Original article

Quality of Life in Patients With Malignant Disease

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Abstract

Aim: To assess the quality of life in patients with a malignant disease.

Research subjects and methods: Research included 105 patients with a malignant disease who were receiving stationary and daily treatment at the Radiotherapy and Oncology Department of the University Hospital Center, Osijek, Croatia. A questionnaire containing various demographic data and including a scale for measuring the quality of life in patients with a malignant disease – the Functional Assessment of Cancer Therapy-General (FACT-G) – was used as a research instrument.

Results: Average score on the scale was 89. Level of satisfaction with social/family relationships was significantly lower in older respondents (p = 0.027), single persons (p = 0.018) and participants with total income under HRK 3,000 (p = 0.031). Regarding family and social relationships, the patients receiving hospital day care expressed a significantly higher level of satisfaction (p = 0.001), as well as the subjects with college/university qualifications (p = 0.007). Patients with malignant disease of the head and neck expressed significantly lower levels of satisfaction on all subscales and with regard to overall health (p = 0.005).

Conclusion: Quality of life in patients with a malignant disease is satisfactory.

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Introduction

Quality of life is an extremely complex concept involving various scientific disciplines. Given the complexity and number of perspectives that the concept reflects on, it is almost impossible to provide an unambiguous definition of the quality of life (1). Its definitions vary, just as the manner in which it is assessed. Raphael et al. state that the assessment method can also influence the definition of the quality of life. Whereas a medical approach emphasizes the impact of illnesses and difficulties on the quality of life, an approach focusing on health draws attention to well-being and the abilities required in everyday life (2). Researchers agree that the concept of quality of life is multidimensional and subjective (3). Krizmanić and Kolesarić state that the quality of life is a subjective experience of one's own life determined by objective circumstances in which a person lives, personality traits affecting the experience of reality and the person's specific life experience (4). The concept of quality of life in each person relates to the experience of satisfaction with the way of life, its course and conditions, perspective, possibilities and limitations (5).

Felce and Perry define the quality of life as an overall, general well-being including objective factors and a subjective evaluation of physical, material, social and emotional well-being. It includes personal development and purposeful activity valued through a personal set of values. They also accentuate the linkage of objective and subjective indicators. It has been found that there is a weak connection between a person's subjective feeling of life satisfaction and a self-assessment of the quality of life and objective living conditions (6). A significant correlation of subjective and objective indicators has been found in situations of poverty and misery, when basic human needs are not met. Regarding life in developed countries, some authors stated that "people in the 1990s are, on average, four and a half times richer than their ancestors from the beginning of the 20th century but are not four and a half times happier" (7). Cella and Tulsky presented the four basic dimensions of the quality of life: physical, business, emotional and social well-being (8).

Patients diagnosed with cancer often experience a reduction in the quality of life to a variable degree, but they can adapt to life with cancer (9). Today, the therapeutic effects in the treatment of patients should no longer be compared and evaluated only on the basis of improvements in laboratory findings and survival of patients, but also on the basis of improvements in their quality of life (10).

Despite a great progress in the treatment of malignant diseases, the course of illness, treatment and psychological challenges have a negative impact on a patient's quality of life (11). Being diagnosed with a malignant disease is in itself stressful. Apart from excessive physical stress caused by illness and its treatment, many patients also experience psychological stress and worry about their diagnosis and prognosis, difficult treatments and decisions, as well as the change in common life functions and roles. A recent study conducted in the United States has shown that long-term cancer survival increases serious psychological difficulties among those who have been disease-free for five-years compared to the general population (12). Malignant disease can lead to major life-style changes; it can cause serious disorders in a person's normal functioning, from illness to severe financial crises (13). It has been found that 33% of individuals diagnosed with cancer experience psychological pain, whereas up to 70% of cancer patients experience some degree of anxiety and depression (14). Regardless of treatment, these individuals experience changes in their physical and emotional integrity, restlessness, pain, changes in appearance, dependence on others and loss of self-confidence, which consequently reduces their quality of life in a short time (15).

People living with malignant diseases have a greater risk of developing various psychological problems. Studies have shown that such patients suffer not only from physical symptoms of a disease, but also from psychological and social stress associated with the diagnosis.

Research has also shown that, apart from the fear of dying, patients feel endangered by interventions, such as chemotherapy or radio therapy, and they worry about losing their physical integrity, independence and social roles (16). Prolonging a patient's life has always been one of the dominating objectives of traditional medicine. As the success in this direction has increased, it has become clear that such a goal is inadequate. Medicine must strive not only to add years of life, but also help ensure the quality of life acceptable and worthy of human being for the years added (15).

Incidence of malignant diseases (cancer) varies across the world and has been changing over time. Cancer is a major public concern in the entire world. In developed countries, it is often the second leading cause of death after cardiovascular disease. According to the latest international data, 12.7 million new cases are discovered annually, 7.6 million people die of cancer, and 28.0 million people live with cancer within 5 years of diagnosis. Cancer is also the second most significant cause of death in Croatia, from which every fourth resident dies. Cancer incidence rates by the 15 most common primary sites in Osijek-Baranja County in 2013 was 472.5/100,000 for men and 383.8/100,000 for women. Also, cancer incidence rates in Croatia have been increasing (17).

The main objective of this study was to examine the quality of life in patients with a malignant disease. Specific goals were focused on the following issue: examining whether there is a difference in the quality of life in patients with malignant diseases regarding the type of malignant disease, age, gender, education level, marital status, financial situation and place of treatment (day hospital, stationary treatment).

Research Subjects and Methods

Research subjects were patients suffering from malignant diseases who were receiving stationary treatment and those treated in the day hospital at the Department of Radiotherapy Oncology, Osijek University Hospital Center. There were 105 respondents participating in the study, of whom 71 were treated in a day hospital and 34 were ward patients. Research was conducted from 18 August to 30 October 2015.

A survey questionnaire containing various demographic data (age, gender, level of education, illness, marital status, and financial situation) was used as a research instrument. Functional Assessment of Cancer Therapy-General (FACT-G) scale was used for the self-assessment of the quality of life in malignant patients. FACT-G questionnaire contained 27 particles. It evaluated the four following areas: physical well-being, social/family well-being, emotional well-being and functional well-being. Physical well-being was covered by GP particles (GP 1, 2, 3, 4, 5, 6, 7), social/family well-being was covered by GS particles (GS 1, 2, 3, 4, 5, 6, 7) and emotional benefit was included in GE particles (GE 1, 2, 3, 4, 5, 6). Functional benefits were covered by GF particles (GF 1, 2, 3, 4, 5, 6, 7). The answer for each particle was evaluated on the Likert scale of 0 – 4 (0 – not at all, 1 – a little, 2 – somewhat, 3 – a lot, 4 – very much). Overall result on the whole scale was 0 – 108. Total result for the area of physical and social/family well-being could range from 0 to 28, whereas the result for the area of emotional and functional well-being could range from 0 to 24. The assessment of the quality of life related to seven days prior to the testing day. In all areas of the questionnaire, higher results represented better quality of life (9). In a literature review, Victorson et al. (2008) reported Cronbach's Alpha reliability coefficients from about 78 published studies. Average FACT-G score reliability was .88 and subscales ranged between .71–.83 (ref). Internal consistency for the sample of participants in this research was very good (Cronbach's alpha 0.72 – 0.85) (18).

Statistical analysis

Category data was represented in absolute and relative frequencies. Numerical data was described by the median and the interquartile range limits.

Normality of the distribution of numeric variables was tested by Kolmogorov-Smirnov Test. Differences between normally distributed numeric variables between the two independent groups were tested by the Mann-Whitney U-Test, and, in the case of 3 and more groups of independent groups, by the Kruskal-Wallis Test. Due to a deviation from the normal distribution, the correlation was estimated by the Spearman coefficient of correlation ρ (rho). All p values were two-sided. Level of significance was set to α = 0.05.

Statistical analysis of the data was done by the statistical program SPPS for Windows (version 16.0, SPSS Inc., Chicago, IL, USA).

Ethical principles

Prior to the research, a written consent of the Commission for Ethical and Vocational Issues of Nurses at the Osijek University Hospital Center was obtained on 29 June 2015, as well as a written consent of the Head Nurse of the Department of Radiotherapy Oncology of the Osijek University Hospital Center.

All respondents were informed about the purpose of the research. They received a written notice for respondents, a document on consent and a statement of consent of the informed interviewee to participate in the research. They all willingly agreed to participate in the research, which they confirmed by their signature.

Research was conducted in accordance with ethical principles and human rights in research.

Results

Study was conducted on 105 respondents with a malignant disease, of whom 71 (67.6%) were treated in a day hospital, and 34 (32.4%) were patients receiving stationary treatment. Average age of respondents was 62 (interquartile range of 55-71), ranging from 33 to 88 years of age. There were more male than female respondents – 58 (55.2%). Regarding the level of education, 60 (57.1%) of respondents had vocational qualifications. There were 71 (67.0%) married respondents and 93 respondents (88.6%) reported having children. Eight subjects (8.0%) had under HRK 3,000 of monthly household income, 55 (55.0%) of them had monthly household income in the amount of HRK 3,000 to 5,000 and only two respondents had income ranging from HRK 12,000 to 20,000. The most common site of malignant disease was the thoracic region and digestive tract.

With regard to whether the subjects were being treated in a day hospital or receiving stationary treatment, the mean values of single particles and of the overall scale were equal, with the exception of social/family relationships: the patients treated in the day hospital responded significantly better – the median was 24.0 (interquartile range of 22.2 to 26.8) (p = 0.001). The mean value on the overall FACT-G scale was 89.0 (interquartile range of 73.3 to 95.5).

The lowest rating on the overall scale was given by those with a lower education level – the median of 80.5 (interquartile range of 64.8 – 94.2). Overall satisfaction was significantly higher in subjects with a college or higher education level – the median of 98.5 (interquartile range 87.1 to 101.8) (p = 0.007). Regarding the particles, these subjects were more satisfied with their physical health (p = 0.009), social/family relationships (p = 0.032), and functional state (p = 0.016), while the emotional state was equal regardless of the level of education (Table 1).

Table 1. Quality of life regarding education level

Subscales	Median (interquartile range) regarding education level				p*
	Unqualified	Vocational	College or Graduate	Total	
Physical health	21.5 (15.5 - 25.3)	23 (18.2– 26.0)	26.5 (22.1– 28.0)	23 (18.0– 26.0)	**0.009**
Social/family relationships	22.1 (17.9 - 26.2)	23 (21.0– 25.0)	25.1 (24.0 - 26.9)	24 (21.0 - 25.9)	**0.032**
Emotional state	19 (12.0– 22.0)	21 (18.0– 23.0)	22 (18.8 - 23.3)	21 (17.0– 23.0)	**0.103**
Functional state	20 (16.0 - 24.3)	21 (16.0– 25.0)	25 (21.0– 27.0)	21 (17.0– 25.0)	**0.016**
Overall FACT-G Scale	80.5 (64.8 - 94.2)	89 (74.2– 95.0)	98.5 (87.1 - 101.8)	89 (73.3 - 95.5)	**0.007**

* Kruskal-Wallis Test

Patients with malignant disease of the head and neck reported the lowest satisfaction with their physical health (p = 0.004), social/family relationships (p = 0.003), emotional state (p = 0.044), functional state (p = 0.021) and overall health (p = 0.005) (Table 2).

Table 2. Quality of life regarding site of malignant disease

Subscales	Median (interquartile range) regarding site of malignant disease					p*
	Head and neck	Thoracic region	Digestive tract	Uro-genital tract	Total	
Physical health	17.5 (16.0– 21.0)	24.5 (20.0– 26.0)	21 (15.5 - 25.0)	26 (22.8 - 27.3)	23 (18.0– 26.0)	**0.004**
Social/ family relationships	18 (16.0 - 22.2)	24 (21.0 - 26.8)	23 (21.0 - 24.7)	24.2 (22.8 - 25.4)	24 (21.0 - 25.9)	**0.003**
Emotional state	14 (12.0– 22.0)	21 (19.0– 23.0)	18 (15.0 - 22.5)	21 (20.0 - 22.5)	21 (17.0– 23.0)	**0.044**
Functional state	16 (13.0– 20.0)	22 (19.0 - 25.2)	20 (17.0 - 24.5)	21 (19.5 – 24.0)	21 (17.0– 25.0)	**0.021**
Overall FACT-G Scale	66.5 (53.0 - 81.2)	90.4 (83.8 - 97.3)	84 (72.0 - 94.9)	92.8 (84.5 - 96.3)	89 (73.3 - 95.5)	**0.005**

* Kruskal-Wallis Test

Respondents up to 50 years of age were significantly more satisfied with social/family relationships – the median of 26 (interquartile range of 23.2 to 28.0). There were no significant differences in other subscales ($p = 0.027$).

Areas of the scale have a significantly positive correlation ($p < 0.001$).

Discussion

According to the research literature, the most common cancer site was the thoracic region, which is in accordance with our research results. Most respondents had secondary education qualifications, were married and had average income in relation to the standard of our country.

The number of deaths due to prostate cancer has been increasing (19). This is also apparent from our research, which found that malignant diseases of the urogenital tract are the third most frequent diseases.

Regarding physical health, the majority of respondents, 59 (57.8%) of them, stated that they had energy. On the other hand, it is interesting to note that a large number of respondents, 24 (23.5%) of them, were quite or very much lacking in energy. Such a finding was expected considering the nature of the disease, especially of malignant diseases in lung and thoracic regions, as described in the available literature (20).

It is also interesting that patients can hardly meet the expectations of their families. This may point to an inadequate psychological assistance provided to cancer patients and their families. Psychological support for patients and their families is extremely important when they go through the shock and stress of being confronted with a diagnosis and being in the state of fear, loneliness and demoralization between therapies, as well as in critical situations when their condition worsens and prognoses are not optimistic. Support can be offered by the persons whom the patients trust and with whom they are close. Family and friends are play a vital role in providing patients with boost and help (5, 21). This is also apparent from our research, which shows that for the largest number of respondents, a great amount of support comes from their family or a person close to them, most commonly a partner. The majority of the respondents agreed that their friends were giving them a lot of psychological help and support.

Sexuality, which unfortunately receives very little attention, is a major issue for severely ill patients. Health care staff is so busy that there is no time to ask the patient about their sex life. Such discussions are often confusing for patients, or they are lacking sufficient knowledge to provide an answer to the questions asked. Most people in our community consider talking about sex life indecent. It is still a taboo topic, even in conversation with healthcare staff (22). The majority of subjects did not want to answer the question of whether they were satisfied with their sex life, while 29 of them (40.3%) responded that they were barely or not satisfied with their sex life. It is apparent from our research that a number of respondents were lacking in sexual rehabilitation, which can no longer be a matter of choice rather than a need.

Most of our respondents were sleeping well and enjoying things they do for entertainment. Compared to other researchers who have dealt with the same topic, our results did not deviate from the values of their results. The results support the fact that physical activity and the inclusion of oncological patients and their families in the community contribute to a more successful outcome of rehabilitation, the objective of which is an increase in the quality of life (5).

The overall value of the quality of life in patients with a malignant disease in our study was 89 (FACT-G), which is similar to the results of other studies (8, 5). The majority of research has shown that patients with a malignant disease have a good quality of life. Good social and family relationships certainly have a significant impact on the quality of life of our respondents.

A higher level of satisfaction with the quality of life has been found in highly educated subjects. Higher education leads to a better knowledge about the illness and the manner of coping with it, as well as to increased availability of

information about the treatment and psychological self-help. In the Martinis' research, the results have shown that men aged between 50 and 59, younger people and people with higher education qualifications are more satisfied in all areas of the quality of life (23).

Results of a research conducted on a large group of Australian women, three and twelve months after early breast cancer surgery, have shown that the impact of illness and treatment on the quality of life varies according to age, education and marital status. Single women and those with a lower education level have rated their quality of life as bad in a number of particles (24, 25). Croatian authors have also suggested that a better quality of life is associated with higher education. Higher level of education is positively associated with a greater degree of satisfaction in certain areas of life, as well as with the achievement of patients' goals, wishes and hopes (26).

The lowest score was given by unmarried respondents, especially on the social/family relationships subscale. This is understandable in terms of the impact of family and friends on caring for such patients. The same results were also described in a study conducted in Queensland, where married or cohabiting respondents had better results in the area of social-family relationships than unmarried respondents (8).

A significantly lower level of satisfaction with the quality of life was found in respondents in less favourable financial situations. Generally, it is expected that patients with a lower total income will be more dissatisfied with the quality of their life because treatment causes expenses due to which they are unable to provide everything they need for the best quality of life.

Patients with malignant disease of the head and neck are least satisfied with the quality of life. Head and neck cancer is specific because the disease itself and the surgical removal of the tumour affect the most visible parts of the body and often have an adverse effect on daily activities, such as swallowing, breathing, speech and appearance (25, 27).

Respondents up to 50 years of age are significantly more satisfied with social/family relationships. With the increase of age, the level of satisfaction with social/family relationships decreases significantly, while the connection with other particles was weak. The overall value of the scale was also higher among the youngest group of respondents. This means that older patients are less satisfied with social/family relationships, i.e. they are emotionally and functionally dissatisfied. Studies have shown that a family-oriented treatment of chronic diseases represents progress in treatment when compared to a traditional disease- or patient-oriented treatment (5). Patients want to be treated as persons with a character rather than persons with a malignant diagnosis. A person's character implies a physical, emotional, and spiritual dimension. Neglecting any of these human dimensions leaves the patient feeling empty and incomplete, which can result in a more difficult and slower recovery. That is certainly not the patient's goal nor should it be the goal of the health care staff (28).

The quality of life of patients with a malignant disease is satisfactory. There is a significant difference in the quality of life of respondents, in all areas of health, in relation to the site of a malignant disease. Patients with malignant disease of the head and neck are considerably less satisfied with physical health, social/family relationships, emotional and functional state and with overall health. There is also a significant difference with regard to the age of respondents in the field of social/family relationships. The quality of life is lowest among the respondents aged 51 to 60.

There is no significant difference in the area of health of respondents and the overall quality of life with regard to gender. There is a considerable difference in the quality of life regarding the level of education of respondents in the areas of physical health, social/family relationships and functional state. A significantly greater overall satisfaction with the quality of life was noticed among the respondents with college or higher education qualifications.

There is a significant difference on the subscale of social/family relationships with regard to the marital status of respondents. The highest rating on the overall quality of life scale was noticed in divorced respondents and the lowest in unmarried respondents, without statistically significant differences.

A significant difference exists in the quality of life of respondents in the field of social/family relationships with regard to their financial situation. The lowest rating was given by the patients whose total income amounted to under HRK 3,000. There is a significant difference regarding the place of treatment in the subscale of social/family relationships. The patients treated in the day hospital are more satisfied with the quality of life.

There is no recent data available for this geographic area on the subject of research.

Conclusion

FACT-G meets all requirements for use in oncological clinical trials, including ease of administration, brevity, reliability, validity and ability to respond to clinical change. Selecting it for a clinical trial adds the capability to assess the relative weight of various aspects of QL from the patient's perspective.

Quality of life based on the health of people with a malignant disease must be the ultimate outcome of the rehabilitation process for each patient with a malignant disease.

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Original article

Employment in Patients With Renal Replacement Therapy

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Abstract

Aim: To determine the prevalence and rate of employment of patients on renal replacement therapy (RRT) for end-stage renal disease (ESRD), to study the difference in the rate of employment between patients on hemodialysis (HD) and those with functioning kidney transplant (FKT) and to compare the rate of employment with patients' opinions about their working ability and determine the possible reasons for the presumed disproportion..

Methods: 220 RRT patients (126 on HD and 94 with FKT) at the University Hospital Centre Osijek were surveyed. We created and used a questionnaire about the level of education, occupation, employment, professional timeline during the course of RRT, personal opinion about working ability and potential reasons for unemployment. Research was conducted during April and May 2017. The data were analyzed using SPSS (version 16.0. Inc., Chicago, IL, USA).

Results: At the time of our research, 13.7% of patients on RRT were employed. Employment of FKT patients prevailed, without significant difference compared with dialyzed patients of working age (15 to 65 years old). 38.3% of patients in that age group felt capable of working. Transplantation did not improve access to employment. Highly educated people were employed more frequently. The main reasons for unemployment were poor health caused by CKD, advanced age, and employers' unwillingness to hire chronically ill persons because of the potential need to adjust working hours.

Conclusion: CKD reduced working ability and employment opportunities. Only a minority of patients on RRT were employed. Kidney transplantation did not increase the rate of employment. Patients should therefore be provided with education, appropriate guidelines and support for finding employment.

