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COVID-19 – Lessons Learned

Biljana Mijović 1

Abstract

Modern society has not forgotten yet epidemics that killed millions in the last millennium and the COVID-19 pandemic caused by the SARS CoV-2 has recently emerged. With the onset of the Wuhan epidemic in the Chinese province of Hubei, the initially called new corona virus due to the similarity of 80 % to the 2002 SARS virus was renamed to SARS CoV-2. The virus was originally isolated from bronchoalveolar aspirate specimens. Viral RNK was detected in 6 of 41 blood samples with clinical signs of infection. A senior Chinese expert told to the media that the median incubation period was 7 days, ranging from 2 -1 2. The International Health Regulations Emergency Committee for Epidemics gives a preliminary estimate basic reproduction number R0 of 1.4 - 2.5. COVID-19 is mainly transmitted by close contact with the infected by drops due to sneezing and coughing. Fever, cough, myalgia and fatigue are the predominant initial signs and symptoms. The clinical picture is non-specific. Exacerbation occurs suddenly, as bilateral interstitial pneumonia that requires admission to intensive care. Initial lethality in hospitalised cases was 15 %, but these estimates had to be taken with reserve as the situation evolved. According to recent data, the global fatality rate is 3.7 %, the lethality rate in China is 3.9 % and in Italy 6.8 %. According to data from the Chinese Centre for Disease Control and Prevention, of 44,672 confirmed cases 1,023 people died, therefore lethality was 2.3 %. In the absence of specific prevention and control measures, mankind is limited to general prevention measures.

Key words: Covid-19, SARS CoV-2, pandemic, lethality.

Introduction

Modern society have not yet forgotten the epidemics that killed millions in the last millennium and the COVID-19 pandemic caused by the SARS CoV-2 virus has emerged. According to the World Health Organization (WHO), as of the end of December 2019, 153,517 persons had been laboratory-confirmed by 15 March 2020, of which 81,048 or 52.79 % of the total number of patients became ill in China. At the same time, 5,735 people died, 3,204 in China.1

The epidemic was first reported in Wuhan, Hubei, China, and quickly spread to 143 more countries. While the epidemic in China is gradually dying out, new hotspots centred in Europe are opening up around the world. Italy was particularly affected with 21,157 patients and 1,441 deaths, as well as about 3,500 patients and over 170 deaths on a daily basis. The situation is continuing to deteriorate in Spain, France, Germany and the United Kingdom.

The first death outside China occurred on 2 February 2020 in the Philippines. On 15 February 2020 France reported the first death outside Asia, a person who had been to China. The first two Europeans to die from COVID-19 were reported in Italy on 23 February 2020.

The first two cases of infection in the Republic of
Srpska were confirmed in a man who returned from Italy, as well as in his school-age son. As of 16 March, 20 cases of COVID-19 were confirmed in the laboratory in the Republic of Srpska. No case with severe clinical picture was recorded yet.

The epidemic is spread across the continents of Europe, Asia, Africa, America and Oceania and the WHO has declared a pandemic. The whole world has come together to fight this global epidemic. All countries implement the same measures, with differences in the implementation of measures and levels of restrictions. It is very important to summarise the known facts on the epidemiological characteristics of COVID-19 disease.

**SARS CoV-2 virus**

With the onset of the Wuhan epidemic in the Chinese province of Hubei, the causative agent was designated as a new corona virus, but over time, after 80% sequence similarity with the 2002 SARS virus mankind is, this virus was designated SARS CoV-2. Coronaviruses were are known since the mid-1960s. It has been known that they have ability to infect humans and many birds and mammals. Two outbreaks involving coronaviruses with animal reservoirs were noted: SARS-CoV in 2002, caused by a Betacoronavirus, subgenus Sarbecovirus, and MERS-CoV in 2012, caused by Betacoronavirus, subgenus Merbecovirus.2

In the previous decade, two pandemic viruses, SARS and MERS, affected several thousands of people with very high fatality ratio. Known facts regarding SARS virus identified in 2002 are: 8,096 affected people, mostly having pulmonary infections, 774 deaths, fatality ratio 10 %.3 Most likely, bats were origin of the virus, which was spread further to Himalayan palm civets, Chinese ferret badgers and raccoon dogs sold for food at the markets of Guangdong, China. The 2012 MERS virus had ever greater fatality rate than SARS, it was around 35.7 %. It was spreading among the people, particularly in healthcare settings. Besides that, dromedary camels were animal reservoirs of the virus.4

In December 2019, a new coronavirus caused pneumonia in three patients connected to the cases of acute respiratory illness from Wuhan. After genetic analyses of the new coronavirus were performed, it was discovered that it was closely related to SARS-CoV and genetic clusters within the genus Betacoronavirus, forming a distinct clade in lineage B of the subgenus Sarbecovirus together with two bat-derived SARS-like strains.5 It was later confirmed that the COVID-19 shares with SARS-CoV a property binding to the angiotensin-converting enzyme-2 (ACE-2), a membrane exopeptidase that acts not only as the receptor for these viruses, but also enables them to enter into the human cells.6

The virus was originally isolated from broncho-alveolar aspirate specimens.7 Virus RNA was detected in the blood samples in six of 41 cases in a study on the clinical characteristics of infection.8 At present, it remains unknown whether the virus is excreted in faeces or urine.

**Incubation and infectiousness**

Since first identified, the epidemic scale of the recently emerged novel coronavirus (2019-nCoV) in Wuhan, China, has increased rapidly, with cases arising across China and other countries and regions. Using a transmission model, a basic reproduction number $R_0$ of 3.11 (95 %CI, 2.39-4.13) was estimated.9 The mean incubation period was 5.2 days (95 % confidence interval [CI], 4.1 to 7.0), with the 95th percentile of the distribution of 12.5 days. In its early stages, the epidemic doubled in size every 7.4 days. With a mean serial interval of 7.5 days (95 % CI, 5.3 to 19), the basic reproduction number $R_0$ was estimated to be 2.2 (95 % CI, 1.4 to 3.9).10 Linton and co-workers11 used the best-fit lognormal distribution method and showed that the incubation period was approximately 5 days (95 % CI 4.1 to 7.0 days).

However, these estimates are still only preliminary and will be updated when more information becomes become available. The infection correlates with the onset of symptoms and the severity of the clinical picture.8
Pathogenesis

In patients with more severe clinical imaging requiring intensive care, a Th1 immune response is enhanced. However, production of IL-4 and IL-10 was also enhanced that stimulates the Th2 immune response that was not observed in SARS and MERS. For researchers in this emerging situation, it remains to be investigated how the use of corticosteroids will affect this balance between Th1 and Th2 immune responses.8

Ways of transmission

COVID-19 is mainly transmitted by close contact with the infected person by droplets in sneezing and coughing. It was shown in a study by Ghinai and colleagues12 that a person-to-person transmission of COVID-19 had occurred between a symptomatic and uninfected subject after a prolonged, unprotected exposure. No further spread of infection was detected, despite the active symptom monitoring and testing with symptomatic and some asymptomatic contacts. Contagiousness is greatest in the period of symptoms. Air is also possible as a transport route. Faecal transmission is not excluded but is unlikely. Transmission is possible through contaminated hands in contact with eyes and saliva of nose and mouth.13,14

Clinical characteristics

Fever, cough, myalgia and fatigue are the predominant initial signs and symptoms. The clinical picture is extremely nonspecific with a wide range of symptoms, bilateral pulmonary infiltrations and sudden worsening followed by dyspnoea and admission to the intensive care unit. Huang and co-workers8 described that the median time since the onset of symptoms to hospital admission had been 7 days, until occurrence of dyspnoea 8 days, until occurrence of acute respiratory distress syndrome 9 days and 10 to 15 days until admission to the intensive care unit.

Age and gender distribution of diseased

Among the first published studies on the epidemic in China there was a study published by Li et al.10 In a sample of 425 confirmed cases in Wuhan, China, in January 2020, the median age of patients was 59 years (range 15 to 89 years). Out of the total number of patients, 240 (56 %) were men and there were none under the age of 15. This age distribution can be explained by the fact that the first cases consisted of a cluster of non-specific pneumonias. Further follow-up revealed that the disease manifested in children with a lighter clinical picture, so these cases were not registered until the detection of the pneumonia cluster. The slightly milder representation of men can be explained by the exposure to the wholesale market in Wuhan.

According to the Chinese Centre for Disease Control and Prevention, as of 11 February 2020, men represented 63.8 % of the sample of 44,672 confirmed patients. Children between the ages of 0 and 9 accounted for 0.9 % of the total number of patients, but there were no fatalities. The lethality increased with age, thus at the age of 80 and over it was 14.8 %.15 According to the same source, it was concluded that the number of diseased health workers increased with the increase in the number of patients and accounted for 3.8 % of the total number of patients with a lethality of 0.3 %. Hubei patients accounted for 95.7 % of the total number of patients, an indication of well-implemented control measures in the province and halting further spread across China. Comorbidities were significantly represented, such as hypertension (39.7 %), diabetes (19.7 %), and cardiovascular diseases (22.7 %). In 80.9 % of the confirmed cases, the clinical picture was mild and 4.7 % critical. Wei and co-workers8 described 9 diseased new-borns (7 girls and 2 boys) in their study who had a mild clinic picture.

Lethality

Initial lethality in hospitalised cases was 15 %,17 but these estimates had to be taken with reserve.
as the situation evolved and changed. According to recent data, the global fatality rate is 3.7 %.
According to the same source, the lethality rate in China is 3.9 % and in Italy 6.8 %. According to data from the Chinese Centre for Disease Control and Prevention, of 44,672 confirmed cases, 1,023 people died, so lethality was 2.3 %. In the same sample confirmed in China, 416 cases of children between the ages of 0 and 9 were registered, with no deaths. Higher lethality in Italy may perhaps be explained by the fact that the Italian population is older than the population in China. Further statistic monitoring and analysis are needed to accurately assess the COVID-19 lethality.

Yang et al have reported that the median time for a radiological confirmation of pneumonia since the onset of symptoms was 5 days (ranging 3-7 days); since the symptom onset until admission to the intensive care unit it was 11 days (ranging 7-14 days) and from admittance to the intensive care unit to death it was 7 days (ranging 3-11 days). The median time from pneumonia confirmation to death was 13 days. In the study of Linton and colleagues, an average of 13 days elapsed from the onset of the disease to death.

In a letter to the editor Wilson et al reported on the case-fatality risk estimates by means of a lag time for fatality methodology. Hubei Province was excluded from the clause. The case-fatality risks, when adjusted for a 13-day lag from reporting to death, was 3.5 % in China (0.8% in China, excluding Hubei Province), 4.2 % in the group of 82 countries, territories and areas and 0.6 % for the passengers and crew on a cruise ship.

**Conclusion**

In the absence of specific prevention and control measures, healthcare systems are limited to general prevention measures. Key measures include limiting travel, quarantining the exposed, minimising social contact, sanitary and hygiene measures, personal hygiene measures and the proper use of personal protective equipment on the exposed. The Chinese authorities pointed to the importance of all these measures.

**Acknowledgements**

None.

**Conflict of interest**

None.

**References**


Pharmacologist's View of the New Corona Virus

To the Editor

Despite many advances in the prevention and treatment of infectious diseases, the global spread of infections is accelerated by close contact among concentrated populations. A pandemic caused by a new type of coronavirus (SARS-CoV-2) continues to spread across the globe. This new disease attributed to COVID-19 emerged a century after the Spanish flu pandemic, which affected one-third of the world's population, killing more than 50 million people. The disease, caused by the H1N1 virus, has been around for less than two years and it is still unclear what contributed to its termination; possibly a mutation of the virus reduced its viral strength. Since information about biological sciences and human medicine is far more advanced today than at the beginning of the 20th century, it is expected that the current pandemic will be contained, regardless of the potential for viral mutation. Scientists and doctors now face an urgent task - how to treat numerous sick people and stop the spread of this infection. The pandemic urgently requires identification of preventive measures, along with optimum means of diagnosis and treatment and data on dissemination of the infection, duration of incubation, clinical features of the disease, along with the best means of diagnosis and treatment for a very large number of people, and ultimately the long lasting consequences of the disease. In addition, we must identify pathways for the spread of the virus, its persistence on various surfaces and means of its inactivation.

Epidemiology and basic sciences

Since no cure exists, as yet, for this viral disease, and it will take time to produce a suitable vaccine, strict epidemiological measures were implemented initially. This resulted in the largest quarantine in history – between 50 and 60 million people in several Chinese cities were isolated, group meetings were cancelled, schools were closed, and travel was prohibited. Later evaluation will show whether all of these epidemiological measures were justified. When analyses of deaths due to infection were made, the lowest mortality due to COVID-19 infection was found in patients with lung disease, compared to individuals who suffer from the heart diseases or diabetes. The mortality of hypertensive individuals due to the new coronavirus is similar to that of patients with lung disease.

Researchers almost immediately, in January 2020, identified the genome of the new virus, which made it possible to compare this new SARS-CoV-2 with an older coronavirus, SARS-CoV. The SARS-CoV, which originated in bats, caused a viral epidemic in 2002-2003. The genetic similarity of old and new corona viruses has been shown to be greater than 70 %. Thus, the new corona virus is a combination of the bat virus with an unknown virus source. The snake virus seems to be the reservoir of that combined virus. Earlier, it was found that the older coronavirus (SARS-CoV) binds to the receptor for the angiotensin converting enzyme-2 (ACE-2), which is found at the airway cells localised in the alveoli.

Usage of the approved medications

Doctors quickly looked to see if any existing medicines could affect the COVID-19; the fastest way for treatment would be to use approved medicines proven to help resist invading pathogens. Development of specific drugs to treat this new illness may take several years and will require clinical trials; this will require time that we do not have. Enlistment of existing, safe medications, such as atorvastatin, met-
formin, glitazones, cyclosporine, interferon beta-1, ribavirin, antiviral peptides, nutrient supplements (especially zinc, which has anti-viral activity) should help patients resist the invading virus. In addition, biologics that reduce immunopathology and stimulate immune responses can be employed.

The Chinese reported efficacy of chloroquine, an antimalarial drug for treatment of the infection. Then, French researchers found, in a small group of patients with COVID-19 infection, that combined administration of hydroxychloroquine and azithromycin had produced even better results. Although that study was insufficient, it indicated a path to treatment, regardless of some serious side effects of azithromycin (severe diarrhoea due to *Clostridium difficile* and prolonged Q-T interval on the ECG). Despite these drawbacks, mortality due to infection with the new virus could be significantly reduced.

Some pharmacologists believe that because certain growth-inhibiting agents, such as cepharanthin, selamectin or mefloquine hydrochloride, affect replication of coronaviruses *in vitro*, these agents might be used to treat COVID-19 infection *in vivo*, but it will first be necessary to check their clinical efficacy. Cepharanthin (an ingredient in the local plant *Stephania cephalantha*) has been used in Japan since almost 70 years ago for a number of acute and chronic diseases because it has various pharmacological effects, including anti-inflammatory, anticancer, and antiviral effects.

There is a considerable debate among biomedical scientists and physicians as to whether patients should stop taking ACE inhibitors (ACEI: enalapril, lisinopril, ramipril and others) and angiotensin receptor blocking drugs (ARB: losartan, eprosartan, valsartan and others) due to their increase of ACE-2 activity and rise of the ACE-2 receptors. These drugs help thousands of patients with high blood pressure, chronic heart failure, diabetic kidney damage and other vascular disorders. Discontinuing these medicines could have many serious consequences, although medical opinions remain divided. On the one hand, there is an indication that these renin-angiotensin blockers could exacerbate coronavirus disease, but on the other hand, some preliminary data show that they are beneficial. Controlled clinical trials will establish whether these drugs should be temporarily discontinued (and in which patients) or whether they should even be given to individuals infected with COVID-19. For now, no one should omit taking any of these medicines.

**Epilogue**

It is worth noting that since the year 1940 until now, around 400 new infectious pathogens have been identified, providing an opportunity for scientific researchers to better treat and eliminate new infections. The production of vaccines and vaccination of a significant part of the population has proven successful in previous dangerous infections, but it is a time-consuming procedure. It remains imperative for us to quickly identify drugs that will, in the meantime, reduce mortality and prevent the spread of infection.

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References


9. Igić R. [Pharmacology of the renin-angiotensin system]. Banja Luka: Faculty of Medicine, University of Banja Luka; 2014.


Abstract

**Background/Aim:** The relation of social isolation (SI) to global cardiovascular health (CVH) is not clear. In this paper the association of CVH metrics to SI within US adults was investigated.

**Methods:** Using the US National Health and Nutrition Examination Survey, the association of SI with American Heart Association’s Life’s Simple 7 (LS7) CVH components (smoking, body mass index, physical activity, total cholesterol, diet, blood pressure, fasting blood glucose) was examined. The sum of the components (scored 1 for poor, 2 for intermediate and 3 for ideal) created a composite CVH score. Multiple logistic regression provided the odds of SI according to levels of the LS7 components adjusted for age, sex, and ethnicity.

**Results:** A total of 3,528 adults aged ≥ 40 were studied. The mean age, sex and ethnicity-adjusted composite CVH scores were 14.1 vs 14.6 in those with vs without SI (p < 0.01). Multiple logistic regression examined individual LS7 components in relation to SI and showed the adjusted odds for SI for those with ideal vs poor smoking status to be 0.57 (range 0.38-0.85), ideal vs poor physical activity 0.53 (range 0.37-0.76) and ideal vs poor fasting glucose 0.65 (range 0.47-0.91).

**Conclusion:** This study generally shows lower levels of CVH in those with vs without SI, with non-smoking status; ideal physical activity and ideal glucose levels were all less likely associated with SI, suggesting the potential value for screening for SI in identifying those at potential cardiovascular disease risk.

**Key words:** cardiovascular health, social isolation, risk factors.

Introduction

Social support is a key psychosocial factor that has a prominent and powerful impact on physical and mental health. Previous research has indicated that there is a decreased risk of mortality in those with significant quality or quantity of social connections compared to those with low quality or quantity of social networks, when adjusted for baseline health status. Subsequently, data show that psychosocial factors, including low social support, are correlated to greater risk of cardiovascular disease (CVD). The impact of social relationships on cardiovascular health has also been shown to be comparable with that of standard traditional risk factors. In addition, social connections have been shown to be associated with the development and progression of CVD.

The American Heart Association (AHA) had set a goal to improve cardiovascular health (CVH) of the general American population by 20% by the year of 2020. The AHA promotes and encourages primary prevention of CVD by describing 7 cardiovascular and behavioural health factors or Life’s Simple 7 (LS7) including fasting glucose, blood pressure, total cholesterol, body mass index (BMI), smoking, diet and physical activity as
Methods

A total of 3,528 adults aged ≥ 40 from the National Health and Nutrition Examination Survey (NHANES) were examined. NHANES is a cross-sectional, multistage, stratified, clustered probability sample of the US non-institutionalised population conducted by the Centers for Disease Control and Prevention’s National Center for Health Statistics (NCHS). Participants underwent a clinical examination including blood collection and answering a household interview; the precise methods and study design for NHANES have been previously described.20, 21 The study was conducted using data from 2007-2008, which is the last wave of data that includes social support questionnaires. Participants aged < 40 years were excluded, as they were not included in the sample given social support questionnaires. All participants gave a written informed consent. The current analysis utilised de-identified data from NHANES and was thus exempt from institutional review board approval.

The primary measure of social support was self-reported through a household questionnaire, which queried the participant as to whether there is anyone to provide adequate social support for both male and female participants. Participants who answered negatively for this question were indicated as being socially isolated.

Based on the American Heart Association’s Life’s Simple 7, each of the 7 health factors and behaviours including diet, BMI, blood pressure, total cholesterol, fasting blood glucose, smoking status, and physical activity were classified as poor, intermediate, and ideal; specific and detailed descriptions of individual metrics are presented in Table 1.

<table>
<thead>
<tr>
<th>Poor</th>
<th>Intermediate</th>
<th>Ideal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smoking</td>
<td>Former or quit ≤ 12 months prior</td>
<td>Never or quit &gt; 12 months prior</td>
</tr>
<tr>
<td>Body Mass Index</td>
<td>25.0-29.9 kg/m²</td>
<td>≤ 25.0 kg/m²</td>
</tr>
<tr>
<td>Physical Activity</td>
<td>1-149 min/wk moderate intensity</td>
<td>≥ 150 min/wk moderate intensity</td>
</tr>
<tr>
<td>Diet</td>
<td>0-1</td>
<td>≥ 150 min/wk moderate + vigorous</td>
</tr>
<tr>
<td>Total Cholesterol</td>
<td>200-239 mg/dL or treated to goal</td>
<td>≤ 200 mg/dL untreated</td>
</tr>
<tr>
<td>Blood Pressure</td>
<td>Systolic ≥ 140 or Diastolic ≥ 90 mmHg</td>
<td>&lt; 120/≤80 mmHg untreated</td>
</tr>
<tr>
<td>Fasting Blood Glucose</td>
<td>100-125 mg/dL or treated to goal</td>
<td>≤ 100 mg/dL untreated</td>
</tr>
</tbody>
</table>

Participants scoring poor for an individual measure were given a score of 1, intermediate score of 2, or ideal score of 3, respectively. Aggregating the scores assigned for the 7 metrics, a cumulative Life’s Simple 7 score (LS7 score) was calculated from the sum of all 7 individual risk factor scores, resulting in a cumulative range of 7 to 21. A score of 7 correlated to the poorest health and 21 corresponded to optimal health.

