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Behaudin Selmanović (1915-1972), "Still Life", 1957, oil on canvas, 800x650 mm. Courtesy of the International gallery of portrait Tuzla.

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First-year dental students' motivation and attitudes for choosing the dental profession

Nadya Avramova[1], Krassimira Yaneva[2], Boyko Bonev[3]

[1]Medical University – Sofia, Bulgaria
Faculty of Dental Medicine, Department of Dental Public Health
[2]Medical University – Sofia, Bulgaria
Faculty of Dental Medicine, Department of Dental Public Health
[3]Medical University – Sofia, Bulgaria
Faculty of Dental Medicine, Department of Dental Public Health

Corresponding author:
Nadya Avramova
Faculty of Dental Medicine
1 Georgi Sofiiski Str.
1431 Sofia, Bulgaria
avramova_nadia@abv.bg
Tel.: + 359 89 860 9286
Fax.: + 359 29 521 506

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Objective. To determine first-year dental students' current motivation and attitudes for choosing the dental profession at the Faculty of Dental Medicine, Medical University – Sofia, Bulgaria. **Material and methods.** An anonymous questionnaire, consisting of 12 questions about students' socio-demographic profile and their motivation for choosing dentistry, was administered to 119 first-year dental students at the Faculty of Dental Medicine of the Medical University of Sofia. The study was conducted at the beginning of the 2012-2013 academic year. The data was processed and analyzed with the following software: Microsoft Windows Server 2008 R2; Microsoft SQL Server 2008; Internet Information Server 7.5.; Microsoft SharePoint Server 2010. **Results.** The majority of the students (73%) were self-motivated for choosing dentistry as a career; 61% of them did not have relatives in the medical profession; 43% chose dental medicine because it is a prestigious, humane and noble profession; 50% – for financial security; 59% – because of the independence that it provides. There were no significant differences in the motivation between males and females. **Conclusion.** Independence, financial security and 'prestige' were the predominant motivating factors in this group of first-year dental students. Determining the reasons for choosing dentistry has important implications for the selection and training of students as well as for their future job satisfaction.

Key words: Dental students, Dentistry, Motivation, Job satisfaction.

Introduction

The reasons for choosing a particular profession are very complex, and the choice of dentistry is no exception (1). Many factors influence the choice of a profession: an individual's strengths and weaknesses, personal interests and desires, willingness and financial ability (or inability) to endure a long period of training related to a specific profession, the social prestige of the profession; the actual type of work that is inherent in the profession, working conditions, financial compensation associated with the profession and the availability and attractiveness of alternative occupations (1, 2).

A critical look (over time and across different countries) of the research literature on the motivation of people, who chose dentistry as a career, shows a wide variety of motivating factors (1, 3-7). Most studies were

based on surveys and used various methodologies. The results of these studies are not easily compared. There is common agreement, both on the large number of motivating factors and the fact that dominant motivational factors can vary significantly, over time and between countries. Moreover, from a sociological point of view, the emerging workforce today has very different expectations from those of previous generations (8).

Scarbecz and Ross (1) examined the motives of first-year students (University of Tennessee, USA) for selecting the dental profession, demographic data and future professional plans. Their study identified four key factors or groups of reasons for choosing the dental career:

- A financial motive – focuses on the financial and professional stability;
- A people -oriented or caring motive – refers to interpersonal relationships and the ability to take care of and help other people;
- A flexibility motive – dentists have greater freedom and flexibility in planning their work time compared with other professions connected or not connected to protecting public health;
- A business-oriented motive – focuses on the extent to which students believe that independence and self-management of their activities are important reasons for choosing the dental profession.

In this study the authors present and analyze data on the socio-demographic profile and reasons for the professional choice of first-year dental students in the Faculty of Dental Medicine, Medical University – Sofia for the 2012-2013 school year, as well as their premises regarding the nature of their future profession.

The aim of this study was to determine first-year dental students' motivation and preliminary attitude for choosing the dental profession at the Faculty of Dental Medicine, Medical University – Sofia, Bulgaria.

Materials and methods

Respondents

This study was approved by The Ethical Committee of the Medical University of Sofia (number 6506/18.12.2013). The research was carried out in compliance with the Helsinki Declaration. The study was conducted among newly enrolled dental students at the beginning of the 2012-2013 academic year at the Faculty of Dental Medicine of the Medical University of Sofia, Bulgaria. The total number of first-year dental students enrolled at the beginning of each academic year is 120 persons (60 males and 60 females).

Data collection

After sufficiently clear and detailed information about the purpose of the study had been given to the students, they were given the questionnaire (Appendix) and were asked to respond to this survey anonymously. The completion of the questionnaire by each student was taken as a form of individual consent to participate in the study.

Material

The self-administrated questionnaire (Appendix) contained 12 questions which were related to: Students' socio-demographic characteristics; Students' motivation for choosing the dental profession; Students' opinion and preliminary attitudes towards the dental profession. Some of the questions required the students to respond merely with a "yes" or "no", such as the question, "Do you have any close relative (mother, father, siblings, etc) in the medical profession?". Other questions offered one or several possible answers and the respondents had to choose one or several, for example, the questions related to the basic motives for choosing dentistry as a career. This type of

questions also had a blank space that was left for writing other answers if the students had any. The third group were open questions with an option for writing different answers. For example: "How many times did you apply for dentistry?".

Data Processing

The information was processed and analyzed using the following software: Microsoft Windows Server 2008 R2; Microsoft SQL Server 2008; Internet Information Server 7.5; Microsoft SharePoint Server 2010.

Results

A total of 119 students (99.2%) responded to the survey. Of these, 62 (52.1%) were women and 57 (47.9%) – men, aged 18 to 32 years (Figure 1). As shown in Figure 1, the number of students aged 19 years prevailed – 69 (57.9%). There were 31 students aged 18 (26.1%) and 10 aged 20 (8.4%). There was a small number of students aged 21, 22, 23, 25, 28 and 32 years – respectively 2 (1.7%), 2 (1.7%), 2 (1.7%), 1 (0.8%), 1 (0.8%) and 1 (0.8%).

The majority of the students graduated from high school: 58 (48.7%) students graduated from language high schools, 34 (28.6%) from mathematics high schools and 21 (17.7%) – from ordinary high schools. Two students (1.7%) graduated from technical schools, 2 (1.7%) from other universities, 1(0.8%) from dental technician college and 1 (0.8%) from nursing college (Figure 2).

More than half the newly enrolled students – 72 (60.5%) reported that they did not have relatives – a mother, father, brother or sister – in the medical profession. For 87 (73.1%) of the students the choice of dentistry was a result of their own decision. A much smaller number of students were influenced by their parents – 28 (23.5%), by a dentist – 16 (13.5%), friends – 3 (2.5%) or teachers – 1 (0.8%). For some respondents the decision was a result of several factors (Figure 3).

According to the results, ¾ of the students – 89 (74.8%) – decided to apply for dentistry in high school, 22 (18.5%) – before high school, and only 8 (6.7%) – after completing their secondary education.

Students were asked two questions related to their eagerness to study dentistry. To

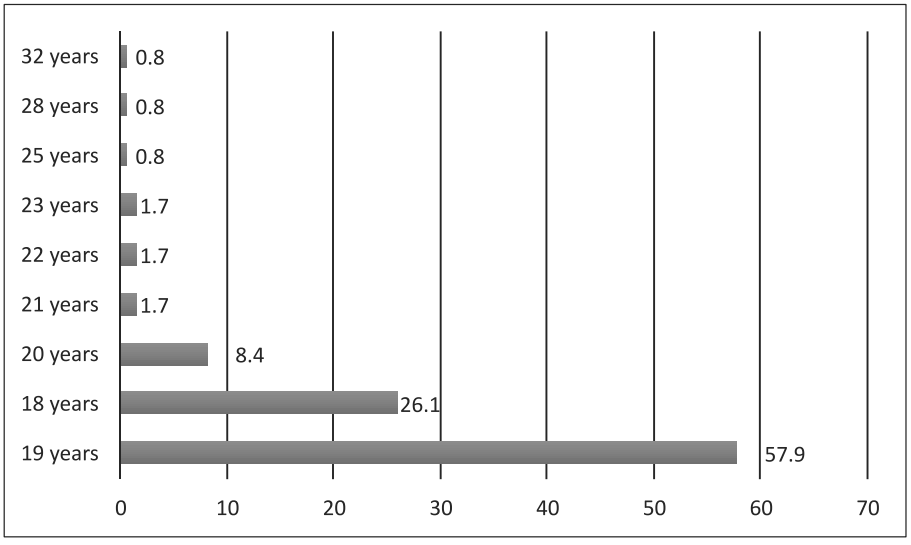

Figure 1 Distribution (%) of students by age groups.

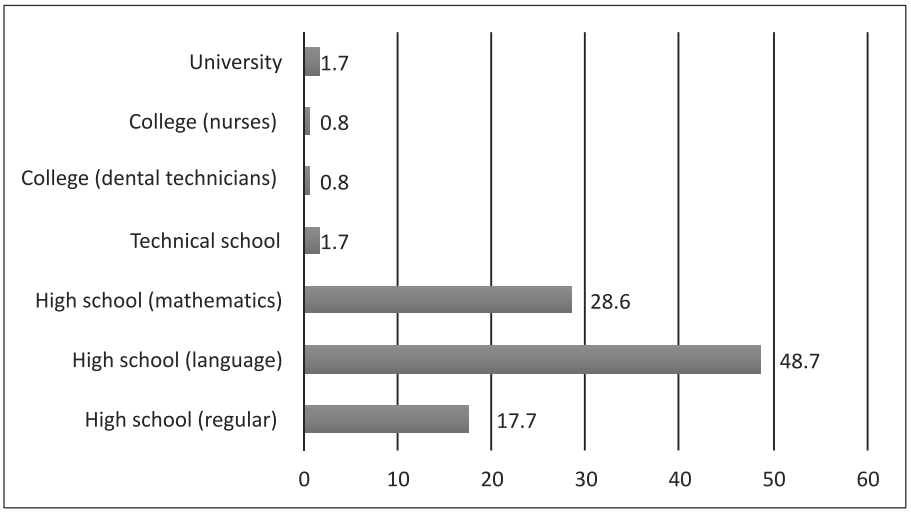

Figure 2 Distribution (%) of the students according to the type of school they graduated from.