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KEYWORDS: employment; chronic kidney disease; kidney transplantation; renal replacement therapy; work capacity

Introduction

Chronic kidney disease (CKD) often develops slowly and without specific symptoms. Consequently, many patients are diagnosed in the advanced stages of the disease (1). CKD is accompanied by numerous complications: anemia, mineral and bone disorders, inflammation-induced progression of atherosclerosis, malnutrition, dyslipidemia, cardiovascular disease (CVD), infections, immune system disorders, gastrointestinal disorders, neurological disorders, etc. (2). Patients with end-stage renal disease (ESRD) are treated by renal replacement therapy (RRT) (1). RRT is performed by dialysis or renal transplantation (TX). Today, more than a million patients with CKD worldwide are treated by hemodialysis (HD). During and after the HD procedure, numerous complications such as hypotension, cramps, nausea, vomiting, headache, tingling, sweating or hypoglycemia may occur (3). Hypotension is one of the most common complications (4). TX is the best protocol for treating ESRD (5). Successful kidney TX improves quality of life (QoL) and reduces the risk of death for most patients, when compared with the risk present for patients on dialysis (6, 7, 8). The term "health-associated QoL" has been in use (HRQOL, Health-Related Quality of Life) for several decades. This term encompasses the effect of health, i.e., of the illness and of treatment for the illness on the patient's physical, cognitive and social functioning. CKD significantly affects QoL. HD is a complex procedure that requires frequent visits to the dialysis center, usually 3 times per week, which takes time and necessitates changes in the lifestyle. Therefore, HD patients have impaired QoL, both from symptoms of ESRD and from the psychological and physical burdens of HD. Patients who develop ESRD are in poor physical condition, they develop anemia, get tired quickly, often have sleep disorders, and develop depression (9, 10). Although QoL cannot be measured directly, QoL questionnaires are designed according to determinants that are important to the patients. QoL questionnaires survey the physical and mental condition and the characteristics of the primary renal disease which affect QoL. Studies using such or similar questionnaires have shown that QOL of HD patients is significantly worse than that of the general population, especially in regard to the physical component of HRQOL (9). TX, the best form of RRT, improves QoL, but it could be hard to find a suitable graft and to have the graft successfully accepted (11, 12). Likewise, lifelong immunosuppressive therapy after TX carries risks of infection, malignancy, and CVD. Working ability is considered a significant indicator of well-being and health (13). Although patients have significantly improved health after TX, in practice, transplanted patients frequently face unemployment. We thus presumed that treatment of CKD with dialysis or TX prevents patients from gaining employment and that kidney TX, as the best form of RRT, does not improve employment access for patients. The aims of the study were to determine the prevalence of employment of patients on and during the course of RRT, to evaluate whether there was a difference between the prevalence of employment for patients on HD and those after TX, to compare employment with patients' opinions about their working ability, and to establish the possible reasons for the presumed disproportion.

Patients and Methods

The research was conducted at the Department of Nephrology of the Clinic for Internal Medicine at the University Hospital Centre Osijek during April and May 2017, with approval of the Ethics Committee for Research at the Faculty of Medicine, Josip Juraj Strossmayer University in Osijek, Croatia. 220 patients were selected as the subjects of our study: 126 were treated by HD and 94 by functioning kidney transplant (FKT). At the time of our research, the sample included cooperative patients that could and did fill in the questionnaire (the initial eligible group consisted of 150 HD and 200 FKT patients). Out of 126 patients who were treated by HD during the research period, 12 had undergone a kidney transplant. 133 (60.5%) of all participants were male and 87 (39.5%) were female. The median age was 63, ranging from 31 to 88 (IQR 52 – 71). 80 (63.5%) of the 126 participants treated by

dialysis were male and 46 (46.5%) were female. The median age of patients on HD was 69, ranging from 32 to 88 (IQR 59 – 76). Of 94 patients with FKT, 53 (56.4%) were male and 41 (43.6%) female. The median age of patients with FKT was 57, ranging from 31 to 78 (IQR 50 – 63). 120 patients were of working age (15 – 65 years old). Chronic glomerulonephritis and diabetic nephropathy were the two most common causes of renal failure in patients on RRT. The third most common basic renal disease was arterial hypertension, followed by interstitial nephritis and autosomal dominant polycystic kidney disease. Patients were asked to fill in a questionnaire about their formal education, occupation, employment, professional timeline during the course of RRT and possible reasons for unemployment. The questionnaire was specifically created for our study by the researcher and it included data on finding employment and carrying out work along the following timeline: before CKD diagnosis, after CKD diagnosis, prior to starting dialysis, a year subsequent to starting dialysis, before TX, a year after TX and at the time of the research (Figure 1).

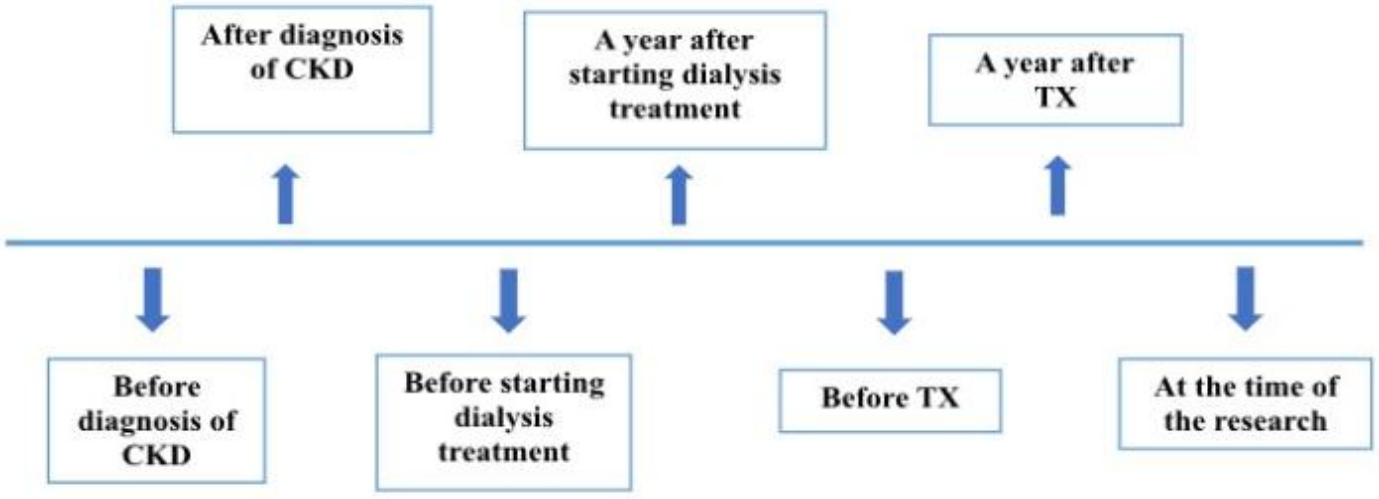
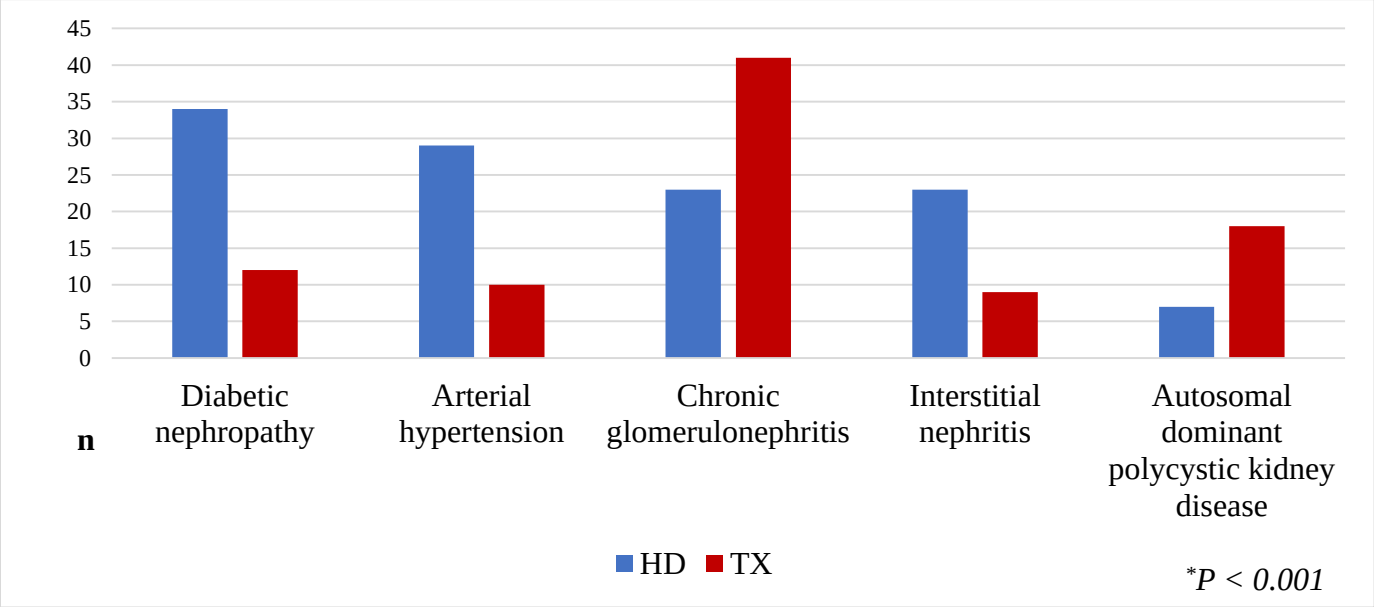


Figure 1. Time points for which the participants were asked about their employment

Demographic data, time data about the dialysis and TX, as well as data about the primary renal disease and concomitant morbidity were taken from medical records. Data were statistically analyzed using SPSS (version 16.0. SPSS Inc., Chicago, IL, USA). Descriptive statistics included the median with interquartile range (IQR) for numeric data. Absolute and relative frequencies were used for nominal data. Differences between two unrelated groups were obtained by the Chi-Square or Mann-Whitney Test, depending on the data type. Marginal Homogeneity was used for the difference between two related samples. Statistical significance was accepted if P was < 0.05.

Results

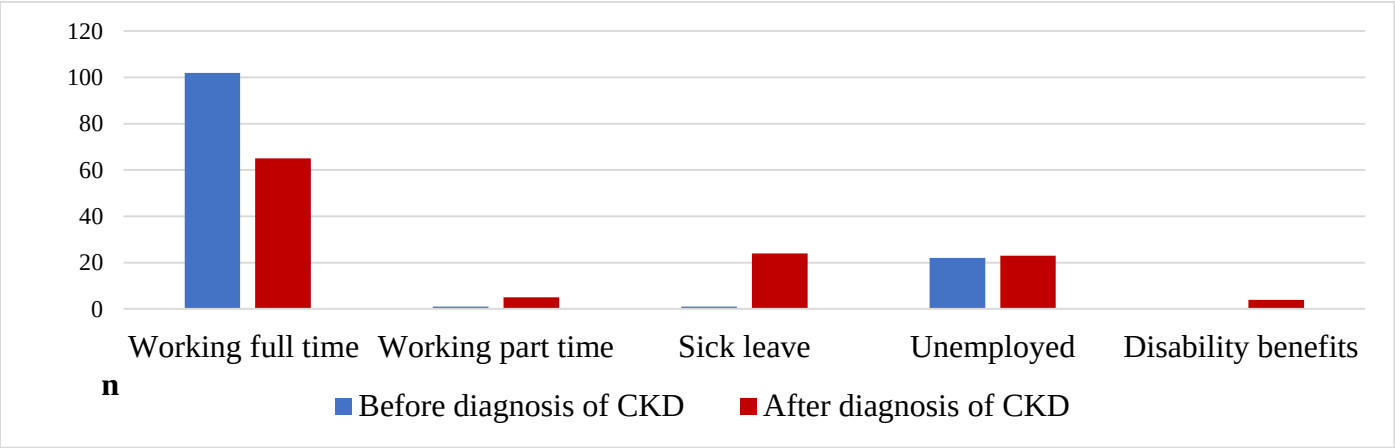
220 patients were included in the research, 126 of which were treated with HD and 94 with FKT. FKT patients were significantly younger than patients on dialysis at the time of the research ($P < 0.01$; Mann-Whitney test). Diabetic nephropathy (27%) and arterial hypertension (23%) were two of the most common causes of renal failure in patients on HD. Chronic glomerulonephritis (43.6%) and autosomal dominant polycystic kidney disease (19.1%) were two of the most common causes of renal failure in patients with FKT (Figure 2).



*Chi-Square Test

Figure 2. Causes of renal failure in patients on hemodialysis (HD), n = 126, and with functioning kidney transplant (FKT), n = 94

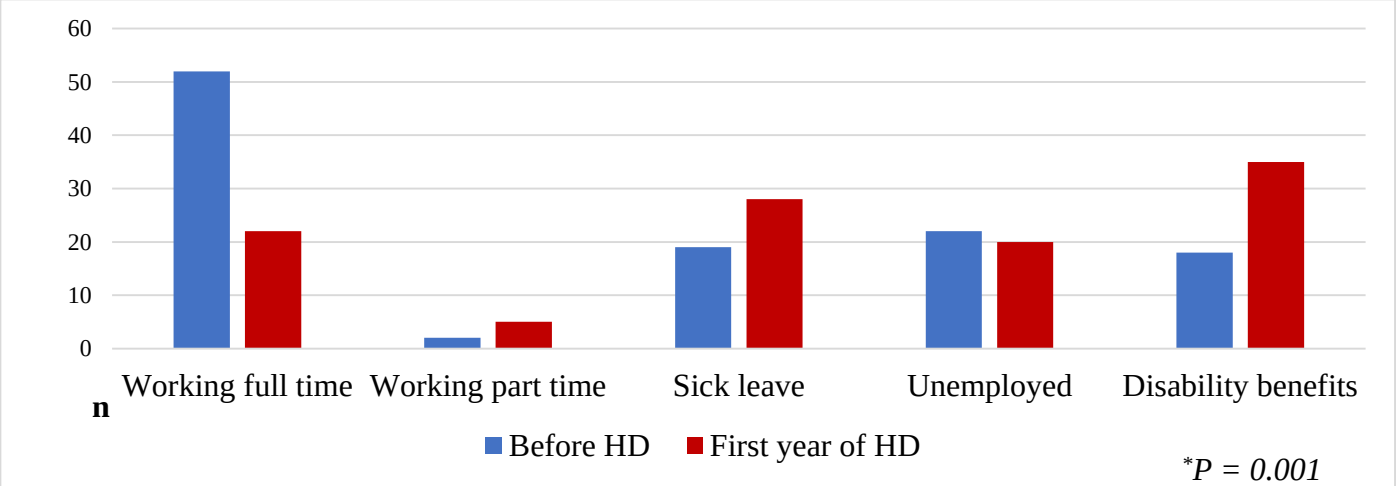
Of the 220 participants, 71 completed only primary education or less (32.3%), 118 finished high school (53.6%), and 31 had a higher education qualification or a university degree (14.1%). After receiving the diagnosis of CKD, employment among the working patients decreased by 32.7% (103 were working before the diagnosis, 70 after). Overall employment decreased by 4.7% (47.4% before the diagnosis, 42.7% thereafter). The number of subjects on sick leave (for more than a month) increased 24 times (1 before the diagnosis, 24 immediately thereafter) and 4 people started receiving disability benefits because of CKD immediately after the diagnosis (Figure 3).



*The marginal homogeneity test

Figure 3. Employment before and after diagnosis of chronic kidney disease (CKD)

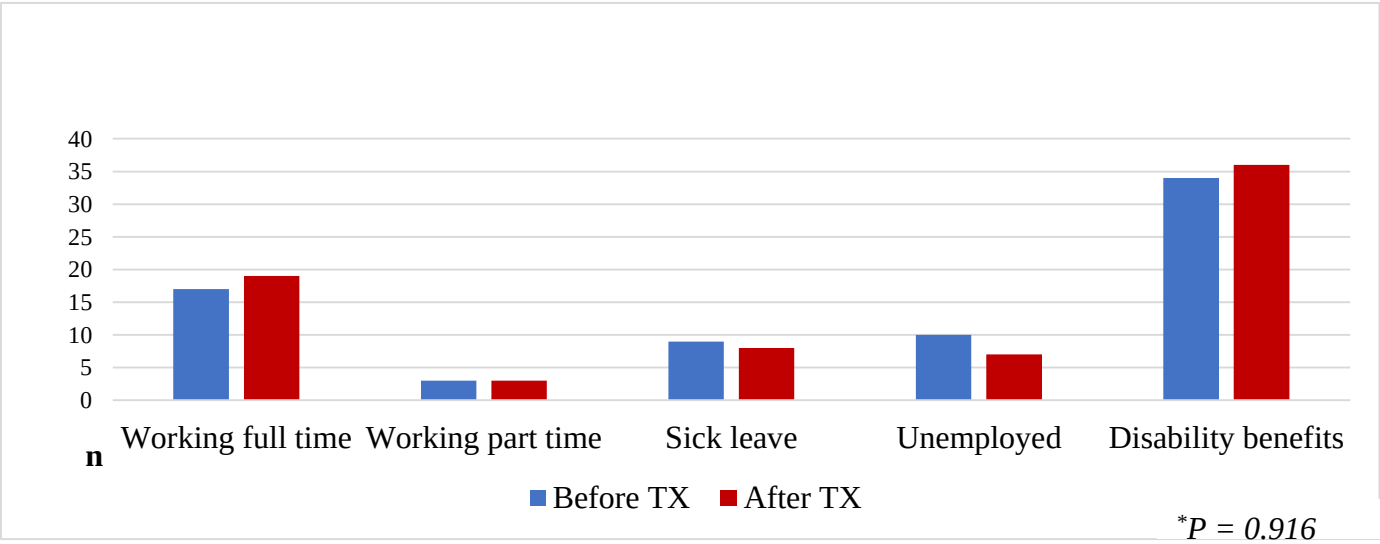
Immediately after the diagnosis of CKD and before the beginning of HD, the number of working patients decreased by 22.9%, and overall employment decreased by 9.6% (42.7% after the diagnosis of CKD, 33.1% before HD). The number of recipients of disability benefits increased by 4.5% (4 after the diagnosis of CKD, 18 before the start of HD). The proportion of employees decreased by 50% after the start of HD treatment when compared to the period before HD (54 employed before HD, 27 a year after the start of HD). Overall employment decreased by 8.1% (33.1% were employed before HD, 25% a year after starting HD). Out of all employed patients, 50.1% were on sick leave for more than a month after the beginning of HD. The number of recipients of disability benefits increased by 7.7% compared to the period before dialysis (Figure 4).



*The marginal homogeneity test

Figure 4. Employment before starting dialysis treatment and within the first year of dialysis

The number of patients who were working a year after TX increased by 9.1% compared to the period before TX (20 before TX, 22 after TX). Overall employment increased by 0.9% (27.3% were employed before TX, 28.2% a year after TX; Figure 5).



*The marginal homogeneity test

Figure 5. Employment before and a year after kidney transplantation (TX, n = 94)

At the time of the research, the number of employed participants had decreased by 36.4% compared to the time point a year after TX (22 after TX, 14 at the time of the research; Figure 6).