Analysis of variance (ANOVA) was performed to compare the means of LS7 among subjects with and without SI, while adjusting for age, gender, and ethnicity. Additionally, logistic regression was used to calculate the odds of SI, comparing ideal and intermediate versus poor levels for all the LS7 measures adjusting for age, gender, and ethnicity. Any and all statistical analyses and/or computation of weighted estimates for the general US population were performed using SAS version 9.0.3 (SAS Institute Inc.).
Results

A total of 3,528 adults aged ≥ 40 were included (50.9 % females, 18.8 % African American, 26.2 % Hispanics). Of the total study population, 90.4 % were classified as being absent of SI (having social support) and 9.6 % being socially isolated. The mean adjusted composite CVH score was 14.6 without vs 14.1 in those who were socially isolated (p < 0.01). There were significantly lower proportions of persons with ideal levels of physical activity and fasting blood glucose in those who were socially isolated (p < 0.01). Among those who were socially isolated compared to those who were not, there were higher proportions who were Hispanic (26.1 % vs 8.6%) but lower proportions who were African American (7.7 % vs 9.7 %) (both p < 0.01) (Table 2).

<table>
<thead>
<tr>
<th>Measures</th>
<th>Social Isolation Absent (n=3191, 90.4 %)</th>
<th>Social Isolation Present (n=337, 9.6 %)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mean ± SD)</td>
<td>56.9 ± 0.33</td>
<td>57.5 ± 0.68</td>
<td>0.46</td>
</tr>
<tr>
<td>Female (%)</td>
<td>6.2 M (52.9%)</td>
<td>4.0 M (49.5 %)</td>
<td>0.44</td>
</tr>
<tr>
<td>African American (%)</td>
<td>11.3 M (9.7%)</td>
<td>0.6 M (7.7 %)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Hispanics (%)</td>
<td>6 M (8.6%)</td>
<td>2.1 M (26.1 %)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Total Cholesterol, mg/dL (mean ± SD)</td>
<td>202.4 ± 1.07</td>
<td>208.7 ± 3.53</td>
<td>0.119</td>
</tr>
<tr>
<td>HDL-Cholesterol, mg/dL, (mean ± SD)</td>
<td>52.9 ± 0.54</td>
<td>51.3 ± 1.26</td>
<td>0.176</td>
</tr>
<tr>
<td>Systolic Blood Pressure, mmHg (mean ± SD)</td>
<td>125.8 ± 0.49</td>
<td>127.8 ± 1.27</td>
<td>0.116</td>
</tr>
<tr>
<td>Diastolic Blood Pressure, mmHg (mean ± SD)</td>
<td>71.9 ± 0.42</td>
<td>72.1 ± 0.79</td>
<td>0.835</td>
</tr>
<tr>
<td>Body Mass Index, kg/m² (mean ± SD)</td>
<td>28.8 ± 0.14</td>
<td>28.5 ± 0.49</td>
<td>0.44</td>
</tr>
<tr>
<td>AHA Life’s Simple 7 Measures (%):</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>18.8</td>
<td>24.9</td>
<td>0.086</td>
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<tr>
<td>Past</td>
<td>1.97</td>
<td>3.73</td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>79.2</td>
<td>71.3</td>
<td></td>
</tr>
<tr>
<td>Obesity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Obese</td>
<td>35.3</td>
<td>33.3</td>
<td>0.714</td>
</tr>
<tr>
<td>Overweight</td>
<td>36.7</td>
<td>40.1</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>27.9</td>
<td>26.5</td>
<td></td>
</tr>
<tr>
<td>Total Cholesterol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poor</td>
<td>17.6</td>
<td>23.2</td>
<td>0.323</td>
</tr>
<tr>
<td>Intermediate</td>
<td>48.0</td>
<td>43.3</td>
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<tr>
<td>Ideal</td>
<td>34.3</td>
<td>33.5</td>
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</tr>
<tr>
<td>Blood Pressure</td>
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</tr>
<tr>
<td>Poor</td>
<td>21.9</td>
<td>25.4</td>
<td>0.492</td>
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<tr>
<td>Intermediate</td>
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<tr>
<td>Ideal</td>
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<td>26.7</td>
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<tr>
<td>Diet</td>
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<tr>
<td>Poor</td>
<td>73.5</td>
<td>70.1</td>
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<tr>
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<tr>
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<td>6.32</td>
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<td>Exercise</td>
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<tr>
<td>Poor</td>
<td>26.8</td>
<td>37.9</td>
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<tr>
<td>Intermediate</td>
<td>46.3</td>
<td>41.0</td>
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<tr>
<td>Ideal</td>
<td>26.9</td>
<td>21.0</td>
<td></td>
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<tr>
<td>Fasting Blood Glucose</td>
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<td></td>
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<tr>
<td>Poor</td>
<td>12.2</td>
<td>18.5</td>
<td>0.0092</td>
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<tr>
<td>Intermediate</td>
<td>16.8</td>
<td>17.9</td>
<td></td>
</tr>
<tr>
<td>Ideal</td>
<td>71.0</td>
<td>63.5</td>
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</table>

* AHA Life’s Simple 7 Score (mean ± SD) 14.6 ± 0.09 14.1 ± 0.11 0.003
Regression (Table 3) shows that compared to participants those at poor levels of health metrics, the age, ethnicity, and gender-adjusted odds (95 %) for social isolation among those at ideal fasting blood glucose were 65 % (p < 0.05) as likely to be socially isolated. Compared to non-smokers, current smokers were 57 % (p < 0.01) as likely to be socially isolated. Those who were at an ideal level of physical activity were 53 % (p < 0.001) as likely and intermediate levels of physical activity to be 73 % as likely (p < 0.05) to be socially isolated. While age and sex did not relate significantly to the likelihood of social isolation, in adjusted analyses, compared to whites, Hispanics were 4 times more likely and other races 2.9 times more likely to be social isolated (both p < 0.01).

Table 3. Multivariable logistic regression odds of social isolation among AHA’s LS7 measures

<table>
<thead>
<tr>
<th>Measures</th>
<th>Odds Ratio (95 % CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (per 10 years)</td>
<td>1.09 (0.95-1.26)</td>
<td>0.2113</td>
</tr>
<tr>
<td>Female (vs. Male)</td>
<td>0.81 (0.58-1.13)</td>
<td>0.2148</td>
</tr>
<tr>
<td>Black (vs. White)</td>
<td>0.88 (0.51-1.53)</td>
<td>0.6460</td>
</tr>
<tr>
<td>Hispanics (vs. White)</td>
<td>4.00 (2.94-5.44)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Other Race (vs. White)</td>
<td>2.94 (1.67-5.20)</td>
<td>0.0002</td>
</tr>
<tr>
<td>Diet 2 vs. 1</td>
<td>1.01 (0.67, 1.51)</td>
<td>0.9727</td>
</tr>
<tr>
<td>Diet 3 vs. 1</td>
<td>1.49 (0.58, 3.77)</td>
<td>0.4026</td>
</tr>
<tr>
<td>BMI 2 vs. 1</td>
<td>1.21 (0.86, 1.69)</td>
<td>0.2688</td>
</tr>
<tr>
<td>BMI 3 vs. 1</td>
<td>1.06 (0.70, 1.61)</td>
<td>0.7710</td>
</tr>
<tr>
<td>Blood Pressure 2 vs. 1</td>
<td>0.88 (0.63, 1.24)</td>
<td>0.4629</td>
</tr>
<tr>
<td>Blood Pressure 3 vs. 1</td>
<td>0.92 (0.60, 1.42)</td>
<td>0.7251</td>
</tr>
<tr>
<td>Total Cholesterol 2 vs. 1</td>
<td>0.72 (0.46, 1.12)</td>
<td>0.1439</td>
</tr>
<tr>
<td>Total Cholesterol 3 vs. 1</td>
<td>0.76 (0.42, 1.39)</td>
<td>0.3807</td>
</tr>
<tr>
<td>Fasting Blood Glucose 2 vs. 1</td>
<td>0.77 (0.49, 1.21)</td>
<td>0.2646</td>
</tr>
<tr>
<td>Fasting Blood Glucose 3 vs. 1</td>
<td>0.65 (0.47, 0.91)</td>
<td>0.0117</td>
</tr>
<tr>
<td>Smoking 2 vs. 1</td>
<td>1.30 (0.39, 4.88)</td>
<td>0.6112</td>
</tr>
<tr>
<td>Smoking 3 vs. 1</td>
<td>0.57 (0.38, 0.85)</td>
<td>0.0061</td>
</tr>
<tr>
<td>Physical Activity 2 vs. 1</td>
<td>0.73 (0.54, 0.99)</td>
<td>0.0446</td>
</tr>
<tr>
<td>Physical Activity 3 vs. 1</td>
<td>0.53 (0.37, 0.76)</td>
<td>0.0006</td>
</tr>
</tbody>
</table>

\[3 = \text{ideal}; 2 = \text{intermediate}; 1 = \text{poor (reference)}\]

Discussion

This study is the first to examine and analyse the relation between AHA’s LS7 metrics and social isolation (also defined within the literature as "lack of social support"), in a representative sample of the US population. The primary finding of this study was that lack of social isolation (presence of social support) was associated with greater levels of cardiovascular health measured by LS7. In particular, it was found that among the LS7 components, social isolation is associated with poorer fasting blood glucose, smoking, and physical activities. Moreover, Hispanics were four times as likely as whites to be socially isolated.

Smoking is strongly associated with social isolation and current smokers are more likely to be socially isolated. Although there are discrepancies in the findings, social support has been shown to be associated with successful smoking cessation. With a smoking prevalence of 15.2 % among adults age ≥ 18 in 2015, the presence of social support will be quite beneficial at the reduction of smoking prevalence among the population thus contributing to an improved general cardiovascular health. While smoking is a critical risk factor, the reduction of smoking prevalence in the recent years has been counterproductive by the increase of poor blood pressure, BMI, and absence of healthy diet resulting in the need to consider other factors for primary prevention of CVD.

Physical activity is an important factor of LS7 and hold a significant correlation with SI where those with intermediate and ideal level of physical activities are less likely to be socially isolated. Many prior studies have established that physical activity is positively related to social support. Females with high physical activity social support were twice as likely to be active at least 30 minutes for 5 or more days per week. Absence of friend or family support has been shown 23-55 % more likely to be inadequately active comparing to those with high family or friend support. The influence of physical activity on other factors such as blood glucose are rather essential in the primary prevention of CVD, thus the presence of social support influencing physical behaviour may be advantageous in the reduction of blood glucose indirectly through the increase of physical activity.

Previous studies have indicated that social support is associated with better diabetes self-management. Furthermore, social support at higher levels are related with improved glycaemic control, enhanced adherence of treatment, and strengthened life’s quality; although the relationship is controversial and debated. While the relation between social support and blood glucose is indirect, the significance of social support in diabetes care remains a prominent factor as it has been shown to be valuable in diagnosis acceptance, adjusting emotionally, and stress alleviation.
The clinical implication of this study is that the promotion of social support is essential to CVH. These findings may help to explain the link between SI and increased CVD risk, but further study is warranted to confirm this. The individualist culture within the US population may interfere with effective and sufficient prevention of diseases such as CVD. The findings should be an encouragement for the general public to seek out and maintain strong social relationships, as it yields improvement with respect to quality of life. Additionally, clinicians may be able to improve patient outcomes by assessing social support and intervening more aggressively in those who are socially isolated.

This study only assessed the presence of SI and not the degree or specific type of SI, thus future study is needed to examine the relation between the type of social support or SI and CVH. To some individuals, having a family member is considered as social support but others may find having a peer equally, if not more, beneficial; the discrepancies between the relationships pose potential challenges when it comes to quantifying social support. Depending on the individual’s personality and preferences, some may prefer to have a large group of friends while others enjoy a small intimate group of peers, but both are perceived as sufficient support. Quantity of social connections does not convey quality.

This study has strengths and limitations. The key strength of the National Health and Nutrition Examination Survey is the inclusion of a US-population representative sample of adults with data generalisable to the greater US population. However, due to the limitation of the NHANES social support variable, there was no ability to analyse quality vs quantity of social support, thus warranting future studies. Furthermore, the measure of SI used in this study was only based on one NHANES question with respect to self-reported presence of social support due to significant missing values. This information was only available from the 2007-2008 NHANES survey and not more recent ones; however, the association between social isolation and cardiovascular health factors is not anticipated to depend on the survey year (unlike assessing prevalence information of risk factors). Finally, the cross-sectional nature of the study remains a limitation of the study. This warrants further longitudinal studies in individuals initially free of SI at baseline to determine the prospective relation of CVH such as LS7 with the actual development of SI.

Conclusion

This study shows a higher level of CVH in those with social support compared to those with social isolation, with non-smoking status, ideal physical activity, and glucose levels components independently associated a lower likelihood of SI, warranting the consideration of screening for SI as a possible determinant of CVD risk. Prospective study should examine how social isolation may lead to a declining cardiovascular risk factor profile.

Acknowledgments

None.

Conflict of interest

None.

References

34. Schafer LC, McCaul KD, Glasgow RE. Supportive and nonsupportive family behaviors: relationships to adherence and metabolic control in persons with Type I diabetes. Diab Care 1986;9:179-85.
Pharmacokinetic and Drug Absorption Profiles of the Anti-Hyperglycaemic Agent Gliclazide in Oral Tissue-Targeted Microcapsules in Rats

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Abstract

Background/Aim: Gliclazide is one of the most commonly prescribed oral anti-hyperglycaemic therapies in type 2 diabetes mellitus (T2D). Recently reported additional beneficial pharmacological properties of gliclazide, including immunomodulatory and anticoagulant activities, suggested its potential application in treatment of type 1 diabetes mellitus (T1D). However, following oral administration, gliclazide was shown to have poor and variable absorption directing research into development of novel pharmaceutical delivery systems of gliclazide suitable for T1D. Since bile acids have previously demonstrated stabilising and controlled-release effects on microcapsules, their use for preparation of microcapsules of gliclazide may lead to improvements in gliclazide release, absorption and antidiabetic effects. This investigation was aimed to evaluate drug absorption profiles and hypoglycaemic effects of alginate-based microcapsules of gliclazide, prepared together with or without cholic acid, in healthy rats.

Methods: Thirty healthy Wistar rats with confirmed normal glucose blood concentration were allocated into five groups and administered with a single dose of either vehicle microcapsules, gliclazide in suspension, gliclazide microcapsules, gliclazide in suspension together with cholic acid or microencapsulated gliclazide in combination with cholic acid. Following respective gliclazide dose, blood was sampled over next 10 hours and blood glucose levels and gliclazide serum concentrations were measured.

Results: This analysis demonstrated altered effects of different gliclazide formulations in healthy rats with the highest gliclazide absorption mirrored by the most profound hypoglycaemic effect being achieved after its oral administration as a suspension (p <0.01) compared to any other investigated pharmaceutical formulation.

Conclusion: When conducting pharmacokinetic characterisation of novel pharmaceutical formulations of antidiabetic drugs, it is of utmost importance to select the appropriate research model and consider the possible role of gut-metabolic activation on their hypoglycaemic effects.

Key words: gliclazide; capsules; bile acids; hypoglycaemic agents; rats.

Introduction

Gliclazide, a second-generation sulphonylurea, is the second most commonly prescribed oral antihyperglycaemic agent in the treatment of type 2 diabetes mellitus (T2D), after metformin.1 It is the preferred therapeutic option because of its selective binding to the pancreatic β-cell sul-
phosphorylurea receptor (SUR1) and subsequent stimulation of insulin release, as well as for its unique antioxidant properties\(^2\) and other beneficial haematological effects.\(^3\) \(^4\) Furthermore, gliclazide was shown to restore peripheral insulin sensitivity, decrease hepatic glucose production and skeletal muscle glycogenesis, independent of its insulin-mediated effects.\(^5\) Due to its known extrapancreatic action, gliclazide may exert conceivable potential in type 1 diabetes mellitus (T1D) treatment, especially when used in combination with other agents that exhibit hypoglycaemic effects, including certain bile acids and probiotics, as previously reported.\(^6\) \(^8\)

Despite the clinical experience with gliclazide being extensive and its pharmacological and pharmaceutical applications well-documented, poor and variable absorption after oral administration has limited its potential applications in T1D due to the inconsistent dose-response effects, necessitating research into novel pharmaceutical delivery systems of gliclazide suitable for T1D.\(^9\) Therefore, besides available immediate and modified release formulations of gliclazide,\(^10\) novel pharmaceutical approaches that utilise high-scale techniques, such as drug microencapsulation are needed for enhancement of its oral absorption and potentially maintain tight blood glucose levels in diabetic patients.\(^11\)

Multiple recent studies reported on the development and characterisation of novel alginate-based microencapsulated formulations of gliclazide mixed with different bile acids. Notably, these new gliclazide formulations exhibited stable colon-targeted delivery and its enhanced pharmacokinetic and pharmacodynamics effects in T1D rats when cholic, deoxycholic or taurocholic acids were added to the microencapsulated gliclazide formulation.\(^12\) \(^17\)

However, given the novelty of the designed microencapsulated gliclazide formulations, basic in vivo pharmacokinetic and pharmacodynamic studies should be also conducted in a sufficient number of clinically healthy animals, in order to validate the in vitro and in silico approaches needed to gain a more comprehensive understanding of its efficacy and safety profiles in diabetic models and ultimately patients.

Hence, this study was aimed to investigate the hypoglycaemic effects of alginate-based microcapsules of gliclazide, prepared together with or without cholic acid, in healthy rats. Focusing on the effects of microencapsulation and addition of cholic acid on gliclazide systemic absorption in healthy animals, this study complements the ongoing work aimed at investigating potential bile acids use and microencapsulation technology in the delivery and absorption of antidiabetic agents.

### Methods

#### Reagents and Materials

Gliclazide (99.9%), Na-alginate (98%), and cholic acid (95%) were supplied by Sigma Chemical Co, USA. Anhydrous calcium chloride was purchased from Scharlab S.L, Australia. The suspending gel for ultrasound-aided manipulation was obtained from Australian Medical Association, Perth, WA, Australia. Test tubes for oral gavage were purchased from Instech Laboratories, Inc., USA. Acetonitrile and HPLC-grade water were delivered by Fisher Chemical, Australia. All other chemical reagents and solvents were supplied by Merck, Australia.

#### Microcapsules Preparation

Fresh stock of gliclazide (40 mg/mL) and cholic acid (4 mg/mL) were prepared by dissolving the respective powders in 10 % ultrasonic suspending gel, while the 2 % CaCl\(_2\) stock solution was prepared by adding CaCl\(_2\) powder to HPLC-grade water. Each stock preparation was stored at 4\(^\circ\) C and used within 12 hours. Sodium alginate-based microcapsules of gliclazide and cholic acid were prepared as previously described, according to the established protocols.\(^15\) \(^17\)

#### Animal Procedures and Blood Glucose Measurements

The conducted experiments were approved by the local Ethics Committee for Experimental Animals Welfare Protection of the University of Novi Sad, Serbia (approval number: 01-31/4-1/2014-01). All experiments were performed in alignment with ethical principles and guidelines for welfare of the laboratory animals. Male Wistar rats, 8–10 weeks old, were obtained from the Animal Farming Facility of the Military Medical Academy, Belgrade, Serbia and were allowed a 7-day acclimati-
sation period and given access to food and water ad libitum. Following allocation into 5 separate groups with 5 rats per cage, animals were treated via oral gavage with a single dose of either vehicle microcapsules (control group), gliclazide suspension (G), microencapsulated gliclazide (G (MC)), gliclazide plus cholic acid suspension (G+CA) or gliclazide plus cholic acid microcapsules (G+CA (MC)), with identical doses of gliclazide and cholic acid of 40 mg/kg and 4 mg/kg, respectively across all treatment groups. After a single dose administration of the respective treatment, blood was drawn from tail vein at following time-points: 0, 5, 10, 20, 40, 60, 90, 120, 180, 240, 360, 480 and 600 min post-dose, for blood glucose and gliclazide concentrations measurements. Blood glucose levels were directly measured in collected samples, using ACCU-CHEK Glucose Advantage Meter (Roche).