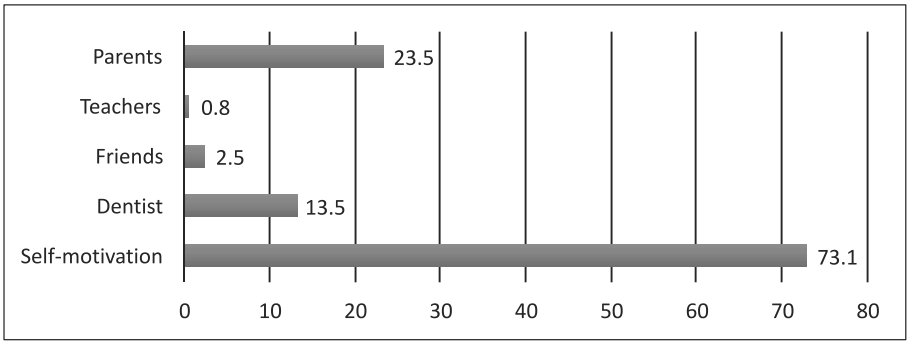

Figure 3 Distribution (%) of students according to the source of their motivation to study dentistry.

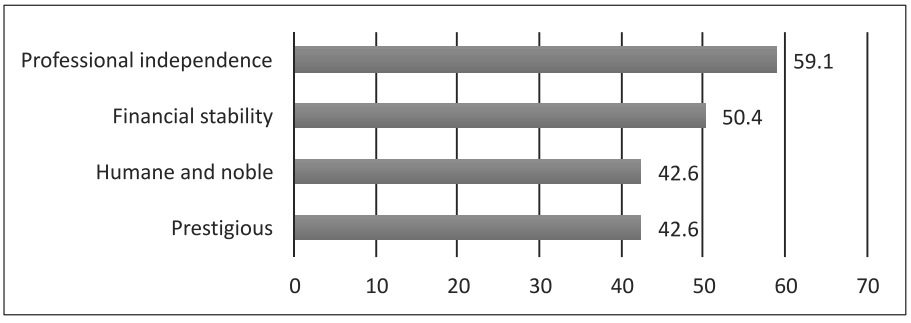

Figure 4 Distribution (%) of students according to their motives for choosing dentistry.

the first question: "How many times did you apply for dentistry?" 101 (84.9%) responded that it was their first attempt, 16 (13.5%) had applied for a second time and 2 (1.7%) – for a third time. A total of 116 respondents answered the second question: "What was your first choice?" The results showed that 110 (94.8%) of the students indicated dental medicine as their first choice and only 6 (5.2%) chose medicine.

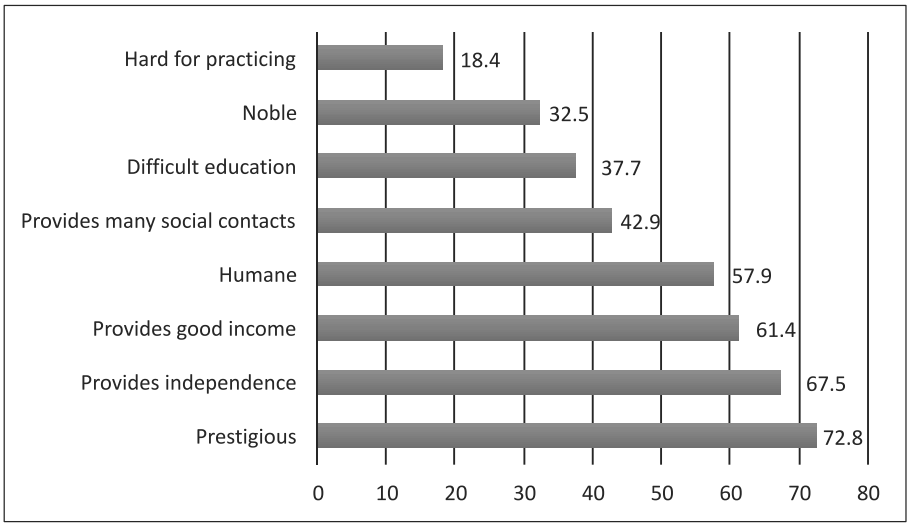

Figure 5 Distribution (%) of students according to their attitudes towards the dental profession.

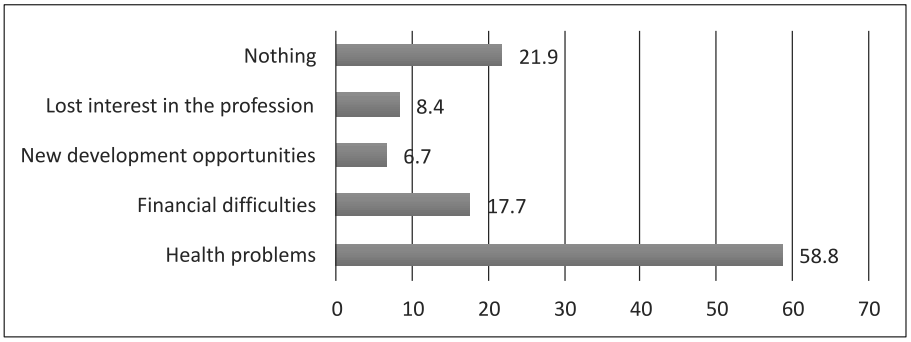

Figure 6 Distribution (%) of students according to the reasons for terminating their education.

No one chose pharmacy as an option. The students' preliminary opinion regarding the dental profession was expressed in the answers to questions № 9 and 10. 115 students explained their choice to become dentists by giving more than one answer: 49 (42.6%) of them chose dentistry as a prestigious profession; also 49 (42.6%) because it is humane and noble; 58 (50.4%) for financial stability, and 68 (59.1%) because it provides autonomy to the practitioner (Figure 4).

Determining the students' attitudes and perceptions about the dentist's profession was also important for the purpose of this study. 114 respondents answered the question: "What do you know about the chosen profession?". 83 (72.8%) of them believed it to be a prestigious profession, 77 (67.5%) that it provides autonomy, 70 (61.4%) that it provides a good income, 66 (57.9%) that it is humane, 49 (42.9%) that it is a profession that provides a large number of social contacts, 43 (37.7%) that it is difficult to study, 37 (32.5%) considered that the dental profession is noble, and only 21 (18.4%) thought that it is hard to practice (Figure 5).

The students again gave more than one response to this question. The last two questions were related to the students' opinion about the potential changes in their future education. The answer to the question "What could make you terminate your education?" is presented in Figure 6.

For 70 (58.8%) of the respondents, health problems were a serious reason for interrupting their education, 21 (17.7%) considered that financial difficulties would interrupt their education, 8 (6.7%) would leave the university if new opportunities for development arose and 10 (8.4%) if they lost interest in the profession. Nothing could make 26 (21.9%) of the students give up their chosen education. Only 2 (1.7%) of the respondents would try to transfer to another university. The remaining 113 (94.9%) responded negatively to the question: "Will you try to transfer to another university?" and 4 students (3.4%) did not answer it.

Discussion

The discussion of issues related to students' motivation and attitudes towards their future profession cannot be considered separately from the specific social, economic and political environment. Moreover, it should be constantly adapted to changes in the profession, health policy and increased demands of society (9). The fact is that the reasons for choosing the dental profession have the potential to change over time (3). However, personal views and concepts about the profession are major influencing factors for choosing dentistry as a career (10). Therefore it is essential to clarify whether the choice of a profession is a matter of an individual's decision or whether it was influenced by other people.

Students' motivation and their preliminary attitude to the dental profession are associated with a dentist's future job satisfaction. Considering the Two-Factor Theory of job satisfaction (11), there are intrinsic-motivation and extrinsic-hygiene factors. The presence of intrinsic motivation facilitates higher satisfaction and performance, whereas the absence of extrinsic factors helps mitigate against dissatisfaction (12). Both components of the theory, intrinsic and extrinsic, are essential for dentists, but the presence of intrinsic motivating factors has the most positive impact on job satisfaction (12).

The present findings indicated that the majority of the students (73%) were self-motivated to study dentistry. Yolov (13) conducted a survey of professional satisfaction among 327 dentists, and found that the majority of them (83.5%) made the decision to apply for dentistry on their own. The remainder, 16.5%, were influenced by relatives, or because their exam score was not high enough for their first choice (medicine or pharmacy). The author reported that the proportion of dissatisfied dentists was mainly formed by those who were influenced by relatives or those who were not practicing their preferred profession (13).

There were similar results from a survey conducted in Australia by Marino et al. during the 2009-2010 academic year (14). According to this survey, 85.3% of the students were mostly self-motivated to pursue a dental career (14). According to the results of the current study, the influence of the students' parents was significantly less (24%) than their self-motivation. Furthermore, over half of the students did not have relatives in the medical profession (61%). Conversely, the results of a study conducted by Tanalp et al. (15), concerning the future expectations of the students enrolled to study at the first private dental school in Istanbul (Turkey), presented the key role of the students' relatives (mother, father, brother, sister, etc.). According to this study, students' fathers and mothers had a similar impact on forming their choice (respectively 45.9% and 46.6%) (15).