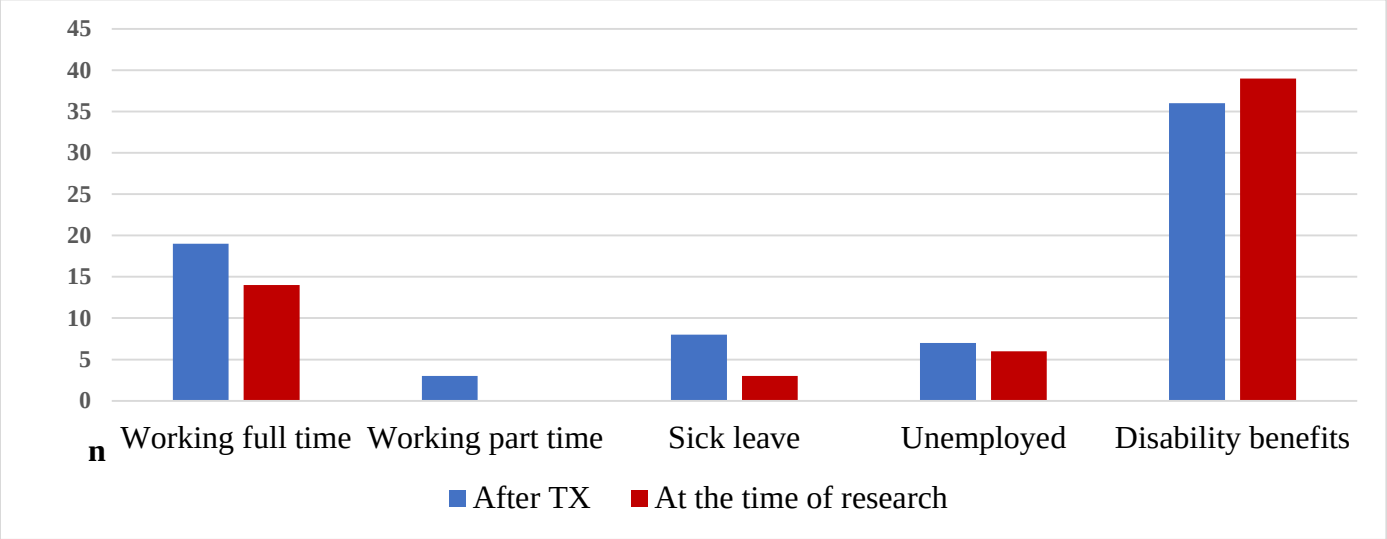


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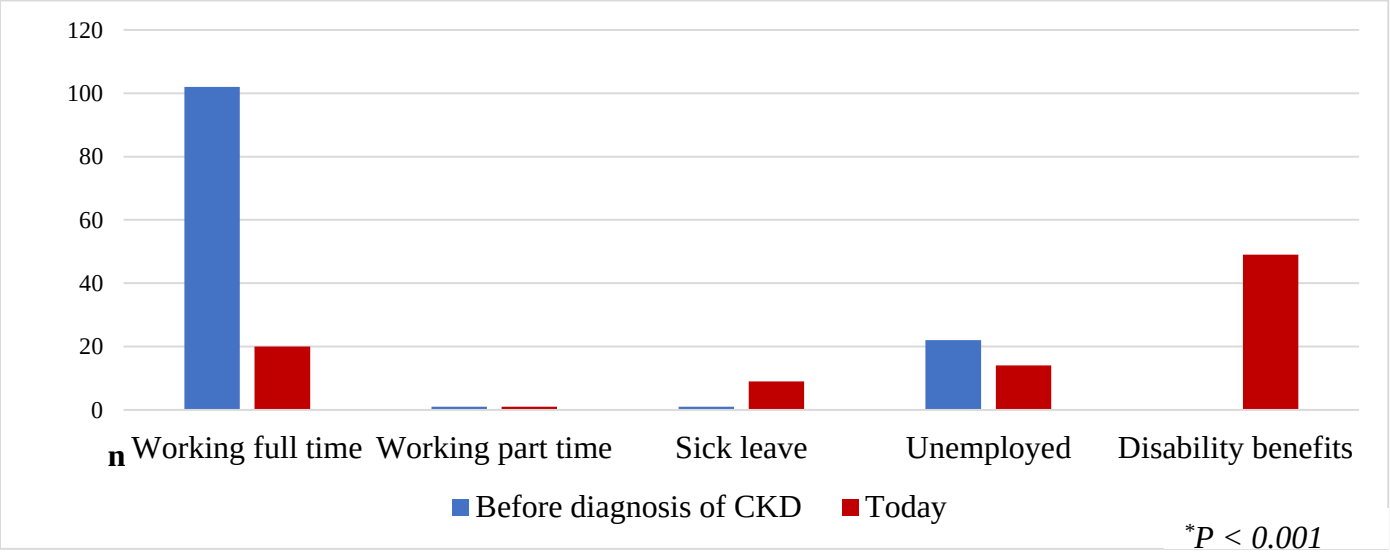
Figure 6. Employment a year after transplantation (TX) and at the time of research (n = 94)

Overall employment decreased by 13.8% (31.9% after TX, 18.1% at the time of the research). The number of subjects using sick leave decreased by 5.3% in the same period and the number of disability benefits increased by 3.2%. At the time of the research, overall employment of all patients had decreased by 33.7% compared to the period before the diagnosis of CKD (47.4% of the participants had been employed before the diagnosis, 13.7% were employed at the time of the research). Out of all patients who were employed at the time of the research, 31% had been on sick leave for more than a month (Figure 7).



*The marginal homogeneity test

Figure 7. Employment before diagnosis of chronic kidney disease (CKD) and at the time of the research (N = 220)

The number of working patients on dialysis at the time of the research decreased by 82.5% compared to the period before CKD diagnosis and overall employment decreased by 21.3% (31.7% before diagnosis, 10.4% at the time of the research). Out of all patients on HD who were employed at the time of the research, 6 were on sick leave for more than a month (46.2%). Ten participants (7.9%) were receiving disability benefits because of CKD, 94 (74.6%) were retired due to age, disability caused by another illness or were military retirees. The number of working patients with FKT at the time of the research decreased by 77.8% compared to the period before the diagnosis of CKD and overall employment decreased by 50.1% (68.2% before the diagnosis, 18.1% at the time of the research). Out of all FKT patients who were employed at the time of the research, 3 were on sick leave for more than a month (17.6%). 39 (41.4%) were receiving disability benefits, 23 (24.5%) were retired due to age, disability caused by another illness or were military retirees. Of all participants of working age (15–65), 30 were employed (25%) while 46 felt capable of working (38.3%) at the time of the research (Figure 8).

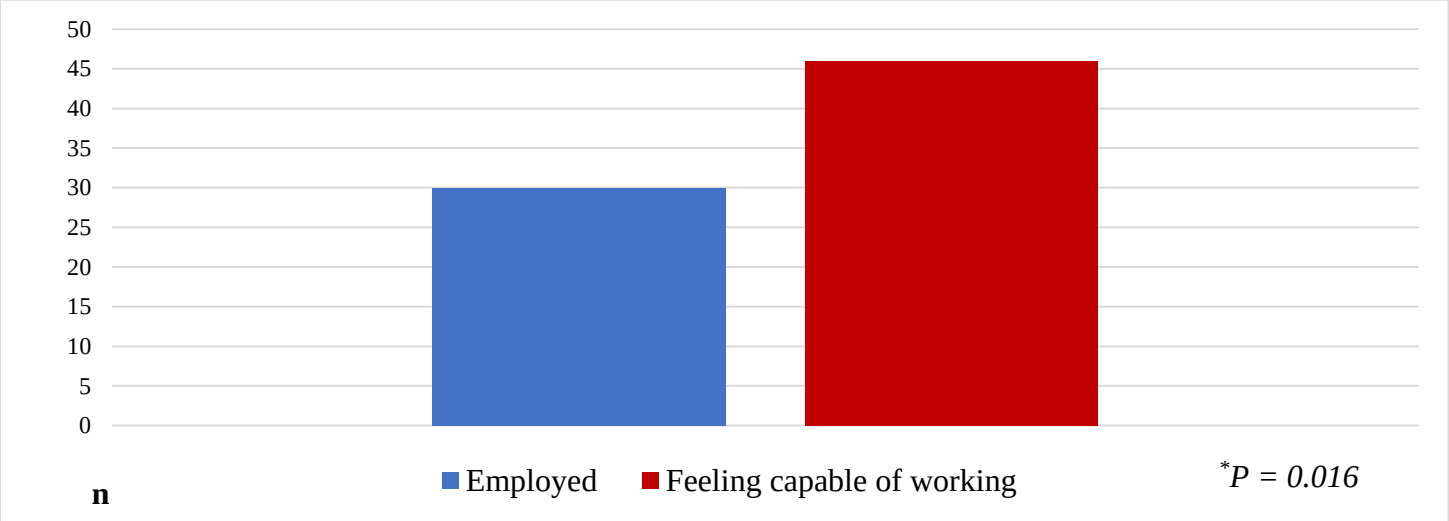


Figure 8. Employment and feeling capable of working in patients of working age (15 to 65, n = 120)

Thirty percent of all employees in that age group were on sick leave for more than a month at the time of the research. 17 FKT patients aged 15 to 65 (22.3%) were employed, while 32 of them (47.9%) felt capable of working. Out of the 17 patients employed at the time, 3 had been on sick leave for more than a month. 12 patients on HD aged 15 to 65 (26.1%) were employed at the time while 14 felt capable of working (30.4%). Out of 12 employed patients, 6 had been on sick leave for more than a month. Almost a quarter (24.2%, n = 22) of the unemployed patients aged 15 to 65 on RRT felt capable of working at the time of the research. 18.2% of those patients were retired, 4.6% were housewives, 36.4% were unemployed and 40.9% were receiving disability benefits because of CKD. As the main reason for their unemployment, the majority of respondents, 45.8%, reported poor health due to CKD, followed by advanced age (44.2%). The third main reason for unemployment was that employers did not want to hire someone with CKD because of the potential need to adjust working hours (5.8%). Only 2.8% of patients with completed primary education were employed at the time of the research, in comparison with 14.4% of those with high school education and 35.5% of those with a university degree.

Discussion

Employment of patients on RRT worsened with the progression of CKD. Overall employment decreased by 4.7% after diagnosis of CKD, and the number of persons on sick leave increased from 1 to 24. Four people started receiving disability benefits immediately after the diagnosis. HD takes time, leads to a certain degree of disability, and often threatens

employment within a particular profession. Accordingly, as patients started with HD, the number of employed patients decreased. Within the first year of HD treatment, overall employment decreased by 8.1% when compared with the period before HD. 25% of patients were employed, and 50.1% of patients were on sick leave for more than a month after starting HD. The number of recipients of disability benefits continued to increase. Within the first year after TX, the rate of employment of patients did not change significantly. If we compare the prevalence of employment after TX and at the time of the research, we see that overall employment decreased by 13.8%, although it would be logical to expect an increase in the number of people employed after TX, especially a year after TX, when patients have recovered from the kidney transplant procedure, since TX improves QoL and working ability (14). Patients with FKT have better health than those on HD, but overall employment of patients with FKT exhibited a twofold decrease in comparison with the period when they were on dialysis, during the RRT timeline from diagnosis of the disease to the present. Despite that, at the time of the research, the cross-sectional prevalence of employment for FKT patients (18.1%) was higher than for those on HD (10.4%). This can be explained by the fact that current FKT patients were younger and more of them were employed at the beginning of CKD. This was also confirmed by the prevalence of employment of FKT and dialyzed patients of working age, 15 to 65. Prevalence of employment was almost the same in both groups, but there was significant disproportion in the personal opinion about working ability between FKT and dialyzed patients: 30.4% of patients on HD and 47.9% of FKT patients felt capable of working. The number of FKT patients receiving disability benefits because of CKD was five times greater than that of dialyzed patients at the time of the research. Almost three quarters (74.6%) of dialyzed patients had retired due to age, disability caused by another illness, or were military retirees. The ratio of disability- and age-related retirement between FKT and dialyzed patients was in accordance with the age differences between those groups. A large number of disability benefits can be the result of availability of disability benefits and lack of support for patients on RRT who are willing to work. Today, 13.7% of RRT patients are employed, which is a third (33.7%) less than was the case before they received the diagnosis of CKD. Out of the total number of unemployed patients, almost a quarter felt capable of working. At the time of the research, a quarter of all patients aged 15 to 65 were employed, and almost 40% of the patients felt capable of working. 30% of the total number of employees in the group were on sick leave for more than a month, which means that the number of people working at that time was even smaller. We wondered what caused the disproportion between employment and feeling capable of working. As the main reason for unemployment, the majority of respondents reported poor health due to CKD, followed by advancing age and the fact that employers did not want to hire someone with CKD because of the potential need to adjust working hours. Higher level of education had a favorable impact on the employment of our respondents. A similar research had been conducted in the Rijeka University Hospital, where the working ability of patients on HD was surveyed. That research showed that 22.4% of the patients had been employed at the beginning of HD. During the first year of HD, 36.1% of patients who had jobs at the beginning of dialysis were forced to retire, and there was also a disproportion between the number of employed patients and those who felt capable of working (15). Based on our research and on the research conducted in Rijeka, we can conclude that the number of working patients in the Republic of Croatia significantly decreases with the start of HD treatment. There is a large number of publications devoted to this topic – QoL, working ability and possibility of employment of patients with CKD (14, 16, 17, 18). A study from the Netherlands showed that TX significantly increased the employment rate of patients, which reverted to almost the same rate as prior to diagnosis of CKD. At the time of the study, 67% of FKT patients aged 15 to 65 were employed, as opposed to the situation in Osijek, where only 22.3% of FKT patients from the same age group

were employed. It is important to note that 45% of patients employed after TX in the Netherlands had part-time jobs, while none of the transplanted respondents in Osijek had a part-time job (14). The question is: are patients in Croatia given the opportunity to work after TX or is retirement the only option? Nowadays, the goal of medical treatment is not just survival, but also improvement in QoL. Important determinants of QoL are the ability to work and access to employment. CKD disrupts QoL, but patients who accept RRT do not give up on life; in fact, they do just the opposite – they choose to live. Many patients with ESRD, faced with limitations of health status and requirements of their treatment, reasonably decide to quit their jobs. This decision is made easier by the availability of disability benefits due to CKD. However, other patients who have the desire and opportunity to work find that guidance and support for employment are limited (19). These patients should be involved in public life and motivated to continue with their normal lives. A potential method for achieving higher employment of patients on RRT could be motivating the employers with financial incentives or other rewards.

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Competing interests. None to declare

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Original article

Awareness About Information Security And Privacy Among Healthcare Employees

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Abstract

Aim: The aim of this study was to analyze healthcare employees' knowledge of information security and potentially risky behavior on the Internet considering demographic parameters and in comparison with the standardized behavioral norms among Internet users in Croatia.
Methods: The study was conducted as a cross-sectional study. Healthcare employees from three hospitals in different geographical areas (Osijek, Pula and Zagreb) were included in this study. The validated UISAQ (Users' Information Security Awareness Questionnaire) was used for data collection. The questionnaire contains 33 questions, grouped in two scales and six subscales, and participants were self-evaluated using Likert scale. The time period of data collection was the summer of 2017.
Results: Surveyed healthcare employees show significantly less risky behavior and overall better knowledge than the average Internet user in Croatia. Female participants display online behavior that is less risky than that of the male participants; participants with a university degree are better at PC maintenance, while participants with a high school diploma are more skeptical in regard to loss of personal or professional data. Older people are significantly more careful and lend their access data to other colleagues at work less often.
Conclusion: Healthcare employees included in this study display partially better results than the average Internet users in Croatia when it comes to their knowledge and potentially risky online behavior. However, their average estimations are only partially better than referent estimations and their scores are not very high, especially when it comes to their awareness measured in the "Security in Communications" and "Secured Data" subscales. As there is high risk of losing data because of the nature of business protocols, healthcare employees need more education and training in order for their awareness regarding the importance of information security and privacy to increase.

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KEYWORDS: information security, privacy protection, risky behavior, Internet, UISAQ

Introduction

Since the Internet has become an integral part of human life, more and more opportunities are being created, both in the positive sense for the advancement of technology and communication between people as well as in the negative sense, which refers to the existence of risks regarding personal security and privacy. Therefore, there exists a great need to protect personal data in order to reduce the risk of theft of information from users of all age groups, from the youngest to the oldest. New services on the Internet (applications, electronic healthcare, shopping, etc.) that are becoming increasingly necessary and involve more and more users require users to disclose some of their personal information. With this potential risk increasing and seeing as numerous users are ignorant when it comes to the information and communication systems involved, they accept imposed rules and readily start using new services as soon as they appear in the digital market. Since previous research has shown that a person as an information system user may be the most critical security element in said system (1 4), the issue of privacy and user protection will most likely never be solved, even though program security, security procedures and backup automation are at a high level. It is certain that the above is not enough to fully protect the user. Responsibility and conscientious use of Internet services by the user are also required. Therefore, reducing said risk is possible, and one of the best ways of doing so is increasing user awareness by educating them about the various types of unwanted events like frauds and privacy loss on the Internet. For example, installing additional apps or divulging a small piece of personal data may ultimately result in financial or other, less significant loss, which was by no means the intention of the user who installed or provided said information (5).

In general, data protection, not just on the Internet, is carried out in order to prevent data theft or data manipulation. There are two reasons for protecting electronic data: the possibility of their loss and the possibility of unauthorized use of data by an unreliable person with malicious intent. There are several ways to protect data, and the most common one is the use of antivirus programs that protect your computer's operating system from different kinds of malware. Before using the computer's operating system, it is useful to update both the antivirus program and its virus definitions to secure the personal data stored on the computer. Malicious people who want to cause harm to computer users and software or operating system manufacturers tend to do so in order to prove that manufacturers did not create the application, program or operating system with sufficient protection mechanisms. Malicious people do not benefit greatly from developing and producing viruses and other types of malware (6, 7).

Healthcare employees are users and integral parts of a hospital's information and communication system. The Hospital Information System (HIS) is a unique information system within a hospital that combines medical and non-medical data created at various hospital departments for a better and more effective way of exchanging information and more successful way of communicating with patients (8). Modernizing medicine with accessible information technology in patient management systems provides many benefits, but it is possible to manipulate and abuse the privacy. Patient's personal data availability is important to medical staff in order to provide better medical care, healthcare and treatment (9). The protection of personal data, in particular data relating to the health of persons, is primarily carried out with the aim of protecting the right to privacy of personal and family life, which is one of the personal rights protected by our legislation.

Similar empirical studies were conducted on the subject using the UISAQ, but they focused on other groups of Internet users (10 12). The latest study was conducted at the national level as part of the EU project under agreement number INEA/CEF/ICT/A2015/115320. Those results are used as referent values for comparison with results of this study (13).

Therefore, the aim of this study was to analyze knowledge on information security and potentially risky behavior on the Internet among healthcare employees considering demographic parameters and in comparison with the standardized behavioral norms among Internet users in Croatia.

Methods

The authors used the validated Users' Information Security Awareness Questionnaire (UISAQ) for data collection in three Croatian hospitals located in different geographical areas: Osijek, Pula and Zagreb. The data were collected during the summer of 2017. The study was conducted as a cross-sectional study.

The UISAQ has two major scales with three subscales each; each subscale contains five or six items (questions). Associated abbreviations are used in the subsequent text and tables:

• Potentially Risky Behavior (PRB; k = 17)

o Usual Behavior (UB; k = 6)

o Personal Computer Maintenance (PCM; k = 6)

o Access Data Lending (ADL; k = 5)

• Knowledge and Awareness (KA; k = 16)

o Security in Communications (SC; k = 5)

o Secured Data (SD; k = 5)

o Backup Quality (BQ; k = 6)

These subscales describe the user's behavior, knowledge and awareness (5, 11). Participants were asked to estimate how much they agree with a statement on a 5 point Likert type scale, where five means excellent, from the aspect of information security. At the end of the UISAQ, two additional questions about behavioral security of users were given, as well as a section for the provision of demographic data.

Statistical Analysis

The statistical software tool MedCalc 14.12.0 was used for statistical analysis in this paper. Statistical significance, when comparing differences in estimations among groups, was defined as $P < 0.05$ using Student's T-test and one way ANOVA with post hoc Scheffé test. Correlations with age were tested using the Spearman's rank correlation test.

Results

The surveyed healthcare employees were 38.5 ± 11.3 ($x \pm SD$) years old, mostly female (83.8%, $P < 0.001$, Chi-square Test) and mostly with a high school diploma (94.1%, $P < 0.001$, Chi-square Test). Average estimation in the "Usual Behavior" subscale (Student's T-test, $P = 0.03$) was significantly higher for women than for men. There was no significant difference between the genders in the case of other subscales used to describe behavior or the subscales that assess the level of knowledge (Table 1).

Table 1. Gender differences among healthcare employees

Scales and subscales	Arithmetic mean (standard deviation)		P*
	Male (n = 45)	Female (n = 242)	
PRB	3.99 (0.42)	4.03 (0.36)	0.51
UB	4.29 (0.59)	4.48 (0.50)	**0.03**
PCM	3.10 (0.98)	2.92 (0.85)	0.22
ADL	4.59 (0.43)	4.70 (0.38)	0.10
KA	3.10 (0.52)	3.20 (0.50)	0.20
SC	3.16 (0.98)	3.33 (0.83)	0.22
SD	2.12 (0.74)	2.30 (0.86)	0.18
BQ	4.02 (0.80)	4.00 (0.70)	0.86

**Student's T test*

In regard to qualifications, average estimation regarding personal computer maintenance was significantly higher for highly educated participants (one way ANOVA, P = 0.01), while participants with a high school diploma were the group that was most aware of the importance of data protection and of the risk of loss of personal and professional data, money or identity on the Internet (one way ANOVA, P = 0.03), as seen in Table 2.