**Gliclazide Serum Concentrations Measurements**

Gliclazide concentrations in serum were measured using high pressure liquid chromatography (HPLC) techniques based on the previously established methods. Shortly, 40 µL of serum samples were extracted with acetonitrile in a 2:1 ratio, vortexed for 30 seconds and centrifuged (10,000 rpm/min) for 5 minutes. The supernatant was injected into the HPLC system, together with respective standards and quality control samples and analysed in the same manner. The used HPLC system was HPLC-DAD (Dionex, USA) with ODS Hypersil analytical column (100 mm x 2.1 mm, 5 µm; 120 Å) from Agilent with the appropriate ODS precolumn (Agilent; 20 mm x 2.1 mm, 5 µm). The mobile phase was 49% acetonitrile and 51% water, v/v (pH 2.7). The flow rate was 0.4 mL/min, and the analysis was performed using the wavelength of 229 nm. Limit of detection (LOD) was 0.4 µg/mL, limit of quantification (LOQ) was 0.5 µg/mL, whereas the recovery of 85.83 ± 4.02% and linearity of 0.8 - 500 µg/mL was noted.

**Statistical Analysis**

The D'Agostino-Pearson omnibus test was used to test for the normality of data (p > 0.05). Linear regression and one-way ANOVA analysis followed by a post hoc Tukey’s multiple comparison test was performed. Data are represented as mean ± standard deviation (SD). GraphPad Prism version 5.0 (GraphPad Software, Inc., USA) was used and p values reported where significant (p < 0.05) or highly significant (p < 0.01).

**Results**

**Blood Glucose Measurements**

According to the D'Agostino-Pearson omnibus test, data passed the normality test (p > 0.05), indicating normal distribution of data. All administered gliclazide formulations, with or without added cholic acid resulted in significantly lower blood glucose concentrations in healthy rats compared with control vehicle microcapsules, therefore indicating a strong and significant hypoglycaemic effects of orally administered gliclazide (Figure 1). However, the reduction in blood glucose levels was more profound (p < 0.01) compared with control group when rats were treated with gliclazide suspension than with gliclazide microcapsules (p<0.05). On the other hand, when cholic acid was added to gliclazide, microencapsulated combination of gliclazide and cholic acid exerted stronger hypoglycaemic effects compared to the combination administered in suspension.

**Gliclazide Serum Concentrations**

According to the D'Agostino-Pearson omnibus test, data passed the normality test (p > 0.05), indicating normal distribution of data. When comparing the effect of microencapsulation technology on gliclazide serum concentrations, it is observed that microencapsulation of gliclazide resulted in its lower concentrations in serum with maximum serum concentration (C max) of 135.00 ± 86.73 µg/mL, compared with non-microencapsulated gliclazide with peak concentration in serum of 358.50 ± 92.88 µg/mL (Figure 2). Moreover, the time needed for gliclazide to...
reached its peak concentration in serum ($T_{\text{max}}$) was significantly prolonged ($p < 0.05$) with microencapsulated gliclazide formulation compared to gliclazide suspension, with $T_{\text{max}}$ values being $276.00 \pm 116.96$ minutes and $138.00 \pm 58.48$ min, respectively. Addition of cholic acid to gliclazide microcapsules resulted in significantly higher concentrations in serum compared to non-microencapsulated combination of gliclazide and respective bile acid ($p < 0.05$).

### Discussion

It has been previously shown that diabetes may influence the pharmacokinetic, as well as pharmacodynamic properties of various oral antidiabetic compounds, including gliclazide, presumably due to the pre-systemic drug elimination or altered gastric emptying and gastrointestinal motility in diabetic individuals.$^{18, 19}$ In the present study, healthy rats exhibited increased gliclazide bioavailability compared to that previously reported in diabetic rats treated with same pharmaceutical gliclazide formulations,$^{17}$ regardless of its administered formulation. However, Stětinová et al.$^{20}$ reported that even when biological gliclazide bioavailability was similar in both healthy and alloxan-induced diabetic animals, the hypoglycaemic effect of gliclazide was not equally distributed and was less pronounced in animals with alloxan-induced hyperglycaemia (23 % decrease at 60 min) compared to healthy animals (36 % decrease at 60 min). Interestingly, at later time points, this gliclazide hypoglycaemic effect was maintained in hyperglycaemic animals, while healthy animals demonstrated a reversal of the hypoglycaemic effect.$^{20}$

It was shown here that in healthy normoglycaemic animals, gliclazide microencapsulation resulted in its significantly lower $C_{\text{max}}$ and almost doubled $T_{\text{max}}$ values, compared with non-microencapsulated gliclazide. On the other hand, the addition of cholic acid to the alginate-based gliclazide microcapsules increased its serum concentrations and extended the time needed to reach its peak concentration in serum compared to the combination of gliclazide plus cholic acid that was not microencapsulated (Figure 2). Similar findings have previously been reported for alloxan-induced diabetic animals.$^{17}$ This was also confirmed by the in vitro stability and release kinetic studies of the same gliclazide formulations, indicating possible suitability of microencapsulated gliclazide formulations for its sustained and targeted delivery to the lower intestine.$^{12, 15}$

Having a closer look at the maximum blood glucose level drops ($E_{\text{max}}$) compared to its initial values before gliclazide administration ($E_0$) in healthy rats, it was observed that gliclazide suspension lowered the glucose levels by 69 %, microencapsulated gliclazide formulation by 51 %, combination of gliclazide suspension and cholic acid by 46 % and microencapsulated combination of gliclazide and cholic acid by 46 %. Interestingly, in the previously reported study in diabetic rats,$^{17}$ the greatest glucose levels reduction from the initial values was observed after the administration of the microencapsulated gliclazide and not with gliclazide suspension as it was the case in healthy rats. Moreover, even though statistical test showed non-significant difference between healthy and diabetic rats, a tendency towards higher values of $C_{\text{max}}$ and shorter $T_{\text{max}}$ for gliclazide were observed in healthy rats within this study compared with alloxan-induced diabetic rats from the previous study,$^{17}$ regardless of the administered gliclazide pharmaceutical formulation.

Linear regression analysis of blood glucose levels throughout time and gliclazide concentrations in serum administered in different pharmaceutical formulations in healthy rats revealed disproportionate association of gliclazide suspension treatment and resulting glucose levels in blood. Even
though gliclazide and cholic acid microcapsules also resulted in consistent reduction in blood glucose levels, these changes were not significantly different compared with control (Figure 3).

Mikov et al have reported decreased bioavailability of gliclazide administered in suspension in alloxan-induced diabetic rats compared to healthy controls, possibly due to gastrointestinal motility disorder related to diabetes. However, in the same study, when gliclazide was administered together with sodium 3α,7α-dihydroxy-12-keto-5β-cholanate (MKC), its bioavailability was further increased in healthy animals, but no significant differences were observed in terms of glucose levels. On the other hand, gliclazide bioavailability being substantially lower in diabetic rats was not altered by this bile acid salt, but the hypoglycaemic action in diabetic rats of the combination of gliclazide and MKC was significantly greater in comparison to the situation when gliclazide was administered alone.

Differential drug permeation-enhancing and hypoglycaemic effects of various bile acids are further supported by a recently reported study that evaluated the glucose lowering potential of novel microencapsulated gliclazide pharmaceutical formulation with gliclazide alone or in combination with a taurocholic acid (TCA). Mathavan et al reported that microencapsulated gliclazide alone failed to exert the hypoglycaemic effect in diabetic rats, whereas the addition of TCA into gliclazide microcapsules resulted in enhanced gliclazide absorption and significant hypoglycaemic effects compared with diabetic untreated controls. However, the data on the effects of this microencapsulated gliclazide formulation in healthy animals is not reported.

Another study that investigated pharmacokinetics and pharmacodynamics of gliclazide in immediate and modified release formulation tablets showed non-significant difference of evaluated parameters between healthy and streptozotocin-induced diabetic rats for either formulations. Even though the differences were not significant, a tendency towards slower elimination and higher mean residence time (MRT) in both healthy and diabetic rats treated with modified release gliclazide versus its immediate release formulation was found, confirming the sustained release mechanism of the modified release drug formulations. Resztak et al also reported significantly higher reduction of blood glucose level with immediate-release gliclazide formulation than with one with modified release, with the highest pharmacodynamic efficacy of gliclazide being observed in the healthy animals following treatment with the immediate-release gliclazide tablets, whereas the hypoglycaemic effect of the drug was reduced in diabetic animals. Therefore, the authors suggested that hypoglycaemic effects of gliclazide in healthy subjects might not be a suitable approach for characterising antidiabetic drugs.

Conclusion

This study demonstrated the impact of gliclazide pharmaceutical formulations on the systemic absorption and hypoglycaemic effects in healthy rats, revealing that altered pharmacokinetic/pharmacodynamic effects were due to the modified physiological outcomes in alloxan-induced diabetic rats. The highest gliclazide absorption was achieved after its administration as a suspension and was mirrored by the most profound hypoglycaemic effect versus any other investigated pharmaceutical formulation, unlike in diabetic rats where gliclazide hypoglycaemic effects were found to be independent of its absorption and serum concentrations. Taken together, these data indicate the importance of selecting the appropriate model for pharmacokinetic characterisation of novel pharmaceutical formulations of antidiabetic drugs and the possible role of gut-metabolic activation on their hypoglycaemic effects.
Acknowledgments

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These data were previously presented as a part of a PhD thesis of Jelena Calasan; Title: The effect of alginate microcapsules pharmaceutical formulation on gliclazide absorption in rat gastrointestinal tract.

Conflict of interest

None.

References

Beneficial Effects of Pomegranate Peel Extract Treatment on Anthropometry and Body Composition of Overweight Patients With Diabetes Mellitus Type-2: a Randomised Clinical Trial

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Abstract

Background/Aim: Polyphenol compounds obtained from pomegranate have beneficial pharmacological activities in the treatment of diabetes mellitus type 2 (DMT2). Most of DMT2 patients are overweight or obese and obesity by itself is very much related to insulin resistance and abnormalities in insulin secretion. This clinical study aimed to evaluate the pomegranate peel extract (PoPEx) activity on anthropometric parameters and body composition of overweight patients with DMT2.

Methods: Sixty patients with DMT2 on continuous metformin therapy were involved in this double-blind, placebo-controlled, randomised clinical trial. Patients from the study group (n=30) were treated with capsules containing PoPEx (250 mg) twice a day for 8-week period, while those ones from the placebo group (n=30) received placebo capsules for the same period. Anthropometric characteristics (body weight, waist circumference, fat mass percentage, visceral fat level) were measured at the beginning and at the end of the study.

Results: Eight-week treatment with PoPEx resulted in significant changes in BMI (mean value ± standard deviation: 0.18 ± 0.30 kg/m²) and body mass (0.48 ± 0.93 kg). The intake of PoPEx produced a significant decrease in waist circumference (z = -4.613, p < 0.001, r = 0.60) indicating a large effect size using Cohen’s d-test, and a non-significant decrease in the level of visceral fat. The results showed a non-significant reduction in fat mass percentage in PoPEx group (-0.58 ± 2.21 %, p = 0.159) compared with the placebo group (0.14 ± 1.24 %, p = 0.546).

Conclusion: The eight-week supplementation with PoPEx had a beneficial effect on anthropometry and body composition of overweight diabetic patients.

Key words: pomegranate peel extract; overweight; obesity; diabetes mellitus type 2; anthropometry.

Introduction

Nearly half a billion people worldwide or 9.3 % of all adults globally have diabetes mellitus (DM). The most common type of DM belongs to type 2 (DMT2), accounting for about 90 % of all diabetes cases and the prevalence of this disease is rising.1 Hyperglycaemia is the most common clinical sign of DMT2 that occurs as a result of decreased insulin secretion and the inability of the body to fully respond to insulin, known as insulin resistance.1, 2

Obese patients are at higher risk for non-communicable diseases including DM.3 Most DMT2...
patients are overweight or obese and obesity by itself is linked to insulin resistance and abnormalities in insulin secretion.\textsuperscript{3, 4} One of the most significant risk factors for metabolic disorders and its predisposition to DMT2 is abdominal distribution of fat.\textsuperscript{5} Having in mind that DM is one of the leading causes of death worldwide and that DMT2 and obesity together increase the mortality, it is not surprising that the prevention and treatment of DM and obesity are important public health measures.\textsuperscript{1, 6}

Diabetes can be managed by different approaches, including antidiabetic medication, nutrition, physical activity or herbal remedies.\textsuperscript{7, 8} There is an increasing interest in identifying herbal compounds that have lipid-lowering activities or properties to reduce obesity.\textsuperscript{9} According to some studies, polyphenols exert very potent anti-inflammatory effects and can improve metabolic conditions.\textsuperscript{8} Pomegranate is an excellent source of polyphenols (flavonoids, condensed tannins and hydrolysable tannins) with beneficial pharmacological properties and potential for treatment of various disorders including DM.\textsuperscript{10-13} According to the literature data, pomegranate peel extract (PoPEx) can affect adipocyte differentiation.\textsuperscript{9} Adipocytes, on the other hand, are very important in the development of metabolic disturbances that are related to obesity and DM.\textsuperscript{9, 14} A recent clinical study, performed in obese patients with DMT2, clearly showed that PoPEx, containing ellagitannins had a very potent hypolipaemic, hypoglycaemic and anti-hypertensive effects, but had no effect on body mass index (BMI), body weight, fat mass and fat-free mass.\textsuperscript{15} However, the animal and cell culture studies suggest that dietary polyphenols may have a pronounced anti-inflammatory effect associated with a reduction of body weight and FM.\textsuperscript{16, 17}

Therefore, this clinical trial, as an arm of the existing clinical investigation, was aimed to study the effects of PoPEx on anthropometry and body composition in overweight patients with type 2 diabetes mellitus. Patients were recruited at the Department Endocrinology of the University Clinical Centre of the Republic of Srpska, Banja Luka. All participants (40 - 65 years of age) were overweight (BMI ≥ 25 kg/m\textsuperscript{2}), had poor glycaemic control (glycosylated haemoglobin, HbA1C ≥ 6.5\%) and were treated with metformin for the period of at least one year before being enrolled in the study. Patients not enrolled in the study were those with inflammatory diseases, with chronic kidney or liver disease or those on hormone replacement therapy and antioxidant supplements. Patients on insulin treatment were not considered for the study.

### Ethical Considerations

All the subjects interested in participating in this study had to sign an informed consent. They were informed about the study purpose and protocol, as well as on the possible risks or benefits of treatment. This clinical study was approved by the Ethics Committee of the Faculty of Medicine, University of Banja Luka No 01-9-604-2/17 and the study was conducted according to the Declaration of Helsinki.

### Study design and medication

After randomisation, sixty patients were allocated into two groups. The study group (n=30) received capsules (250 mg) containing PoPEx twice daily for 8-weeks period, and the placebo group (n=30) received capsules containing the same quantity of placebo. Participants had to follow the study protocol without changing their dietary habits, physical activities and medication regimens during the study period. They participants

![Figure 1: The study design and the participants flow diagram](image)
were provided with a fixed number of capsules needed for the course of treatment. The participant flow diagram and study design are presented in Figure 1.

Pomegranate peel extract
PoPEx was provided by Institute for Medicinal Plant Research "Dr Josif Pančić", Belgrade, Serbia. Pomegranate fruits were obtained from Herzegovina, a southern region of Bosnia and Herzegovina. After being separated from the fruit, peels were dried at room temperature (4 - 6 days). The dried peels were grounded with a laboratory mill to obtain the powder. Powdered pomegranate peel was extracted with 50% ethanol. After filtration, the extract was evaporated to dryness and put in capsules. Each capsule (250 mg) contained polyphenols (punicalagin, punicalin, ellagic acid, and gallic acid) in defined quantities. The detailed methods of preparation and quantification of phenolic compounds of PoPEx were described in detail in a recently published paper. The chemical formulae of these polyphenols are presented in Figure 2.

Energy and nutrient intake
Using a 3-day food diary records, the dietary intake was assessed at the beginning and at the end of the treatment period. The energy and nutrition intake were estimated every day using the Serbian Food Composition Database, harmonised with the European Food Information Resource (EuroFIR) standards and integrated into the EuroFIR Food Platform and Balkan Food Platform. The participants were advised not to change their usual daily diet.

Statistical analyses
For statistical analyses, the IBM SPSS 20 software was used (Chicago, IL, USA). All results were expressed as mean ± standard deviation and \( p < 0.05 \) was considered significant. For comparisons between the groups, Student t-test and Mann-Whitney U test were used. The distribution of variables was assessed by Shapiro-Wilk's test. For the power of statistical significance, Cohen's test was used, and the analysis of differences was performed by paired sample t-test or Wilcoxon Signed Rang test. Pearson and Spearman correlation coefficients were used to assess the correlation between body mass change.

Results
Two patients (3.33 %), out of 60 enrolled in the study, did not complete the study. At baseline, there were no differences in age and DM and metformin therapy duration between the PoPEx group and the placebo group (57.9 ± 6.1 years...
versus 56.9 ± 6.7 years; 74.00 ± 49.2 months versus 74.8 ± 53.0 months; 56.3 ± 38.0 versus 64.1 ± 49.8 months, respectively). In both study groups, no adverse effects were observed during the follow-up period. The baseline characteristics of the PoPEx and the placebo groups are presented in Table 1. There were no significant differences in anthropometric variables between the PoPEx and the placebo group at baseline.

Mean energy intake at baseline was similar in both groups (2333.6 ± 307.9 kcal and 2265.6 ± 343.4 kcal, respectively). The 3-day diary food intake showed no significant changes in the energy and macronutrient intake during the study period. The mean fat energy intake was 920.2 ± 99.3 kcal in the PoPEx group and 881.1 ± 83.8 kcal in the placebo group, accounting for nearly 40% of fat energy. The average daily intakes of saturated fat energy were 289.6 ± 31.5 kcal and 296.7 ± 30.6 kcal in the PoPEx group and the placebo group, respectively (Table 2).

After the intervention period, significant increase in BMI (0.18 ± 0.30 kg/m²) and body mass (0.48 ± 0.93 kg) were noticed in the PoPEx group; z = -2.646, p = 0.008 (r = 0.34) and z = -2.391, p = 0.016 (r = 0.30), respectively. However, at the same time, the intake of PoPEx produced a significantly decreased WC, z = -4.613, p < 0.001 (r = 0.60), indicating a large effect size using Cohen’s test and a non-significant decrease in the level of visceral fat. In the placebo group, there were no statistical differences in the anthropometric characteristics at the end of the intervention period (Table 3).

The relation between the bioelectrical impedance analysis (BIA) and skinfold thicknesses (SF) caliper measurements are illustrated in Figure 3. The correlation factor was high in the total sample (r = 0.887; p < 0.001), but it was lower.
This clinical study was performed to examine the effects of the 8-week treatment with PoPEx on body composition and anthropometric parameters in overweight subjects with DMT2. The results indicated that PoPEx had a beneficial effect on WC, but the effects on body mass and anthropometric characteristics were not consistent. Compared to the placebo group, a significant effect on BMI increase and WC decrease was noticed in the PoPEx group. However, the percentage of FM was decreased (-0.58 %) but the change was statistically not significant. Moreover, the recent results of an additional arm of this study undoubtedly demonstrated hypolipaemic activity of PoPEx treatment with a beneficial effect on fatty acid composition indicating a strong influence on lipid metabolism.15

Life-style management, including balanced nutrition and physical activity, are very important keys for improving glucose control in the context of DM self-management.19 Having that in mind, at the beginning of this study, all participants completed a 3-day food diary and all participants were asked not to change the nutritional pattern. The results showed that diabetic patients had a similar proportion of macronutrients in the diet as the rest of the population which is in accordance with the 2019 Consensus Report.20 Nearly 40 % of calories taken originated from fat and saturated fat energy and it was was higher than recommended. Epidemiological studies suggested a positive relationship between the saturated fat intake and plasma cholesterol levels.21 Furthermore, a meta-analysis of randomised controlled clinical trials on modification of dietary fats on cardiovascular disease (CVD) risk suggested that saturated fat energy reduction might reduce cardiovascular events by 14% in patients with DMT2.22 In diabetic patients replacing 2% saturated fat energy with polyunsaturated fatty acids (PUFA) energy was associated with a 12 %-decrease in CVD mortality rate.23 The results of this study clearly showed that at the end of the intervention period, the energy intake and energy macronutrient proportion remained the same in both study groups.