In the current survey it was found that the influence of other people rather than relatives was less. 13% of the future dentists were influenced to choose this profession by a dentist, 3% by friends and only 1% by a teacher. A study conducted in Nevada, USA

by Hawley et al. (16) involving 152 first-year students and aiming to clarify their attitudes to the dental profession, showed that a large proportion of the respondents (52.6%) indicated their family dentist as the person who had the greatest influence on their decision.

The process of decision making about the future career requires the availability of sufficient time for understanding the priorities and future consequences related to the profession. The majority (75%) of the Bulgarian students surveyed made this decision while studying in high school. The data obtained did not differ significantly from those of other studies on similar topics (7, 14, 16).

The philosophy associated with education, the requirements for university admission and the procedures related to students' selection, undoubtedly affect their sociodemographic profile (7). Therefore the majority of Bulgarian freshmen students were aged 18, 19 or 20 years, which means that they applied for dentistry immediately after completing their secondary education. They were also representatives of the leading schools in Bulgaria (Language and Mathematics High Schools). Moreover, almost all the students (95%) indicated dentistry as their first choice and 85% of them had applied for this specialty for the first time. These facts prove the students' eagerness to explore the aspects of the dental profession: to work with and for people, to be independent and financially secure, and to be satisfied with their profession (5, 7).

The features of the dental profession, as mentioned above, are present in the students' perception and attitudes about the nature of their future profession. The majority of them (73%) believe that it is a prestigious occupation; 68% consider that it provides independence to the practitioner and for 61% of the students the dental profession is related to a good income. However, the awareness about the nature of the future profession could (and should) not be the same as the reasons for choosing it. It was detected that, in contrast, the students' motives regarding their choice of career showed that only 43% chose dentistry because it is a prestigious, humane and noble profession; the financial stability associated with the profession, appeared in second place (50%) and the main and leading motive for choosing the dental profession indicated by the students, was the independence they would have as practicing dentists (59%).

Undoubtedly concepts such as "helping others", financial stability, independence and many others, have different meanings to different people (4). The authors found only one study in which the financial motive was the first for choosing dentistry. Aguiar et al. (17) examined 1,024 students in Pernambuco (Brazil), to establish the factors leading to choosing dentistry as a profession. The results of this study showed that 73.5% of the students chose the dental profession mainly due to economic reasons (17).

There were more sources where the leading reasons for choosing dentistry were indicated, such as: the respect and prestige associated with the dental profession; the fact that "I help others"; job satisfaction, etc. According to students in Johannesburg (South Africa), Sydney (Australia) and Amman (Jordan) the independence and financial stability that the dental profession provides were not so important reasons for choosing dentistry as a career (3, 5, 9, 18).

Conclusions

From the conducted study it can be concluded that:

1. The majority of Bulgarian students were self-motivated for choosing dentistry as a career;
2. According to the Bulgarian first-year dental students, the leading motives for choosing the dental profession are as follows: a) The profession "dentistry" provides

independence for the practitioner; b) It provides financial stability; c) It is a prestigious, humane and noble occupation;
3. Students' perception about their future profession is associated with the prestige, independence and financial stability characterizing the dental profession.

Determining the preliminary attitude to the profession, as well as the leading reasons for pursuing it are closely linked to the practitioners' future job satisfaction. Not so precisely clarifying the nature and characteristics of the future profession is a major prerequisite for further disappointment with it. Therefore, students' motivation and attitudes for choosing the dental profession should be considered in the selecting and training of students, if job satisfaction in dental practice is to be achieved.

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Appendix

Dear colleagues,
This survey is being conducted in order to determine your attitudes and motivation for choosing the dental profession. Please, answer the questions honestly, and fill in or tick the correct answer.

1. **Your age is……..……..**
2. **Your gender is:**

☐ Male
☐ Female

3. **You graduated from:**

☐ High school (regular)
☐ High school (foreign language)
☐ High school (mathematics & nature sciences)
☐ Technical school
☐ College for dental technicians
☐ College for nurses
☐ Other university

4. **How many times did you apply for dentistry? ……..……..……**
5. **When did you decide to choose dentistry as a profession?.**

☐ Before high school
☐ In high school
☐ After high school

6. **Who influenced you to choose dentistry?**

☐ Parents
☐ Teachers
☐ Friends
☐ Dentist
☐ It is my own decision

7. **Do you have any close relative (mother, father, siblings, etc) in the medical profession?**

☐ Yes
☐ No

8. **What was your first choice?**

☐ Dentistry
☐ Medicine
☐ Pharmacy

9. **Which are your basic motives for choosing dentistry?**

"Dentistry" is:
☐ Prestigious
☐ Humane and noble
☐ Provides financial stability
☐ Provides independence for the practitioner
☐…….

10. **What do you know about the chosen profession?**

☐ Humane
☐ Noble
☐ Prestigious
☐ Provides good income
☐ Provides independence
☐ Difficult education
☐ Hard to practice
☐ Provides many social contacts
☐……

11. **What could make you terminate your education?**

☐Health problems
☐ Financial difficulties
☐ New development opportunities
☐ Losing interest in the profession
☐……

12. **Will you try to transfer to another university?**

☐ Yes
☐ No

Original article

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The effects of diabetes mellitus and hypertension on work productivity

Vesna Krstović-Spremo[1], Maja Račić[2], Bojan N. Joksimović[3], Vedrana R. Joksimović[2]

[1]Department of Occupational Medicine Faculty of Medicine, University of East Sarajevo, East Sarajevo, Bosnia and Herzegovina
[2]Family Medicine Department, Faculty of Medicine, University of East Sarajevo East Sarajevo, Bosnia and Herzegovina
[3]Department of Pathological Physiology Faculty of Medicine, University of East Sarajevo, East Sarajevo, Bosnia and Herzegovina

Corresponding author:
Bojan N. Joksimović
Department of Pathological Physiology
University of East Sarajevo
East Sarajevo
Bosnia and Herzegovina
joksimovic_bojan@yahoo.com
Tel.: + 387 65 373 507
Fax.: + 387 58 210 007

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Objective. The primary objective of this paper is to examine the impact of diabetes mellitus on the ability to work in patients with diabetes mellitus. The second objective of this paper is to examine the differences in the ability to work between patients with diabetes mellitus and patients with other chronic diseases, such as hypertension. **Material and methods.** A study was conducted in 10 family medicine practices from two primary health care centers, Pale and East Sarajevo, in the period between July 2009 and May 2010, utilising a retrospective medical records review and a cross sectional survey. The outcomes used to portray respondent's health status included functional measures and ability to work. Functional measures were analyzed using SF-36 and a general questionnaire. Absenteeism and productivity loss were retrospectively analyzed for the past ten years from a regional sick-leave database and the administrative records of the Commission for the assessment of work capacity for the Pension and Disability Insurance Fund of the Republika Srpska respectively. **Results.** Out of the total number of patients with diabetes, 24.6% had some form of disability. A statistically significant difference was found between the two groups; patients with diabetes mellitus were much more likely to have problems meeting the required standards at the workplace due to emotional and physical health issues compared to hypertensive patients. **Conclusion.** Diabetes mellitus appears to reduce an individual's ability to work in comparison to patients with hypertension. There is a need to set up a diabetes mellitus prevention program and to develop and implement effective targeted intervention to help workers to manage their disease better.

Key words: Diabetes mellitus, Work productivity, Disability.

Introduction

Work is a basic human activity through which every individual realizes their own livelihood. It is closely associated with the categories of health and quality of life. Diabetes mellitus, as a disease with a high prevalence of growth in all countries, threatens to become a global epidemic risk, and thus the question of the ability to work of this category of patients is extremely important in terms of professional orientation, professional selection, work absenteeism and disability evaluation (1).

Lost productivity at work is an important concern for employees, employers, and so-

ciety. Moreover, the complications related to diabetes mellitus are major cause of disability, reduced quality of life, and death. Employees with diabetes mellitus may stop working prematurely and may experience unemployment, which could translate into a reduction in earned income and savings, and loss of self-esteem. For employers too, lost productivity due to absenteeism, disability and early retirement is an important economic issue (2-9).

Work ability assessment is a continuous process that in ideal social circumstances should accompany a person throughout their entire life. Basically, it has to answer the question of whether there is a match between a man's psychophysical ability and the demands of working conditions and the work environment.

The most common causes of work absenteeism are: disease, occupational disease, and injury at work, injury outside of work, care for a family member or some other reason provided by law (10). According to estimates by the International Labour Organization (ILO) for sick leave, about 5% of the total employed labour force is absent from work every day. The average number of sick days per employee in the EU was 4.6 days per year (11).

Diabetes mellitus is a common cause of absenteeism in the population. An estimated 171 million people were suffering from diabetes mellitus in 2000, and this number could total 366 million by 2030 (12). Type 2 diabetes mellitus accounts for more than 90% of all diabetes cases, and it often appears in middle age. In 2010, the prevalence of diabetes mellitus in the U.S. was 11.3 and 26.9% among individuals aged 20 years or over and 65 years or older, respectively (13). Data provided by the Public Health Institute of Republika Srpska show that the prevalence of diabetes mellitus is 42% and for hypertension it was 14% for 2012, in Republika Srpska, Bosnia and Herzegovina (14).