Table 2. Qualification differences among healthcare employees

Scales and subscales	Arithmetic mean (standard deviation)			P*
	High school diploma (n = 176)	Bachelor's degree (n = 69)	Master's degree (n = 42)	
PRB	4.01 (0.37)	4.02 (0.36)	4.09 (0.39)	0.52
UB	4.47 (0.55)	4.47 (0.49)	4.35 (0.42)	0.43
PCM	2.85 (0.87)	2.99 (0.84)	3.29 (0.88)	**0.01**†
ADL	4.72 (0.39)	4.62 (0.39)	4.61 (0.37)	0.08
KA	3.21 (0.54)	3.16 (0.48)	3.16 (0.41)	0.75
SC	3.29 (0.87)	3.39 (0.81)	3.18 (0.87)	0.46
SD	2.37 (0.90)	2.08 (0.70)	2.14 (0.75)	**0.03**‡
BQ	3.97 (0.77)	4.00 (0.63)	4.16 (0.63)	0.29

**One way ANOVA*
†between high school diploma and master's degree (Scheffé test)
‡between high school diploma and bachelor's degree (Scheffé test)

A relatively low, statistically significant positive correlation was found between age and the "Usual Behavior" subscale, meaning that older participants display more secure online behavior on the Internet (Spearman's Correlation Test, rho = 0.29, P < 0.001). Likewise a very low, but statistically significant positive correlation was found between age and the "Access Data Lending" subscale (Spearman's Correlation Test, rho = 0.13, P = 0.03), which mostly means that older participants lend their access data to other colleagues at work less often (Table 3).

Table 3. Age differences among healthcare employees

Scales and subscales	Age of healthcare employees (n = 287)		
	rho	95% CI	P*
PRB	0.09	-0.03 to 0.20	0.13
UB	0.29	0.18 to 0.39	**< 0.001**
PCM	-0.08	-0.20 to 0.03	0.16
ADL	0.13	0.01 to 0.24	**0.03**
KA	0.10	-0.01 to 0.21	0.09
SC	0.03	-0.08 to 0.15	0.58
SD	0.06	-0.05 to 0.18	0.30
BQ	0.10	-0.01 to 0.22	0.09

*Spearman's Correlation Test

Average estimations per scale and subscale are shown in the first column of Table 4. The lowest average estimation for participants was 2.27 ± 0.84 (x ± SD) for the "Secured Data" subscale, which measures awareness of privacy, while the highest average estimation 4.68 ± 0.39 (x ± SD) was for the "Access Data Lending" subscale, which measures risky behavior (Table 4).

Table 4. Differences between healthcare employees and standardized behavioral norms among Internet users in Croatia

Scales and subscales	Arithmetic mean (standard deviation)		P‡
	Healthcare employees (n = 287)	Standardized behavioral norms* (n = 4859)	
PRB	4.03 (0.37)	4.00 (0.42)	0.24
UB	4.45 (0.52)	4.16 (0.59)	**< 0.001**
PCM	2.95 (0.87)	3.27 (0.83)	**< 0.001**
ADL	4.68 (0.39)	4.66 (0.49)	0.50
KA	3.19 (0.51)	3.07 (0.53)	**< 0.001**
SC	3.30 (0.85)	2.93 (0.82)	**< 0.001**
SD	2.27 (0.84)	2.32 (0.87)	0.34
BQ	4.00 (0.72)	3.83 (0.78)	**< 0.001**

*average Internet user in Croatia (25)
‡Student's T test

The results of the comparison between the surveyed healthcare employees and the standardized behavioral norms among Internet users in Croatia collected in a national project (N = 4859) show statistically significant differences for the majority of the subscales, often in favor of healthcare employees (Table 4). The surveyed healthcare employees achieved better results in the "Usual Behavior" subscale (Student's T test, $P < 0.001$), in the "Security in Communications" subscale (Student's T test, $P < 0.001$), and in the "Backup Quality" subscale (Student's T test, $P < 0.001$). They achieved better results in the overall "Knowledge and Awareness" scale (Student's T test, $P < 0.001$), but significantly worse results in the "Personal Computer Maintenance" subscale (Student's T test, $P < 0.001$).

Discussion

Analysis of the results has shown that healthcare employees included in this study are statistically better than the average Internet user in Croatia when it comes to their knowledge about digital security and their potentially risky online behavior. However, their average estimations per scale and subscale are better than referent estimations only in part and are not very high, especially when it comes to awareness measured in the "Security in Communications" and "Secured Data" subscales.

Female users are the most skeptical of the surveyed healthcare employees, so they are more careful in their online behavior. The results were similar for older participants. Both results are in accordance with previous studies based on the same questionnaire (11, 12).

The results that show that participants with a high school degree are the most skeptical in regard to data protection and the risk of loss of personal and professional data, money or identity on the Internet are also partially in accordance with those of previous studies. On the other hand, participants with higher education achieved better results when it comes to their personal computer maintenance.

Hospitals' business information systems include both business and private data, i.e. digital data about healthcare processes and patients' private information. Since the user is often the weakest element of the information and communication system when it comes to data protection (1 4), healthcare employees have an obligation to be familiar with and behave in accordance with security protocols at their workplace. Employees typically need additional education courses organized by their institution and alert messages sent on a regular basis by system administrators (14 17) in order to acquire better knowledge regarding security threats and to increase their own awareness regarding the importance of information security for the system, for the patients and for themselves (18, 19).

Some general recommendations for more secure behavior on the Internet that may help healthcare employees and all other Internet users are presented below (13):

- Limit posting personal data on the Internet because what is once posted on the Internet remains permanently recorded!
- Different systems are not equally secure or equally dangerous; you should exercise increased caution in an unknown setting!
- User access data are personal and they are used to verify identity on the Internet. They should be handled with extreme caution because they represent the electronic identity of a user. Users should know that NO ONE should EVER request that he/she disclose their access data, neither a system administrator nor a bank clerk!
- The Internet is similar to the real world and public spaces. Maintain a healthy dose of mistrust when communicating with strangers ¬– creating a fake identity on the Internet is much simpler than in the real world!
- Keep your operating systems, applications you use, and particularly antivirus protection up to date, both on personal and portable devices, as well as on mobile phones.

- Back up important documents and files periodically and copy them to another location, removed from the original data.
- Try to differentiate business communication from private communication.
- A high-quality password significantly increases the level of security. Use a combination of capital and small letters, numbers and special characters.

Some limitations of this study are the relatively small number of healthcare employees surveyed in only three hospitals. In addition, the participants were compared to standardized behavioral norms in Croatia. However, those referent values do not refer to secure behavior and extensive knowledge of data protection, but to the average Internet user. In a future research, a comparison is planned between healthcare employees and different target-groups, such as students, employees in the government sector or in the banking sector.

As there exists a high risk of data loss because of the nature of business protocols, healthcare employees need more education and training in order for their awareness regarding the importance of information security and privacy to increase.

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Review article

Aortic and Cerebral Aneurysms: Link with Genetic Predisposition, Risk Factors, and Aortopathies

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Abstract

Routine cardiology practice includes diagnostic algorithms for thoracic aortic aneurysm detection at varying degrees of clinical significance. Standard procedures for evaluation and follow up involve screening for standard atherosclerotic risk factors, including hypertension, dyslipidemia, diabetes mellitus, obesity, smoking history and family history without genetic testing, as well as cardiac imaging techniques, such as echocardiography, computed tomography or magnetic resonance imaging. According to the latest reports, thoracic aortic aneurysms can present concomitantly with intracranial aneurysms, although the exact etiopathogenic mechanisms are not yet known. There is evidence that connects these two conditions with genetic predisposition, risk factors, and aortopathies. Routine practice does not include screening for other aneurysm locations. This review will highlight existing knowledge in this area and the need for further investigations.

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Introduction

An aneurysm is defined as a focal dilatation of the arterial wall. The cause and pathogenesis of aneurysms remain unknown. The initial arterial dilatation appears to be caused in part by degeneration of a portion of the arterial wall, often medial elastin, followed by smooth muscle. In recent years, there has been greater evidence regarding the concomitant incidence of intracranial aneurysms (IAs) and aortic aneurysms (AAs). The prevalence of unruptured IAs in the general population is estimated to be 3.2%. The prevalence of AAs, both thoracic aortic aneurysms (TAAs) and abdominal aortic aneurysms (AAAs), is around 1%–2% in the general population. The prevalence is higher in older populations at approximately 10% (1, 2).

The precise mechanisms of initiation, enlargement, or rupture of IAs, TAAs, and AAAs remain unknown. Biomechanical factors play fundamental roles in aneurysmatic development. An assessment of rupture potential should include geometry (shape and thickness), mechanical properties (anisotropy and strength), and applied loads (hemodynamic and perivascular), without primarily relying on maximum dimension. Early loss of elastin and subsequent loss of smooth muscle, with collagen structure change controlled by cellular responses as a result of hemodynamic and intramural stresses, can initiate thinning of the arterial wall and cause enlargement (3).

Congenital conditions, such as bicuspid aortic valve (BAV) and coarctation of the aorta (CoA), frequently occur with dilated aortic roots with a predisposition for aortic or cervical dissection. IAs are also more prevalent in patients with BAV or CoA, implying that IAs and aortic pathologies share a common developmental defect. The tunica media of both arteries has the same embryological origin, neural crest cells (NCCs), and a similar structure of cross-linked elastin and collagen (4, 5).

Another important and proposed pathway for aneurysm formation involves endothelial dysfunction and/or injury, inflammation, vascular smooth muscle cell (SMC) response, extracellular matrix remodeling, vessel wall ageing, and degeneration with end-stage death (6). These processes are common with atherosclerosis etiopathogenesis to a degree, but their relationship with IAs and AAs is still unknown. The most recent epidemiological, clinical, and biological evidence suggests that atherosclerosis and IAs/TAAs are more distinct than traditionally thought (7).

This review will focus on the concomitant incidence of IAs and TAAs, their etiopathogenesis, risk factors, genetic predisposition, and aortopathies.

Artery – anatomy, histology, physiology and pathophysiology

Arteries are the main conductive blood vessels that deliver blood from the heart to all parts of the body. The arterial wall consists of three layers: a) intima – innermost layer, consisting of endothelial cells attached to a basement membrane composed of type IV collagen and laminin; b) media – middle layer, built from SMCs embedded in extracellular matrix composed of elastin, collagen, and proteoglycans; and c) adventitia – the outermost layer, consisting of fibroblasts and type I collagen with admixed elastin. The largest arteries contain the vasa vasorum, a network of small vessels that supply large blood vessels (3). The cells and matrix of each layer are built to provide a structural and functional support to blood vessel walls (6).

The main difference in the structure of systemic arterial vasculature is the composition of the tunica media. According to structure, arteries can be divided into elastic and muscular arteries. The common carotid artery is an elastic artery, while intracranial arteries are muscular arteries. The absence of an external elastic lamina makes intracranial arteries more vulnerable to aneurysmatic formation and rupture compared to other muscular arteries. Each arterial site may possess a different degree of durability and vulnerability as a result of

variance in pathophysiological signals, such as pressure increase (8).

Although it may occur in any blood vessel, the most common aneurysms according to location are aneurysms of the brain (Circle of Willis), TAAs, and AAAs (3). Also, arterial bifurcations are locus minoris resistentiae for cerebral aneurysm formations because of hemodynamic stress resulting from oscillations in blood flow (8). According to shape, aneurysms can be classified as saccular or fusiform aneurysms. The pathogenesis of aneurysm formation has not yet been clarified, but it is assumed that initial dilatation is first caused by elastin degeneration in the media, followed by smooth muscle degeneration (3).

Embryology

The vascular system starts its development early in the third week of gestation when the first blood islands first appear in the umbilical vesicle. Blood islands are composed of mesodermal cells that are induced by fibroblast growth factor (FGF2) to differentiate into hemangioblasts, which are then stimulated by vascular endothelial growth factor (VEGF), so they become endothelial cells and bind into the first blood vessels. This is the process of angiogenesis (9). Another way in which blood vessels develop is vasculogenesis, which can best be explained as sprouting from pre-existing ones. The earliest formed primitive blood vessel is the dorsal aorta, which soon after becomes surrounded by SMCs originating from the splanchnic mesoderm (10). Slightly later in development, primary SMCs derived from the splanchnic mesoderm are gradually replaced by a secondary population of SMCs originating from NCCs (11-13). The thoracic aorta almost fully replaces its primary SMCs with neural crest-derived SMCs (11). Conversely, the abdominal aorta retains its mesodermal-derived SMCs. SMCs derived from NCCs tend to produce a higher amount of elastin but at the same time express lower contractility function compared to those of mesodermal origin (14). Unique responses of NCCs to various cytokines and growth factors are firm evidence of the different embryonic origins of the thoracic and abdominal aortas. Neural crest-derived SMCs from the thoracic aorta show increased DNA synthesis and collagen production by transforming growth factor-β1 (TGF) as well as enhanced proliferation, along with synthetic activity, when stimulated by homocysteine. Contrary to that, mesodermal SMCs show no response to either of these (7, 8). Further support for these findings includes studies showing a causative link between the loss of signaling through TGF-β receptors I and II and development of familial TAAs and dissection (15-17). The proposed underlying cause for this kind of smooth muscle distribution in the aortic wall is that these neural crest-derived SMCs can better endure the higher pulse pressure in the thoracic aorta by laying down more elastic lamellae during development and growth (10).

The embryological link between the ascending aorta and the intracranial arteries lies precisely in the neural crest origin of SMCs. The cerebrovascular structures of the face and forebrain are derived from pericytes and the musculo-connective wall of arteries from NCCs (18). Malposition or malfunction during the perturbation of these cells (neurocristopathy) could be a plausible cause for the simultaneous occurrence of AAs and IAs (19). Considering the different embryological origins of SMCs, Shin et al. showed a site-specific relationship between aneurysms of the aorta and intracranial arteries. Ascending aortic aneurysms occur more commonly with aneurysms of the anterior and middle cerebral arteries, in contrast to AAAs, which occur more often with internal carotid artery aneurysms (20). Besides the development of vascular structures, NCCs play an important role in the development of head structures, skin melanocytes, a great part of the peripheral nervous system, some endocrine cells, and other structures (21, 22). Waldo et al. have contributed to the knowledge about the importance of NCCs in the septation of the truncus arteriosus (12). Co-occurrence of pathologies, such as a BAV (23), CoA (24), aortic root dilatation, cystic medial wall degeneration, and cervical arterial dissection (23) all fall into the category of neurocristopathy phenotypes (23,

24). Studies have also shown higher incidence of IAs in neurocristopathy diseases, such as neurofibromatosis type 1 (25), and diseases that are reminiscent of neurocristopathies, such as Moyamoya disease, fibromuscular dysplasia, and cervical dissection (26). Through these types of head, neck, and heart pathologies, the importance of the participation of NCCs in vascular remodeling is emphasized.

Genetic predisposition

Given the knowledge of the common embryonic origin, numerous studies have focused their genetic research on diseases related to neurocristopathy phenotype (Table 1, Figure 1).

Table 1. Gene mutations and clinical presentation on IAs2, TAAs3 and AAAs4

MUTATIONS	CLINICAL REPERCUSSION	MOLECULAR LEVEL
Mutations in the genes encoding the TGFBR1 and TGFBR2 receptors	Loeys-Dietz syndrome	TGF-β[5] pathway
SMAD3 gene	Aneurysm osteoarthritis syndrome	TGF-β[1] pathway (a gene encoding a downstream signaling mediator TGF-β via the TGFBR1 and TGFBR2 receptors)
COL3A1 gene	Ehlers-Danlos syndrome type IV	TGF-β[1] pathway (a gene for the collagen type III pro α-1 chain)
PKD1 gene (85%), PKD2 gene (15%)	Autosomal dominant polycystic kidney disease	Polycystin-1 and polycystin-2, when healthy, likely work together to help regulate cell growth and division (proliferation), cell movement (migration), and interactions with other cells
3p24–25 and 5q for gene TGFBR2 and CSPG2 gene	Possible loci for IAs[6] and TAAs[7]	TGF-β[1] pathway, pathology of the arterial wall
4q32–34	Effect on aneurysms of the abdominal aorta and intracranial vessels	
11q24	Significant role in all 3 sites of aneurysm formation	
19q	Effect on aneurysms of the abdominal aorta and intracranial vessels	
9p21, 2q33	Found in patients with IAs[2] and AAAs[8]	
18q11 and 15q21	Strong connection of IAs[2] and TAAs[3] with AAAs[4]	

1 Transforming Growth Factor β
2 Intracranial Aneurysms
3 Thoracic Aortic Aneurysms
4 Abdominal Aortic Aneurysms

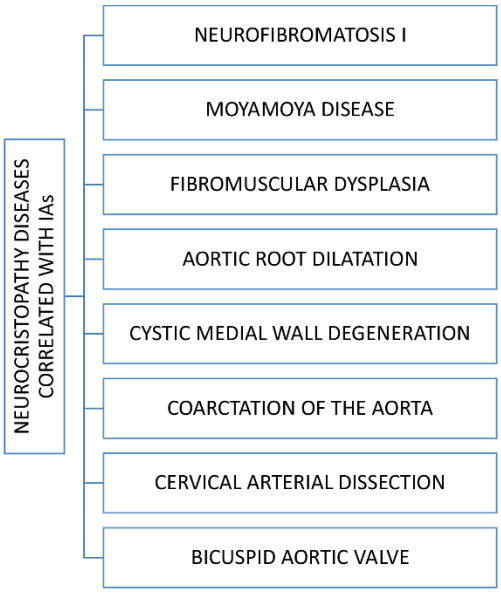

Figure 1. Correlation of IAs with neurocristopathy diseases

Pathology in the septation of heart outflow tracts, such as BAV, CoA, and dilated aortic root, was shown to co-occur with cerebral aneurysms (24, 27, 28). As evidence for the same pathological origin, a study done by Rosenquist et al. showed impaired initiation of elastogenesis in the great vessel walls after the ablation of NCCs (29).

Considering the existing knowledge in embryology about the importance of TGF-β signaling in the development of arterial walls, it is easy to understand that diseases with defects in the TGF-β pathway show a tendency of aneurysm development in multiple vascular beds. Loeys-Dietz syndrome (mutations in genes encoding the TGFBR1 and TGFBR2 receptors) (30), aneurysm-osteoarthritis syndrome (mutations in SMAD3, a gene encoding a downstream signaling mediator TGF-β via the TGFBR1 and TGFBR2 receptors) (31, 32), and Ehlers-Danlos syndrome type IV (mutations in the gene for the collagen type III pro-α-1 chain [COL3A1]) are prone to forming both TAAs and IAs (33). Nurmonen et al. showed a higher occurrence of multiple IAs in patients with autosomal dominant polycystic kidney disease. Likewise, in terms of age of occurrence of aneurysmal subarachnoid hemorrhage, in people with IAs it occurs 10 years earlier than in the general population (34). A study of 669 patients with fibromuscular dysplasia showed a significantly higher prevalence of IAs (35).

Linkage studies of the entire genome carried out on predefined loci for three sites of aneurysm formation (intracranial, abdominal, and thoracic aorta) found five overlapping chromosomal regions at 3p24-25, 4q32-34, 5q, 11q24 and 19q. 3p24–25 and 5q for the CSPG2 gene are possible loci for IAs and TAAs. 3p24–25 is the locus for the TGFBR2 gene, which was already mentioned in relation to diseases affecting TGF-β signaling, while CSPG2 seems to have an effect on the pathology of the arterial wall as well. For the other regions, there is no known candidate gene yet, but 11q24 seems to have a significant role in all three sites of aneurysm formation, while 4q32–34 and 19q have an effect on aneurysms of the abdominal aorta and intracranial vessels (36).

In 2016, a mega-analysis of four previously published aneurysm cohort studies was performed (two intracerebral aneurysm cohort studies from the Netherlands and Finland, an AAA cohort study from the Netherlands, and a TAA cohort study from the United States). It detected a single nucleotide polymorphism at four loci (9p21, 2q33, and two chromosomal regions, 18q11 and 15q21) which showed a strong connection between IAs and TAAs with AAAs (37). In a study by Shin et al., while searching for evidence of innate aortopathy in patients with IAs, the scientists noticed that short stature was common among the respondents (24). The aforementioned NCCs have a number of roles outside the development of the vascular system, including a role in the development of the pituitary, which can have an effect on the growth of bone and cartilage (21, 38). Further research on other potential, unknown causes could highlight this problem and provide new insights in this area.