The 8-weeks consumption of PoPEx induced a significant reduction in WC in the treatment group. WC is an independent predictive factor of chronic disease including DMT2.24 According to Lou et al,25 a change in WC can decrease the risk for DMT2, despite the lack of change in BMI. Several previous studies showed a significant influence on body mass after pomegranate extract consumption.26, 27 Our findings are not in accordance with the results obtained from previous animal studies, which have shown that pomegranate induces a weight loss. Intake of pomegranate leaf extracts had demonstrated a significant loss of body weight and a percentage of FM, decreased lipid profile and a decrease in the intestinal fat absorption in the animal model. Lei et al26, 27 have indicated that one of the possible mechanisms by which leaf extract affects the body mass is similar to the mechanism of drug orlistat (Xenical) causing the decrease in the activity of intestinal lipase, fat absorption, and increase of fat excretion. The administration of the whole pomegranate extract significantly decreased the body mass in overweight people, while there were no changes in the placebo group.28 Another study noted that intake of 120 mL of pomegranate juice during the 30 days led to a decrease in body mass and total body fat percentage.29 Discrepancies in outcomes of these studies could be explained by the differences in study protocols and treatment regimens (different study designs, different duration of the interventional period, variations among subjects, dosages and forms of pomegranate used).30 The phase angle value has been reported previously as a biomarker of fat-free mass and changes of phase angle value and fat-free mass in the PoPEx group confirm that assumption.31, 32

Concerning the body composition changes in the PoPEx group, average weight gain was 0.48 ± 0.9 kg, but the percent of FM was decreased by 0.58 ± 2.2 %. For the assessment of body composition in this study, two measurement tools were used, including BIA and SF caliper. The results showed a highly significant correlation between BIA and SF caliper (p< 0.001, r =0.887).
In DMT2 patients, atherosclerotic CVD is the major cause of morbidity and mortality. Although multiple factors play key roles in the development of CVD in DMT2, obesity is one of the factors that can be modified by dietary interventions. Previous studies investigating the effects of the pomegranate consumption on BMI and body composition are inconsistent. According to Ghefalti et al., supplementation with pomegranate extract showed a tendency to exert a beneficial effect on weight and the percentage of FM. Some in vivo and in vitro studies suggested that pomegranate had regulatory effects on dyslipidaemia and adipose tissue metabolism in human and animal adipose tissue. Polyphenols as functional food components exert a potential anti-obesity effect through an impact on white adipocyte browning and activation of the brown adipose tissue. Induction of the beige adipocytes may be mediated via the adrenergic membrane receptors, resulting in the stimulation of lipolysis and thermogenesis.

Conclusion

The results of this study showed that eight-week supplementation with PoPEx had a beneficial effect on anthropometry and body composition of overweight diabetic patients. Further studies are needed to explore the potential effect of PoPEx on adipokines and adipocyte functions in obese patients.

Conflict of Interest

None.

Acknowledgements

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References

D-Dimer: a Role in Ruling out Pulmonary Embolism in an Emergency Care Department

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Abstract

Background/Aim: Pulmonary embolism (PE) is a diagnostic challenge, particularly in prehospital care. The aim of this study was to determine to what extent the evaluation of D-dimer value helps physicians with differentiation of PE and whether D-dimer values are in correlation with the values of revised Geneva score.

Methods: Data have been collected for the patients whose D-dimer has been evaluated at the Emergency Care Department of the City of Banja Luka in 2018. Gender, age, symptoms, working diagnosis and D-dimer value have all been recorded and also the fact whether the patient was referred to hospital treatment or not. For each patient the revised Geneva score was determined.

Results: Sixty-eight tests were done in 2018. Out of 68 tests, 41 were negative (60.3 %). D-dimer results helped in making decisions about referring patients to the hospital or not ($\chi^2 = 36.32$, $p < 0.001$). Patients with elevated D-dimer levels, especially where the values were four times higher than the reference ones typically were referred to hospital treatment, whereas 67.5% patients with negative D-dimer results were sent home after giving a treatment and advice. In the elderly patients D-dimer was statistically more positive ($F = 10.82$, $p < 0.001$). Values of D-dimer were not significantly different regarding gender ($\chi^2 = 2.19$, $p = 0.33$). According to the results of the revised Geneva score, 5.1 % of patients had high risk of PTE, while moderate and low risk had 47.5 % each. Although it has been found that the values of D-dimer were slightly more elevated at higher values of the revised Geneva score and that the difference was not statistically significant ($\chi^2 = 7.71$, $p = 0.10$).

Conclusion: Values of D-dimer considerably helped in differentiation of PE in the Emergency Care Department. D-dimer has a high negative predictive value and should be used to exclude PE diagnosis for patients with low clinical probability of PE.

Key words: D-dimer, pulmonary embolism, revised Geneva score, emergency care department.

Introduction

Pulmonary embolism (PE) is one of the most difficult conditions to diagnose, particularly in the prehospital care. It represents the most serious clinical manifestation of venous thromboembolism (VTE), which represents the third most common cardiovascular disease. The main cause of PE-related deaths is the undiagnosed PE during lifetime (59 %), followed by sudden fatal PE (34 %). It is estimated that only 7 % of patients who died from PE had PE diagnosed on time. Frequency of PE is hard to determine because it can sometimes remain asymptomatic and therefore go unsuspected, while on the other hand PE is often accidentally diagnosed as an incidental finding.
PE is one of the most urgent conditions in medicine. The success of treatment largely depends on timely diagnosis. PE primarily needs to be thought of, and early diagnosis and treatment of patients is possible if PE is suspected on the basis of detailed anamnestic data. Many risk factors can raise suspicion about PE, however it can happen without any of the predisposing factors (up to 30%). Clinical manifestation of PE is also nonspecific, so dyspnoea, as the most common symptom/sign of PE, occurs in only 50% of the clinically confirmed PEs.10, 11 A clinical probability for PE is estimated on the basis of clinical presentation by using the Wells clinical decision rule or the revised Geneva score.12-16

The electrocardiographic (ECG) changes are miscellaneous and usually nonspecific (most common is sinus tachycardia, followed by the right bundle branch block - RBBB, turning of heart axis to the right, SI QIII TIII, P pulmonale, simultaneous inversion of T waves in inferior and right precordial leads), but in 18% of patients ECG is normal.17, 18 Nonspecific changes or lack of them can also be found during the physical check-up, chest X-ray and laboratory tests.7, 19, 20 Gold standard for the diagnosis of PE is computed tomography (CT)-pulmonary angiography and/or ventilation-perfusion (VQ) scan.21, 22 D-dimer is a fibrin degradation product, a small protein fragment present in the blood after fibrinolysis. Role of D-dimer is to exclude the PE diagnosis.23-25 D-dimer plasma levels are elevated in patients with acute thrombosis because of the simultaneous activation of coagulation and fibrinolysis.26 Negative predictive D-dimer value is high (95-98%) and PE with normal values of D-dimer is unlikely. On the other hand, fibrin is produced in other conditions including cancer, inflammation, bleeding, trauma, necrosis and surgical intervention.27-29 Therefore, positive predictive D-dimer value is low and elevated D-dimer levels are not useful in confirmation of PE.30, 31 This implies recommendations that the patients with highly suspected PE should immediately undergo CT-pulmonary angiography and skip the D-dimer testing.6, 9

The aim of this study was to determine to what extent D-dimer values help to differentiate PE in emergency care departments and therefore help in making a decision to transfer patients to a higher referential level or not. Furthermore, the aim was to determine whether the D-dimer values correlated with the values of revised Geneva score.

Methods

The protocol of the study was approved by the Ethics Committee of the Primary Medical Centre Banja Luka and all the efforts were undertaken in order to keep the anonymity of the included patients.

Following the protocol of the Emergency Care Department of the City of Banja Luka, data were found for all the patients in whom the D-dimer was tested in 2018. Gender has been recorded (female/male), age, symptoms, D-dimer level (mg/L), working diagnosis, and data whether the patient was referred to the hospital treatment or not. Patients’ anonymity was preserved, while only gender and age were recorded, excluding other personal data.

The D-dimer values have been analysed by a quantitative, latex-enhanced immunoturbidimetric immunoassay on Cobas h-232 system. Although D-dimer has only one cut-off value, the authors were interested in finding to what extent a D-dimer value affected the physician’s decision, ie, if there was a difference whether a D-dimer value was slightly elevated or if it was four times or more higher than referential value. Therefore, a value of D-dimer was recorded in two ways, as a numeric value and as a value in one of three categories: < 0.5 mg/L, ≥ 0.5-2 mg/L and ≥ 2 mg/L.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Points</th>
</tr>
</thead>
<tbody>
<tr>
<td>RISK FACTORS</td>
<td></td>
</tr>
<tr>
<td>Age 65 or over</td>
<td>1</td>
</tr>
<tr>
<td>Previous DVT or PE</td>
<td>3</td>
</tr>
<tr>
<td>Surgery or fracture within 1 month</td>
<td>2</td>
</tr>
<tr>
<td>Active malignant condition</td>
<td>2</td>
</tr>
<tr>
<td>SYMPTOMS</td>
<td></td>
</tr>
<tr>
<td>Unilateral lower limb pain</td>
<td>3</td>
</tr>
<tr>
<td>Haemoptysis</td>
<td>2</td>
</tr>
<tr>
<td>CLINICAL SIGNS</td>
<td></td>
</tr>
<tr>
<td>Heart rate: 75-94/min</td>
<td>3</td>
</tr>
<tr>
<td>≥ 95/min</td>
<td>5</td>
</tr>
<tr>
<td>Pain on deep palpation of lower limb and unilateral edema</td>
<td>4</td>
</tr>
<tr>
<td>CLINICAL PROBABILITY</td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>0-3</td>
</tr>
<tr>
<td>Intermediate</td>
<td>4 - 10</td>
</tr>
<tr>
<td>High</td>
<td>≥ 10</td>
</tr>
</tbody>
</table>

Using the anamnestic data, symptoms, clinical signs, age and gender of the patient, for each patient the revised Geneva score was calculated. The parameters set for Geneva score are listed in the following table.
Table 1. Based on the values of Geneva score, patients were allocated into one of three categories: with low, intermediate or high clinical probability for PE.

Results were processed by using IBM SPSS 21.0 software. Categorial data were analysed by Chi-square test and age, after distribution uniformity was verified, by one-way analysis of variance (ANOVA). The average D-dimer values were distributed by the Man-Whitney U test, or the Kruskal-Wallis test.

Table 2. Distribution of gender, age and D-dimer values

<table>
<thead>
<tr>
<th>Variable</th>
<th>N (%)</th>
<th>D-dimer</th>
<th>Test</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GENDER</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>30 (44.12 %)</td>
<td>0.77 (0.99)</td>
<td>0.40-1.14</td>
<td></td>
</tr>
</tbody>
</table>
| Female     | 38 (55.88 %) | 0.80 (0.93) | 0.49-1.10 | U = 479.50*
|            |         |         |          | 0.264   |
| AGE (years)|       |         |          |         |
| < 40       | 12 (17.65 %) | 0.47 (0.93) | 0.11-1.06 |          |
| 41-65      | 25 (36.76 %) | 0.55 (0.59) | 0.32-0.79 |          |
| > 65       | 31 (45.59 %) | 1.21 (1.14) | 0.75-1.66 | χ² = 12.96** | 0.002 |
| TOTAL      | 68 (100.00 %) | 0.79 (0.95) | 0.55-1.02 |          |

*Man-Whitney U test;
**Kruskal-Wallis test showed general statistical significance; further post-hoc analysis showed statistical significance for all groups.

D-dimer, the frequent D-dimer was significantly more positive in elderly people (One-way ANOVA: F = 10.82, p < 0.001).

There were 30 men (44.12 %) and 38 women (55.88 %). The ratio of positive and negative results of the D-dimer test was without significant difference with respect to gender (Chi square test: χ² = 2.19, p = 0.33), as was the average value of the D-dimer itself (Man-Whitney U test: U = 479.50, p = 0.264).

The most common symptoms/signs in patients for whom D-dimer level were determined were: dyspnoea (37.3 %), chest pain (25.4 %), heart rate over 90 per minute (20.39 %), cough (15.25 %), pain and leg oedema (15.25 %), nausea (11.86 %), fever (11.86 %) and syncope (10.17 %). Anamnestically, 3.36 % of patients had previous surgeries and 3.36 % of patients had deep vein thrombosis or pulmonary embolism.

According to the Geneva score, 5.08 % of patients were at high risk of PTE and 47.46 % at intermediate or low risk each. Although the D-dimer values were found slightly higher at the higher Geneva score values, the difference was not statistically significant (χ² = 7.71, p = 0.10) (Table 3).

Half of the patients analysed for the D-dimer were not referred for further hospital treatment (48.48 %). On suspicion of PE, 27.27 % of patients were referred for further hospital treatment and other patients were referred, but under a different diagnosis. The D-dimer values helped in making the decision whether to refer a patient to hospital treatment or not (χ² = 36.32, p < 0.001). The correlation between the revised Geneva score and D-dimer values is shown in Table 3.

Patients who were found to have high D-dimer values, especially where they were four times more the cut-off values, were generally referred for hospital treatment, while 67.50 % of patients with negative D-dimer values were returned home after receiving treatment and advice (Table 4).
Discussion

In 2018, 68 D-dimer analyses were performed at the Emergency Care Department of the City of Banja Luka. The assumption is that the need for analyses was greater, but unfortunately analyses are not continuously available. For this reason, it was decided to analyse only patients who had done the D-dimer test, because if all patients who were suspected on PE or with dyspnoea had been analysed, an unrealistically low percentage of patients in whom D-dimer was determined would had been obtained.

Two thirds of the tests were negative (< 0.5 mg/L). A high percentage of patients who are D-dimer-negative is logical, since the D-dimer is primarily used to rule out the diagnosis of PE and deep vein thrombosis. The D-dimer was significantly higher in elderly patients, which is in accordance with the results of other studies. There are also recommendations to adjust the cut-off value of the D-dimer according to age by adding 0.1 to 0.5 mg/L for every 10 years of age for people over 50. This is thought to increase the specificity of the D-dimer in the elderly.

Clinical studies indicate that the D-dimer values are slightly higher in women, which is in accordance with the results of this study. As was the case with the results of other researchers, the difference was not significant in the present study either. The difference in D-dimer values in men and women is considered to be of no clinical significance and it is not recommended to correct the cut-off values based on gender. However, some researchers believe that D-dimer values should be adjusted for both gender and age, with multiple cut-off values, in order to significantly improve the specificity of D-dimer testing. In contrast to the D-dimer values, PE itself is slightly more common in men. The results of this study show that women were slightly more frequently diagnosed with PE (30 % : 20 % of patients), which could be explained by the fact that no cut-off corrections were made for women and therefore there was a higher percentage of false-positive tests in female patients.

The Wells score and the Geneva score were introduced in an attempt to adequately suspect or exclude PE based on history, symptoms and clinical presentation. Clinical trials indicate that the significance of the revised Geneva score is primarily in the exclusion of PE combined with low D-dimer values. The results of this study indicate that the revised Geneva score values did not influence physicians' decision not to refer such patients for further treatment, but only the D-dimer values. Analysis from other studies also implies underuse of clinical decision rules. There was a correlation between the revised Geneva score and the D-dimer, but it was not statistically significant. However, given a relatively small sample, it would be assumed that with a sufficiently large sample statistical significance would be reached. It is also the authors’ belief that when deciding whether to refer a patient for further hospital treatment or not, physicians should consider the revised Geneva score, especially if its value is < 3. Intermediate-risk PE patients present a diagnostic and therapeutic dilemma. Besides, research results indicate that in older, high-risk patients, the Wells scores are more in correlation with the diagnosis of PE than the revised Geneva score.

The D-dimer values significantly aided the physicians' decision to refer the patient to further hospital treatment under the diagnosis of PE. When D-dimer values were < 0.5 mg/L, two-thirds of patients were not referred for further hospital treatment and the others were referred under some other diagnosis. Two patients, despite the negative D-dimer values, were referred under the diagnosis of PE and this is, in fact, an example of poor clinical practice. The reason is that if the low D-dimer value does not alter the physician's opinion that it is PE or not, the D-dimer should not been tested at all. On the other hand, half of the patients with D-dimer values > 0.5 and all patients with values > 2 mg/L were referred for further treatment and diagnosis.

Conclusion

The very purpose of the D-dimer and revised Geneva score is to exclude PE and reduce unnecessary imaging diagnostic procedures, such as CT-pulmonary angiography and similar. The fact that the test results are available after 20 minutes should also not be overlooked, given that the speed of diagnosis and patient treatment is one of the key factors in working in an emergency care department.
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None.

Conflict of interest

None.

References


Association of Body Mass Index With Progression and Prediction of Multiple Sclerosis

Daliborka Tadić,1, 2 Viado Đajić,1, 2 Sanja Grgić,1, 2 Siniša Miljković1, 2

Abstract

Background/Aim: Multiple sclerosis is a disease whose aetiology involves multifactorial interactions among genetic and environmental factors. Obesity is one of the most important environmental factors conducive to the onset and progression of the disease. The aim of the study was to determine the value of body mass index (BMI) in a population of patients with multiple sclerosis compared to the general population, in order to assess the relation between the BMI and physical disability in patients with multiple sclerosis and the influence of the BMI on the course and progression of the disease.

Methods: A cross-sectional study was performed in 100 patients suffering from multiple sclerosis (experimental group) and 50 healthy people (control group). In order to determine the degree of physical disability, the Expanded Disability Status Scale (EDSS) was used. Clinical and demographic data and values of the BMI in both studied groups were collected. Statistical analysis included the descriptive statistics, t-test, chi-square test, analysis of variance, correlation and regression analysis.

Results: Mean body weight and BMI were significantly higher in the control group (p< 0.05). There was no significant correlation between EDSS and BMI (p = 0.574). There was a correlation between the course of MS and the fact whether BMI was abnormal or normal (p = 0.031).

Conclusion: BMI is an environmental factor that significantly correlates with the progression and prediction of multiple sclerosis, but not to the degree of physical disability.

Key words: multiple sclerosis, BMI, progression, prediction, physical disability.

Introduction

Multiple sclerosis (MS) is a chronic demyelinating disease of the central nervous system (CNS) that often occurs in a population of young adults, predominantly female.1 Pathophysiological mechanisms of this disorder include a degenerative and an inflammatory component that covers both grey and white matter of the CNS. These mechanisms are basis for a very heterogeneous relapsing or progressive clinical course of MS, where longer duration of the disease leads to physical and cognitive disability. A reliable long-term individualised prognosis has not yet been possible and represents a subject to intensive research.2 The aetiology of MS involves multifactorial interactions among genetic and environmental factors. Genetic predisposition is only a part of the risk of MS, while lifestyle and environmental factors are key participants in the development of the disease.3 Proven risk factors for the development of the disease are
female gender, smoking, low level of vitamin D, dietary habits, Epstein Barr virus infection and obesity in childhood and adolescence.4, 5

According to the previous studies, there is an obvious connection between the disability in MS and obesity. Increased level of disability decreases the level of physical activity and the frequency of overweight or obese status is higher and vice versa.6 This may be associated with loss of mobility, but also with other disorders that accompany MS such as depression, pain, fatigue, loss of social contacts and associated chronic diseases.7 However, this interdependence also exists at the molecular level. Since obesity is associated with latent inflammatory reactivity, it leads to the release of inflammatory cytokines that influence the immune response and is associated with a possible risk for the development of MS.8 Adipose tissue secretes hormones that affect the functioning of the immune system, including leptin and interleukin-6, known inhibitors of T-cell activity. Elevated leptin levels in obese people are inversely proportional to the function of T cells in individuals with MS. Another potential mechanism is related to the fact that obese people have lower levels of serum 25-hydroxy vitamin D correlates with leads to an increased risk for MS. Inflammasome is a protein complex that participates in the inflammatory response and it is found in the adipose tissue. Recent studies have shown its significant role in the pathogenesis of autoimmune and inflammatory diseases and demonstrated that elevated levels of these proteins leads to the progression of these diseases, which is particularly applicable to diabetes, atherosclerosis and MS.9-11

So far, in several studies the body composition has been studied as a factor influencing the onset and progression of MS, which was mostly related to the body mass index (BMI), total body fat and lean body mass.12, 13 In well-designed studies, in populations of Sweden and the United States of America (USA), the interactions between HLA genotype and BMI of 20-year-old people were investigated, where it was shown that obesity was associated with an increased risk of MS compared to people with normal weight body.14

The influence of obesity on MS was confirmed by a large longitudinal study, which included nurses in the USA (Nurses Health Study and Nurses Health Study II). The results of this study showed that obesity at the age of 18 years was related to a doubled risk of developing MS, compared to the individuals with normal BMI, while such a correlation was not found in the adult population.15 Munger et al16 in a long-term cohort study also examined children from 7-13 years of age and the results showed that obesity in this age carries a higher risk for the development of MS.