The primary objective of this paper is to examine the impact of diabetes mellitus on the ability to work of people with diabetes. The second objective of this paper is to examine the differences in the ability to work between patients with diabetes mellitus and patients with another chronic disease, such as hypertension. These two chronic diseases were chosen because of their high prevalence in the community and similar hazard effect on the cardiovascular system and overall health.

Materials and methods

This study was conducted in 10 family medicine practices from two primary health care centers, Pale and East Sarajevo, in the period between July 2009 and May 2010, utilising a retrospective records review and a cross sectional survey.

The sample size for the population of 2326 patients with diabetes mellitus included in the regional Diabetes Registry, with a confidence interval of 6.63% and a confidence level of 95%, was calculated to be 200. A specially established audit team randomly selected the medical files of 200 patients with diabetes mellitus from the Diabetes Registry administered by all ten family medicine teams' databases. Patients were registered as patients with diabetes mellitus if they had two fasting plasma glucose levels above 7.8 mmol/l or two random plasma glucose levels above 11.1 mmol/l. and/or were treated with insulin and/or oral hypoglycemic agents. Then, the team randomly selected medical files of 200 patients with arterial hypertension from the Hypertension Registry administered by the same family medicine team database. Patients were registered as patients with arterial hypertension if they had blood pressure ≥140/90 and/or were treated with antihypertensive agents. Exclusion criteria for the patients with diabetes were the presence of other chronic diseases

such as associated hypertension, established cardiovascular disease, renal failure, obesity, pulmonary diseases and being unavailable to complete the questionnaire. Exclusion criteria for patients with hypertension were presence of other chronic diseases such as associated diabetes mellitus, established cardiovascular diseases, renal failure, obesity, pulmonary diseases and being unavailable to complete the questionnaire.

All respondents who were included in the study were invited to see their family physician at the scheduled time. During their visit to the family physician, respondents were informed about the aim of the study and their written informed consent was sought and obtained. The outcomes used to portray the respondent's health status included functional measures and the ability to work.

Functional measures of the respondents' health status were assessed during the visit. The respondents were asked to complete two questionnaires. A standardized questionnaire was used to collect current data regarding the respondents' characteristics such as sex, age, place of residence, marital status, education, occupation, duration of diabetes mellitus and hypertension, respondents' perception of their own ability to work and their quality of life. In evaluating the impact of diabetes mellitus and hypertension on respondents, a generic instrument, the self-administered, linguistically validated Medical Outcomes Study Short Form-36 Health Survey (SF-36) was used. SF-36 is the most widely used generic instrument to quantify health-related problems. It is composed of 36 questions and standardised response choices, and is organised into eight multi item scales: physical function (PF – 10 items); role physical, referring to limitations in performing important life roles due to physical health (RP – 4 items); bodily pain (BP – 2 items); general health perceptions (GH – 5 items); vitality (VT – 4 items); social functioning (SF-2 items); role emotional, referring to limitations in performing important life roles due to emotional problems (RE – 3 items) and mental health, referring to the absence of anxiety and depression (MH – 5 items). All scale scores are linearly converted to a 0-100 scale, with higher scores indicating a better health-related quality of life (HRQoL). Extensive background information on SF-36, as well as standard scoring algorithms and interpretations guides, are available elsewhere (15, 16). We used the cross-culturally validated Serbian version of SF-36. The reliability of SF-36, as measured by Cronbach's alpha coefficient was 0.78. SF-36 mean scores were calculated for both groups (17).

The second outcome was the ability to work. It included absenteeism and productivity loss. The data about absenteeism was retrospectively analyzed reviewing the patients' medical records and regional sick-leave database for past ten years. Productivity loss was also retrospectively analyzed using administrative records of the Commission for the assessment of work capacity for the Pension and Disability Insurance Fund of the Republika Srpska for the past ten years.

Ethical statement

The obtained data were compared between patients with diabetes and patients with hypertension. In the analytical database, personal identifiers were removed to preserve confidentiality, and access to the database was controlled by the Committee for Science and Research of the Medical Faculty Foča, University of East Sarajevo. The study was conducted in accordance with the World Medical Association Declaration of Helsinki of 1975, as revised in 1983, with the approval of the Ethical Committee of the Medical Faculty of Foča, University of East Sarajevo

Statistical analyses

Statistical analyses were carried out using SPSS 17.0 (SPSS Inc., Chicago, IL, USA). Means and standard deviations (SD) for continuous variables and frequency and percentages for categorical variables were used to describe data. The mean scores of SF-36 were calculated for the different groups and the normality of their distributions was tested by the Kolmogorov-Smirnov test. Differences between groups means were analyzed by one way analysis of variance (ANOVA). To compare the difference in answers on their own ability perception between the patients with diabetes mellitus and patients with hypertension, we used a Chi-square statistical test. The correlation between SF-36 and the ability to work were analyzed with Spearman's rho (q) correlation coefficient. Multivariable analysis using linear regression was performed to identify independent factors for the social functioning domain of health related quality of life. The data were presented in tables, and a p-value <0.05 was considered to be statistically significant.

Results

The study included the medical files of 191 adult patients with diabetes mellitus and 100 patients with hypertension. Nine patients with diabetes mellitus and 100 patients with hypertension were excluded from the study due to the presence of associated chronic disease.

The patients in both groups were mainly of male gender, 63.4% in the diabetes mellitus group and 53% in the hypertension group, respectively. The average age of the patients with diabetes mellitus was 55.08 years, with a range from 25 to 78 years. The average age of patients with hypertension was 54.9 years, with range from 35 to 65 years. The majority of the patients in both groups were town dwellers. About 86% of the patients with diabetes mellitus and 75% of hypertensive patients had either a university or high school degree and a significantly higher proportion of them were employed in blue-collar than white-collar jobs. Approximately, 36% of patients had had diabetes mellitus from 2 to 5 years, while the majority of hypertensive patients had had the disease for 5 years or longer (Table 1).

Forty patients (21%) with diabetes mellitus were employed at the time of research, as well as 56 (56%) patients with hypertension ($\chi 2=31.22$, $p<0.001$). However, it was found that the number of retirees was higher in diabetes mellitus group (46.6%). Of those patients who were retired, 53% were receiving disability pensions due to diabetes mellitus, and 47% had retired due to their age. In the hypertension group 19% of patients were receiving old-age pension and none due to disability (Table 1). Fifty-three per cent of patients used oral anti-diabetics while 47.6% used insulin. Patients with diabetes mellitus felt significantly more disabled than patients with hypertension ($\chi 2=13.46$, $p<0.001$) (Figure 1).

A statistically significant difference between two groups was found in exposure to occupational hazards. The total number of patients with diabetes mellitus exposed to some type of hazard at their work place was 28. The largest number of patients were exposed to a physical hazard (57.1%), followed by chemical (28.6%) and biological hazard (14.3%). In the group of patients with hypertension, 20 patients were exposed to some sort of occupational hazard (Table 2).

Out of the total number of patients with diabetes mellitus, 24.6% had some kind of productivity loss. This group of patients was divided into two subgroups. One subgroup was composed of 13 (6.8%) patients who completely lost the ability to work and the other subgroup of 34 (17.8%) patients who partially lost their ability to work. The patients from both groups took (early) disability retirement (Table 2). In the group of pa-

Table 1 Characteristics of the patients with hypertension and diabetes mellitus (n=291)

Characteristic	Patients	
	DM (n; %)	HT (n; %)
Gender		
Female	70 (36.6)	47 (47.0)
Male	121 (63.4)	53 (53.0)
Age (years)		
20 to 29	1 (0.5)	0 (0.0)
30 to 39	8 (4.2)	2 (2.0)
40 to 49	28 (14.7)	24 (24.0)
50 to 59	93 (48.7)	48 (48.0)
60 to 69	57 (29.8)	30 (30.0)
70 to 79	4 (2.1)	0 (0.0)
Place of living		
Town	123 (64.4)	67 (67.0)
Village	68 (35.6)	33 (33.0)
Marital status		
With partner	134 (70.2)	74 (74.0)
Without partner	57 (29.8)	26 (26.0)
Education		
Elementary school	40 (13.7)	25 (25.0)
High school	131 (45.0)	30 (30.0)
University degree	120 (41.2)	45 (45.0)
Occupation		
Blue collar jobs	20 (10.5)	32 (32.0)
White collar jobs	5 (2.6)	14 (14.0)
Farmer	8 (4.1)	7 (7.0)
Retiree	89 (46.6)	19 (19.0)
Retiree (due to age)	42 (47)	19 (100)
Retiree (due to disability)	47 (53)	0 (0.0)
Black market job	7 (3.6)	3 (3.0)
Supported by family member	19 (9.9)	9 (9.0)
Unemployed duo to the lack of job	43 (22.7)	16 (16.0)
Duration of the disease (years)		
<2	57 (30.0)	28 (28.0)
2 to 5	70 (36.4)	32 (32.0)
>5	64 (33.6)	40 (40.0)
Smoking		
Smoker	51 (26.7)	21 (21.0)
Non smoker	129 (67.5)	75 (75.0)
Ex-smoker	11 (5.8)	4 (4.0)
Alcohol consumption		
Yes	24 (12.6)	19 (19.0)
No	167 (87.4)	81 (81.0)

DM=Diabetes melltus; HT= Hypertesion.