Atherosclerosis

Atherosclerosis correlates with arterial aneurysmatic occurrence. It is still unknown if it is a causal relationship, or if they share common risk factors (39). Most studies agree that

atherosclerosis is mainly correlated with AAAs, but not with TAAs and IAs (3, 39-41). There is a shared genetic background for both AAAs and atherosclerosis (39, 42, 43), as well as common risk factors, including hypertension, obesity, smoking, HDL, family history, and thrombosis (2, 20, 42, 44-51). On the other hand, some markers differ, such as LDL (no correlation with AAAs) and diabetes (negative correlation with AAAs) (42, 50, 51).

There is no conclusive evidence about the correlation between the development of IAs and atherosclerosis. Rouchaud et al. showed that there is no correlation between atherosclerosis and IAs (46). Wang et al. even asserted that atherosclerosis is a protective factor against IA rupture (52), while Hokari et al. suggested that middle cerebral artery aneurysms positively correlate with hypertension and cerebrovascular disease, which are common comorbidities in patients with atherosclerosis and have a higher incidence in stroke patients (41). Another type of IAs, paraclinoid aneurysms, were also the subject of the same study. Paraclinoid aneurysms are defects of an internal carotid artery wall that are located between the superior part of cavernous sinus and the entrance for the posterior communicating artery (53), detected with three dimensional time-of-flight magnetic resonance angiography (3D TOF MRA) in stroke patients, as well as in healthy adults volunteering for asymptomatic brain disease evaluation (41). The results showed that paraclinoid aneurysms have a lower incidence in stroke patients than in healthy research participants (41). Likewise, paraclinoid aneurysms are more affected by hemodynamic stress and unchangeable risk factors than middle cerebral artery aneurysms (54). These results might not mean that IAs are "immune to atherosclerotic risk factors", but just that they are not equally affected regarding the localization.

Inflammation

Genetics, hemodynamic changes, and environmental factors are the main factors in aneurysm development. In recent years, there has been considerable evidence for inflammation as a leading factor in the pathogenesis of IAs (6, 39, 55).

Initially, in response to hemodynamic stress, development of endothelial dysfunction is followed by an inflammatory reaction. Macrophages release proinflammatory cytokines that lead to recruitment of additional inflammatory cells and release matrix metalloproteinases (MMPs), which cause degradation of the extracellular matrix and activate other proteinases (55). According to a study by Aoki et al., mice with lower expression of monocyte chemoattractant protein-1 (MCP-1) and decreased accumulation of macrophages had a lower risk of developing cerebral aneurysms (56). Likewise, according to another study by Aoki et al., the application of MMP inhibitor in rats reduced the progression of IAs (57).

MMPs cause apoptosis of SMCs, which are mostly concentrated in the media, leading to the thinning of this layer. Tumor necrosis factor-α (TNF-α) modulates SMCs by inhibiting collagen synthesis, which is a predisposition for aneurysm formation, progression, and rupture (55). T cells, mast cells, and cytokines also participate in inflammation and aneurysm formation (8). TNF-α and interleukin-6 (IL-6) are the best explored cytokines involved in the development of inflammation and aneurysm formation. Complement activation has been found in patients with IAs, but it is still necessary to explain how it contributes to aneurysm formation (58).

According to a study by Shikata et al., intestinal microbiota in mice can affect the formation of IAs by modulating inflammation through depletion of macrophage infiltration and lowering of inflammatory cytokine levels in blood vessel walls (59).

Understanding all aspects of the role that inflammation plays in the formation of aneurysms is essential because there are many sites that can potentially present target sites for pharmacological treatment of aneurysms. Some agents were studied for inhibiting aneurysm

development, such as statins, aspirin, and inhibitor of phosphodiesterase-4 (Ibudilast), but their role in the treatment has not yet been conclusively proved (6).

Aorthopathy

According to recent evidence, there are correlations between IAs and AAs, especially TAAs, which are based on an aortopathy of a different cause (Figure 2) (4, 5, 20, 46, 47, 60, 61).

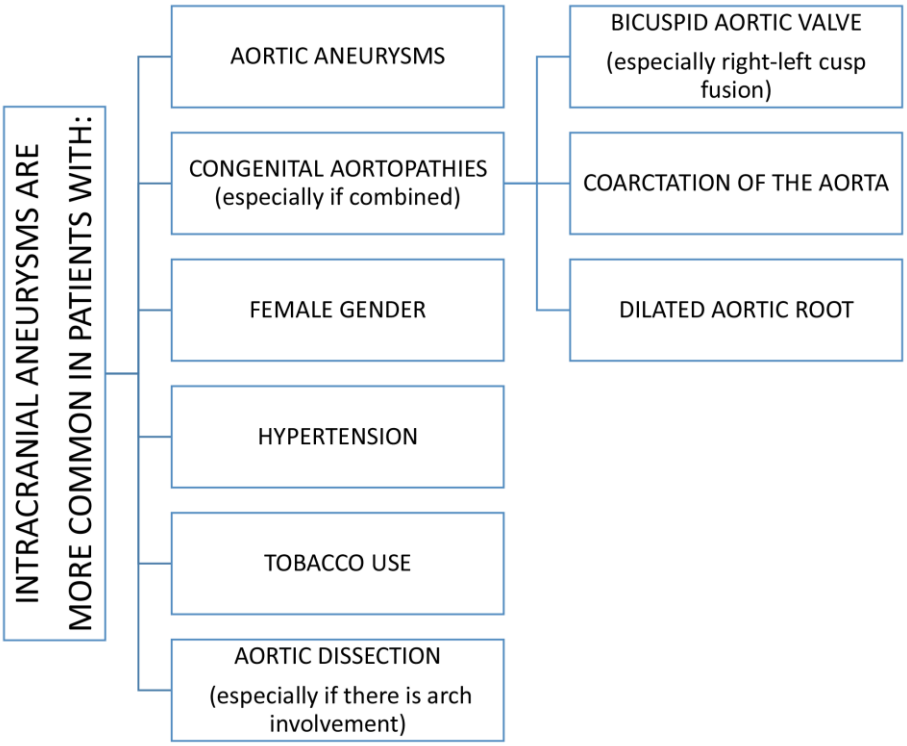

Figure 2. Algorithm for relation of IAs with risk factors and aortopathies

Regarding the prevalence of IAs, studies have shown similarities in groups of patients already known to suffer from AAs (4, 5, 20, 46). Rouchaud et al. conducted a study on 1081 patients with AAs and found the prevalence of IAs to be 11.8% (46). Likewise, Shin et al. carried out a study on 611 patients with AAs and showed the prevalence of IAs to be 11.6% (20). In addition, Jung et al. searched for IAs in patients with dissecting AAs, and the prevalence was 13% (4). Furthermore, research by Kuzmik et al. conducted on 212 patients with TAAs showed a prevalence of 9.0% for IAs (60). These results show a much greater prevalence of IAs in patients with AAs, with the rate of IAs four times higher in patients who also have AAs (4, 20, 46) than in the general population.

TAAs and IAs do not correlate with atherosclerotic risk factors (2, 20, 45-49). Research by Rouchard et al. showed that coronary artery disease and hyperlipidemia actually lowered the odds of IAs (46). Multiple aneurysms in patients with IAs occur in 15–45% (5, 62, 63) of cases, which may suggest that another underlying cause increases susceptibility to aneurysms.

Another study by Shin et al. focused on congenital aortopathy. They identified the presence of BAV and CoA (5), already known conditions that are connected with greater

prevalence of IAs (64-66). These are conditions that are usually associated with a dilated aortic root (5). The study showed that a group of people older than 55 who had large IAs (more than 7 mm in diameter), multiple aneurysms, or ruptured aneurysms had a significantly higher prevalence of dilated aortic roots (5). Another group in this study consisted of patients without the high-risk factors mentioned for the first group (5). The second group had a significantly higher percentage of cerebrovascular risk factors, such as hypertension, diabetes mellitus, and hyperlipidemia, as well as strokes (5). Similarly, a study by Egbe et al. conducted on 678 patients with BAV showed an IA prevalence of 7.7% (66). In this study, the prevalence of IAs in patients with both BAV and CoA was 12.9% (66). In addition, the presence of right-left cusp fusion in patients with BAV was found to be a significant risk factor for developing IAs (66). A study by Schievnik et al. conducted on patients with BAV found that 9.8% of patients had IAs (65). In a study of 117 patients with CoA, Curtis et al. found the prevalence of IAs to be 10.3% (48). A similar study by Connolly et al. conducted on 100 patients with CoA determined that 10.0% of patients had IAs (64).

Aortic dissection is another condition that has been studied to evaluate its co-occurrence with IAs. As mentioned above, a study by Rouchaud et al. conducted on 71 patients found the prevalence of IAs to be 13% in patients with dissections (4). That is about four times higher than in the general population (4). These patients had hypertension and a history of cerebrovascular disease in numbers significantly higher than in the control group (4). This study found a significant correlation between the presence of IAs and the arch involvement seen in aortic dissection (4).

An additional important variable with influence on IA formation in patients with AAs is gender (4, 5, 20, 46). A few studies have shown that women are more prone to aneurysms compared to men (20, 46, 66), but this finding requires further research.

Conclusion

According to available data in the areas of genetics, pathophysiologic findings, risk factors, and aortopathies, there are strong relationships and predispositions for concomitant aneurysmatic formation in different loci. The embryological link between the ascending aorta and the intracranial arteries lies in the origin of SMCs or NCCs. At the developmental level, the septation of heart outflow tracts, such as a BAV, CoA, and dilated aortic root, is in direct relation with IAs as a result of impaired initiation of elastogenesis. Atherosclerosis is not a unique risk factor for IAs and TAAs, but is directly related to AAAs as well.

There are still many unanswered questions in this area in regard to the following: a) genetic predisposition; b) structural and mechanical properties: the extensive differences in histology and pathophysiology, the cause of different shapes of IAs (saccular) and TAAs (fusiform), and difference between vessel wall structures; c) epidemiological risk factors between these three conditions (IAs, TAAs, and AAAs); d) gender differences; e) the impact of ageing; f) the need for further research and gene detection.

Co-occurrence of aortic and cerebral aneurysms exists in multiple conditions. It is not likely that a single genetic disorder will be found to explain the co-occurrence of both aneurysm sites. When it comes to explaining this complex dual pathology, the most probable pathophysiological explanation is that several levels of genetic defects, plus a multifactorial environmental effect, result in arterial wall weakening and the formation of aneurysms in multiple locations.

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Review article

Nutrition Patterns in Prevention and Treatment of Neurodegenerative Diseases: Alzheimer's Disease

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Abstract

Background: The population of the elderly is increasing, as is the number of people suffering from neurodegenerative diseases. Since the cause of those diseases is unknown, there is no appropriate medical treatment. The purpose of this systematic review is to present papers on consumption of certain foods, supplements or introduction of dietary restrictions that promote healthy brain aging or possibly delay the onset of disease.

Methods: The PubMed, ScienceDirect, and SpringerLink databases were used for the research. 24 studies with a total number of 10 445 participants were selected as satisfying the final criteria.

Results: Mediterranean diet, dietary supplements and natural nutrients with recently discovered pharmacological properties are of interest to numerous scientists.

Conclusion: Since the results of the studies are inconsistent, we concluded that a large, carefully controlled long-term interventional study would be required in order to investigate the effect of nutrition on prevention of disease and cognitive decline.

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KEYWORDS: Alzheimer's disease, inflammation, amyloid plaques, berries, supplements

Introduction

The number of people who are over 65 is growing and it is estimated that said age group will constitute up to 30% of the entire world population by the end of 2050. Aging includes changes to all organs and body systems, as well as to the brain. In 2001, there were 24 million people in the world older than 60 and suffering from dementia. It is estimated that this number will double every twenty years and that by the end of 2040 80 million people will be suffering from it (1). The economic burden of care and treatment of neurodegenerative diseases also increases the need for seeking measures for prevention or reversing of age-related disorders (2). A neurodegenerative age-related disease like Alzheimer's (AD) appears to be the consequence of stress and of the same environmental factors that are responsible for the aging of other organs and body systems. The clear cause of the disease is still unknown, but some key changes, including membrane/synaptic degeneration and abnormal protein processing resulting in the formation of amyloid-β plaques and neurofibrillary tangles, have been detected (3). Oxidative stress and inflammation are thought to play a significant role in the early stage of the disease, forming a vicious circle of damaging the sensitive brain cells. Epidemiological studies point out that anti-inflammatory, antioxidant and neuroprotective agents found in food or plants may have a positive effect by strengthening the neuronal antioxidant defence. The Mediterranean diet has also been linked with a lower incidence of neurodegenerative diseases such as AD (4). Neurodegenerative diseases cannot be prevented using specific medication or treatment, so identifying a new natural compound with pharmacological properties is the subject of interest to a large number of scientists. Chronic neurodegenerative diseases like AD and Parkinson's disease are related to obesity and diabetes, not just in the developing countries, but in the developed countries as well. Dietary restrictions, including a diet low in saturated fats, cholesterol and carbohydrates and the consumption of berries, nuts and neuroprotective plant supplements, might have a positive effect on healthy brain functioning (5, 6).

Burgener et al. (2008) state that some people live to be 90 and have adequate cognitive functioning while some develop symptoms of AD in their late 50s. The difference might be due to interaction between genetics and environment (1). Since we cannot fully affect genetics, this review will focus on actions promoting healthy brain aging and possible delay of the onset of the disease, including the consumption of certain foods, supplementation and introduction of dietary restrictions.

Methods

Literature research took place in January 2018. Databases used in the search were PubMed, ScienceDirect and SpringerLink. Keywords neurodegenerative diseases, prevention, Mediterranean diet, nutrition, supplementation were entered to identify papers dealing with neurodegenerative diseases and nutrition. The PubMed database yielded 2 articles. Another 102 articles were found after using the option "similar results" with the first paper and 98 articles were found for the second paper.

Inclusion Criteria

All articles containing the terms Alzheimer's disease, neurodegenerative diseases, dementia, cognitive decline, mild, Mediterranean diet, nutrition, vitamin B, C, E, D, antioxidants, supplementation, MUFAs, PUFAs, ω-3, ω-6, olive oil, prevention, cohort, prospective in the title or abstract were included in the review. If terms physical activity, cancer, cardiovascular diseases, respiratory problems, drugs appeared in the title or abstract of the article, they were excluded. Other exclusion criteria were if a paper included animal research (dogs, rats), if it tested medicinal dietary supplements or if it could not be fully accessed.

The criteria were ultimately met by 24 articles regarding studies conducted on 10 445 participants.

Results

The final results obtained after applying filters are shown in Table 1.

Table 1. PubMed search methodlogy and results

FILTERS	PAPER 1	PAPER 2
similar results	102 new articles	98 new articles
from 2000 until 2018	97	98
on humans	92	96
English language	85	87
persons 65+	16	21
review of abstracts and title of the articles based on including criteria	8	7

The same keywords were used to search the ScienceDirect database and 430 articles were identified initially. This number was narrowed to 372 after setting the timeframe to include articles published from 2009 to 2018. The number of articles was further reduced to 69 by using the option "type of article – research" and to 11 by using the option "publication title". They were published in the journals Clinical Nutrition, Alzheimer's & Dementia and Food Research International. After reading the titles and abstracts, 4 articles were selected.

The above-mentioned keywords were used for searching the SpringerLink database, where 264 articles were identified. After applying the filter article, the initial number was reduced to 177 and then to 23 after using the filter "biomedicine". Further restriction of results was accomplished using the options "Neurosciences and English", which led to 10 articles. Titles and abstracts were reviewed, and 3 articles were selected.

Discussion

Neurodegenerative diseases and nutrition

Dry matter in the brain mostly consists of fat, 20% of which is made up of ω-3 and ω-6 polyunsaturated fatty acids, which significantly influence the structure and composition of the neuronal membrane. Adequate nutrition is the source of those acids. Aging brings changes in the taste and smell of food, the basal metabolic rate decreases, and due to possible swallowing impairment and impaired digestion, dietary intake is also reduced. Unsaturated fatty acids and vitamins are thus in deficit. Neuronal membrane lipids change and influence homeostasis necessary for membrane fluidity and function, as well as for prevention of loss of synaptic plasticity, apoptosis and neurodegeneration (3). Since the cause of neurodegenerative diseases is still unknown, numerous researchers have made speculations regarding the connection between dietary habits, food intake and aging as the possible link for prevention or delay of the onset of neurodegenerative diseases, especially AD (7). Particular attention was given to diets such as the Mediterranean diet, food supplements and introduction of dietary restrictions.

Mediterranean diet

Notably, there are few published studies dealing with primary or secondary prevention of AD through nutritional interventions. Most of them have focused on the effects of single nutrient supplementation (3). However, studies should focus on the entire dietary pattern and not just on a single nutrient in order to avoid neglecting important interactions between food components since people do not eat only single

nutrients (7). After analysing a number of dietary patterns, the Mediterranean diet was selected. This type of diet is characterized by consumption of fruit and vegetables, legumes, fish and complex carbohydrates on a daily basis. Olive oil is the main source of fat, and moderate drinking of red wine at meals is recommended (7). One of the first studies linking the Mediterranean diet to a lower risk of developing AD was conducted in 2006 in New York on 2258 non-demented participants. After 4 years of follow-up, the results showed that higher adherence to this type of diet lowers the risk of developing AD, which indicates that that such diet may have a protective effect (8). Anther cohort study, the Washington Heights/Hamilton Heights Columbia Aging Project (WHICAP) conducted in 2012 on 1219 non-demented people, found that higher levels of ω-3 polyunsaturated fatty acids from fish, poultry, nuts and margarine were significantly associated with lower levels of plasma Aβ42, which suggests that they play an important role in lowering the risk of AD and slowing cognitive decline (9). Consumption of at least 100 grams of olive oil per day as one of the sources of polyphenols also slows cognitive decline (1). Morris et al. (2015) studied the connection between the MIND diet, a hybrid of the Mediterranean diet, and the occurrence of AD. The MIND diet contained 15 nutrients, 9 of which were linked to healthy brain aging (leafy greens, nuts, berries, legumes, whole grains, fish, poultry, olive oil, red wine) and 6 to unhealthy brain aging (red meat, margarine, cheese, pasta, sweets, fried food). The study was conducted on participants of the Rush Memory and Aging Project (MAP) in Chicago from 2004 until 2014, which included 923 people aged 58 to 98. The results showed that even moderate adherence to the MIND diet has a protective effect, significantly lowering the number of AD cases. This does not necessarily mean that the MIND diet is specifically effective for AD, but that it has a protective effect on the brain and general functioning (10). Fruit and vegetable consumption typical for the Mediterranean diet showed a link with lower risk of AD and dementia in women, while consumption of fish and seafood significantly correlated with lower incidence of dementia in general among the people of southwestern France (11). The Mediterranean diet, alone or in combination with other dietary patterns, represents the most effective approach to slowing the progression of AD with the fewest possible adverse effects (11). Persons who adopt this type of diet are less likely to develop AD than their peers who do not (12).

Supplements and their role

Vitamins are important for normal functioning of the whole body, including the brain. Therefore, the American Food and Drug Administration Agency mandated fortification of grains with folate from 1996 until 1997 to prevent vitamin deficiency (13). It is believed that a deficiency of folic acid and other B vitamins, along with vitamin C and E deficit, can contribute to the development of AD (14). However, results of vitamin supplementation are inconsistent. A longitudinal cohort study conducted on 965 people over 65 years in New York (northern Manhattan) suggests that lower risk of AD development is associated with total folate intake from both food and dietary supplements, rather than intake from a single source, while intake of vitamins B6 or B12 is not linked to or significant for the risk of AD (13). Similar results were obtained in a cross-sectional study conducted on 1219 cognitively healthy people older than 65, where no significant link was found between lower risk of AD and vitamin B9, B12, C, E and β-carotene supplementation (9). A longitudinal cross-sectional study conducted on 4470 participants (the Cache County Study) aimed to determine the link between antioxidant supplementation and the risk of AD development. Previous studies reported that vitamins C and E act neuroprotectively and lower the risk of AD by scavenging free radicals and other reactive oxygen species (15). A longitudinal study in Sweden involving 1810 people older than 75 (Kungsholmen Project), conducted from 1987 to 2000, identified that lower levels of vitamin B12 and folic acid, probably due to malnutrition or malabsorption in the elderly, double the risk of AD (16). Vitamins B9 and B12, unlike vitamins C and E, regulate

homocysteine levels in the blood, which are associated with the risk of AD. A multicentre randomized double-blind controlled clinical trial conducted in America (ADCS) from 2003 until 2006 on 340 participants reported that high dosage of vitamin B6, B12 and folic acid supplementation did not delay cognitive decline, nor did it improve the clinical status of persons with mild or moderate AD (17). The discrepancy of results may be due to the different mechanisms of action of vitamins in prevention of AD, the number of participants and their age, the type and duration of the study, pre-existing vitamin deficiency and the type of prevention involved (primary, secondary or tertiary). Berti et al. (2015) conducted a cross-sectional study on 52 participants at risk of developing AD from 2013 to 2014 to clarify how nutrients are associated with healthy brain aging and prevention of AD. The objective was to identify how different patterns of nutrition affect the formation of Aβ plaques (initial sign of pathological changes), glucose metabolism (indicator of neuronal activation) and grey matter volume of the brain (sign of atrophy). The results showed that higher intake of vitamins B12, D and Zn reduces Aβ plaque load in the brain, as well as increases neuronal activity and grey matter volume. Higher activation of neurons was registered during higher intake of vitamin E, ω-3 and ω-6 polyunsaturated fatty acids, vitamins A, C and whole grains. Intake of vitamins B 1, 2, 3, 6, 9 and minerals Ca, Fe, Mg, P, K, Se showed no significant effect on the formation of Aβ plaques, neuronal activation and grey matter atrophy, while higher intake of saturated fatty acids, food high in cholesterol and sodium had a significant impact on lowering neuronal activation and grey matter volume (18). Results obtained from a small number of carefully screened subjects in another large cohort study (Three-City Study) conducted in three French cities from 1999 and 2000 until 2012 on 9294 people, only 666 of whom were non-demented, confirmed that there is a strong link between lower intake of vitamin D, carotenes and polyunsaturated fatty acids, higher intake of saturated fatty acids and long-term risk of AD and dementia. Future interventional research on primary prevention of AD and dementia should focus on the optimal combination of nutrients based on the results obtained so far (19).