A large multinational EnvIMS study that included populations in Italy and Norway has shown that an increased body weight, in particular at the age of 20-25, poses a risk for the MS in Norwegian population, which partly also applies to the Italian population, but without reaching statistical significance. These results are compatible with low levels of vitamin D and a chronic inflammatory condition in the obese, which may originate from the differences in protective exposure to the sun.17 These data are significant, because in other studies lower levels of vitamin D metabolites in obese human subjects were observed and, among other things, this is why overweight status in infancy may be a risk factor for MS.18-21 Most recent studies confirmed the impact of obesity on the occurrence of MS in paediatric patients as well as on a weaker response to the drugs of the first-line therapy in the obese patients.22 It is also important to know that certain genetic structure is a common predictor for the elevated BMI before the onset of MS as well as for the occurrence of the disease.23

Why are these findings important? The survey data show that in the period from 2009 to 2010, 16.9 % of children and adolescents in the USA were obese. Although genetic and some other causes that lead to the disease cannot be affected, it is important to influence the environmental factors that are suitable for modification.24

The aim of this study was to determine the value of BMI in the population of people with MS compared to the general population, to estimate the association of BMI and the degree of physical disability in patients with MS and to estimate the possible influence of BMI on the course and the progression of the disease.
Methods

This cross-sectional study was conducted at the Clinic of Neurology, University Clinical Centre of the Republic of Srpska, Banja Luka. The sample consisted of 100 MS patients and 50 healthy people (control group) from general population, matched according to sex and age and who were not blood-related to the MS patients, who did not have an inflammatory disease of the CNS nor a cerebrovascular disease and did not use statins (control group). The duration of the study was twelve months.

The research was performed upon prior approval of the Ethics Committee of the University Clinical Centre of the Republic of Srpska, Banja Luka. For this study a general questionnaire, consisting of questions related to the demographic and clinical characteristics of patients and the questionnaire for risk factors for vascular diseases in patients with MS, which was created for the scientific purposes at the Institute of Epidemiology, Faculty of Medicine, University of Belgrade were used. In order to determine the degree of physical disability, the Expanded Disability Status Scale (EDSS) was used.\textsuperscript{25} BMI was defined as the body weight (BW) in kilograms divided by the surface area measured in square meters.\textsuperscript{26} BMI was determined in both groups of participants and based on its value participants were classified in following groups: reduced BMI (less than 18.5 kg/m\textsuperscript{2}), normal BMI (18.5-24.9 kg/m\textsuperscript{2}) and people with increased BMI (more than 25 kg/m\textsuperscript{2} - overweight status and over 29.9 kg/m\textsuperscript{2} - obesity).

Statistical analyses included methods of descriptive statistics, $\chi^2$ test, Student t-test, variance analysis, correlations and regression analysis.

Results

From a total of 100 patients with MS, 25 % were male and 75 % female. The average age of participants at the beginning of the study was 41.9 ± 10.1 years in the MS group, while participants from the control group were slightly older (average age 42.1 ± 12.3 years). The distribution of patients according to the clinical form of the disease is shown in Figure 1.

The average value of the EDSS score in the group of patients was 3.7. Based on the quotient of these values and the duration of the disease, the mean value of the index of disease progression was obtained, which in this patient group was 0.9.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>MS patients (n = 100)</th>
<th>Control group (n = 50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>41.9 ± 10.1</td>
<td>42.1 ± 12.3</td>
</tr>
<tr>
<td>Body weight (BW, kg)</td>
<td>67.1 ± 13.2</td>
<td>76.1 ± 16.3\textsuperscript{*}</td>
</tr>
<tr>
<td>Body mass indeks (BMI, kg/m\textsuperscript{2})</td>
<td>22.7 ± 3.1</td>
<td>36.0 ± 4.4\textsuperscript{*}</td>
</tr>
</tbody>
</table>

\textsuperscript{*}p<0.05 versus the MS group

In both groups parameters necessary for calculation of the BMI–BW and body height – were registered (Table 1).

Although the MS patients and their controls were matched by age, the average BW and BMI were significantly lower in the MS group. Values of BMI fell within a normal range in the MS group, while they were increased in terms of obesity in the control group.

The group of patients with MS was analysed according to the form of the disease. In order to establish whether there is a correlation between the clinical types of the disease and the BMI (classified in the pathological and normal level), the chi-square test was used and showed a statistically significant correlation (p = 0.031). More specifically, there was a correlation between the clinical types of MS and the fact whether BMI has pathological or normal values. BMI was a factor that changed during the disease progression and there was a higher incidence of lower values of this parameter with the progression of the disease, ie the transition from relapsing-remitting (RR) in the secondary progressive (SP) form (Figure 2).
Among the patients with RR form, 68.1% had a normal BMI, 25% increased and 6.9% decreased. In the SP group, 66.7% of patients had a normal BMI, 22.2% increased and 11.1% decreased. One patient, suffering from a primary progressive (PP) form of the disease, had decreased BMI.

Using logistic regression methods (univariate analysis) the parameters that could be predictors of MS and thereby determined that the coefficient for the normal BMI was positive, i.e. that normal values of BMI increase odds ratio and the probability that the patient has MS were analysed.

### Discussion

So far, there have been a series of studies involving the assessment of BMI in the population of patients with MS. Our results are in agreement with the hereinafter mentioned best-designed studies conducted on this topic in other environments. A highly significant difference in the values of BMI (p<0.05) in the group of MS patients compared to the control group (average value of BMI in the experimental group was within the normal range, whereas it was increased in the control group) is a consequence of the lower BW of this group of patients.

The study of Markianosa et al.27 showed that women suffering from MS had a lower BMI compared to age- and gender-matched controls, while substantial differences were not found among male patients. Their results showed that patients suffering from MS were overweight at a younger age and later on even had a lower BMI, a fact to which attention should be paid, in the context of a possible weight loss with the disease progression in certain populations.

The study of Nortvedt et al.28 observed a gender-mixed population (75% female) of MS patients compared to healthy individuals, where the average values of the BMI were 23.5 ± 3.6 kg/m², with 11% of subjects with a BMI below 20 kg/m². Low BMI values was observed in several studies in the population of MS patients compared to healthy controls, wherein there was also a significant, inverse correlation between the high BMI and the risk of developing MS.29-34

The study of Pike et al.35 found an average BMI of 23.9 kg/m² for the tested MS population from five European countries, which is within the normal range of values.35 In some other studies, BMI values in patients with MS were in the range of those in the general population.36, 37

A large meta-analysis of clinical trials conducted on this subject also showed a significantly lower BMI in the group of MS patients than in healthy controls and the same results were obtained for patients who had the RR form of the disease.38 However, some other studies have shown conflicting results to those previously shown and also to this study’s results. This may be the case because these investigations were conducted in certain regions of the world where the prevalence of obesity in the general population was higher due to the nutrition style, socio-economic conditions and less physical activity. Besides, some of these studies were performed as retrospective analyses of data from the registries dating from the 1980s, when the awareness of the need for prevention of factors affecting the cardiovascular and other diseases was significantly lower than today.39
A study in patients from the NARCOMS registry, the largest registry of MS worldwide, showed that over 50% of patients with MS were overweight.\textsuperscript{39} Similar data on the increased frequency of obesity in the population of MS patients have been shown in other several studies.\textsuperscript{40-45}

In the present study, in the experimental group, a lower mean BMI value (although within a normal range) compared to the control group can be interpreted in the context of the application of immunomodulatory therapy. Namely, a significant number of patients had been treated with interferon beta-1b (34%), whose one of the possible adverse effects is a reduction of BW.\textsuperscript{12, 46}

The results obtained in the present study can be explained by the fact that MS patients were under a constant medical supervision, in touch with their therapists who gave them advice on proper diet, need for physical activity and other measures of hygienic and dietetic regimens, which led to the levelling of body weight, as opposed to healthy the individuals in the control group.

Regarding the physical disability and its dependence on the value of the BMI, the results of clinical trials conducted so far were inconsistent. In the present study, there was no correlation between these two parameters, which can be interpreted by the fact that the patients from the experimental group had a higher percentage of normal values of BMI compared to the control group. However, in several studies conducted in a population of MS patients, there was a positive correlation between the degree of physical disability and the BMI.\textsuperscript{47-49}

The inverse impact of the BMI on physical disability was also recorded in the research of Flauzino et al\textsuperscript{50} In several studies as well as in the present one, it was shown that BMI may be considered as a factor that can change during the progression of the disease and that there was an increased incidence of lower values of this parameter with the progression of the disease ie with the transition from RR to SP form.\textsuperscript{39}

Obesity, among other factors such as gender, age, genetic profile and smoking, has a disease-modifying effect by forming its phenotypic presentation and contributes to the occurrence and progression of MS.\textsuperscript{51, 52} Research performed at the molecular level showed that high BMI negatively affected the course and form of MS, since the existence of obesity leads to a modulation of the number of monocytes through the ceramide-induced DNA methylation of the antiproliferative genes.\textsuperscript{53} MRI studies showed that lifestyle factors, including obesity, influenced the acceleration of cerebral atrophy, and the appearance of new lesions in patients with MS.\textsuperscript{54}

Prediction of the MS course is nowadays one of the foci of considerable research. In this study, normal BMI values were shown as a predictive factor for the onset of the MS. However, in other investigations conducted on this topic results were inconsistent. In one of them, BMI has shown a correlation with the progression of the disease and a higher frequency of relapses but was in no correlation with the prediction of the conversion from a clinically isolated syndrome to MS.\textsuperscript{55} In other studies it was shown that in a population of MS patients during the one year follow-up there was an increase in physical disability, but there were minimal pieces of evidence that BMI was a predictive factor for this change.\textsuperscript{56}

Taiwanese researchers have shown that the BMI values below the normal range with more than four demyelinating lesions are a strong predictor for the conversion from a clinically isolated syndrome to MS.\textsuperscript{57} It was also confirmed that the BMI values affected the prediction of cognitive disorders in patients suffering from MS.\textsuperscript{58}

So far, in several studies, the BMI has been studied as a factor influencing the onset of MS. Results of this study showed that BMI affected the progression and prediction of the disease, but not the degree of physical disability of patients with MS. To prevent these undesirable effects, it is very important to emphasise that this environmental factor is suitable for modification.

### Conclusion

Prediction of the MS course is nowadays one of the foci of considerable research. In this study, normal BMI values were shown as a predictive factor for the onset of the MS. However, in other investigations conducted on this topic results were inconsistent. In one of them, BMI has shown a correlation with the progression of the disease and a higher frequency of relapses but was in no correlation with the prediction of the conversion from a clinically isolated syndrome to MS.\textsuperscript{55} In other studies it was shown that in a population of MS patients during the one year follow-up there was an increase in physical disability, but there were minimal pieces of evidence that BMI was a predictive factor for this change.\textsuperscript{56}

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### Acknowledgements

None.

### Conflict of interest

None.
References


57. Ro LS, Yang CC, Lyu RK. A prospective, observational study on conversion of clinically isolated syndrome to multiple sclerosis during 4-year period (MSNEO study) and plasma lipid profiles of patients with multiple sclerosis. Mult Scler Relat Disord 2012;1(3):139-44.
Anthropometric Characteristics and Health Self-Assessment of Female University Students

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Department of Physiology, Faculty of Medicine, University of Banja Luka, Banja Luka, the Republic of Srpska, Bosnia and Herzegovina.
Faculty of Medicine, University of Banja Luka, Psychiatric Clinic, University Clinical Centre of the Republic of Srpska, Banja Luka, the Republic of Srpska, Bosnia and Herzegovina.
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Abstract

Background/Aim: An important period in becoming a young woman is studying at the University. It is a period of taking responsibility for yourself, your health and social relationships. The abilities formed during this period directly affect one’s physical and mental health. The study was undertaken in order to determine anthropometric characteristics and to evaluate the health of female students aged 19 to 22.

Methods: An analytical population study based on a survey using a standardised SF36 self-report health test and measurement using an objective bioimpedance method with Omron BF 511 estimated 408 female students of the University of Banja Luka aged 19 to 22, based on inclusion criteria.

Results: The mean body mass index (BMI) ± standard deviation of female students was 22.1 ± 3.2 kg/m², with 31.80 ± 6.47 percent fat and 28.15 ± 2.81 percent muscle mass. The mean values ± standard deviation of the SF 36 for mental health (MH) questionnaires was 60.7 ± 24.95 and for physical health (FH) 72.21 ± 25.89.

Conclusion: Female students have ideal BMI values. Physical health self-assessment reached higher values than the mental health self-assessment, which is probably related to mental distress and problems during studying.

Key words: student health, anthropometry, SF36 questionnaire, physical and mental health.

Introduction

Student population belongs to the young adult population and is in the post-adolescence period when a person has reached full maturity and development of all biological functions of the organism.1 Moving from younger to older adolescence, significant changes affect the body and psychosocial well-being of young people.2-3 Research records the occurrence of risky behavior of young people in the period of study and in a new and unfamiliar environment.4-7 During this period, young people are considered "healthy" and they do not consider their own engagement in health promotion as their priority. The transition from high school to college education is a period of taking responsibility for oneself, one's health and social relationships.4-8 During this period, young people often resort to different types of risky behaviors such as smoking, drinking, unprotected sex and poor eating habits.9-11 Healthcare professionals care about the health of their patients, but they also play a role in health promotion in different parts of the population and especially in the student population. The most important stages of life for physical and mental health are childhood and adolescence. It is a period when young people develop autonomy, self-control, social interaction and learning and the abilities formed during this period directly affect their physical and mental health for the rest of their lives. Half of all mental health problems in adulthood occur during or
before adolescence.\textsuperscript{2, 12, 13} Self-assessment of the existence of mental or physical changes or presence of certain symptoms, attempts to find out to what extent one is experiencing health problems or complaining of impaired physical functioning.

The aim of this study was to determine the anthropometric characteristics and to evaluate health of female students aged 19 to 22 at the University of Banja Luka.

**Methods**

This observational analytical study included a survey and measurement of a sample of 408 female 1st- and 2nd-year students from various faculties of the University of Banja Luka. All students were healthy volunteers aged 19-22. Students with musculoskeletal inherited and acquired diseases, traumas and deformities of the body, acute and chronic diseases as well as with a special diet were not included. The research was approved by the Ethics Committee of the Faculty of Medicine of the University of Banja Luka (Approval Certificate No 18/4.56/18, dated 2 November 2018). All the participants were provided with a detailed oral and printed explanation of the research plan and programme and their written consents were obtained. In this research, the applicable regulations in compliance with the ethical principles of the Declaration of Helsinki were followed. For the purposes of the research, a short socio-demographic questionnaire was used. It provides personal information on the students’ age, year of study, general health status. A standardised test was then used: the SF-36 self-report health questionnaire and the OMRON BF 511 digital scale for anthropometric measurements. SF-36 is a multifunctional health self-assessment questionnaire consisting of 36 questions (particles).\textsuperscript{14-16} Individual responses to all of the particle items are scored according to pre-established empirical norms, given the diagnostic value of the examiner’s specific response. The health change particle is displayed separately, by frequency distribution. The SF-36 measures the subjective sense of health across the eight different dimensions of health: physical functioning, restriction due to physical difficulties, physical pain, perception of general health, vitality, social functioning, restriction due to emotional distress, mental/mental health. The SF-36 questionnaire is based on two general health concepts: physical and mental health, although the dimensions selected represent multiple health indicators, including: behavioural function and dysfunction, suffering and well-being, objective reports and subjective evaluations, and self-evaluation of favourable and unfavourable general health statuses.

OMRON BF 511 is a high-precision digital medical scale: after entering height, age and gender data, the subject stands barefoot without excess clothing on the scale measuring body mass and calculating the body mass index (BMI), body fat percentage, visceral fat (adipose tissue around the internal organs) and the percentage of skeletal muscle. Based on the measurement of the body bioimpedance, the body composition is calculated and recorded on the display. The accuracy of the device when measuring body mass from 40.0 to 150.0 kg is ± 1 %. The measurement results are categorised as follows: a) BMI is divided into 6 categories: malnutrition (BMI ≤ 18.5); ideal mass (18.5 to 24.9); overweight (BMI: 25-29.9); mild obesity (BMI: 30-34.9); severe obesity (BMI: 35-39.9); extreme obesity (BMI ≥ 40); b) Skeletal muscle and adipose tissue percentage of the total body mass, according to the known percentage of adult females aged 18-39, is divided into 4 categories: low percentage 0 to <24.3 %; normal percentage: 24.3 - 30.3 %; high percentage: 30.4 - 35.3 % and with very high percentage ≥ 35.4 % of total body mass. The measurement was always carried out by the same researcher, using the same instrument to reduce the erroneous measurements and the respondent verified that she had not consumed food and drink for at least 3 hours before the measurement, without intense physical activity for the last 12 hours, with a urinary bladder emptied.

For statistical data processing, descriptive and analytical statistics using SPSS 21.0 for Windows was utilised. Statistical significance was adopted at a probability level of $p < 0.05$.

**Results**

The sample consisted of 408 female students aged 19-22 who attended the summer semester of the 1st or 2nd year of the University of Banja Luka (2018/2019 school year). The results of this research were divided into several segments.
A. Results of a basic socio-demographic questionnaire

A total of 37.7% of girls drink alcohol periodically, 19.8% regularly consume tobacco and 7.1% use marijuana. The menstrual cycle was established at the earliest age of eight (0.7%) and at the latest at the age of twenty (0.2%), while in 29.2% of cases it was established at the age of 13. From the demographic questionnaire, the question was: what is your current physical and mental health compared to the pre-study status? The following answers were received: 41.4% of students said that physical health was worse than at the beginning of studying and 45% that mental health is now worse. The usage of psychostimulants was 0.2%, hallucinogens 0.2%, while 2.7% had a lifetime psychostimulant use.

Table 1: Unhealthy behaviors adopted by the female students

<table>
<thead>
<tr>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>SELF-ASSESSMENT OF PHYSICAL HEALTH 1 YEAR</td>
<td>a better 32 7.8</td>
</tr>
<tr>
<td>LATER</td>
<td>the same 207 50.7</td>
</tr>
<tr>
<td>SELF-ASSESSMENT OF MENTAL HEALTH 1 YEAR</td>
<td>a better 40 9.8</td>
</tr>
<tr>
<td>LATER</td>
<td>the same 184 45.1</td>
</tr>
<tr>
<td>ALCOHOL CONSUMPTION</td>
<td>yes 154 37.7</td>
</tr>
<tr>
<td>no 254 62.3</td>
<td></td>
</tr>
<tr>
<td>TOBACCO CONSUMPTION</td>
<td>yes 81 37.7</td>
</tr>
<tr>
<td>no 327 62.3</td>
<td></td>
</tr>
<tr>
<td>MARIJUANA CONSUMPTION</td>
<td>yes 29 7.1</td>
</tr>
<tr>
<td>no 379 92.9</td>
<td></td>
</tr>
<tr>
<td>PROFESSIONAL</td>
<td>yes 29 7.1</td>
</tr>
<tr>
<td>PSYCHIATRIC HELP</td>
<td>no 379 92.9</td>
</tr>
<tr>
<td>SUICIDAL ATTEMPT</td>
<td>yes 5 1.2</td>
</tr>
<tr>
<td>no 403 98.8</td>
<td></td>
</tr>
<tr>
<td>USE OF PSYCHOACTIVE SUBSTANCE</td>
<td>yes 1 0.2</td>
</tr>
<tr>
<td>no 407 99.8</td>
<td></td>
</tr>
<tr>
<td>USE OF SEDATIVES</td>
<td>yes 3 0.7</td>
</tr>
<tr>
<td>no 405 99.3</td>
<td></td>
</tr>
<tr>
<td>USE OF HALLUCINOGENS</td>
<td>yes 1 0.2</td>
</tr>
<tr>
<td>no 407 99.8</td>
<td></td>
</tr>
</tbody>
</table>

Significant difference (p ≥ 0.001) in levels between 5 BMI categories except for body height (Table 1 and Table 2).

Based on the results of the descriptive analysis of demographic data, a description of a typical female student of the first two years of the University of Banja Luka was obtained as a person with an average height of 168.65 ± 6.01 cm and a body mass of 63.09 ± 9.9 kg, which corresponds to a BMI value of 22.1 ± 3.2 BMI, with body fat percentage of muscle tissue of 21.93 ± 4.96% and adipose tissue of 7.1 ± 1.64%.