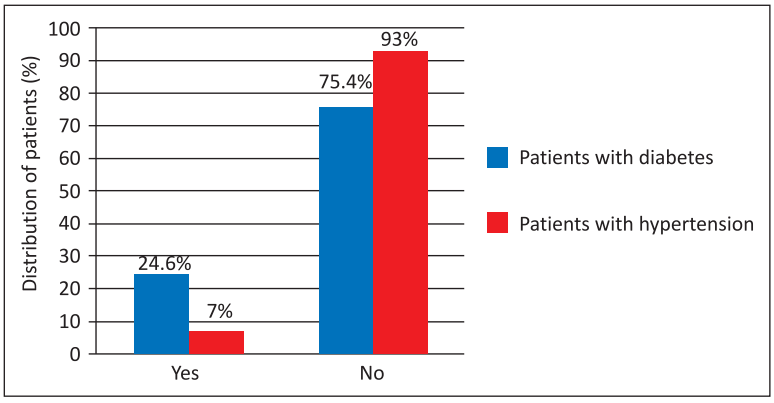

Figure 1 Distribution of patients according to their perception of disability.

Table 2 Distribution of patients by the type of hazard they were exposed to at their work place, fulfilment of labour norm, category of disability, work absenteeism, time spent on sick leave and subjective assessment of quality of life in patients with diabetes mellitus and hypertension

Subject	Offered response	Patients		χ^2	p
		DM (n; %)	HT (n; %)		
Type of damage	Physical hazard	16 (8.4)	9 (9)	24.36	0.001
	Chemical hazard	8 (4.2)	2 (2)		
	Biological hazard	4 (2.1)	9 (9)		
Fulfilment of labour norm	Yes	16 (8.4)	4 (4)	10.04	0.002
	No	43 (22.5)	61 (61)		
	Not working currently	132 (69.1)	35 (35)		
Category of disability	Lost work ability	13 (6.8)	1 (1)	0.431	0.512
	Partial work ability	34 (17.8)	6 (6)		
	No Disability	144 (75.4)	93 (93)		
Have you been on a sick leave in the past year?	Yes	25 (42.4)	19 (29.2)	2.33	0.127
	No	34 (57.6)	46 (70.8)		
Duration of sick leave	Up to month	14 (56)	10 (52.6)	1.17	0.512
	Up to four months	6 (24)	5 (26.3)		
	More than 4 months	5 (20)	4 (21.1)		
Subjective assessment of quality of life	Good	25 (13.1)	35 (35)	24.32	0.001
	I am satisfied	103 (53.9)	51 (51)		
	Bad	63 (33.0)	14 (14)		
Presence of anxiety and stress	Yes	154 (80.6)	53 (53)	24.40	0.001
	No	37 (19.4)	47 (47)		0.001

DM=Diabetes mellitus; HT=Hypertension.

tients with hypertension, 93% had preserved ability to work. Table 2 shows that 8.4% of patients with diabetes mellitus, and 4% of patients with hypertension had a perception of the inability to work. The patients with diabetes mellitus were significantly more likely to experience problems in their work place than patients who were suffering from hypertension ($\chi 2=10.04$, $p<0.002$). When it comes to work absenteeism (sick leave), out of 59 employed patients with diabetes mellitus, 42.4% used sick leave over the past year, compared to 28.2% of hypertension patients. However, no statistically significant

difference in terms of work absence between patients with diabetes mellitus and hypertension was found ($\chi 2=2.33$,$p=0.127$) (Table 2). The largest number of patients with diabetes mellitus (56%) used sick leave in the past year for up to one month, while 20% used sick leave of up to 4 months (Table 2).

Statistically significant differences in the perception of quality of own life were found between the two groups ($\chi 2=24.32$, $p<0.001$). More than half (53.9%) of all patients with diabetes mellitus estimated that the quality of their daily life was satisfactory, 13.1% said that their quality of life was good and 63 (33%) of all patients suffering from diabetes mellitus said that their quality of life was very bad. In the hypertension group, 51% of patients said that they were satisfied with their quality of life and 35% said is was good.

The majority of patients with diabetes mellitus stated that they felt anxiety daily, compared to patients with hypertension (53%). A high statistically significant difference regarding the presence of anxiety was found between the groups ($\chi 2=24.40$, $p<0.001$) (Table 2).

Mean scores for the subscales of SF-36 for study patients are shown in Table 3. Patients with hypertension had significantly lower quality of life (QoL) scores than patients with diabetes for physical functioning, role physical, general health, social functioning and role emotional (Table 3).

Patients with diabetes mellitus were significantly more constrained in the past 4 weeks in terms of work or other activities, as a result of physical health, compared to the patients with hypertension. The difference between these two groups here is statistically highly significant ($\chi^2=29.1$, $p<0.01$). Patients with diabetes mellitus experienced more difficulties in performing work or other activities over the past 4 weeks due to emotional problems compared to the hypertensive group ($\chi^2=14.4$, $p=0.006$). Eleven per cent of patients with diabetes mellitus and 3% of patients with hypertension were constrained to perform work or other activities "all the time".

The correlation between the QoL instrument (SF-36) and the clinical severity of diabetes mellitus is shown in Table 4. Spearman's correlation coefficients between SF-36 and three parameters (perception of disability, productivity loss and absenteeism) ranged from 0.077 to 0.408 determining a weak relationship. The highest correlations were found between productivity loss and the bodily pain scale of SF-36 ($r=0.408$; $p<0.01$), and between productivity loss scale and the role emotional scale of SF-36 ($r=0.366$; $p<0.05$).

A significant negative correlation was observed between the perception of disability and physical functioning ($r=-0.356$; $p<0.05$), social functioning ($r=-0.382$; $p<0.01$) and

Table 3 SF 36 mean scores for patients with diabetes mellitus and hypertension

Variable	Patients		p
	DM (n=191)	HT(n=100)	
Physical functioning	72.4**	52.5	0.004
Role physical	78.3*	62.8	0.037
Bodily pain	65.1	58.0	0.268
General health	82.8*	67.3	0.026
Vitality	57.0	54.5	0.562
Social functioning	71.6*	64.6	0.027
Role emotional	75.4**	60.9	0.008
Mental health	73.2	71.1	0.236

DM=Diabetes mellitus; HT=Hypertension; *p<0.05; **p<0.01.

Table 4 Interscale correlation between SF-36 and clinical severity of disease (DM; n=191)

Variable	PF	RP	BP	GH	VT	SF	RE	MH
Perception of disability	-0.356*	0.251	0.077	0.161	-0.182*	-0.382*	0.154	-0.148
Productivity loss	-0.153*	0.173	0.408**	0.168	0.238	-0.187*	0.366*	-0.206
Absenteeism	0.209	-0.147	0.137	-0.202*	-0.170	0.158	0.115	0.234

DM=Diabetes mellitus; PF=Physical functioning; RP=Role physical; BP=Bodily pain, GH=General health; VT=Vitality; SF=Social functioning; RE=Role emotional; MH=Mental health; *p<0.05; **p<0.01.

Table 5 Multivariable model for the Social functioning domain of the SF-36 questionnaire of patients with diabetes mellitus and hypertension (n=291)

Socio demographic, occupational and psychological characteristics of patients	B	95% CI		p
		Lower bound	Upper bound	
Age	-0.082	-0.261	0.117	0.459
Gender	0.097	-0.095	0.288	0.322
Education	0.365	0.082	0.628	0.011
Occupation	0.980	0.620	1.341	<0.001
Ability to work	0.267	0.031	0.502	0.027
Diabetes	0.567	0.303	0.729	<0.001
Hypertension	0.212	-0.246	0.657	0.372
Duration of disease	-0.180	-0.368	0.008	0.060
Depression	1.042	0.448	1.614	0.001
Anxiety	0.986	0.620	1.341	<0.001
Smoking	0.129	-0.084	0.295	0.284
Alcohol consumption	-0.096	-0.419	0.291	0.722

B=coefficient for the constant.

vitality (r =- 0.182; p<0.05). We also found a significant negative correlation between productivity loss and social functioning scale (r=- 0.187; p<0.05) (Table 4).

A multivariate analysis showed that the independent factors associated with the score of SF-36 were the presence of diabetes, depression and anxiety, education, occupation and ability to work. Hypertension and disease duration were not significant determinants of SF-36 score (Table 5).

Discussion

This study showed that patients with diabetes mellitus were more likely to face problems with work productivity and being constrained in terms of work and other activities. Also, diabetes mellitus patients reported a significant decline in all daily activities due to emotional and physical health problems. Besides diabetes mellitus, education, occupation, ability to work and the presence of anxiety or depression had a significant influence on their quality of life.

These findings are consistent with other studies. The systematic review by Breton et al., (9) included 23 studies investigating the impact of diabetes on ability-to-work outcomes. Studies were conducted in many countries using different study designs and involving different settings (general population or specific population of workers) and age groups. In addition, outcomes definition of productivity measures, recall periods, statistical analyses and variables used for adjustment differ considerably across those studies that assessed the same outcomes. The effects of diabetes mellitus on absenteeism, productivity loss, and early retirement

are generally consistent across studies with high methodological quality. In the majority of studies, diabetes mellitus had a significant negative impact on the ability-to-work outcomes considered. Studies focusing on presenteeism are not considered to have low risk of bias (2, 6, 8, 9, 18, 19).

The number of days lost annually from work per employee that reported in the studies included with high methodological quality ranges between 5.4 and 18.1 days for employees with diabetes and between 3.4 and 8.7 for those without diabetes mellitus. Individuals with diabetes mellitus have between two and ten days absences per year more than those without diabetes mellitus. This result suggests that the associated economic burden could be high for employers. Finally, individuals with diabetes retired 0.7 years earlier compared with individuals without diabetes mellitus (9).

From the results of different studies it is evident that the working ability of the population is an extremely important issue for each country because it involves the labour force as an element of economic power, so it is not only an individual, but also a social category. The active population or labour force of a country (ages 15-65 years), according to a WHO report comprises approximately 50% to 60% of the population whose labour produces all its economic and material values, ensuring the socio-economic development of the country (20).

