of expectant mothers gained weight. However, no statistical difference was found in mothers of male or female infants.

Of the 11 investigated myths, the only myth that produced a significant difference between mothers of male and female infants is the one

stating that mothers of male infants report higher incidences of glossy hair during pregnancy (45%), compared to mothers of female infants (34.1%). It is challenging to propose a physiological process, but some researchers have suggested that testosterone has a positive anabolic effect on hair growth (12), and some researchers reported higher maternal peripheral testosterone levels during the first half of the pregnancy in mothers with male foetuses (13).

Regarding the connection between infants' hair density and incidence and duration of morning sickness, some interesting results were obtained through our study. We found that women who describe their infant as having "a lot of hair" reported severe heartburn in 45.9% of cases and those with "scarce or no hair" reported severe heartburn in 34.5% of cases. The difference is statistically significant (P = 0.03). Surprisingly, this finding is consistent with Costigan et al (14) who reported a simple linear relationship between maternal heartburn severity and infants' hair volume. In addition, they have found that women who reported moderate or severe heartburn gave birth to babies with average or above average amounts of hair. Conversely, most women reporting no heartburn had babies with less than average or no hair. The authors hypothesize that individual variations in pregnancy hormone levels that have been implicated in the relaxation of the lower oesophageal sphincter and resultant reflux are also independently associated with hair growth of the foetus (14).

We found that the most common pregnancy myths had no empirically validated results. However, an interesting relationship was found between maternal heartburn intensity and maternal perception of the infant's hair density. Also, we found statistically significant results related to the myth stating that mothers with male infants reported glossier hair during pregnancy.

The main limitation of this study is that the mothers' perception related to some of the statements that were made could be attributed to the fact that these statements were examined after the sex of the newborn was already known. Consequently, in retrospect, some claims could be reinforced and others discarded (e.g. heartburn intensity, shape of the belly, etc.).

We hope that the results of this study will not diminish the enthusiasm of expectant parents related to common pregnancy myths regarding sex prediction of the foetus, especially since pregnancy folklore tales and myths play an important part in the lives of expectant parents.

Acknowledgement. None.

Disclosure

Funding. No specific funding was received for this study.
Competing interests. None to declare.

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Short Review Article

Laboratory Animal Welfare Approach in Science

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Abstract

The term laboratory animal welfare has been generating a huge amount of controversial questions in science since the very beginning. Humans use animals that are suited to their needs, be they of a psychosocial, therapeutic, official, scientific or nutritional nature. Due to their similarities to human and short life span, the percentage of rodents used for science will correspond to the percentage of similarity between our respective genomes. The era of genetic manipulation led to accelerated and unnatural manipulation of life forms, which was quite shocking for the generations of the last millennium. Yet, it is completely normal today that genetic engineering is applied to living creatures or virtually.

Biomedicine is looking for insights into mechanisms of health preservation, clarity when it comes to development of diseases, prevention of and treatment methods for diseases, researching the environment, and improving the quality of life for mankind. Science observes the phenomena of life in action, and animals change quickly, which makes them appropriate for our objectives. The sheer number of chosen animals with known parameters and bodily responses that can be used in biostatistical analysis, which are expected to help us obtain our results, calls for an approach to animal welfare in science. Alternative methods are being developed through teamwork of researchers and veterinarians in order to find methods that will overall decrease animal pain and suffering to the lowest degree possible, while increasing the possibility of valuable research contributions. Legislative background is available as a starting point and gives researchers basic guidelines for animal welfare categorization.

(Broman* I. Laboratory Animal Welfare Approach in Science. SEEMEDJ 2020; 4(1); 102-105)

Received: Sep 17, 2020; revised version accepted: Jan 7, 2020; published: Apr 27, 2020

KEYWORDS: laboratory animal, animal welfare, 3Rs

Laboratory animal welfare approach in science

The term laboratory animal welfare has been generating a huge amount of controversial questions in science since the very beginning. The idea that a being deliberately changes another being, who is at present healthy and content, into a being that is not sounds controversial as such. On the other hand, one must ask oneself if laboratory animal science is in fact the worst possible mode of animal exploitation for human purposes. The attitude depends greatly on the situation in which the thinker finds themselves and on their knowledge of the animal's actual needs, ensuring that human demands are fulfilled, factors that justify man's involvement and money that one spends on animal maintenance, care and breeding.

Animals do not need humans. If humans had never appeared on the evolutionary tree, a healthy assumption is that none of the animals would ever think: "If only there were people around!" We are the ones who need them; we have absolute control over their exploitation and we place them in isolation from others of their kind in conditions that are suited to our needs, be they of a psychosocial, therapeutic, official, scientific or nutritional nature. If we take a shortcut from synapsids (323 million years ago) to the Cretaceous-Paleogene extinction event (around 66 million years ago), which wiped out most of ectothermic/poikilothermic organisms on Earth, there was a little, primitive rodent that somehow managed to survive (1). Later on, it spread all over the Earth, evolved and, providing that we subscribe to the theory, became human. The most likely relative (Protungulatum donnae) of these ancient humans (1) had a placenta and all other parts that contemporary rodents have, as well as the rest of the animals that we share common ancestry with, which are genetically similar to us. Their bodily responses, reproduction methods, weaning, instincts, curiosity, and nutritional needs are quite comparable to ours.