Table 2: Descriptive statistics of the anthropometric characteristics of the sample

<table>
<thead>
<tr>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>Min</th>
<th>Max</th>
<th>Median</th>
</tr>
</thead>
<tbody>
<tr>
<td>age</td>
<td>408</td>
<td>20.50</td>
<td>0.72</td>
<td>19.0</td>
<td>22.0</td>
</tr>
<tr>
<td>height</td>
<td>408</td>
<td>168.67</td>
<td>6.02</td>
<td>152.0</td>
<td>185.0</td>
</tr>
<tr>
<td>weight</td>
<td>408</td>
<td>63.09</td>
<td>1.00</td>
<td>42.6</td>
<td>106.1</td>
</tr>
<tr>
<td>BMI TOTAL</td>
<td>408</td>
<td>22.10</td>
<td>0.72</td>
<td>16.0</td>
<td>21.60</td>
</tr>
<tr>
<td>BMI</td>
<td>408</td>
<td>21.71</td>
<td>0.56</td>
<td>16.0</td>
<td>18.4</td>
</tr>
<tr>
<td>weight</td>
<td>408</td>
<td>63.05</td>
<td>1.46</td>
<td>41.7</td>
<td>25.0</td>
</tr>
<tr>
<td>muscle</td>
<td>408</td>
<td>28.15</td>
<td>2.81</td>
<td>21.0</td>
<td>46.8</td>
</tr>
<tr>
<td>CATEGORY</td>
<td>BMI</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. BMI (&lt;18.5)</td>
<td>height</td>
<td>33</td>
<td>169.49</td>
<td>5.82</td>
<td>156.5</td>
</tr>
<tr>
<td>weight</td>
<td>33</td>
<td>51.23</td>
<td>4.55</td>
<td>42.6</td>
<td>65.5</td>
</tr>
<tr>
<td>Malnutrition</td>
<td>yes</td>
<td>33</td>
<td>169.49</td>
<td>5.82</td>
<td>156.5</td>
</tr>
<tr>
<td>no</td>
<td>33</td>
<td>51.23</td>
<td>4.55</td>
<td>42.6</td>
<td>65.5</td>
</tr>
<tr>
<td>2. BMI (18.5-24.9)</td>
<td>height</td>
<td>311</td>
<td>168.75</td>
<td>5.88</td>
<td>152.0</td>
</tr>
<tr>
<td>weight</td>
<td>311</td>
<td>61.12</td>
<td>6.45</td>
<td>45.5</td>
<td>84.1</td>
</tr>
<tr>
<td>Ideal mass</td>
<td>yes</td>
<td>311</td>
<td>21.40</td>
<td>5.88</td>
<td>5.88</td>
</tr>
<tr>
<td>no</td>
<td>311</td>
<td>21.40</td>
<td>5.88</td>
<td>5.88</td>
<td>32.40</td>
</tr>
<tr>
<td>3. BMI (25-29.9)</td>
<td>height</td>
<td>53</td>
<td>168.49</td>
<td>5.46</td>
<td>152.0</td>
</tr>
<tr>
<td>weight</td>
<td>53</td>
<td>76.42</td>
<td>7.25</td>
<td>65.0</td>
<td>92.3</td>
</tr>
<tr>
<td>Overweight</td>
<td>yes</td>
<td>53</td>
<td>168.49</td>
<td>5.46</td>
<td>152.0</td>
</tr>
<tr>
<td>no</td>
<td>53</td>
<td>76.42</td>
<td>7.25</td>
<td>65.0</td>
<td>92.3</td>
</tr>
<tr>
<td>4. BMI (30-34.9)</td>
<td>height</td>
<td>8</td>
<td>162.62</td>
<td>11.35</td>
<td>152.0</td>
</tr>
<tr>
<td>weight</td>
<td>8</td>
<td>86.40</td>
<td>3.18</td>
<td>78.0</td>
<td>93.3</td>
</tr>
<tr>
<td>Mild obesity</td>
<td>yes</td>
<td>8</td>
<td>162.62</td>
<td>11.35</td>
<td>152.0</td>
</tr>
<tr>
<td>no</td>
<td>8</td>
<td>86.40</td>
<td>3.18</td>
<td>78.0</td>
<td>93.3</td>
</tr>
<tr>
<td>5. BMI (35-39.9)</td>
<td>height</td>
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<td>187.66</td>
<td>5.69</td>
<td>163.0</td>
</tr>
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<td>95.6</td>
<td>110.1</td>
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<td>5.69</td>
<td>163.0</td>
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<td>3</td>
<td>100.33</td>
<td>4.73</td>
<td>95.6</td>
<td>110.1</td>
</tr>
</tbody>
</table>

B. Results of the anthropological analysis of the sample

The Kruskal-Wallis test confirmed that for all measured parameters there was a statistically significant difference (p ≥ 0.001) in levels between 5 BMI categories except for body height (Table 1 and Table 2).

C. Results of Sample Analysis by SF36 Scale

The Health Survey Questionnaire (SF-36) was used to assess the health status (Table 3 and Table 4). Descriptive analysis was calculated for eight parameters: physical function, role function physical, bodily pain, general health, vitality, social function, role function emotional, and mental health.
health scales (physical functioning), the role of physical restraint, physical pain, general health, vitality, social functioning, the role of emotional restriction and mental health). The reliability of the SF-36 scale was analysed with an alpha Cronbach coefficient whose values ranged from 0.81 to 0.69, which indicated a very good reliability and internal agreement for this sample, with the exception of items of social functioning and general health whose alpha Cronbach values were 0.65 and 0.69, respectively. Descriptive analysis of the SF-36 test (Table 3), which assesses 8 domains of health, shows that the lowest score has the Fatigue variable (mean = 50.10; standard deviation - SD = 17.83), which refers to the feeling of fatigue compared to the previous year and indicates that students are often not "full of energy and life." The highest score is perceived pain (mean = 77.57, SD = 20.89) and physical limitation (mean = 77.02, SD = 33.21).

Table 3: BMI analysis (5 BMI categories) Kruskal-Wallis test

| Height Weight BMI FAT Muscule Viscfat CHI-SQUARE DF ASYM SIG. |
|-----------------|-----------------|-----|-----|--------|-------------|---|-----------------|---|-----------------|---|-----------------|---|-----------------|
| 3.440           | 4               | 4   | 4   | 0.487  | 0.000      | 0.000        | 0.000 | 0.000          | 0.000 |
| 181.580         | 225.672         | 194.757 | 125.896 | 206.465 | 225.672    | 0.000      | 0.000        | 0.000 | 0.000          | 0.000 |
| 194.757         | 225.672         | 194.757 | 125.896 | 206.465 | 225.672    | 0.000      | 0.000        | 0.000 | 0.000          | 0.000 |
| 125.896         | 206.465         | 125.896 | 206.465 | 0.000   | 0.000      | 0.000        | 0.000        | 0.000 | 0.000          | 0.000 |

Table 4: Descriptive analysis of sample with SF-36 scale

<table>
<thead>
<tr>
<th>CRONBACH A</th>
<th>Mean SD</th>
<th>Min Max Median</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHYSICAL FUNCTIONING</td>
<td>0.948 73.90 32.50 0.00 100.00 90.00</td>
<td></td>
</tr>
<tr>
<td>ROLE FUNCTIONING/ PHYSICAL</td>
<td>0.813 77.02 33.21 0.00 100.00 100.00</td>
<td></td>
</tr>
<tr>
<td>ROLE FUNCTIONING/ EMOTIONAL</td>
<td>0.848 60.38 42.65 0.00 100.00 66.67</td>
<td></td>
</tr>
<tr>
<td>ENERGY/ FATIGUE</td>
<td>0.805 50.10 17.83 0.00 100.00 55.00</td>
<td></td>
</tr>
<tr>
<td>EMOTIONAL WELL-BEING</td>
<td>0.868 60.84 18.05 4.00 100.00 64.00</td>
<td></td>
</tr>
<tr>
<td>SOCIAL FUNCTIONING</td>
<td>0.655 71.48 21.29 0.00 100.00 75.00</td>
<td></td>
</tr>
<tr>
<td>PAIN</td>
<td>0.832 77.57 20.89 0.00 100.00 77.50</td>
<td></td>
</tr>
<tr>
<td>GENERAL HEALTH</td>
<td>0.695 66.62 16.97 10.00 100.00 70.00</td>
<td></td>
</tr>
</tbody>
</table>

Table 5: Spearman correlation of SF-36 questionnaire scores (N = 408)

<table>
<thead>
<tr>
<th>SCORE SF-36</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHYSICAL FUNCTIONING</td>
<td>0.322 0.138 0.047 0.068 0.109 0.068 0.177</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ROLE FUNCTIONING/ PHYSICAL</td>
<td>0.364 0.230 0.258 0.338 0.313 0.312</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ROLE FUNCTIONING/ EMOTIONAL</td>
<td>0.444 0.455 0.461 0.127 0.214</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ENERGY/ FATIGUE</td>
<td>0.742 0.469 0.245 0.366</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EMOTIONAL WELL-BEING</td>
<td>0.595 0.223 0.365</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SOCIAL FUNCTIONING</td>
<td>0.272 0.334</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAIN</td>
<td>0.395</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GENERAL HEALTH</td>
<td>0.395</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 The table shows the values of the correlation coefficient (ρ): ** Significance of correlation at 0.01 level
   * Correlation significance at 0.05 level

The relationship between individual scores within the SF-36 questionnaire was investigated by using Spearman’s rho non-parametric correlation. From Table 4 it can concluded that high statistical significance (p = 0.001) with positive correlation among the variables were found for vital energy (fatigue) and mental health (emotions), with coefficient of determination $r^2 = 0.55$ (55%) and mental health and social functioning with $r^2 = 0.36$ (36%). By using the Spearman non-parametric test (Table 4), the values of the correlation coefficient and the significance of the correlation were shown and the strength of the association between the subscales of the SF-36 questionnaire was determined. As expected, most of the correla-

by using Spearman’s rho non-parametric correlation. From Table 4 it can concluded that high statistical significance (p = 0.001) with positive correla-

tions showed a statistical significance of different levels, in a positive direction, given the fact that all subtrees belong to the same type of test, intended to reveal different aspects of the physical and mental health of the subjects. According to the value of the correlation coefficient, the essential clinical significance was shown primarily by the positive correlations of Vital energy (SF-36 fatigue) and Mental health (SF-36 emotions) ($\rho = 0.742; p < 0.01$) as well as General mental health (SF-36 emotions) and Social functioning (SF-36 soc. funct.) ($\rho = 0.595; p < 0.01$), which are also mental health categories. The absence of significant correlations was reported for Physical functionality paired with Near vital energy, Mental health and Pain. In the realm of physical health, the associated sub-axes: physical functioning, physical limitation, physical pain, and general health perception are low positive correlations with high statistical significance for p<0.05, ex-
Discussion

This research was aimed to demonstrate how and to what extent the initial years of studying at the university affect the health of female students through the aspect of self-assessment of physical and mental health as a significant moment in the life of a young woman. By going to college, students face the challenge of changing their previous habits in order to cope with the academic workload and stress that their schooling carries. This often implies lack of time for rest and sleep, physical and recreational activities, but also changed health habits leading to malnutrition, excessive alcohol consumption, smoking and consumption of psychoactive substances, which is confirmed in the current study. Psychostimulants were used by 0.2 % of female students, hallucinogens by 0.2 %, sedatives by 0.7 %; while 35 % of students drank alcohol periodically, tobacco was consumed by 15.9 % regularly, while 7.1 % used marijuana. In the Netherlands, the prevalence of smokers has been shown to be relatively low, only 6 %, but with 46 % of students consuming alcohol and over 6 % of students using stimulants or sleeping remedies in the last 30 days. Among medical students in Germany there were 21 % smokers, with 34 % of students confirming the use of psychoactive substances. Research in Poland has shown that sixth-year medical students have a very cursory knowledge of the harmful health effects of alcohol consumption and smoking, with 26 % of female students being smokers. A study conducted among students in Toulouse, France, also confirmed that approximately the same percentage of smokers among students in this European country was around 23 %. More than half of students use some of psychoactive substances. A study in Istanbul among students of the Faculty of Physical Activity and Sports showed that 25 % were smokers and that at the age of 18 most girls started smoking.

The results of the descriptive analysis of demographic parameters provided data on the anthropological characteristics of female students of the first two years of the University of Banja Luka. These are young persons, 20.5 ± 0.7 years old, averaged 168.65 cm ± 6.01 in height, 63.09 ± 9.9 kg in body weight, corresponding to BMI 22.1 ± 3.2 kg/m² and thus characterised as persons with an ideal BMI, which is a common result in studies by authors from neighbouring countries. The University of Banja Luka students are higher than their female counterparts in Canada, Greece, and Croatia, while female students from Brazil are higher than the students from the University of Banja Luka. According to the measurements performed in this study, the proportion of adipose tissue was 31.80 ± 6.47 %, ranging from 17.2 % to 51 % of the total body mass, while the mean values of muscle mass were 28.15 ± 2.81 %, ranging from 21 % to 36.80 % of the total body mass. Similar research was carried out by Mašina (2019), where the average value of adipose tissue in the total sample of 596 girls of the same age was 20.36 ± 6.66 % (range 12.8-54.1 %). At the same time, the value of muscle mass was 28.80 ± 3.35 % (range 13 % to 42 %). In the present study, a detailed sample analysis indicated that there was a slight difference in the average value of body height compared to belonging to the BMI categories and that female students in the first BMI category had the highest body height. With the increase of BMI, as expected, the proportion of muscle mass was constantly decreasing and the values of all other parameters (body mass, presence of subcutaneous and visceral adipose tissue) were continuously increasing.

Self-assessment of health is the individual perception and evaluation of one's own health through standardised questionnaires. The SF-36 Health Survey Questionnaire was used to assess health status. Physical Health (PH) assessment covers the four scales of the SF-36 questionnaire, namely: physical functioning, physical limitations, physical pain, and general health. The calculated PH value (mean ± SD) was 72.21 ± 25.89. A high score that is appropriate for the student population implies a person's condition without restriction in performing daily activities and a person on this scale described their health as excellent. Assessment of Mental Health (MH) includes the remaining four scales of the SF-36 questionnaire: vitality, social functioning, the role of emotional restriction and mental health. This summary measure of MH had slightly lower value of 60.7 ± 24.95. This relatively lower
score on this MH scale indicates the presence of psychological pain and significant limitations in social functioning, resulting in stress and a degree of psychological distress. A high score on the MH scale is more appropriate for the student population as it relates to a positive emotional state and complete social activities. Achieving a high score on this scale is associated with frequent feelings of happiness and life satisfaction and a person perceives their health as excellent. The "vitality" subscale, which is one of the sub-categories of SF-36, was the lowest in all subsamples, which may be a sign of population fatigue. Normative data for the SF-36 questionnaire are available in many countries. Compared to the SF-36 values of students from other countries, students who participated in this study had better SF-36 scores than students at a Turkish university (FH 66.54 ± 25.39; MH 58.81 ± 27.46). SF-36 scores of the female students that participated in this study were lower than those of the Belgrade University students (FH 78.4 ± 14.0; MH 68.6 ± 19.1), a university in Italy (FH 83.68 ± 24.85; MH 67.88 ± 25.89), and a university in Croatia (FH 72.54 ± 8.24; MH 68.71 ± 10.97).

Conclusion

The results of the study of anthropological characteristics of the students showed the ideal values of the total BMI. A lower percentage of muscle mass indicates a possible lack of physical fitness. The results of the self-assessment of health show that this population of young women has higher values of physical health compared to mental health, which may be a reflection of stress and a degree of psychological distress during studies.

Acknowledgements

None.

Conflict of interest

None.

References

10. Thompson K, Wood D, MacNevin PD. Sex differences in the impact of secondhand harm from alcohol on student mental health and university sense of belonging. Addict Behav 2019;89;57-64.
15. Čuljić Seršić D, Vuletić G. Psychometric evaluation and establishing norms of Croatian SF-36 health sur-


Frequency of Common Complications During Treatment of Patients with Benign Prostate Hyperplasia

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Department of Surgery, University Clinical Centre of the Republic of Srpska, Banja Luka, the Republic of Srpska, Bosnia and Herzegovina.
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Abstract

Background/Aim: Benign prostatic hyperplasia (BPH) is a very common disease in older men. BPH involves the presence of signs of hyperplasia of the stromal and epithelial elements in the prostate with consequent enlargement of its volume. The aim of this study is to analyse the frequency of typical complications in the treatment of patients with benign prostatic hyperplasia and the effect of medicamentous treatment.

Method: Patients diagnosed BPH were included in the prospective, one-year study. They were divided into 2 groups. The first group (30 patients) consisted of those ones, whose prostate volume was equal to or over 50 cm³, while and the second group (30 patients) comprised the subjects with prostate volume less than 50 cm³. The complications of BPH analysed are residual urine (RU), symptomatic urinary infection, haematuria, thickening of the detrusor wall, diverticulum in the bladder, ureterohydronephrosis, renal failure, bladder stone and acute urinary retention (AUR).

Results: The majority of patients in both groups were aged 60-69. There was a statistically significant difference in the average value of RU between the groups at the first, second and fifth check-ups (p < 0.05), as well as the complication of symptomatic urinary infection, since the same occurred earlier in the first group than in the second group (p < 0.05). There was no statistically significant difference in complications: haematuria, detrusor wall thickening and diverticulum (p > 0.05). Complications: ureterohydronephrosis, renal failure, bladder stone, and AUR were not diagnosed in patients in either group.

Conclusion: Relevant medical therapy leads to a reduction of RU and reduces the risk of complications caused by its presence. Other complications of BPH were rare or absent in both groups, suggesting that appropriate and timely applied medical therapy affects the course of BPH and reduces the risk of complications and the need for surgical treatment. Benefit from medicamentous therapy is equally represented in both analysed groups of patients.

Key words: prostate; benign hyperplasia; prostatic obstruction; complications.

Introduction

Benign prostatic hyperplasia (BPH) is a histological term for benign prostatic enlargement and it is usually based on the gland size. The exact aetiology of BPH is unknown. It is common in older men, and it is characterised by direct obstruction of the bladder outlet (the static com-
ponent) as well as increased muscle tone within the gland (the dynamic component). There are several synonyms and descriptions of several forms for the same one. These are: benign prostatic enlargement (BPE), benign prostatic obstruction (BPO) or benign outlet obstruction (BOO). It is also described through lower urinary tract symptoms (LUTS), which is a term that refers to a group of symptoms that originate from the lower urinary tract.

Prostate enlargement is a key clinical sign of the BPH and it is often accompanied by symptoms labelled as LUTS, and which significantly affect the quality of life of these patients. However, it is known that prostate size itself does not correlate well with the intensity of the BPH symptoms. Isolated prostate enlargement and the consequent compression of the posterior urethra are not the only reasons for the appearance of clinical symptoms. Detrusor dysfunction caused by various age-related changes plays an important role. At the same time, the obstruction itself (in this case caused by enlargement of the prostate) causes neural changes on the detrusor that further contribute to the formation and development of symptoms of clinical (symptomatic) BPH.1-6

The aim of this study is to analyse the frequency of typical complications in the treatment of patients with benign prostatic hyperplasia and the effect of medicamentous treatment in groups patients with two different stages of BPH.

Methods

Patients who were diagnosed with BPH were included in the prospective, one-year study. Participants were divided into 2 groups. The first group (30 patients) consisted of patients whose prostate volume was equal to or over 50 cm³ and the second group (30 patients) consisted of subjects with prostate volume less than 50 cm³.

BPH was diagnosed based on patient history, digital rectal examination, prostate specific antigen (PSA) and ultrasound (US) examination of the urinary tract. The following laboratory parameters were monitored simultaneously: urea, creatinine, C reactive protein (CRP), PSA, erythrocyte sedimentation (ES) and blood count (BC) and urine sediment and urine culture (UC) with antibiogram. The complications of BHP analysed were residual urine (RU), symptomatic urinary infection, haematuria, thickening of the detrusor wall, diverticulum in the bladder, uroterohydronephrosis, renal failure, bladder stone and acute urinary retention (AUR).

All participants were given standard medical therapy. Patients with prostate less than 50 cm³ were given only alpha-adrenergic blocker (tamsulosin). Patients with a prostate equal to or larger than 50 cm³ were given an alpha-adrenergic blocker (tamsulosin) and a 5-alpha reductase inhibitor (finasteride). Patients were followed for 12 months with check-ups every 3 months and determination of PSA and digital rectal examination after 6 and 12 months. They were instructed to report immediately for check-up in case of acute complications (symptomatic urinary infection, acute urinary retention, haematuria) or significant exacerbation of the problems. Statistical data processing was performed by using χ² test, analysis of variance (ANOVA), Mann-Whitney U test and Student t-test.

Results

Table 1 shows the distribution of patients by age decades.