This study showed that diabetes mellitus has a major impact on work productivity and the early occurrence of disability in people with this illness. Due to the inability to meet requirements at the workplace, a large number of patients try to obtain a disability pension, but they are often rejected by the Commission for assessment of disability, so they continue working at the same job in spite of the difficulties they have already experienced. In Bosnia and Herzegovina and almost all countries in the Balkans, the number of applications for disability pensions is quite high (21).

In the study conducted in Montenegro (2005-2006), on a sample of the 3055 workers, who were referred for assessment of their working ability, it was found that diabetes mellitus was in third place of the overall causes of disability (45%) (22). In the study by Šljivić et al. (23) ,which included an analysis of 9,313 individuals who were referred to the Committee for disability of Serbia for working ability assessment, it was shown that only 30.86% were categorized as invalids of the first category (equivalent to today's assessment of "the loss of working ability") (22, 23).

According to the results pertaining to the SF-36 quality of life questionnaire, patients with diabetes mellitus were significantly more constrained in terms of work or other activities, as a result of physical health than the other group of patients, with hypertension. This result was not unexpected since it is known that diabetes mellitus can lead to the occurrence of pain in the legs and the inability for prolonged walking or standing. Diabetes is a risk factor of the appearance of atherogenic plaques because chronic hyperglycaemia is a direct cause of the process of atherosclerosis. A high percentage of patients with diabetes mellitus stated that they had had problems in the past four weeks, at work or in other activities as a result of emotional problems such as anxiety. However, scores on SF-36 questionnaire were also determined by education, occupation, the ability to work of the patients, as well as the presence of psychological disturbances. This is consistent with studies that show that people with diabetes mellitus have a higher incidence of psychological disturbances, about one and a half times greater than the rest of the population (24).

From the literature it is known that certain psychological disorders maybe the result of not only primary psychiatric dis-

orders, but may be the result of some metabolic disorders, such as diabetes mellitus. Quality of life in people with diabetes mellitus is substantially dependent, not only on the sphere of good metabolic control, but also on a stable psychological status and other elements of the patient's context (25). Psychological symptoms in patients with diabetes are reflected in the appearance of the burden of disease, which may be accompanied by weaker concentration, a sense of guilt and other disorders ranging from anxiety and nervousness, to burn out syndromes that occur in connection with diabetes (26).

Different studies showed that work disability is significantly higher for individuals with diabetes mellitus than for those without diabetes at all ages, and results in a significant decrease in earnings (27, 28). Our study showed that there is a significant correlation between productivity to work, work absence or perception of disability and patients' functional status. There is the evidence that diabetes mellitus affects patients, employers, and society not only by reducing employment but also by contributing to work loss and health-related work limitations for those who remain employed. Its effects on employment and work productivity are likely to become more pressing for society (29). Even after controlling for other factors presumed to be relevant to the decision to work, such as other chronic health conditions and job characteristics, it was found that diabetes reduced the absolute likelihood of working. The economic burden associated with diabetes mellitus is likely to increase as diabetes mellitus becomes more prevalent. Since diabetes mellitus is a progressive disease, one may speculate that the occurrence or progression of diabetes mellitus complications may lead to functional impairment or limitations to performance and these individuals to stop working. Therefore, the prevention of both diabetes mellitus and its complications through medication, diet, and exercise, is likely to yield economic benefits, in addition to preserving the health status and quality of life of individuals who are at risk for developing or who already have diabetes (29-31).

This study does however, have some limitations. This was a cross-sectional study so it cannot determine cause and effect, but it can identify potential associations. Secondly, we are aware that the sample size achieved in the study was lower than that calculated and hence this affects/limits generalizations from the data to a wider population. Larger and more longitudinal data are needed to provide a better assessment of the causes and effects of diabetes on ability-to-work outcomes.

Nevertheless, the results of this study indicate that there is a substantial impact on the ability to work and patients' HRQoL associated with diabetes. In fact, they suggest that employers, insurers, and decision makers should pay attention to ability to work because of diabetes mellitus and could help employers better manage services overseen by various managers of human resources and employee benefits programs, such as paid sick days, medical insurance, and education or intervention programs.

Conclusion

Diabetes mellitus appears to reduce an individual's ability to work. Patients with diabetes mellitus experienced more difficulties in performing work or other activities due to emotional problems compared to the hypertensive group. The majority of patients with diabetes mellitus stated that they feel anxiety daily, compared to patients with hypertension (53%). There is a need for setting up diabetes prevention programs and to develop and implement effective targeted intervention to help workers better manage their disease. Otherwise this diabetes mellitus-related burden could worsen in the

working-age population. Efficient employer-implemented intervention programs to improve the physical health and well-being of their workers with diabetes could be a good strategy for controlling productivity-related costs.

Authors' contributions: Conception and design: VKS, MR; Acquisition, analysis and interpretation of data: MR, BNJ, VRJ; Drafting the article VKS, MR; Revising it critically for important intellectual content: BNJ, VRJ.

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Original article

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Prognostic factors of overall survival for patients with stage II colon cancer

Harsha Trivedi, Ushasree Chamarthy, Luciano Dicarlo, James Herman, Gordan Srkalovic

Sparrow Cancer Center, Sparrow Health System, Lansing, Michigan, USA

Corresponding author:
Gordan Srkalovic
College of Human Medicine
Michigan State University, East Lansing, MI
Gordan.Srkalovic@sparrow.org
Tel.: + 517 364 2809
Fax: + 517 364 3687

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Objective. To analyze factors influencing survival of patients with stage II colon cancer treated at our cancer center (Sparrow Hospital) from February 1996 through December 2006. **Patients and methods.** Survival analyses on 197 patients' age 71.1±0.9 years (29 to 97) were done using SAS system (V9.3, Cary NC). Analysis included age, gender, stage, surgery type, number of examined lymph nodes, pathological grade, tumor size and the use of adjuvant chemotherapy. **Results.** Mean follow up length was 48.1±2.3 months (0.1-133) and 56±3.3 (0.2-133) for survivors. The average number of removed lymph nodes was 18±13 (1-103). Adjuvant chemotherapy treatment (5-FU± leucovorin) was given to 49 patients, while others (148) were followed expectantly. There were 90 deaths during follow up. Only age exhibits a statistically significant relationship to survival (Hazard Ratio (HR) =1.06, 95% CI=1.03-1.08, p<0.001). Adjuvant chemotherapy possibly reduced the risk of death by 42% approaching a borderline advantage for survival (HR=0.58, CI=0.33-1.03, p=0.06. The number of removed lymph nodes also showed a possible relationship to survival (HR=0.98, CI= 0.62-1.56, p=0.07). Other investigated factors (gender, type of surgery, etc.) were not significant correlates. **Conclusion.** In this study we found that the most important factor for survival of patients with Stage II colon cancer is the patient's age. Adjuvant chemotherapy showed a borderline significance while the number of resected lymph nodes seemed to be an important survival factor. However, in our study statistical significance was not achieved.

Key words: Stage II colon cancer, Chemotherapy, Survival rates, Prognostic factor.

Introduction

For more than 20 years, adjuvant chemotherapy has been shown to improve disease-free survival (DFS) and overall survival (OS) in advanced colon cancer. Although it is agreed that patients with stage III disease benefit from adjuvant treatment, whether all patients with stage II disease should receive such treatment remains controversial. At the center of this controversy are the contradictory conclusions derived by two large investigators' groups. The National Surgical Adjuvant Breast and Bowel Project (NSABP) concluded that the relative benefits of treatment were largely the same for stage II and stage III tumors (1), whereas the International Multicenter Pooled Analysis of B2 Colon Cancer Trials (IMPACT B2) failed

to demonstrate a statistically significant benefit for stage II tumors (2, 3).

FOLFOX has been approved in the adjuvant setting for stage III disease however the value of adjuvant therapy for stage II disease has been debated for decades. Recent data from large trials as well as pooled analyses seem to support its use (4-11). FU-based adjuvant chemotherapy in stage II patients is associated with a 2% to 4% benefit in 5-year DFS compared with surgery alone. On the basis of the MOSAIC results, adjuvant therapy for stage II patients with FOLFOX provides an improvement of 2.7% in 3-year DFS and 3.8% in 4-year DFS compared with optimized (infusional) FU/LV therapy (6, 7). If we postulate a 6% gain in 3-year DFS with FOLFOX in unselected stage II patients, this would translate an additional 1,250 patients free of disease every year in the United States (12). In the US Surveillance, Epidemiology and End Results (SEER) Medicare population based study it was noted that treatment is being administered to a significant proportion of patients with low risk stage II colon cancer (13).

For stage II colon cancer, several well-known pathological and clinical factors are reported for instance: the age of the patient at diagnosis, tumor staging, number of involved lymph nodes, lymphovascular invasion, tumor grade and presence of bowel obstruction are associated with a higher risk of recurrence or death from the cancer. Identifying these factors is important to determine which patients may benefit the most from adjuvant therapy.

In the present study we sought to examine retrospectively the data of patients with Stage II colon cancer seen and treated at Sparrow Hospital from February 1996 through December 2006. There were 197 patients. Our aim was to 1) to find out if we can find an association of the reported factors with overall survival and disease free survival in our patients, 2) we sought to do this as our oncologists also face a similar complex decision of treatment versus no treatment for stage II colon cancer patients and 3) finally we wanted to compare the survival of patients at our center to the national statistics for USA based on the factual information from our data outside the context of controlled clinical trials.