A species that kept its Pleistocene form until today, and may also be a direct intact living ancestor to our own species is the endemic species, the Balkan snow vole (2). The Balkan snow vole today inhabits high-altitude areas of the Dinarides, in Croatia among other countries, and is categorized as "vulnerable" in IUCN's red list of threatened species (3). Today, as will most likely also be the case for many years to come, the percentage of small rodents used for science will correspond to the percentage of similarity between our respective genomes.

By using selection, we have gained absolute control over the majority of species, in fact over all species that were of any interest to us; it further changes according to fashion and refinement of methods, as well as upgrading of breeding methods (4, 5). The era of genetic manipulation led to accelerated and unnatural manipulation of life forms, which was quite shocking for the generation of the last millennium, yet it is completely normal today that genetic engineering is applied to living creatures or virtually.

If we follow the crossroads towards the science branch, there is a rise of multiple interested parties in search of their own object of research. Biomedicine is looking for insights into mechanisms of health preservation, clarity when it comes to development of diseases, prevention of and treatment methods for diseases, researching the environment, avoiding and preventing the spreading and development of factors that can lead to disease, improving the quality of life for mankind.

A pathological state is any state that differs from the homeostatic state, which would fit the age, gender, psychophysical factors and uninhibited possibility of movement, with the ability to fulfill the basic needs of life. Science requires living animals in order to understand the mechanisms that are preventing those differences. In order to discover as many reasons behind such differences as possible, one must understand the functions of various mechanisms, as well as the integration of said mechanisms on different levels. Molecular

mechanisms are constantly in an active state of integration, power and separation in certain cycles, influences and feedbacks. Scientists stick to the parameters that they can measure and explain by the known laws of physics, biology and other branches of science that observe the phenomena of life in action. By observing life in action, researchers develop methods as tools to prove the accuracy of their hypotheses, which proof also has to be reproducible and applicable to certain mechanisms or groups of mechanisms that are present in their current field of work. By developing methods, scientists produce results, or more accurately, enhance the clarity of further control and manipulation of changes, which can have a larger or smaller impact on the welfare and sustainable development of humanity.

Laboratory animals are placed in the category of materials used in biomedical research. Studying their body mechanisms and inducing controlled changes in them by using different methods leads to various scientific contributions. The choice of certain species, which are chosen by selection methods or by direct influence on their genome, is described in research protocol. Competent bodies and institutions evaluate material, financial institutions decide whether and to which degree obtained results are worth their while. If they are, the project is approved. Justification for extracting the animal from nature (it does not have to be a green or snowy picturesque landscape, it could be the city sewer system, if the purpose is to collect ecological samples) calls for animal welfare in science (6). A controlled lab experiment is done with a familiar animal with a health and breed certificate and produces definite results. Methods like gene modification, drastic, fast, aim applicable or slower selective methods, number of chosen animals with known parameters and bodily responses that can be used in biostatistical analysis, are expected to give us purpose in our results.

Animal welfare can be approached in numerous ways; it has applicable and non-applicable measurements of value, it promotes various debates, inspires blogs and it's even used as a cover for terrorist attacks (7). The role of veterinary medicine does in biomedical science is to ensure that the animals are healthy and the environment is as harmless as could be at the given moment, and it tries to engage in activities with the aim of decreasing the damage in a given situation for all parties involved. This means developing methods through teamwork with competent researchers in order to find methods that will decrease animal pain and suffering to the lowest degree possible, while increasing the possibility of valuable research contributions.

The scientific and professional approach attempts to preserve the balance and the degree to which genetic modifications are used in order to achieve scientific results. The same balance is of use to 3Rs (9) (reduction, replacement, refinement in animal research), so genetics in biomedical research gets huge points in animal welfare, as well. Genetics-related research serves the purpose of developing healthcare, including prevention, diagnostics and treatment, as does the occasional funding for that scope of projects.

From now on, every scientist who declares laboratory animals as a necessary material for their work and cannot achieve their result by using known alternative methods, needs to start with research and personal progress now and consider animal welfare from the start. This offers the possibility of involvement in the right way and through proper channels, because, during future research, it will lead to the work having far better quality, direction and recognition in the end. There are unavoidable guidelines on animal welfare sites, which are being refined on a daily basis and which help researchers express the integrative plan of future research. Refined sites list legislative background to start from and give the researcher the basic starting guideline categorization.

.**Acknowledgement.** None.

Disclosure

Funding. No specific funding was received for this study.

Competing interests. None to declare.

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