<table>
<thead>
<tr>
<th>Decade</th>
<th>First group</th>
<th>Second group</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>From 50 to 59</td>
<td>11.1%</td>
<td>33.3%</td>
<td>44.4%</td>
</tr>
<tr>
<td>From 60 to 69</td>
<td>19.2%</td>
<td>60.0%</td>
<td>79.2%</td>
</tr>
<tr>
<td>From 70 to 79</td>
<td>61.1%</td>
<td>26.7%</td>
<td>87.8%</td>
</tr>
<tr>
<td>80 and more</td>
<td>13.3%</td>
<td>10.0%</td>
<td>23.3%</td>
</tr>
<tr>
<td>Total</td>
<td>100.0%</td>
<td>100.0%</td>
<td>100.0%</td>
</tr>
</tbody>
</table>

There was a statistically significant difference ($\chi^2 = 39.067; p < 0.05$) in the distribution of patients by age. Patients from aged 60 to 69 were the most common.
Results and statistical comparisons of differences in the frequency of typical complications in the treatment of patients with benign prostatic hyperplasia: residual urine (RU), symptomatic urinary infection, haematuria, detrusor wall thickening, and diverticulum are presented in Tables 2-5.

Table 2: Decrease in the average residual urine size in patients with benign prostate hyperplasia

<table>
<thead>
<tr>
<th>Check-up</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean value</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First</td>
<td>20.00</td>
<td>150.00</td>
<td>34.33</td>
<td>23.00</td>
</tr>
<tr>
<td>Second</td>
<td>10.00</td>
<td>150.00</td>
<td>27.00</td>
<td>24.37</td>
</tr>
<tr>
<td>Third</td>
<td>10.00</td>
<td>140.00</td>
<td>24.00</td>
<td>23.17</td>
</tr>
<tr>
<td>Fourth</td>
<td>10.00</td>
<td>130.00</td>
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<td>21.44</td>
</tr>
<tr>
<td>Fifth</td>
<td>10.00</td>
<td>120.00</td>
<td>21.00</td>
<td>19.63</td>
</tr>
<tr>
<td>Residual urine – patients of the second group</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>First</td>
<td>20.00</td>
<td>150.00</td>
<td>34.33</td>
<td>23.00</td>
</tr>
<tr>
<td>Second</td>
<td>10.00</td>
<td>150.00</td>
<td>27.00</td>
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</tr>
<tr>
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<td>140.00</td>
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</tr>
<tr>
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<td>130.00</td>
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<td>21.44</td>
</tr>
<tr>
<td>Fifth</td>
<td>10.00</td>
<td>120.00</td>
<td>21.00</td>
<td>19.63</td>
</tr>
</tbody>
</table>

In the first group of patients, the average residual urine value was highest at the first check-up and lowest at the fifth check-up. There was no statistically significant difference between the check-ups compared to the average RU in the first group (ANOVA test: (F = 1.701; p = 0.153; p > 0.05), but there was a statistically significant difference between the check-ups in the average RU in the second group (F = 12.501; p = 0.000; p < 0.05).

Table 3: Symptomatic urinary infection in patients with benign prostate hyperplasia

<table>
<thead>
<tr>
<th>Check-up</th>
<th>First</th>
<th>Second</th>
<th>Third</th>
<th>Fourth</th>
<th>Fifth</th>
</tr>
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<td>0</td>
</tr>
<tr>
<td></td>
<td>% 0.0</td>
<td>6.7</td>
<td>0.0</td>
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<td>0.0</td>
</tr>
<tr>
<td>No</td>
<td>N 30</td>
<td>28</td>
<td>30</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>% 100.0</td>
<td>93.3</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Total</td>
<td>N 30</td>
<td>30</td>
<td>30</td>
<td>30</td>
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<tr>
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<td>% 100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Table 4: Occurrence of haematuria in patients with benign prostate hyperplasia

<table>
<thead>
<tr>
<th>Check-up</th>
<th>First</th>
<th>Second</th>
<th>Third</th>
<th>Fourth</th>
<th>Fifth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
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<td></td>
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<td>30</td>
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<td>100.0</td>
<td>96.7</td>
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<td>100.0</td>
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Table 5: Haematocrit in patients with benign prostate hyperplasia

<table>
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<tr>
<th>Haematocrit</th>
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<th>Second</th>
<th>Third</th>
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<td>0</td>
<td>0</td>
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<td></td>
<td>% 6.7</td>
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<tr>
<td></td>
<td>% 100.0</td>
<td>100.0</td>
<td>100.0</td>
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</tr>
</tbody>
</table>

Symptomatic urinary infection was diagnosed in two patients of the first group at the second check-up (in one patient after 36 days and in another after 61 days) and in two patients of the second group at the third check-up (in one patient after 140 days and in the other one after 141 days) (Table 3). There was no statistically significant difference (Mann-Whitney U test: p > 0.05) between the patients of the first and second groups in the number of symptomatic urinary infections per check-up.

An analysis of the time of diagnosing the symptomatic urinary infection showed a mean value of 48.5 days in the first group and 140.5 days in the second group, with the difference being significant (t-test; t = -7.35; 0.018; p < 0.05).

Haematuria was diagnosed in 3 patients of the first group at the first check-up and in one patient on the fourth one, after 276 days from the first check-up. In the second group of patients on first check-up haematuria was ascertained in two cases and in one patient on the fifth check-up, ie after 365 days (Table 4). There was no statistically significant difference (Mann-Whitney U test: p > 0.05) between the patients of the first
and second groups according to the number of episodes of haematuria per check-up (Table 4).

**Table 5: Changes in detrusor thickness in patients with benign prostate hyperplasia**

<table>
<thead>
<tr>
<th>First group</th>
<th>Check-up</th>
<th>First</th>
<th>Second</th>
<th>Third</th>
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<tbody>
<tr>
<td>Proper wall thickness</td>
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<td>73.3</td>
<td>73.3</td>
<td>73.3</td>
<td>73.3</td>
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<tr>
<td>Thickened wall</td>
<td>%</td>
<td>7</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Total</td>
<td>%</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
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</table>

<table>
<thead>
<tr>
<th>Second group</th>
<th>Check-up</th>
<th>First</th>
<th>Second</th>
<th>Third</th>
<th>Fourth</th>
<th>Fifth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proper wall thickness</td>
<td>%</td>
<td>90.0</td>
<td>90.0</td>
<td>86.7</td>
<td>86.7</td>
<td>86.7</td>
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<tr>
<td>Thickened wall</td>
<td>%</td>
<td>3</td>
<td>3</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>%</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Detrusor wall thickness was found in 7 patients of the first group at the first check-up and in one patient after 90 days at the second check-up. In the second group it was found in 3 patients at the first check-up and in one patient on the third check-up, after 319 days (Table 5). There was no statistically significant difference between the first and second group in the number of patients with thickened detrusor wall on check-ups 1-5, (p > 0.05).

Diverticulum was present in one patient of the first group on first check-up. In the second group there were no patients with diverticulum and there was no statistically significant difference between the patients of the first and second groups according to this parameter. (Mann-Whitney U test: U = 430.000; p = 0.317; p > 0.05). At the same time, complications such as ureterohydronephrosis, renal failure, bladder stone, and acute urinary retention (AUR) were not diagnosed in either of the patient groups.

**Discussion**

BPH is a progressive disease with a frequency that successively increases mainly after the age of 50.1-6 This study showed that patients aged 60 to 69 were predominant. Complications of BPH can occur immediately, but they can also occur after many years of the onset of the first symptoms. Residual urine (RU) is an important factor in the evaluation of pathology of the bladder neck, prostate or urethra. Adequate RU measurement can only be performed after spontaneous voiding. A full bladder causes distension of the wall and reduces contractility of the detrusor, making it difficult for the bladder to complete voiding.

Measurement of RU can be expressed in absolute values, in millilitres, based on measurement of bladder diameter or relatively, as a subjective estimate of RU relative to full bladder capacity. Accurate measurement of RU is achieved by measuring the largest transverse, longitudinal and anteroposterior diameter of the bladder and since the bladder is usually oval in shape, the volume is calculated by multiplying all 3 diameters by 0.5. A residue measured in this way is of importance if less urine is left behind. Subjective data on the reduction of bladder volume before and after voiding is more significant in case when is RUT larger.3-10

In patients from the first group, the average RU value was highest at the first check-up and the lowest at the fifth check-up. There was no statistically significant difference between the check-ups compared to the average RU in patients of the first group, but there was statistically significant difference in the average value of the RU in patients of the second group. RU was higher in the first group at all check-ups than in the second group and there was no statistically significant difference between the first and second group of patients in the rate of decrease of the average RU. This is expected since the patients from both groups were initially included an alpha-adrenergic blocker, acting by relaxing the smooth muscles of the bladder neck, urethra and prostate and it acted to reduce RU equally in patients of both groups. At the first, second and fifth check-ups there was a statistically significant difference in the average value of residual urine between the patients of the first and second groups, while at the third and fourth check-ups there was no statistically significant difference.

Urinary infection in BPH patients is a product of the presence of RU and reduced body resistance. The infection in patients with BPH and significant RU is much more serious than in patients with anatomically and functionally normal urinary tract. The bacteria causing the infection...
often come ascending from the urethra and then multiply in the bladder. Bladder is infected, significantly less frequently, by haematogenous, lymphogenous or per continuitatem route from the surrounding foci (from the prostate in chronic bacterial prostatitis). Nephrolithiasis and diverticulosis of bladder also support the persistence of the infection.3-10 The infection can be limited to the prostate or bladder or it can affect the kidney. According to the resistance of the organism and adequate antimicrobial therapy, the infection recovers relatively quickly.3-12

In this study, there was no significant difference between patients of the first and second group regarding the number of episodes of symptomatic urinary infections per check-up. There was a (statistically significant) difference between the first and second groups when it came to the time of diagnosing these complications. In the first group, symptomatic urinary infection occurs in a shorter period of time than in the second group. This may be partly explained by higher RU in patients of the first group, which is a precipitating factor for urinary infections.

Haematuria is a common complication of BPH. It can be microscopic or macroscopic. Sometimes it is so extensive, that causes bleeding. The causes are different. Spontaneous haematuria is explained by severe congestion of the hyperplastic prostate and sclerotic changes of blood vessels. The cause is sometimes the prostate infarction. In patients of both groups, no significant difference in the number of episodes of haematuria per check-up and the mean time of finding complications was found. Haematuria did not occur in large numbers because the patients regularly used prescribed medication therapy with the proposed diet, thus reducing the possibility of congestion of adenoma and occurrence of significant hematuria.4-14

Bladder wall thickening, occurrence of trabeculae and diverticulum are changes in the bladder that occur due to obstruction. In the analysis presented here, the occurrence of complications in the form of thickening of the detrusor was monitored. The same occurs earlier in the development of complications on the bladder wall than the diverticulum, so the incidence is higher. Detrusor thickening was also found to be more common in patients in the first group than in patients in the second group, at all check-ups. This finding is expected since a larger volume of the prostate indicates prolonged, more intense subvesical obstruction. Detrusor thickening was verified if the ultrasonographically measured detrusor thickness was over 5 mm.4-14

Diverticulum of the bladder is a common complication in the late stage of BPH and it is caused by loosening of the stretched wall. In one study of 300 patients, diverticulum was found in 10 (3.4 %) patients and secondary diverticulosis in 38 (12.6 %) patients.7 In patients from this study diverticulum was present in only one patient of the first group.

Renal insufficiency is often present in the late stages of BPH. It is caused by dilation of the upper urinary tract and subsequent urinary compression on the kidney parenchyma, as well as occasional kidney infections. Ureterohydronephrosis, accompanied by varying degrees of renal insufficiency, occurs in patients with large RU and decompensated bladder. Ureterohydronephrosis and renal insufficiency were not observed during this study. The patients in the present study did not have an AUR and/or RU over 300 mL, so this complication did not develop.6-17

AUR is a very common complication of BPH. It is caused by a conglomerate: sphincter spasm, detrusor hyperdistension, congestion and prostate infarction. It may develop suddenly or gradually, with the progressive enlargement of RU. Acute complete retention may occur at all stages of BPH. The causes of acute retention are complex: hyperdensity of detrusor muscle fibres, congestion and prostate infarction, sphincter spasm.6, 10-20 According to one study performed in 300 patients, 231 (77 %) had different forms of retention during BPH. Complete retention as the first sign of BPH, occurred in 52 (17.6 %) patients.7

AUR was not present in the patients of either groups. As all patients included in the study received medical therapy (alpha-adrenergic blocker or combination therapy of alpha-adrenergic blocker + 5-alpha-reductase inhibitor). Also, the relatively short follow-up time (one year) within the analysed study should be considered.

Bladder calculi is a relatively common complication of BPH. It is mainly secondary, for urinary tract and infection.19 The incidence of this complication in the group of 300 patients was 49 (16.3 %) patients.7 Bladder calculi in patients with BPH occurs due to a urinary stasis com-
bined with infection. These calculi are secondary, mainly due to infection, with a basis that lies in the urinary tract.6,10-20 The bladder calculi were not verified in the patients presented in this study. Given that there were not many patients with a large amount of post-voiding RU and with long-term urinary infection due to the inclusion of adequate antibiotic therapy, as well as a limited time of this study, it is believed that this is the reason why this complication did not occur.

Conclusion

The majority of patients in both groups were aged 60–69. Medicamentous therapy leads to a reduction of RU and reduces the risk of complications caused by its presence. Other complications of BPH were rare or absent in both groups, suggesting that appropriate and timely applied medical therapy affects the course of BPH and reduces the risk of complications and the need for surgical treatment. Benefit from medicamentous therapy is equally represented in both analysed groups of patients.

Acknowledgements

None.

Conflict of interest

None.

References

Disruptive Technologies in Cardiac Surgery and Interventional Cardiology

Jacob Bergsland 1, 2, 3

Abstract

The last several decades have witnessed a huge expansion of surgical and interventional treatment of cardiac disease. Axel Cappelen from Oslo, Norway was one of the first to operate on the heart, something that had until then, been considered foolish and without chance of success. Cappelen dared to operate on the heart in spite of Billroth’s stark warning against such "unwise" attempts as he ligated a bleeding coronary artery caused by a stabbing injury. Most of the innovation in recent years has been within the interventional cardiology domain in contrast to the first 7 decades of the 20th century, when surgeons dominated. Cardiac surgeons have developed less invasive procedures, although the most common surgical incision is based on the time-honoured and large median sternotomy incision. Many surgeons continue to prefer the concept of "Grosse Chirurgen, Grosse Schnitte" and have stayed away from minimisation and continue to use direct vision, usually augmented by magnification glasses, median sternotomy and cardiopulmonary bypass (CPB). However, new technology has made the need for CPB less important. In this article a selection of recently developed devices for cardiac intervention will be described.

Key words: mitral valve; aortic valve; valve surgery; valve intervention.

Introduction

The development of cardiac interventional care has required technological innovation and dedicated and qualified personnel. It was more than 100 years ago that the first coronary bypass procedure was performed on research animals by the Nobel Laureate Alexis Carrell, but it required the development of cardiac catheterisation to diagnose the extent and localisation of coronary lesions and the CPB-machine to operate on a motionless heart. The heart-lung machine made coronary bypass feasible, but again became unnecessary with the development of off the pump bypass surgery (OPCAB). New technology made OPCAB feasible and safe.

Interestingly, many of the common cardiac conditions affecting the heart valves had surgical solutions before CPB was developed and some of these methods have now been revived using catheter-based techniques. Mitral stenosis, one of the most common cardiac conditions before the time of antibiotics and treatment of streptococcal infections, could be effectively relieved with mitral commissurotomy performed with a left thoracotomy and access to the mitral valve through the left atrial appendix. The surgeon would use his finger or an instrument to dilate the stenosed valve, thereby relieving disabling congestive heart failure (Figure 1). In the Western World such closed procedures were mostly replaced by open commissurotomy on CPB and an arrested heart to decrease the risk of embolisation. Later, cath-
eter-based balloon-dilatation, using a transseptal catheter technique, has become a successful method for treating mitral stenosis (Figure 2). In 2003 it was reported that a third of patients with mitral stenosis were treated with balloon dilatation in Europe. Closed, operative dilatation for aortic stenosis without CPB was performed through a transapical technique and a surgical instrument was used to open up the fused aortic leaflets. This procedure was less successful than dilatation of the mitral valve as demonstrated by the experience with aortic balloon valvuloplasty.

For many years valve replacement was the most common invasive treatment for patients with disease of the valves, regardless of whether the pathology caused stenosis, insufficiency or a combination of both. Both tissue and mechanical valves performed well and selection of type depended on age, co-morbidities, surgeon and patient preferences or other factors. The pulmonary and tricuspid valves were treated surgically and to an extent with catheter-based techniques. Focus of development, science and practice in the early, mainly surgical, era was valve-replacement and a number of mechanical valves were developed, including Starr-Edwards, Björk-Shiley, Medtronic Hall and others. These mechanical devices were made of artificial material and required life-long anticoagulation. Alternative devices were made of a combination of biologic and synthetic tissues where the leaflets were biological and less thrombogenic. Anticoagulation could therefore be avoided, which is a major benefit for quality of life. The main disadvantage was the shorter durability of biological valves, making reoperation a common scenario especially for younger patients and pre-menopausal women.

While stenotic lesions of the mitral and aortic valves could be treated with dilatation, regurgitation was a more complex issue. While most surgeons elected to replace the leaking valves, it became increasingly evident that the degenerative valve pathology, especially for the mitral valve, could be repaired, preventing the adverse effects of a prosthesis. Surgical mitral valve repair became, and still is, an excellent choice. Techniques for mitral valve repair were developed by a lifelong dedication by the French surgeon Alain Carpentier and others. His ground-breaking work resulted in further understanding of the mitral valve’s function and pathology and the development of the techniques to repair the valve with excellent results and low rates of reoperation. Aortic insufficiency has been more difficult to repair and surgical therapy has mainly been the valve replacement.

Until the 1970s, cardiology interventionists were mainly involved in diagnosis of cardiac conditions, while surgery was a dominant invasive non-medical therapy. This changed after Andreas Grünzig’s disruptive approach to coronary artery disease when he started performing percutaneous balloon angioplasty. After stents were added to the procedures, it has become safer and is now performed much more commonly than the coronary artery bypass grafting. To most cardiac specialists it seemed unlikely that catheter-based therapy could be a major factor in treatment of valvular conditions, except for dilatation of the mitral and aortic valves.
The development of catheter-based alternatives to surgery for stenotic lesions in coronary arteries and aortic and mitral valves led to further pursuit of new solutions by physicians, surgeons and engineers. The med tech industrial giants and numerous upstart companies saw a huge potential in developing less invasive therapies, mainly based on catheter interventions. In other surgical specialties, like gastrointestinal surgery, minimally invasive techniques have penetrated the Western world and even the remote and poor countries, but unfortunately thoracoscopic and robotic operations for cardiac disease has not gained general acceptance, in spite of clear demonstration of clinical feasibility and excellent results. The majority of surgeons does not possess required skills or acceptance of the paradigm shifts necessary for minimal invasive approaches. However, similar to what occurred regarding valve dilatations, many new ideas for interventional therapy came from surgeons who were used to seeing cardiac pathology "in real life". When surgeons, cardiologists and engineers worked together, interventionalists with imaging- and catheter-expertise could perform intracardiac procedures with good results. In the following a selection of the new devices and methods available and under development will be described. Due to space constraints, a complete review will not be possible.

Aortic valve

Dilatation of the aortic valve to relieve stenosis was attempted in the pre-cardiopulmonary bypass using a transapical approach where a surgical dilator instrument was introduced antegrade through the aortic valve. When CPB became available, aortic valves were mostly replaced using CPB and on an arrested heart. Balloon dilatation through a retrograde approach from the femoral artery continued to be a valuable technique in some cases, but the procedure either did not relieve stenosis adequately or resulted in aortic insufficiency. Implantation of a prosthesis using a catheter-based approach was developed by Cribier and others, who described the procedure and initiated it clinically in Rouen, France. Within a few years several implantable valves were on the market, the most common were the Sapien™, Edwards Inc., USA and Corevalve™ Medtronic Inc., USA. Sapien™ is made of bovine tissue mounted on a cobolt-chromium metal frame and is balloon-expandable (Figure 3), while the Corevalve™ (presently named Evolut™) is made of porcine pericardium mounted on self-expandable nitinol (Figure 4). Extensive, controlled, clinical studies have been undertaken demonstrating benefits compared to surgery of this transcatheter approach first in high- then in intermediate- and finally in low-risk patients. In several markets transcatheter aortic valve implantation (TAVI) is now more common than surgical valve implantation. The high cost of the device and delivery catheters has been a limitation to the implementation of the technique in less prosperous markets. Several new devices are in different phases of development and will probably eventually result in a more affordable price-structure.