Patients and methods

We reviewed data of 197 patients who were examined, surgically treated and followed up by oncologists at Sparrow Hospital in Michigan for a ten-year time spanning from February 1996 through December 2006. The time to progression (TTP) was not calculated due to too few events observed in the follow up. Included in the analysis were: age, gender, stage (IIA vs. IIB), surgery type, number of examined lymph nodes, histological grade (well vs. moderately vs. poorly differentiated), tumor size and the use of adjuvant chemotherapy.

Statistical analysis

All data and survival analysis were performed using SAS statistical software (V9.3 (SAS Institute Inc., Cary, N.C.). Disease free survival (DFS) and Overall survival (OS) experience was characterized by constructing Kaplan-Meier survival curves. Cox proportional hazards models were used to assess differences in survival by baseline characteristics. For Disease free survival only cases with a notation of "Disease Free" were considered. This conservative approach was thought appropriate by the statistician as some cases marked as "Unknown" may well have been disease free. Subjects who died but whose deaths were determined to be unrelated to disease were censored at the time of last contact. A Type I error protection rate of 0.05 was assumed. All *P* values of less than .05 were considered statistically significant.

Ethics statement

This project was reviewed and was deemed as "an Exempt from IRB review status" by the Sparrow Institutional Research Review Committee (IRRC) according to the USA federal regulations. The confidentiality and the privacy of the subjects and the data were maintained as the data was obtained as anonymous from the tumor registry. No attempt was made to identify individuals. All authors have completed the Human subject Training required by the Sparrow IRRC and have adhered to ethical standards of Human subject research.

Results

Analysis was performed on 197 patients with Stage II colon cancer patients age 71.1±0.9 years (range 29 to 97). Of the 197 patients, 114 were female (58%) and 83 (42%) were male. Subtotal colectomy was performed on 142 (72%) patients, while partial colectomy and local resection were done on 38 (19.8%), and 3 (1.6%) patients respectively. Total colectomy was done on 14 patients (7.1%). Out of 197 patients, 51 (25.8%) were Stage IIA and 146 (74.1%) stage IIB. Average number of removed lymph nodes was 18± 13. Most of the cancers (145, 73.6%) were moderately differentiated, 23 (11.7%) well differentiated, 22 (11.2%) poorly differentiated, while one patient had undifferentiated cancer. Eight biopsies (4.1%) were not classified. Tumor sizes were available for 175 patients with a mean size of 5.2±0.2 cm (range 0–16.5). Adjuvant chemotherapy treatment (5-FU±LV in almost all cases) was given to 47 (23.9%) patients, while the rest (150 patients, 76.1%) were followed expectantly.

The mean length of follow up for all patients (197 total) was 48.1±2.3 months (0.1–133) and 56±3.3 (0.2–133) for survivors. There were 90 deaths (45.7%) recorded

Table 1 Results from the Cox regression Univariate analysis for survival

Variable	Categories	p-value	Hazard ratio	CI 95%
Age (years)	29-97	<0.001	1.06	1.03-1.08
Gender	Female (n =114)	0.410	0.84	0.56-1.27
	Male (n=83)			
Type of surgery	Total colectomy (n=14)	0.563 (for any differences by surgery type)	Not done	Not done
	Hemi colectomy (n=142)			
	Partial colectomy (n=38)			
	Local resection (n=3)			
Tumor stage	Stage 2Ac (n=51)	0.311	1.34	0.76-2.35
	Stage 2B (n=146)			
Adjuvant chemotherapy	5FU+/- LV (n=47)	0.056	0.58	0.33-1.03
	Follow-up expectantly (n= 150)			
Number of lymph nodes removed	>12	0.07	0.98	0.62-1.56
	<12			
Histological grade of tumor	Moderate differentiation (n=145)	0.77	0.93	0.58-1.51
	Well differentiated (n=23)			
	Poorly differentiated (n=22)			
	Undifferentiated (n=1)			

Hazard ratio (with 95% confidence interval and p-values) estimates the relative risk of death associated with each variable.

during the follow up. Only age exhibits a statistically significant relationship to the survival (HR=1.06, 95% CI=1.03–0.08, p<0.001) (Table 1).

Treatment with adjuvant chemotherapy showed definite trend toward superior survival with risk of mortality from colon cancer been reduced by 42% (HR=0.58, 95%CI=0.33-1.03). However, statistical significance did not reach previously defined level of confidence (p=0.056). Number of removed lymph nodes also showed possible relationship to the survival, although the results were not statistically significant (HR=0.98, 95%CI=0.33-1.03, p=0.07). Other analyzed factors ; Gender (HR=0.84, 95%CI=0.56-1.27, p=0.41, type of surgery (p=0.563 for any difference in all surgery types), stage of the tumor (HR=1.34, 95%CI=0.76-2.35, p=0.311), histological grade (HR=0.93, 95%CI=0.58-1.51, p=0.77) and the size of the tumor (HR=1.00, 95%CI=0.92-1.09, p=0.93) were not significant survival correlates. At 12 months overall PFS was 94%, 90% at 24 months and 78% at 60 months (Figure 1).

For progression free survival (disease free survival) only cases with a notation of "Disease Free" were considered to be free from progression. This is a conservative approach (but likely appropriate) as some cases marked as "Unknown" may well have been disease free. Subjects who died but whose deaths were determined to be unrelated to disease were censored at that point.

Overall survival (OS) for all patients was 90% at 12 months, 82% at 24 months and 56% at 60 months (Figure 2).

Patients more than 80 years old had 24 and 60 months survival of 66% and 33%, while at same time points, 92% and 78% of patients younger than 63 were still alive (Figure 3)

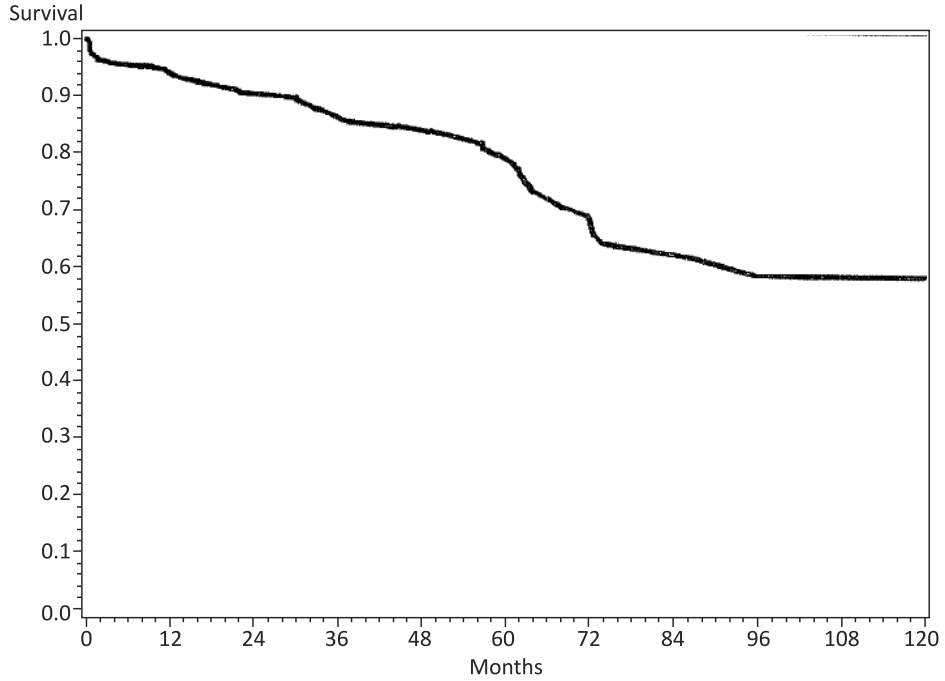

Figure 1 Progression Free Survival. Kaplan-Meier survival curve for 197 patients with Stage II colon cancer treated at Sparrow Hospital from 1996-2006.

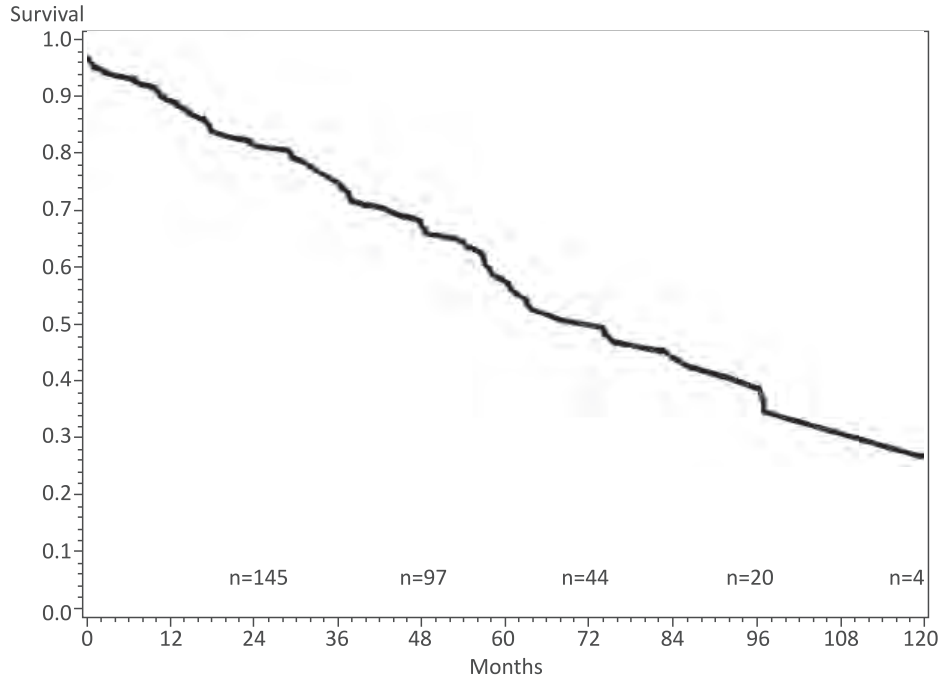

Figure 2 Overall Survival. Kaplan-Meier survival curve for 197 patients with Stage II colon cancer treated at Sparrow Hospital from 1996-2006. Numbers of patients remaining are indicated at 24 month increments.