Polyphenols

This paper did not focus on the effects of polyphenols on animals, only on humans. Vitamin P was first mentioned in 1936, and has been plentiful in the human diet since then. Polyphenols are secondary plant products believed to have a positive effect on human health, particularly for the elderly (20). Polyphenols from different fruit such as blueberries, blackberries, grapes and apples, from green and black tea, wine, coffee, cocoa and from spices such as turmeric and curry possess anti-inflammatory, antioxidant and neuroprotective properties (21). Research on polyphenols in Japan and Singapore has indicated that they have the potential to be neuroprotective (12), although the underlying mechanisms have not been fully clarified. Polyphenols interact with a wide range of neurotransmitters, suppressing neuroinflammation and degeneration through direct, indirect or complex action (22). Since 1936, almost 10 000 articles have been published on the chemical nature and biological activities of polyphenols (20), suggesting that they have a neuroprotective effect. Evidence from first-in-human research has identified that polyphenols have a positive effect, as part of a balanced diet. However, the effects of antioxidants have been disputed in the past few years. It has been suggested that their metabolites do not cross the blood-brain barrier, which brings their neuroprotective effect into question (20).

Caloric restrictions

The average energy requirements for an adult person are 25 kcal/kg/day. They decline after adolescence because the human body stops growing and basal metabolic rate decreases. Between the ages of 55 and 75 energy requirements decrease by 8% for every decade and by an additional 10% for every decade after 75 (23). Caloric restriction as a way of primary

prevention of AD may be used only in those individuals with normal eating patterns. Malnutrition and weight loss are usually associated with the onset of AD; caloric restriction would thus be counterproductive (23).

Discussion

Although no cure for neurodegenerative diseases exists, prevention and delaying the onset of the diseases is important. Large cohort studies have confirmed the protective role of diets like the Mediterranean diet, of a higher intake of polyunsaturated instead of saturated fatty acids, of a higher intake of fish and of vitamin supplementation (24). The role of decreased levels of a single nutrient and its supplementation in prevention of cognitive decline and dementia are eliciting controversy, probably because supplements act in a synergistic way and single nutrient supplementation will not lead to improvement (25). Seven lifestyle risk factors have been identified in connection with AD. Besides low education, physical inactivity, smoking, depression and hypertension, the role of nutrition is the most controversial and the role of sleeping disorders has not been sufficiently researched (26). A carefully controlled long-term interventional study on the effects of diet on prevention and delaying the onset of disease is required (24), with special consideration given to participants, type and duration of study, biomarkers for AD and intermediate outcomes (26).

Conclusion

The most investigated nutrients in regard of AD and cognitive decline are ω-3 and ω-6 polyunsaturated fatty acids from fish, poultry, nuts and margarine, as well as, folic acid, vitamin C and E. Consumption of polyphenol-rich foods like blueberries, blackberries, grapes, apples or beverages such as green and black tea, coffee and red wine may be beneficial. Adherence to Mediterranean diet is recommended because of the protective effect on the brain and general functioning. Caloric restriction may be counterproductive in elderly. Future intervention studies aiming to influence the modifiable lifestyle factors and to reduce the number of people at risk for AD, or at least slow down cognitive decline, are required.

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Original article

Effect of Low Selenium Diet on Glutathione Peroxidase 3 Concentration in Male Sprague-Dawley Rats' Serum

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Abstract

Aim: Determination of antioxidative enzyme glutathione peroxidase 3 (GPx3) serum concentrations after consumption of food which contains different concentrations of selenium (Se).

Research subjects and methods: Four-week-old Sprague Dawley rats consumed food containing different concentrations of Se (food Divan) over a period of 10 weeks. The animals were divided into two groups: 1) normal Se (0.363 mg/kg Se) and 2) low Se (0.030 mg/kg Se). Each animal was weighed at the end of protocol, and serum samples were collected for determining GPx3 concentrations. All experimental procedures were in compliance with the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes and approved by the Ethics Committee of the Faculty of Medicine in Osijek and the Ministry of Agriculture of the Republic of Croatia.

Results: Different concentrations of Se in food did not cause a change in body weight. Food containing the recommended intake of Se according to the guidelines of the World Health Organization significantly increased GPx3 enzyme concentration (13.96±0.42 mg/ml) when compared to low selective Se (12.04 ± 0.33 mg/ml, p = 0.002).

Conclusion: Serum concentration of the antioxidant enzyme GPx3 depends on the concentration of Se in food. It is shown that, in comparison with food with low Se levels, food containing a normal concentration of Se is enriched with the antioxidant GPx3 which, according to numerous studies, has a protective role in the human body.

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Introduction

The most important antioxidant enzymes are catalase, glutathione peroxidase, glutathione reductase and superoxide dismutase, which have harmful oxidative metabolic intermediates. These enzymes require cofactors selenium (Se), zinc, copper, iron, and magnesium for their catalytic activity (1).

Glutathione peroxidase (GPx), which is the subject of our study, is a family of enzymes that metabolize hydrogen peroxide and lipid hydroperoxide into water. They have a binding site for Se, which oxidizes in reaction with hydrogen peroxide (2-4). Glutathione peroxidase is involved in protection against oxidative stress in that it participates in the transfer of amino acids through the plasma membrane, removes the hydroxyl radical and the singlet oxygen, thereby detoxifying the hydrogen peroxide and the lipid peroxide by the catalytic action of GPx. It can replenish the active forms of the most essential vitamins, i.e., vitamins C and E (5).

It is known that oxidative stress is one of the pathogenic mechanisms that cause disorders of the vascular system and contribute to development and progression of various cardiometabolic diseases (hypertension, diabetes, atherosclerosis, obesity). A diet enriched with trace elements, which increase the concentration of antioxidant enzymes, could provide better protection against oxidative stress (6).

As part of GPx, Se is important for the regulation of the oxidative system (7-9). It is an essential trace element which must be ingested in sufficient quantity through food. In many countries, there is malnutrition due to the lack of Se as a micronutrient. Inadequate Se intake causes many different disorders (necrotizing cardiomyopathy, peripheral myopathy, reduced muscle tone, concentration problems, hair loss and nail splitting) (10-12). In our previous animal study, we showed that low dietary Se content affects the function of aorta, reduces ACh-induced relaxation, which is dominantly mediated by NO, and also increases the level of local oxidative stress (13).

Human studies have shown that Se concentrations are inversely related to mortality and occurrence of cancer (14, 15). Se functions as an antimutagenic agent that prevents the transformation of healthy cells into malignant ones; it is assumed that these protective effects are primarily associated with the activity of GPx (16, 17).

Glutathione peroxidase 3 (GPx3) is the only enzyme in the GPx group that functions in extracellular space. The substrates for this enzyme are hydrogen peroxide and phospholipid hydroperoxides, which play a significant role in the antioxidative processes in the blood (18) in that they decrease oxidative stress by reducing H2O2 and organic hydroperoxides to their corresponding alcohols and oxygen (19). Since increased GPx3 activity is found in certain subtypes of tumors, like ovarian tumors (20), it can also be used as a biomarker.

This study aims to determine the level of the GPx3 antioxidative enzyme in serum samples with different levels of Se and to determine whether Se causes a change in body mass.

Materials and Methods

Ethical Approval

Experimental procedures complied with the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Council of Europe No 123, Strasbourg 1986) and were approved by the Ethics Committee of the Faculty of Medicine, University of Osijek (Class: 602-04/14-08/06, No: 2158-61-07-14-05), and authorized by the Ministry of Agriculture of the Republic of Croatia (Class: UP/I-322-01/14-01/90, No: 525-10/0255-15-4). The tests were performed in the Laboratory for Vascular Physiology and Laboratory for Molecular and Clinical Immunology at the Department of Physiology and Immunology of the Faculty of Medicine Osijek, Croatia.

Experimental groups

Food was prepared at the Faculty of Agrobiotechnical Sciences Osijek, Croatia, based on the wheat briquettes recipe developed by Mucedola, Italy. Wheat was specially grown in soil with different concentrations of selenium and zinc, so that the concentration of said micronutrients was adjusted according to the requirements of the experiment. Four-week-old healthy male Sprague Dawley rats (N = 7 per group) consumed the above-mentioned food for ten weeks. The animals were randomly divided into groups:

1) normal Se group (0.363 mg/kg Se) and

2) low Se group (0.030 mg/kg Se).

Rats were housed doubly in shoebox-style cages with free access to food and tap water, housed in a temperature of -21˚C-22˚C, humidity- and light-controlled room, maintained on a 12:12 hour light : dark cycle.

Determination of Se (normal or low concentration) in food is consistent with previous studies (13, 21-24).

After 10 weeks of feeding, the animals were weighed and then anesthetized using a combination of ketanest S 75 mg/kg (Ketanest S 25 mg/ml, 2 ml ampoules, Pfizer) and midazolam 0.5 mg/kg (Midazolam Torrex 5 mg/ml, 3 ml, Torrex Chiesi Pharma). Blood samples were collected immediately after decapitation (arterial and venous blood) in empty tubes without anticoagulants to obtain serum and centrifuged at 3500 rpm for 10 minutes. The separated serums were stored in a refrigerator at -80 ˚ C until analysis.

Glutathione peroxidase 3 (GPx3) determination in serum samples

GPx3 concentrations were determined using the commercially available enzyme immunoassay kit purchased from LifeSpan BioSciences, USA (LSBio Cat. No. LS-F6289). Each well of the supplied microtiter plate was precoated with an antibody. First, 100 μ l of serum samples and standards (in duplicates) were put in their appropriate place on the ELISA plate and incubated for 1 hour at 37 ˚ C. After the first incubation, the unbound standard or sample was washed away, 100 μ l of biotin-conjugated detection antibody was added to each well and the same time and temperature incubation was continued. After that, it was washed 3 times and Avidin-Horseradish Peroxidase (HRP) conjugate was added, followed by a 30-minute incubation at 37 ˚ C. A TMB substrate was then added, which reacted with the HRP enzyme, resulting in color development. At the end of the protocol, stop solution was added to terminate the color development reaction and optical density (OD) was measured at a wavelength of 450 nm on the PR 3100 TSC Microplate Reader in the Laboratory for Molecular and Clinical Immunology at the Department of Physiology and Immunology of the Faculty of Medicine, Josip Juraj Strossmayer University of Osijek.

Statistical analysis

Differences in normally distributed numerical variables between groups were tested with the Student t-test, and in case of deviations from normal distribution with the Mann-Whitney U test (SigmaPlot version 11.2, Systat Software, Inc., Chicago, USA) The level of significance was determined at $p < 0.05$. The sample size was determined using the Sigma Plot version 11.0 program. For the power of the test of 0.8, p value less than 0.05 and the minimum expected difference of 0.25, it was found that at least 4 animals per group were required. Results are presented below as the mean ± standard deviation (SD).

Results

Effect of diet on body weight

There was no difference in body weight between the normal Se and the low Se group ($p < 0.05$) (Figure 1).

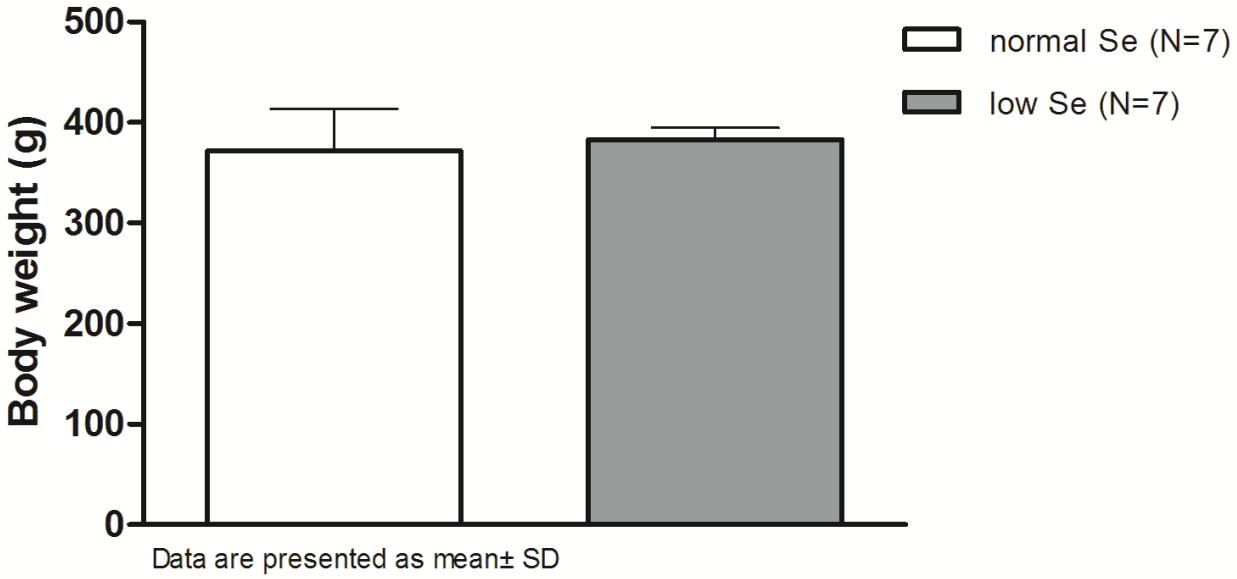

Figure 1. Body weight in experimental rat groups

GPx3 serum concentration

The group of animals that consumed food with normal Se content had significantly increased GPx3 enzyme concentrations (13.96 ± 1.11 ng/ml) compared to the low selective Se content group (12.04 ± 0.89 ng/ml, p = 0.002) (Figure 2).

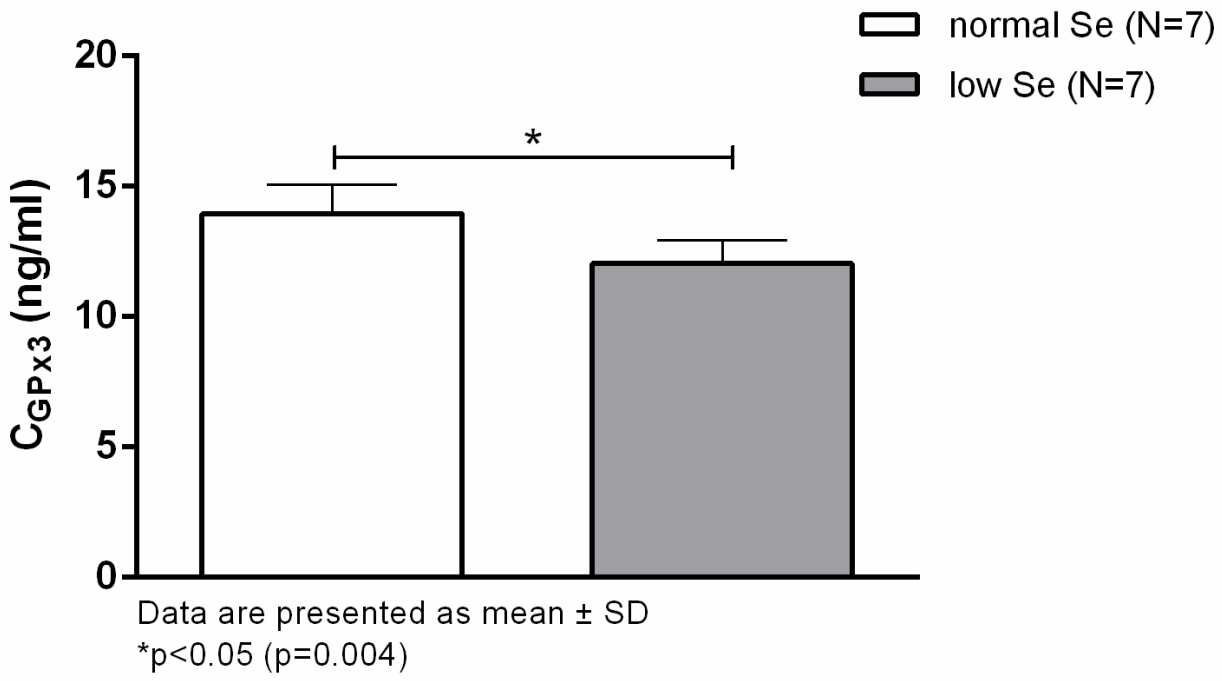

Figure 2. Concentration of the antioxidative enzyme GPx3 (ng/ml) in serum samples

Discussion

We recently showed that low dietary Se content increases oxidative stress, which is possibly caused by decreased gene expression of the antioxidant enzyme GPx1 in thoracic aortic tissue without changes of the same enzyme in serum samples (13). Gene expression of other antioxidative enzymes (Cu/Zn SOD and catalase) did not change significantly. GPx3 is a better isoform of GPx for determination of the antioxidant effect of Se in blood samples, so we decided to measure the more appropriate GPx isoform, which is readily detectable in the blood (25).

The main findings of this study are the following: a) serum concentration of the antioxidant enzyme GPx3 depends on the concentration of Se in food; b) our study once again confirmed that food with sufficient levels of Se (according to the World Health Organization (WHO) and the American Institute of Nutrition (AIN)) is richest with the antioxidant GPx3, which has a protective role in the body; c) different concentrations of Se have no effect on body weight.

Trace elements are essential for living organisms because they are necessary for normal function of the organism, its growth and performance of its metabolic functions. Amounts in the human body range from 1.5 mg to 4.5 g. It is characteristic for trace elements that very small amounts of a trace element affect the condition of the entire organism (26, 27). Levels of Se in food can vary in different parts of the world and countries (28, 29). Intakes of Se are high in America and Japan, and much lower in Europe, particularly Eastern Europe (30).

It was recently shown that Se has an important role in antioxidant selenoproteins used for protection against oxidative stress initiated by ROS and its compounds in different types of cancers, cardiovascular diseases, immune system diseases and aging (31, 32). The World Health Organization published findings that a diet which contains 0.1 mg Se per kilogram of food is sufficient for normal function of the organism (33), while the American Institute of Nutrition (AIN) and the TestDiet® AIN-93 Growth Purified Diet indicate that 0.24 mg Se/kg in food is optimal (21). According to these guidelines, we determined the values of low (0.030 mg/kg Se) and normal (0.363 mg/kg Se) levels of Se for our research. Measuring Se levels in blood samples seems to be a good indicator because plasma and serum contain about 75% of the Se. The level of Se found in these samples is directly related to recent dietary intakes (29).

Our research has shown that consuming food containing sufficient amounts of Se according to the guidelines provides a significantly higher level of GPx3 antioxidants in plasma compared to foods with low Se concentration (Figure 2). Such findings indicate that Se is directly responsible for increasing the antioxidative status of GPx and for lowering oxidative stress.

Se and its protective role against disease are mostly related to the removal of free radicals and the enzymatic breakdown of oxygen metabolites (34). For example, in tuberculosis patients, reduced oxidative stress was caused by ROS generation with Se supplementation (35). Of all types of tissue, the thyroid gland has the highest Se concentration, and in the form of GPx3, Se protects thyroid cells from free radicals and oxidative stress (36, 37). It was shown that in Hashimoto's thyroiditis Se supplementation significantly lowered thyroid peroxidase autoantibody titer after 3 months (38). Furthermore, studies showed that Se has a beneficial effect on lowering the risk for different types of cancer (39- 43). It can reduce oxidative damage and prevent DNA damage (44). It can be used as alternative medicine by cancer patients undergoing radiotherapy (45 - 47). In addition, cellular and molecular processes that might be involved in the anti-cancer effects of Se are stabilization of the immune response, induction of programmed cell death and inhibition of angiogenesis (44).