Mitral Valve

The mitral valve is anatomically more complex than the aortic and mitral stenosis, especially
when less calcified, can usually be successfully treated with balloon dilatation. Mitral regurgitation (MR) is more difficult to treat as it has a number of different aetiologies and variable anatomy, but development has accelerated in spite of technological challenges, strict regulation and financial challenges.

There is much variability among regurgitant mitral valves; the two main categories being degenerative (DMR) and functional (FMR) mitral regurgitation (MR).

DMR is MR caused by a primary defect in the mitral valve itself, while FMR is caused by conditions affecting the left ventricle (LV), causing dilation of the mitral annulus and subsequent (secondary) MR. DMR can in many cases be treated surgically with valve repair and if the repair is performed by an experienced team, outcomes are excellent. For FMR the prognosis is worse and mitral repair or replacement, in spite of symptomatic improvement, do not improve long-term survival unless the LV function recovers.

For both DMR and FMR interventional therapies are being developed to decrease invasiveness and make treatment available for larger groups of patients. A few of the devices and methods in various phases of development are presented here.

a) Mitraclip™, Abbott Inc., USA (Figure 5) was designed to reproduce a surgical procedure invented by Alfieri. With the Alfieri method, the anterior and posterior leaflets of the mitral valve are sutured together, creating a double outlet valve where the two leaflets support each other. Mitraclip can produce the same anatomical result as the Alfieri procedure by fixing the two leaflets together with a patent-deployed catheter approach.

The catheter is introduced through the femoral vein and trans-septally into the left atrium. An animation can be seen on YouTube (link: https://www.youtube.com/watch?v=4-yTPoEaZzg). Mitraclip can be used in DMR by fixing a prolapsing or flail leaflet to the opposite leaflet, thereby eliminating or decreasing MR. The device can also be used in FMR since it improves coaptation of the leaflets, thereby reducing MR. This effect on patients with FMR has been demonstrated in a well conducted randomised study in the US (COAPT-study), which demonstrated lower mortality, less heart failure symptoms and improved LV function compared to the optimal medical treatment.

b) Implantation of annuloplasty ring. Cardio-band™, Edwards Lifesciences, USA, is also used through a transseptal approach. The annuloplasty ring is sutured in place in the mitral valve annulus, using a sophisticated anchoring system. The procedure is explained by this animation (https://www.youtube.com/watch?v=VIAOs31fsc)

This ring is inspired from the surgical annuloplasty procedure and intended to reduce the size of the mitral annulus thereby improving coaptation of the leaflets.

c) Implantation of new mitral chords. Several companies are working on devices that can repair the mitral valve by the placement of artificial chordae tendinae. Such procedures are based on concepts from surgical repair. MR caused by ruptured or degenerated mitral chords can be repaired by placing artificial chordae tendinae.
chords between the prolapsing leaflet and the papillary muscles, thereby increasing coaptation of the anterior and posterior leaflets.

Different concepts for placing such “neo-chords” include transapical approaches, where a small thoracotomy is used to place chords between the prolapsing leaflet and the wall of the LV. The length of the chords can be adjusted under ultrasonic imaging until MR is eliminated. DS1000™, NeoChord Inc., USA (Figure 6A) and Harpoon™ Edwards Lifesciences Inc, USA (Figure 6B) are devices under clinical investigations for trans-apical chordal implantation. DS1000™ is approved in Europe and several reports have been published demonstrating good results.31, 32 Transfemoral chordal implantation has been developed by Cardiomech AS, Trondheim, Norway, Cardiomech MVRSTM (Figure 7). The device is still in preclinical trials and not available for clinical use. Several other companies are in the process of developing transfemoral devices.

d) Surgical mitral valve replacement gives good long-term result for patients with MR, but can contribute to the obstruction of the aortic outlet, but this can usually be prevented by careful planning.34 Several transapical valves have been designed and implemented including Tendyne™, Abbott Inc., USA (Figure 8)35 which has recently received the CE mark after successful human studies.36 Tiara™, Neovasc Inc., Canada37 and Intrepid™ Medtronic, USA48 are transcatheter mitral prosthesis in clinical trials.

Pulmonary Valve
Balloon dilatation has been used for many years for treatment of congenital pulmonary steno-

Tricuspid valve
Tricuspid valve disease frequently occurs secondarily to other valvular disease, which can cause right ventricular (RV) dilation and tricuspid regurgitation (TR). Surgical repair of such TR at the same time as the primary valve operation improves long- and short-term outcomes.41 For patients who have high grade primary TR or TR after a previous valve operation, reoperation may be necessary. Alternatively, a transcatheter repair may in the foreseeable future be performed through a femoral or jugular venous approach.42 Patients with combined disease of mitral and tricuspid valves live shorter than those with only mitral dysfunction. Several devices, some adapted from mitral applications can be used in the tricuspid area.43
Discordance

Cardiovascular disease remains the most common cause of death and disability worldwide. Surgical and interventional therapy save lives and improves quality of life for several hundred thousand patients every year. Classic open surgical operations were for decades dominating such therapy with coronary bypass and valve replacements being the most common procedures. The development of catheter-based therapy for coronary stenosis has led to a paradigm shift. CABG has become relatively less common while the use of acute and elective percutaneous coronary interventions has increased dramatically. The biggest benefit is seen in patients with acute myocardial infarction.

Interventional therapy for valvular disease is now increasing rapidly. Development of new devices are complex and costly and requires long time for regulatory approval. Use of TAVI has increased rapidly after regulatory approval of Sapien™ in 2012. A number of other TAVI valves are now on the market or under development. The development of transcatheter devices for mitral valve repair has been slower, but the Mitraclip™ and NeoChord DS 1000 are already on the market and are expected to be followed by a number of other devices. Similarly, start up and strategic medical device companies are actively pursuing new and innovative solutions.

Conclusion

In conclusion, the trend towards image-guided procedures performed with minimally invasive surgical and interventional therapies is rapidly changing the way patients with cardiovascular disease are treated. The increasing number of devices and competition in the strategic device industry will eventually bring down the costs. The availability of less invasive procedures will make effective therapies available for new patient groups, some of them not eligible for higher risk surgical operations. At the same time, shorter hospital length of stay and lower rates of adverse events will decrease cost to the individual patients and for the society at large.

Conflict of interest

None.

Acknowledgements

The author is one of the founders and the medical director of Cardiomech, AS, Trondheim, Norway.

References


25. Bergsland J, Elle OJ, Fosse E. Barriers to medical de...
Abstract

Kaposiform haemangioendothelioma (KHE) is a rare, locally invasive vascular tumour that is commonly associated with the Kasabach-Merritt phenomenon (KMP). A case of a five-month-old female infant admitted for dyspnoea, stridor, and skin haematoma is presented. Computerised tomography of the chest showed a tumour mass occupying mediastinum and most of the left hemithorax, while laboratory analysis revealed a thrombocytopenia and a consumption coagulopathy. Histology of tumour biopsy was characteristic of KHE with a component of tufted angioma. Corticosteroid treatment initially induced a reduction in tumour size, but progression occurred four weeks later and led to a fatal outcome despite additional chemotherapy. After a literature search, we found only 18 cases of mediastinal KHE published so far, with 21% fatality rate. In the present case several risk factors for adverse outcome were present: onset of disease in early infancy, a large volume of the tumour, mediastinal location, KMP, and partial response to available therapy.

Key words: kaposiform haemangioendothelioma; Kasabach-Merritt phenomenon; mediastinum; infant.

Introduction

Childhood tumours represent just over 1% of all human tumours. They are different from tumours in adolescents and adults regarding histological types, biology, incidence, clinical features, prognosis, and response to treatment. In the era of multimodal anticancer therapy, the majority of malignant tumours in childhood have a favourable prognosis. In contrast, benign tumours at that age may cause death due to their anatomic location and size.1, 2 In a series of more than 900 benign and malignant soft tissue tumours detected in the first two decades of life, 30% were of vascular origin.3 Estimates of the overall prevalence of vascular anomalies range from 6% to 25%.4 The most frequent location is the skin, followed by mucous membranes, deep connective tissue, and internal organs.1 According to the 2014 update of the International Society for the Study of Vascular Anomalies (ISSVA) Classification, vascular anomalies are divided into two main groups, namely vascular malformations and vascular tumours, which are further classified into benign, locally aggressive (borderline), and malignant.5
Here, a case of kaposiform haemangioendothelioma (KHE), a sporadic, locally aggressive, vascular tumour in a very rare extracutaneous, mediastinal location in a female infant is presented. After adding our case, which is, to the best of our knowledge, the 19th published case of KHE in that location, the clinical-pathological aspects of mediastinal KHE were analysed, with the addition of a brief literature review of the cases published thus far.

Case history

A five-month-old girl presented with stridor and dyspnoea. During a course of treatment for bronchiolitis (including systemic corticosteroids), she developed numerous petechiae and haematomas in the skin. Blood analysis revealed severe thrombocytopaenia and she was referred to our tertiary care paediatric hospital in early 2005. Computerised tomography (CT) of the chest showed a soft tissue tumour mass occupying the mediastinum and most of the left hemithorax, measuring more than 20 cm in its largest diameter (Figure 1A). The tumour extended from the base of the neck to the diaphragm, compressing the mediastinal structures (trachea, oesophagus and aorta) as well as the left main bronchus, causing atelectasis of the left lung which was confirmed by fibre-optic bronchoscopy. Laboratory analysis verified a severe consumption coagulopathy with thrombocytopaenia (14 x 10^9/L), hypofibrinogenaemia (0.42 g/L), and elevated D-dimer levels (11.70 mg/L) which was consistent with Kasabach-Merritt phenomenon (KMP). Severe anaemia was also present (haemoglobin 76 g/L) with schistocytes detected in the peripheral blood smear. After the initial stabilisation of the gas exchange using the...
mechanical ventilation, administration of blood products and anticoagulants and antimicrobial therapy, an open surgical biopsy was performed.

Histopathology findings
One piece of tissue measuring 0.9 x 0.8 x 0.7 cm was analysed. Histologically, the tumour growth was in the form of partly coalescing nodules infiltrating the fat and fibrous tissue and surrounded by blood-filled vessels (Figure 1B). Some tumour nodules bulged into the outer crescent-shaped thin-walled vascular spaces, which is a pattern corresponding to the tufted angioma (TA) structures. These tumour components were associated with malformed, thin-walled, lymphatic vessels corresponding to the microcystic lymphatic malformation (Figure 1C). The tumour nodules were densely cellular with plump, spindled cells, arranged in haphazardly interlaced, sometimes tightly coiled, short fascicles interspersed with tiny capillaries and lining slit-like vascular channels filled with erythrocytes (Figure 1D). Immunohistochemical stains showed a diffuse CD31, CD34, and FLI-1 immunopositivity of tumour cells in the well-canalised and spindled areas (Figure 2A). These cells were GLUT-1 and HHV-8 immunonegative, but they expressed podoplanin (D2-40), mainly at the periphery of the nodules (Figure 2B). Tumour cells did not show significant atypia, the mitotic index was low, and around 5% of tumour cells showed proliferative activity marked by Ki-67 immunopositivity. The histology of the tumour was entirely consistent with KHE with the TA component.

Therapy and course of the disease
Severe postoperative bleeding was stopped with intravenous administration of the activated recombinant factor VII (NovoSeven®) over three days. After establishing the diagnosis of KHE, intravenous corticosteroid therapy was instituted (initial pulse dose of 100 mg methylprednisolone, followed by 5 mg/kg/day). During the first two weeks of treatment, a significant reduction of tumour size was verified, along with reduced airway compression, re-expansion of the left lung, and improvement in respiratory function. The coagulopathy also subsided; platelet count rose to $309 \times 10^9/L$, and D-dimer levels decreased to normal. The dose of the corticosteroid was then reduced to 2.5 mg/kg/day. Two weeks later, clinical and radiological signs of tumour progression with the re-appearance of the coagulopathy were observed. Subsequently, chemotherapy with vincristine (single dose of 1.5 mg/m²) and three consecutive daily doses of cyclophosphamide (15 mg/kg) was initiated. Unfortunately, there was no response to this treatment and the infant’s respiratory function deteriorated, leading to the reinstitution of mechanical ventilation. A fatal outcome occurred due to sepsis and respiratory failure within two months after the diagnosis was established. Parental consent for autopsy was not obtained.

Discussion
KHE is a rare, locally invasive, vascular tumour, initially described by Zukerberg et al in 1993 as
an infant skin lesions with ill-defined borders.\(^6\) It is considered as a tumour of intermediate malignancy due to the absence of firm evidence of its metastatic potential.\(^7\)-\(^9\) Unlike considerably more common infantile haemangioma, KHE shows no tendency toward spontaneous regression nor immunohistochemical expression of GLUT-1.\(^5\),\(^7\)

Precise histopathological diagnosis of vascular anomalies and their categorisation according to the current ISSVA classification play a crucial role in the clinical management of patients since different types of malformations and tumours show great variability in response to advanced therapeutic modalities.\(^10\),\(^11\) Several reports suggested the existence of close clinical, histological and developmental relationship between KHE and TA,\(^7\),\(^12\),\(^13\) which was also seen in current patient’s tumour. Presently, these two entities are regarded as a continuum of the same vascular tumour (KHE/TA),\(^5\),\(^14\) sharing also the propensity for KMP and identical immunophenotype with an expression of podoplanin and PROX-1.\(^5\),\(^7\)

Vascular tumours with a predominantly spindle-cell component represent a diagnostic challenge for a histopathologist because of the morphologic overlap between different entities with varying malignant potential.\(^15\) In addition to the striking differences in clinical presentation, each of these tumours is different from KHE in the following histologic details: (1) spindle cell haemangioma has a biphasic composition with cavernous vascular spaces and more solid spindle-cell areas containing cytoplasmic vacuoles; (2) in nodular phase of Kaposi sarcoma there is no lobular arrangement of the spindle cells, which are also invariably HHV-8 immunopositive; (3) spindle cell variant of angiosarcoma is characterised by an infiltrative growth and multilayering of more atypical endothelial cells at the periphery of the lesion.\(^7\),\(^8\),\(^15\) A recently defined entity, kaposiform lymphangiomatosis (KLA), shares overlapping patterns of clinical symptoms (including KMP), anatomical location, imaging features and complications with KHE. KLA has features of both tumours and malformations of the lymphatic vessels, grows diffusely or multifocally, often in the mediastinum and lungs, and its histological hallmark is the presence of poorly marginated clusters and sheets of kaposiform spindled cells oriented in parallel fashion amidst abnormal, dilated lymphatic channels. With diffuse lymphatic markers immunoreactivity, such histological picture is still different from KHE.\(^16\)

Just over two-thirds of patients with KHE/TA develop KMP, which is defined as an accumulation of platelets and coagulation factors within lesions followed by thrombocytopenia and consumption coagulopathy with a consequent life-threatening haemorrhage and a mortality rate estimated at 10 - 30% of infants.\(^6\),\(^9\),\(^13\),\(^14\),\(^17\)-\(^20\) KMP is more common in patients with KHE located in deep tissues extending into multiple anatomic regions (78% of patients) than in patient with superficial lesions (36%).\(^20\) The identified risk factors for KMP also include larger tumour size, multifocality and depth of tumour infiltration, as well as an intrathoracic, intra- and retroperitoneal location.\(^12\),\(^14\),\(^17\)-\(^21\) There is also a higher incidence of KMP in females and younger patients,\(^9\),\(^12\),\(^14\),\(^18\),\(^20\) (79% of infants; 10% of adolescents).\(^14\) KMP is associated with more aggressive KHE and worse disease outcomes,\(^21\) which may be also related to more frequent compression of vital structures.\(^18\)

The strategies used so far in the treatment of KHE can be divided into three groups: (1) resection/interventional procedures, including vascular embolisation or ligation, (2) irradiation, (3) antiangiogenic drugs, chemotherapy agents and anticoagulation substances. The best option for KHE treatment is a complete surgical resection, if it is achievable.\(^9\),\(^12\),\(^14\) According to the literature, the most frequently used medical treatments were, in descending order: steroids, vincristine, interferon alpha, platelet aggregation inhibitors, sirolimus and propranolol.\(^9\),\(^14\) In the case of the patient presented in this paper, the enormous size of the tumour and its relation to vital mediastinal structures, association with severe coagulation disorder and only partial responsiveness to conservative treatment made radical surgery impossible at the time. In the absence of definitive guidelines for the pharmacological treatment of KHE, the most common combination of applied medical therapy was steroids/vincristine,\(^9\),\(^14\) which was also tried in this particular patient, but with the addition of cyclophosphamide. A good therapeutic response could be achieved in 30 - 43% of patients.\(^14\) At the time of this patient’s treatment, there were no reports on the usefulness of the inhibitor of mammalian target of rapamycin (mTOR) sirolimus in the treatment of vascular lesions. Since many studies have demonstrated a very effective response to sirolimus treatment, it is now considered the first-line therapy for KHE and KMP.\(^14\),\(^19\),\(^22\)

So far, two of the most comprehensive KHE studies have included 107 patients from a US centre
KHE is most commonly located in the dermal and subcutaneous tissue of extremities, trunk and neck, face and head region.\(^9, 14, 18, 20\) According to the most significant series of data, KHE extends to more than one region in 7 - 26 % of cases. KHE is noted in the extracutaneous site in 11 - 17 % of patients\(^14, 18, 20\) and 3 - 10 % are found in the intrathoracic location.\(^18, 20\)

Regarding the incidence of mediastinal lesions in childhood, mesenchymal tumours rank second (18.2 % of 137 lesions), after neurogenic (34.3 %), and followed by lymphoid neoplasms (16.8 %).\(^23\) Only 2.2 % of mediastinal lesions are diagnosed as haemangiomas,\(^23\) although in some analyses even lower incidence was reported.\(^24\)

After a systematic literature search in the Medline database (http://www.ncbi.nlm.nih.gov/pubmed/), it was found that only 18 cases describing patients with mediastinal KHE location had been published so far. In 2014, Wallenstein et al\(^25\) summarized 12 previously reported cases of mediastinal KHE and added one case of their own. Following this review, to date, only five new cases of mediastinal KHE were published in the English.\(^9, 17, 26\) In one of these cases, the exact age of patient remained unknown,\(^9\) while in two cases gender of patients was missing.\(^5, 9\) After the inclusion of the present case in the calculation, the age of patients at the time of tumour detection ranged from soon after birth to 60 months, with 15 out of 18 (83 %) patients being in the infant age. A male/female ratio was 7 : 9. KMP developed in all but one patient.\(^9\) Majority of patients had compression of vital structures (trachea, bronchi, large blood vessels) with respiratory failure and some of them had pericardial and/or pleural effusions at presentation. In four patients, the tumour spreading to the neck tissue was registered\(^3, 17, 26, 27\) and in one patient KHE was multifocal.\(^17\) In all patients, the tumour was inoperable at the time of diagnosis. Under the influence of different modalities of medical therapy, the course of the disease was characteristically variable. Four of 19 patients died (21 %). Two died due to KMP-related haemorrhage\(^27\) and respiratory distress, the others due to sepsis which had developed after partial tumour resection.\(^13\) The patient presented in this paper also deceased after tumour biopsy followed by medical therapy. In other patients, regression of tumour mass and KMP was eventually noted, but residual tumour tissue persisted after variable follow-up periods.\(^17, 19, 25, 26\)

The reported mortality rate related to KHE ranges from 10 - 25 %.\(^9\) In the recent retrospective study of Schmid et al \(^9\) % of 191 followed patients died from complications of the disease (bleeding, disseminated intravascular coagulation, aspiration pneumonia, compression of airways, sepsis).\(^14\) Based on the literature data, adverse prognostic factors in patients with KHE significantly overlap with the risk factors for the development of KMP that were previously mentioned in this article.\(^9, 14, 16, 18-21, 28\) However, the result of the present analysis of 19 cases shows that the mortality rate from mediastinal KHE is close to the upper limit of the mortality rate from KHE of all anatomical sites, but does not exceed it. This is consistent with the latest analysis of Ji et al,\(^18\) in which tumour location failed to reach independent prognostic significance in the multivariate statistical analysis.

### Conclusion

It can still be concluded that, in the case the patient presented in this article, several risk factors for adverse outcomes were present: onset of disease in early infancy, large dimensions of the tumour, mediastinal location, KMP and partial response to the available therapy. Although exceptionally rare, KHE must be included in differential diagnostic considerations when dealing with a fast-growing mediastinal mass associated with respiratory problems, coagulopathy and profound thrombocytopaenia, especially in a patient in the first year of life.

### Acknowledgements

None.

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None.
References

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