Lack of the Se causes irreversible brain injury (48). Similar, lower serum Se was also found in children with epileptic seizures or febrile seizures and in adults with epileptic seizures (49, 50).

Se has also been proved to be important for maintaining the immune system. It has been shown that Se in a concentration of 400 μg per day significantly increased the number of T-cells (51), and supplementation with 100 μg per day for 6 months significantly increased the response to antigen challenge (52). Se deficiency may cause a slower immune response because of the negative influence it has on immune cells (53).

ROS is involved in regulating the synthesis of adhesion molecules on endothelial cells, and is essential for inflammatory responses during the early stages of a disease (54, 55). The expression of intercellular and vascular adhesion molecules increases significantly during inflammation and is involved in the firm adhesion of leukocytes and endothelial cells (54). Se nutritional status can directly influence vascular endothelial cell functions, e.g., under low Se conditions, aortic endothelial cells exhibit increased platelet activating factor biosynthesis, which causes vascular disorders that are affected by increased oxidative stress (56). It was also found that the production of prostaglandins was significantly decreased in Se-deficient endothelial cells, which are associated with the pathophysiology of several inflammatory diseases through significantly increased biosynthesis of thromboxane B2 (TXB2) and 15-hydroperoxyeicosatetraenoic acid (15-HPETE) (57, 58). Crucial role of Se in oxidative stress and endothelial influence has been demonstrated by the study Stupin et al. (2017), which showed that increased oxidative stress affects a NO-mediated response in low-Se aortas probably due to decreased NO bioavailability (13). Selenoproteins regulate vascular tone by establishing a balance between superoxide anion and nitrogen oxide (7), which was found to be the case in this study as well.

Numerous studies have shown the important role that Se plays in different health/disease states. Further research is necessary to make use of the benefits of this important element as much as possible. Observing the specific role of Se in the endothelium, glutathione peroxidase was found to have an indispensable role in the maintenance of normal vascular function. Specifically, low Se decreased tissue expression of GPx1 and blood concentration of GPx3, which caused an increase in oxidative stress and disrupted endothelial function (Figure 3).

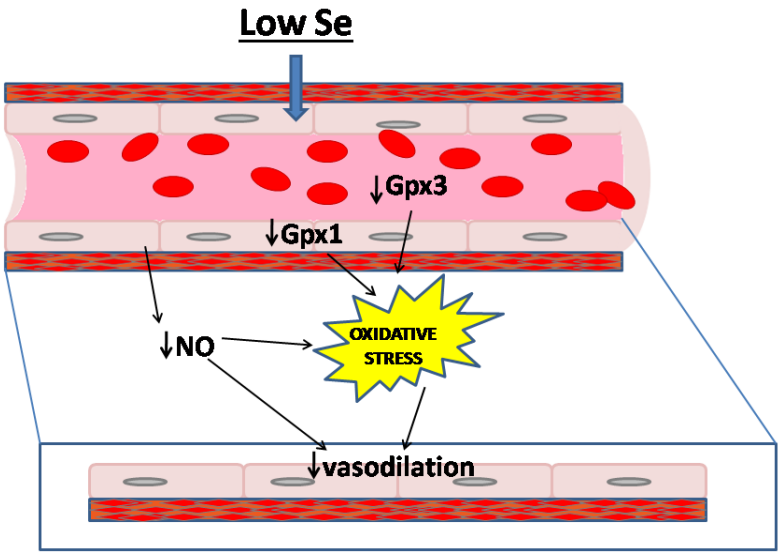

Figure 3. Negative effect of low Se levels on changed vascular antioxidant status and consequent endothelial dysfunction

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Original article

Significance of HbA1c in Monitoring Diabetes at the Public Hospital, Guyana

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Abstract

Aim: To determine the prevalence of baseline/mean HbA1c among patients with suspected diabetes using Bio-Rad for the first time at a public hospital in Guyana.

Methods: A retrospective, laboratory-based, descriptive study examined 1,547 diabetic patients who underwent repeat HbA1c testing at a public hospital laboratory in Guyana between 2010 and 2014. All statistical analyses were performed using SPSS 21.0, JMP and Microsoft Excel. Distributions were used to show frequencies, the bivariate fit test was done to achieve correlations and significance, the $\chi 2$ test was used to compare the differences in proportions and ANOVA was used for differences in mean differences.

Results: A total of 1,547 patients were identified for the study. Mean age and standard deviation (SD) was 59.9 ± 11.47 (95% CI 59.3 - 60.4). Mean value for test 1 HbA1c was 9.2 ± 2.7 (95% CI 9.1-9.3), test 2 HbA1c recorded 9.1 ± 2.5 (95% CI 8.9-9.2), test 3 HbA1c was 9.6 ± 2.6 (95% CI 9.3-9.9) and test 4 HbA1c recorded a mean of 9.6 ± 2.6 (95% CI 9.0-10.2).

Conclusions: The mean HbA1c of the diabetic population was observed to be higher than the baseline in all tests.

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Introduction

Diabetes mellitus is defined by the World Health Organization (WHO) as a chronic disease caused by inherited and/or acquired deficiency in the production of insulin by the pancreas (1). It is estimated that 39 million people are living with diabetes in the North America and the Caribbean (NAC) region (2). In 2014, there were 61,800 cases of diabetes in Guyana and diabetes was the cause of 1,025 deaths in adults aged 20-79. The International Diabetes Federation (IDF) also estimated that there are about 15,400 undiagnosed cases of diabetes (3). With the prevalence of diabetes increasing at an alarming rate both nationally and internationally, diabetes-related complications and deaths tend to increase as well (4-8). It is therefore critical to maintain good glycemic control to keep diabetes in check and reduce diabetes-related complications (9).

HbA1c has been recommended as a test option for the diagnosis of diabetes by both the American Diabetes Association (10) and the World Health Organization (WHO) (11). Both organizations advised that after a result consistent with the diagnosis of diabetes (≥ 6.5% (48 mmol/mol)), testing of HbA1c levels should be repeated in asymptomatic patients within 2 weeks to rule out the rare occurrence of a sample being mislabeled. Not much research has been conducted in the area of repeat testing of HbA1c; however, Driskell et al. (2014) stated that "the optimal testing frequency required to maximize the downward trajectory in HbA1c was four times per year, particularly in those with an initial HbA1c of ≥7% (≥53 mmol/mol), supporting international guidance. Testing 3-monthly was associated with a 3.8% reduction in HbA1c compared with a 1.5% increase observed with annual testing; testing more frequently provided no additional benefit. Compared with annual monitoring, 3-monthly testing was associated with a halving of the proportion showing a significant rise in HbA1c (7–10 vs. 15–20%)" (12). The authors concluded that monitoring frequency is associated with a significant detrimental effect on diabetes control and that to achieve the optimum downward trajectory in HbA1c, monitoring frequency should be quarterly.

There are also reports that suggest that guidelines for testing HbA1c are not necessarily being followed (13-15). This study therefore evaluated for the first time the baseline/mean HbA1c (mmol/mol) among the Guyanese population and aims to determine if a standard international algorithm has been followed in regard to repeat testing of patients with suspected diabetes and of undiagnosed/high-risk population.

Methods

This was a laboratory-based retrospective study conducted between 2010 and 2014 at a level III public hospital in Guyana. All patients who had already performed the HbA1c test to screen for diabetes and those who had complete information were included in the study. Any patient who did not follow the HbA1c algorithm was excluded. Inappropriate ordering of tests was defined as any order for testing a given patient occurring more than 3 months after the previous order. Bio-Rad D-10 was used to monitor HbA1c at the public hospital. Bio-Rad D-10 utilizes the principle of High Performance Liquid Chromatography (HPLC) to determine the percentage of HbA1c. Permission to conduct this research was obtained from the Ethics Committee under the Ministry of Health, Guyana, and from the Director of Medical Services of the public hospital.

Data analysis

The data collected from the database were first entered into a spreadsheet using Microsoft Excel. All statistical analyses were performed using SPSS 21.0, JMP and Microsoft Excel. Distributions were used to show frequencies, the bivariate fit test was done in order to achieve correlations and significance, the $\chi 2$ test was used to compare the differences in proportions and ANOVA was used for differences in mean differences. A p-value of < 0.05 was used to show significance.

Results

A total of 1,547 eligible patients were selected for the study from the database of the GPHC medical laboratory. The patient population analysis had the following baseline characteristics: mean age was 59.9 ± 11.5 years (95% CI 59.3 - 60.4), 5% of the patients were between the ages of 21 and 40, 41% were between the ages of 41 and 60 and 54% were between the ages of 61 and 90.
Figure 1 shows the HbA1c percentage in test 1, the maximum, median and minimum values of 19.2%, 8.9% and 4.2% respectively and a mean and standard deviation of 9.2 ± 2.7. For the HbA1c percentage in test 2, the maximum, median and minimum values were 18.5%, 8.7% and 4.0% respectively, with a mean (SD) of 9.1 ± 2.5. For test 3, 396 patients were tested with the maximum, median and minimum values of 19.7%, 9.4% and 4.1% respectively, and a mean (SD) of 9.6 ± 2.6. Only 82 patients were tested for a fourth time, with the maximum, median and minimum values of 15.1%, 9.2% and 5.2% respectively, and a mean (SD) of 9.6 ± 2.6 (Figure 1).

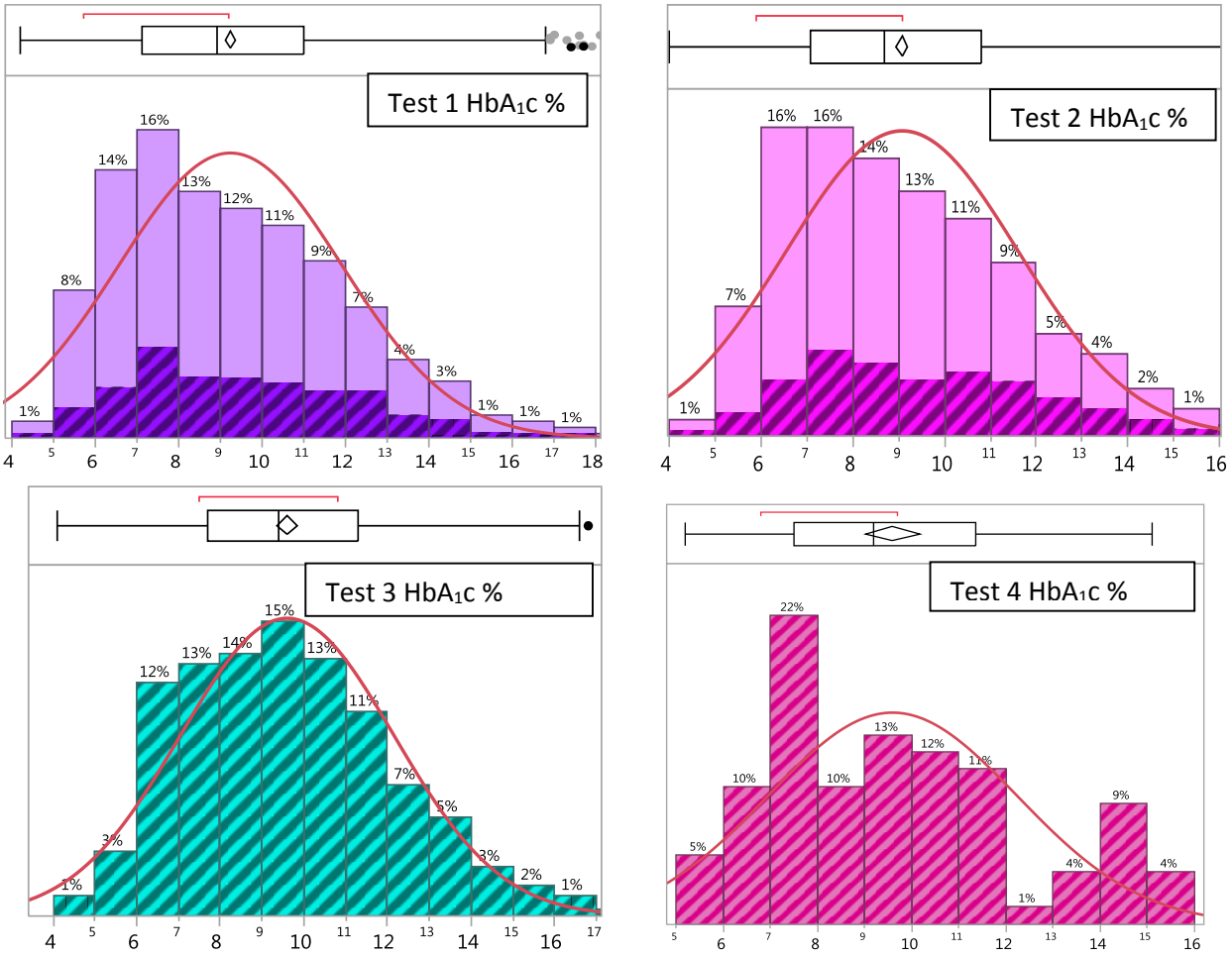

Figure 1. Distribution of HbA1c test results

The mean HbA1c ± standard deviation (SD) of all tests is shown in Table 1. Test 1 had a mean of 9.23 ± 2.6 (95% CI 9.1-9.4), Test 2 had a mean of 9.08 ± 2.5 (95% CI 8.95-9.20), Test 3 had a mean of 9.59 ± 2.6 (95% CI 9.34-9.85) and Test 4 had a mean of 9.59 ± 2.6 (95% CI 9.01-10.17).

Table 1. Mean ± SD of HbA1c in regard to gender

HbA_1c	Male Mean ± SD	95% CI	Female Mean ± SD	95% CI	Total Mean ± SD	95% CI
Test 1	8.9 ± 2.7	8.7-9.2	9.4 ± 2.6	9.5-9.2	9.2 ± 2.7	9.1-9.4
Test 2	8.7 ± 2.4	8.5-8.9	9.2 ± 2.6	9.4-9.1	9.1 ± 2.5	8.9-9.2
Test 3	9.2 ± 2.5	8.7-9.7	9.8 ± 2.6	9.5-10.1	9.6 ± 2.5	9.3-9.8
Test 4	8.8 ± 2.5	7.8-9.8	9.9 ± 2.7	9.3-10.7	9.5 ± 2.6	9.0-10.2

The correlation analysis between each test is shown in Figure 2. The correlation between Test 1 and Test 2, Test 3 and Test 4 was 0.64 (95% CI 0.61-0.67), 0.58 (95% CI 0.50-0.64) and 0.66 (95% CI 0.52-0.77), respectively. The correlation between Test 2 and Test 3 was 0.64 (95% CI 0.57-0.69), and the one between Test 2 and Test 4 was 0.66 (95% CI 0.51-0.76). The correlation between Test 3 and Test 4 was 0.64 (95% CI 0.49-0.75).

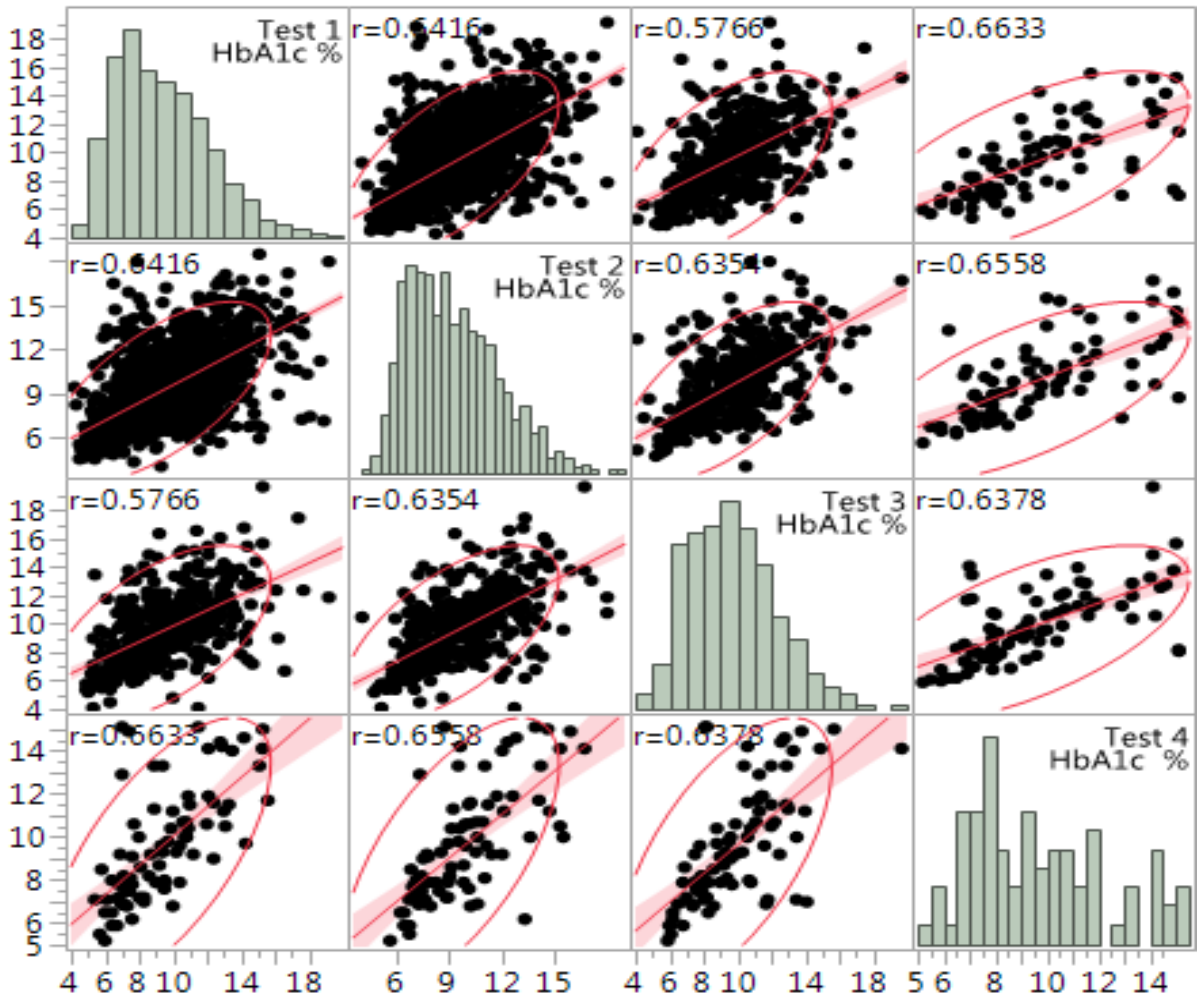

Figure 2. Correlation between individual HbA1c test values

Mean ± SD of each HbA1c test among male and female patients is shown in Table 2. Mean HbA1c was higher among females in all four HbA1c tests. Females were more at risk and all four tests indicated that females were above the baseline when compared to males (30.8% (2 5.86 and p < 0.05)).

Table 2. Prevalence of mean HbA1c and risk factor among male and female population

	Female	Male			Total	
HbA_1c > Mean	**N (%)**	**N (%)**	**P-value**	**RR (95% CI)**	**N (%)**	**95% CI**
Test 1						
No	145 (9.4)	92 (0.1)	< 0.05	1.13 (1.02-1.26)	237 (15.3)	13.6-17.2
Yes	906 (58.6)	404 (26.1)			1310 (84.7)	82.8-86.4
Test 2						
No	147 (9.5)	85 (0.1)	< 0.05	1.04 (0.99-1.08)	232 (15)	13.3-16.9
Yes	904 (58.4)	411 (26.6)			1315 (85.0)	83.1-86.7
Test 3						
No	27 (6.8)	15 (3.8)	> 0.05	1.04 (0.96-1.13)	42 (10.5)	7.9-13.9
Yes	258 (64.7)	99 (24.8)			357 (89.5)	86.1-92.1
Test 4						
No	3 (3.7)	2 (2.4)	> 0.05	1.03 (0.90-1.16)	5 (6.1)	2.6-13.5
Yes	53 (64.6)	24 (29.3)			77 (93.9)	86.5-97.4

The mean difference ± standard error (SE) change between two HbA1c tests is shown in Table 3. The duration between individual tests is shown in Figure 3. Mean duration between test 1 and test 2 was 16.14 ± 11.3 (95% CI 15.6-16.7), with the standard error of 0.28; for test 2 and test 3, it was 12.43 ± 8.83 (95% CI 11.6-13.3), with an SE of 0.44; for test 3 and test 4, it was 10.25 ± 8.8 (95% CI 8.32-12.2), with an SE of 0.96.

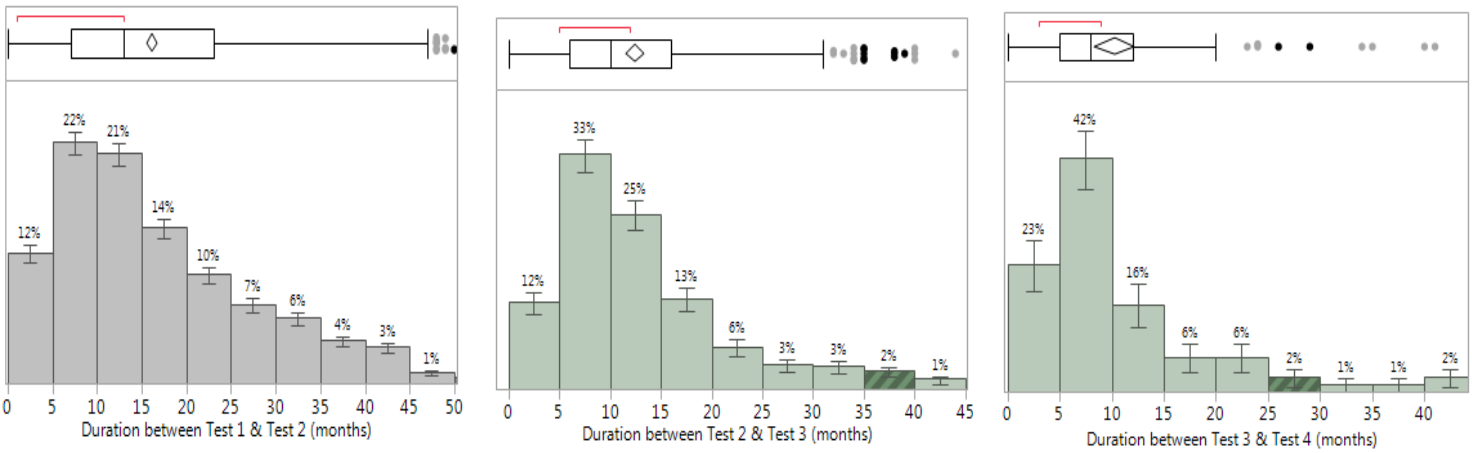

Figure 3. Duration and standard error between individual tests

Table 3. Correlation and mean difference between each test

HbA_1c	Mean difference	SE	95% CI	r
Test 1 - Test 2	-0.15	0.05	-0.26-0.04	0.64
Test 1 - Test 3	0.06	0.12	-0.17-0.29	0.57
Test 1 - Test 4	0.22	0.23	-0.25-0.68	0.67
Test 2 - Test 3	0.11	0.10	-0.10-0.32	0.63
Test 2 - Test 4	-0.23	0.24	-0.70-0.23	0.67
Test 3 - Test 4	-0.35	0.24	-0.83-0.12	0.65

Of the 1,547 patients included in the study, for 828 (53.5%) patients, the HbA1c result decreased in test 2 when compared to test 1. Of the 396 patients who were tested for a third time, the HbA1c result decreased for 171 (43.2%) patients when compared to test 2. Of the 83 patients tested for a fourth time, 46 (56%) patients also experienced a decrease.

Discussion

The main outcome was the proportion of patients undergoing repeat HbA1c testing and the proportion of patients whose baseline HbA1c value decreased. This study used the HbA1c threshold for the diagnosis of diabetes (48 mmol/mol (6.5%)), according to the new diagnostic criteria for diabetes adopted by the American Diabetes Association (16).

This study represents the first attempt to review and analyze the HbA1c data of patients using Bio-Rad at the Georgetown Public Hospital Corporation. Older patients, between the ages of 61 and 90, represented the largest group (54%) in the diabetic population and female patients were above baseline HbA1c in more cases than male patients. A similar pattern has been noted in other studies, where female patients of all age groups and races were found to be at risk (17).

This study revealed that no standard guidelines were followed by the clinicians in terms of repeat testing intervals. There were many variations noted in regard to the duration between tests. Discordant HbA1c results have created confusion among the clinicians. Clinicians need to be aware that significant test-retest variation exists in HbA1c and is sometimes quite large (18). Mean HbA1c for all four tests was recorded above 9.0, which is much greater than the baseline value. Many studies have warned that HbA1c concentrations above 48 mmol/mol (6.5%) are risk factors for development of diabetic retinopathy, macrovascular outcomes and death (18-21).

The International Expert Committee (IEC) (24) and ADA (16) have proposed new diagnostic criteria for diabetes on the basis of HbA1c measurement, in which HbA1c of ≥ 6.5% is defined as diabetes. In the present study, an HbA1c level of 6.5% had a reasonably high specificity (99.1%) and low false-positive rate (0.9%) for the diagnosis of diabetes, which is in complete concordance with IEC and ADA recommendations.

Repeat testing showed a significant decrease in HbA1c levels in a portion of patients. A decrease in a follow-up test is favored because this is indicative of following proper treatment strategies and overall control of the patient's diabetic condition. It is also understood that a decrease should not be expected in every case of repeat testing, since an increased value maybe be accounted for by a number of different reasons. Multiple factors affect the accuracy of HbA1c as an indicator of average

glucose concentration, including abnormal erythrocyte lifespan, assay-related artifacts, fast vs. slow glycosylation, ethnicity, pregnancy, use of drugs and acute illness. Different factors affect the validity of HbA1c, such as iron deficiency, altered hemoglobin structure, erythrocyte lifespan and interracial variability, age (the elderly and children), gender and pregnancy (25, 26).

It is a well-known fact that high levels of HbA1c are associated with an increased risk of atrial tachyarrhythmia and paroxys¬mal atrial fibrillation in patients with type 2 DM (27). Furthermore, it has been reported that an increase of 1% in HbA1c concentration was associated with roughly a 30% increase in all-cause mortality among diabetes patients (28). This shows that the proportion of HbA1c percentage increase is far too high and, as such, stricter measures should be implemented to reduce this percentage.

Conclusion

It is a fact that HbA1c is a test that is accurate and easy to administer for diagnosing diabetes, especially in low and middle income countries like Guyana. However, the mean HbA1c percentage of the diabetic population in Guyana was found to be 9.4%, which is far higher than ADA's established HbA1c criteria for pre-diabetes and diabetes (5.7% and 6.5%). Therefore, the researchers recommend that strict guidelines be followed by physicians in regular and accurate testing of HbA1c.

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Review article

Trefoil Factor Family (TFF): Peptides with Numerous Functions

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Abstract

The trefoil factor family (TFF) consists of a group of small peptides and is highly expressed in tissue that contain mucus-producing cells, predominantly in the mucosa that lines the gastrointestinal tract. Those peptides, which are highly important for epithelial restitution, may act in ways other than using the usual factors responsible for restitution. It was observed that several mechanisms are involved in the TFFs' promotion of restitution. In addition to that, peptides have other functions as well, e.g. they interact with the immune system. Although the TFFs' therapeutic effects have been studied, it is uncertain which of the TFFs' in vitro properties are directly involved when it comes to their in vivo engagement. Observing mice with genetic deletion of TFF peptides can help us discover the function of the peptides that could be indicated by the deletion of the target protein or by adaptive regulation of some other protein that is affected by the deleted gene product. At the very least, a subset of functional networks controlled by a TFF isoform and its downstream effectors can be identified by observing such mice. The discoveries related to the signaling mechanisms of the TFF family leave much to discover about the distinct and shared pathways among those protective peptides.

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Biological Properties of TFF

The trefoil factor family (TFF) includes a group of small peptides, the first member of which was discovered about thirty years ago (1). TFF1 (formerly known as breast cancer-associated peptide pS2), TFF2 (formerly spasmolytic polypeptide SP) and TFF3 (formerly intestinal trefoil factor) are three members of the trefoil factor family (TFF) known in mammals. These three proteins are small compact peptides that have one or two trefoil domains. While TFF1 and TFF3 contain only one trefoil domain, TFF2 contains two trefoil domains. The basic elements of a trefoil domain are 42–43 amino-acid residues. Six cysteine residues form three disulfide bonds, creating a characteristic three-leafed structure (2).

There are several studies that suggest that TFFs can be regulated by cytokines and transcription factors (especially NF-kB) related to the immune system and that TFFs can regulate them in return, but there is also data suggesting otherwise (3-6).

TFFs have been extensively studied in vivo and in vitro, with most data suggesting that these small peptides improve epithelial repair in the gastrointestinal (GI) tract and other body systems (7).

TFF's Role in Mucosal Protection and Repair

Due to their compact structure, TFFs are relatively resistant to proteolytic degradation in the stomach and small intestine (8). TFFs can mostly be found as secreted molecules in the mucus covering normal epithelium. The predominant site of TFF synthesis is the mucin-producing or goblet cells dispersed in epithelia. All three TFFs are expressed in the stomach, being localized to the surface gastric mucosal cells (9). Their mRNA has been found in the brain, lungs, trachea, thyroid gland, salivary glands, prostate, uterus and other organs (10). The genes that code them are located on chromosome 21 (11).

Despite the fact that they have been found in almost all tissue containing mucus-secreting cells, TFFs are predominantly expressed in the gastrointestinal tract. Considering their appearance in mucosal tissue, it can be concluded that their functioning might be related to that of mucins. Nevertheless, TFF2 expression is not so common and there is a possibility that different TFFs have different roles in the protection of the epithelium, which is corroborated by the complementary expression of TFFs in the GI tract and by the simultaneous occurrence of each of them with their unique mucin type (MUC); TFF1 appears with MUC5AC, TFF2 with MUC6, and TFF3 with MUC2 (12-14), although gastric and ocular co-localization of TFF3 with MUC5AC also occurs (15, 16). It is speculated that mucosal defense is improved by direct interaction of TFF peptides and mucins.

The role of TFF peptides in cell migration was observed in several studies (17-20), predominantly as a consequence of the response of a damaged epithelium that strives to restore its continuity. In cases of small discontinuity of the epithelium, where cell proliferation is not required, restitution of the epithelium takes place soon after the injury, with coordination of the removal of damaged cells and the migration of healthy epithelial cells into the injured location (Figure 1). The importance of efficient restitution from the physiological viewpoint is high, as the loss of fluids and electrolytes has to be stopped and the luminal antigens and bacteria have to be prevented from entering the tissue and immune cells of the host. Proliferation occurs instead of restitution when tissue is more severely damaged.

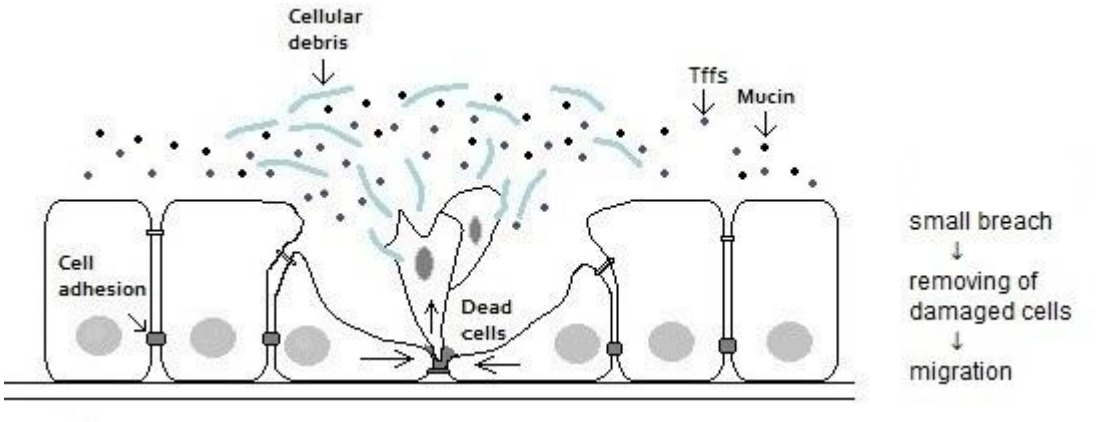

Figure 1. The role of TFF peptides in epithelial restitution

TFF and Energy Metabolism

The influence of trefoil factor proteins on energy metabolism can be observed in mice with TFF3 gene knockout (TFF3-/- mice). The TFF3 knockout mice have a different expression of miRNA associated with the glycolysis and gluconeogenesis metabolic pathways compared to wild-type mice. The TFF3 knockout mice have a significantly lower body weight compared to the wild type (21). A change in the body mass of mice did not occur in the research with increased expression of the TFF3 gene (22). The fatty changes in the liver of mice have been connected with the change of expression of the TFF3 gene (23). Research has shown that the TFF3 protein participates in glucose metabolism. Similarly, a study showed that hepatic TFF3 expression levels were lower in obese (ob/ob) and high-fat-diet-induced obese mice. Cellular glucose output in mice decreased as a consequence of overexpression of TFF3 in primary mouse hepatocytes, which inhibited the expression of gluconeogenic genes. Experiments using the glucose tolerance test and insulin tolerance test showed that adenovirus-mediated overexpression of TFF3 in diabetic or obese mice improved glucose tolerance and insulin sensitivity. The results also showed that TFF3 peptides are a factor in glucose homeostasis and insulin sensitivity. Consequently, it was concluded that said peptide might be a part of successful modern therapies aimed at metabolic disorders related to type 2 diabetes mellitus (24). Increasing concentrations of glucose and insulin treatment boosted the expression of TFF3 in intestinal epithelial cells. In addition to that, insulin treatment caused the upregulation of human sodium/glucose cotransporter 1 (hSGLT1), which additionally increased intracellular glucose levels. Downregulation of TFF3 was observed in diabetes mellitus type 1 patients, but the values were modified by insulin treatment. It was discovered that insulin signaling was important for the optimal expression of TFF3 in intestinal epithelial cells, as it elevates intracellular glucose levels and mediates gene expression (25).

Aberrant energy metabolism in the liver promotes insulin resistance, diabetes, and nonalcoholic fatty liver diseases (26). It was recently shown that liver triglyceride accumulation does not cause cellular injury in the liver; the primary causes of liver injury via increased oxidative stress are free fatty acids or their metabolites (27). Changes in lipid metabolism, especially the increase of saturated fatty acids, are associated with increased endoplasmic reticulum (ER) stress, oxidative stress and liver injury in the course of development of fatty liver disease (28). Sirtuin 1 (SIRT1) plays a key role in metabolic regulation, adaptation and oxidative stress. Acting as a nuclear metabolic sensor and deacetylating a wide range of targets, it leads to epigenetic modifications of histones and modulation of transcription factors or metabolic enzymes (29). In addition to SIRT1, peroxisome proliferator-

activated receptors (PPARs) also have an important role in cell metabolism (30).
In case of TFF3 deficiency, the profile and accumulation of fatty acids (FAs) in the liver are affected (Table 1), with no obvious oxidative stress increase, although the expression/activity of monitored enzymes changes, as does the level of SIRT1 and PPARg protein. Due to the strong downregulation of hepatic TFF3 in diabetic/obese mice, its presence in circulation and its regulation by food/insulin, TFF3 represents an interesting new candidate for research in metabolic relevant conditions (31).

Table 1. Fatty acids in liver of Tff3 -/- mice compared to wild type (elevated ↑, decreased ↓)

Fatty acids	Tff3 -/-
SATURATED	
C14:0 myristic acid	↓
C18:0 stearic acid	↑
C20:0 arachidic acid	↑
MONOUNSATURATED	
C16:1 palmitoleic acid (ω-9)	↓
C18:1 oleic acid (ω-9)	↓
C18:1 vaccenic acid (ω-7)	↓
C20:1 eicosenoic (gondoic acid) (ω-9)	↓
POLYUNSATURATED	
C20:2 eicosadienoic acid (ω-6)	↑
C20:4 (AA) arachidonic acid (ω-6)	↑
C18:3 (ALA) alpha linolenic (ω-3)	↓
C22:6 (DHA) docosahexaenoic (ω-3)	↑
RATIO ω-3/ω-6	↑

TFF's Participation in Defense Against Harmful Agents

Another role of the TFF3 protein is the defense of the organism against harmful agents. Mice that cannot synthesize enough TFF3 protein in their liver are deprived of the protective effect of the TFF3 protein in the serum after myocardial (32) and brain ischemia, which consequentially leads to greater tissue damage. Thus, in such mice, a significantly higher activity of caspase 3 and a higher level of cell death in the ischemic cerebral lesion were observed, together with a larger fraction of cerebral infarcts and a smaller fraction of injuries in the cerebral hemisphere, accompanied by more severe forelimb motor deficits. Since the mice were TFF3-deficient, recombinant TFF3 was administered intravenously and it reversed changes in cerebral injury and forelimb motor function, pointing at the existence of an endocrine neuroprotective mechanism that uses TFF3 from the liver in experimental cerebral ischemia/reperfusion injury (33). TFF3-/- mice have difficulties with regeneration of the mucous membrane of the gastrointestinal tract (34).

High-salt diet (HS) causes endothelial dysfunction and vitiates vascular reactivity to various stimuli. In a recent study, transgenic TFF3-/- mice were introduced as a new model, characterized by a favorable ratio of ω-6/ω-3 free fatty acids and modified metabolism of arachidonic acid (AA). The results showed that acute HS intake has a much smaller impact on FIR (flow-induced response) in TFF3-/- mice

compared to the wild type (WT) (35). The study showed that HS intake does not affect NO production in TFF3-/- mice (36).

According to another study, although the TFF3 peptide is not expressed in an intact corneal epithelium, its expression is extensively upregulated following an epithelial injury. In addition to that, corneal injuries in TFF3-/- mice take much more time to re-epithelialize compared to similar injuries in wild-type mice. In case of alkali-induced corneal wounds, external application of recombinant TFF3 to the wounds speeds up the in vivo and combined in vivo/in vitro model wound healing in both wild-type and TFF3 /- mice. This proves that TFF3 has a key role in the mechanism of corneal wound healing, which opens a possibility of creating new ways of coping with non-healing wounds (37).

TFF in the Respiratory System, Pregnancy and Tumorigenesis

A study (38) describing a murine asthma model found that trans-differentiating Clara cells specifically express TFF1 which is stored in a specific subset of secretory granules. This is proof that TFF1 is an autocrine factor for the trans-differentiation of Clara cells into goblet cells. Another study (39) showed that TFFs play such a role in the differentiation of the airways as well, showing the induction of TFF3 synthesis with the differentiation in in vivo humanized tracheal xenograft and in vitro air-liquid interface culture models. In addition to that, exogenous TFF3 promoted differentiation of ciliated cells in an EGF-receptor-dependent manner. Both studies implied that TFFs may have important roles in different processes of differentiation of airways, making them promising new targets in treatment of severe chronic and acute airway diseases.

Dynamic changes of trefoil factor proteins in a pregnant woman's serum point at their importance in embryogenesis (40). The presence of the TFF3 protein in the cartilage of mice fetuses during endochondral ossification has been identified, while the exclusion of the TFF3 gene causes changes to the histomorphological structure of cancellous bone, as well as hearing disorders and accelerated presbycusis, which indicates that it has a role in morphogenesis of organs (41-43).

The TFF3 gene participates in the proliferation of pancreatic cells – decreased expression of the TFF3 gene leads to decreased proliferation of pancreatic -cells, while increased expression leads to increased proliferation of pancreatic -cells, having no influence on their function (44). The TFF3 protein is also related to angiogenesis, which makes it an important factor in tumor pathogenesis (45). Research has identified increased expression of the TFF3 gene in gastrointestinal and lung tumors, advanced prostate cancer, hepatocellular carcinoma and other tumors (46-49). Expression of the TFF3 gene has a predictive role in breast tumors (50) and is simultaneously identified as a valuable and easily detected biomarker in screening for stomach cancers. Moreover, serum TFF3 might predict gastric cancer more efficiently than the PG test, while the combined testing of serum PG (pepsinogen test) and TFF3 could make gastric cancer screening even more efficient (51).

Conclusions

Despite the fact that not much is known about the TFF signaling pathways, some straightforward benefits of TFF peptides for healthy and damaged tissue have been discovered. TFFs are pivotal for mucosal protection and repair of epithelial surfaces, and they also have a role in cancer development and progression. Trefoil factors can be used as prognostic markers for different types of carcinoma. However, their biological effects are still unknown. Considering that there are not many studies on the influence of the TFF peptides on vascular reactivity, it would be interesting to find out more about their role in it.

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