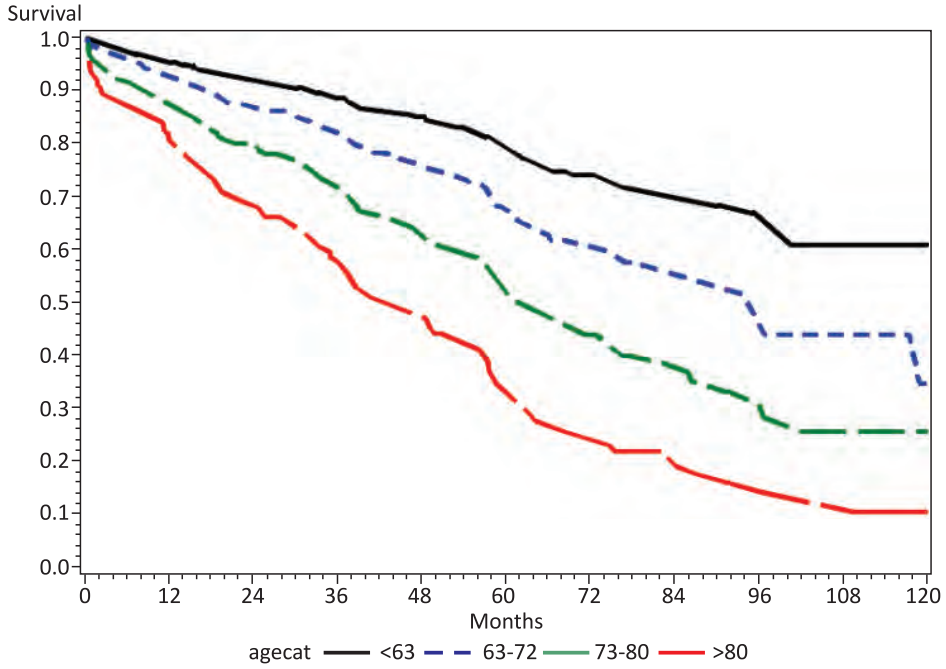

Figure 3 Overall Survival by Age. Kaplan-Meier survival curves for 197 patients with Stage II colon cancer treated at Sparrow Hospital from 1996-2007, stratified by age (<63, 63-72, 73-80 and >80).

Discussion

The role of adjuvant chemotherapy in completely resected, pathological stage II colorectal cancer is an important unresolved area in the clinical care of the cancer patient. It warrants further investigation of survival factors in this population. Randomized controlled trials and meta-analyses have uniformly failed to definitively detect a survival benefit for adjuvant chemotherapy in stage II colon cancer. These trials have included insufficient numbers of patients with stage II disease to ultimately determine whether adjuvant chemotherapy in this population is truly beneficial. Nonetheless, there remains no clinical or biological reason to believe that the clinical behavior of stage II tumors should be different from that of stage III tumors. Analysis of pooled data from 4 NSABP studies and IMPACT B report raised more questions than answers about adjuvant chemotherapy in these patients (1-3). In the IMPACT B2 meta-analysis of stage II subgroups from five adjuvant trials, non-significant trends for improvements in DFS (73% vs. 76%) and OS (80% vs. 82%) were reported (4, 5). A separate pooled-analysis of four consecutive NSABP adjuvant trials demonstrated consistent treatment benefits in both stage II and stage III patients, but interpretation was limited because of the heterogeneity of the treatments and control arms in the included trials (1). A differential magnitude of benefit was observed in a separate analysis done by Gill et al., with proportional reductions in risk of recurrence by 17% and death by 15% for node-negative disease as compared with 40% and 35%, respectively, for node-positive disease (10). A biologic explanation for a differential effectiveness of adjuvant FU-based therapy between node-negative and node-positive colon cancers has not been elucidated. It may be postulated that this effect is associated with Microsatellite instability (MSI), as a greater proportion of node-negative colon cancers observed have MSI-H phenotype (14). Survival of 23,017 patients with Stage II colon cancer from American College of Surgeon Commission on Cancer (CoC) National Cancer Database (NCD) showed that 87.7% were alive at 12 months, 80.1% at 24 months and 61.4% at 60 months (15). Data from this large database are very similar to the findings in our study with 90%, 82% and 56% survival at corresponding times. Observed 5-year survival rates for patients with stage II colon cancer from the same database diagnosed in 2000 were superior with adjuvant chemotherapy when compared to those who were observed (55.7% vs. 36.4%) (15-16). Although several statistically underpowered studies have not shown significant evidence of prolonged survival with adjuvant chemotherapy for patients with stage II colon cancer, a reduced risk of recurrence was seen retrospectively in the MOSAIC trial of adjuvant FOLFOX therapy for patients with high-risk stage II disease. Stage II colon cancer still presents a significant therapeutic challenge. Indeed, expert panels from both the National Cooperative Cancer Network (NCCN) and the American Society of Clinical Oncology (ASCO) have recommended that adjuvant therapy for stage II disease be considered and discussed with patients. Both panels strongly recommended that such treatment be given within clinical trials, when possible. In view of all these controversies more data is needed for patients with stage 2 colon cancer within and outside clinical trials setting.

Despite the limitation of small sample size, our study also showed a tendency toward better survival in the chemotherapy treated group, but the results remained statistically insignificant (p=0.056). In a separate univariate analysis done by Gill et al. (10), improvements with adjuvant chemotherapy did reach statistical significance for 5-year DFS (72% vs. 76%; p=0.0490), but did

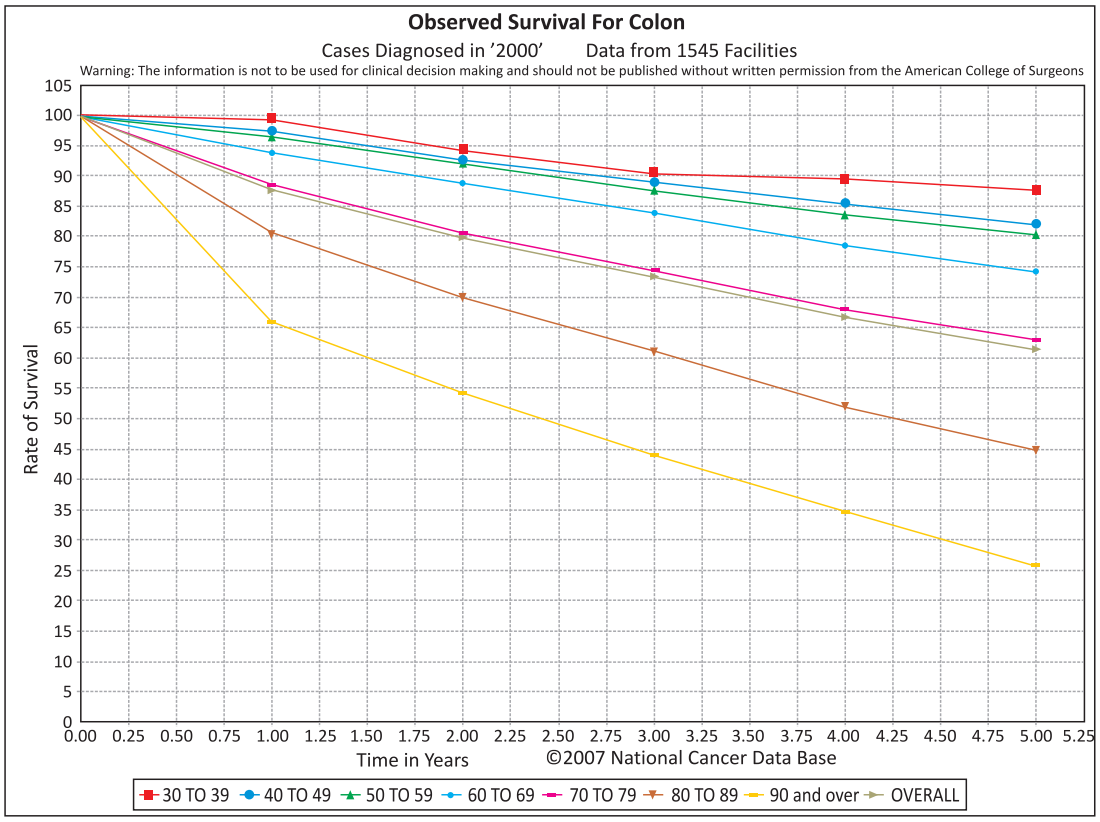

Figure 4 Observed survival in Stage II colon cancer patients diagnosed in 2000 from 1545 medical facilities from CoC Database and stratified by age (30-39, 40-49, 50-59, 60-69, 70-79, 80-89 and >90).

not for OS (80% vs. 81%; p=0.1127). Therefore, adjuvant chemotherapy in Stage II colon cancer still remains a viable option, but an OS benefit is not readily evident for all subgroups of patients.

Our study is based on the retrospective analysis of the data and as such it has limited power to predict prospective outcomes. It is also limited by the small sample size (197 patients) and the non-availability of all the literature reported risk factors. It is another, albeit small, contribution to the data examining the still unresolved question of the role of the adjuvant chemotherapy in the treatment of stage II colon cancer.

Our findings that age of the patients represents the most important prognostic survival factor in this disease are consistent with data from CoC NCD. There was evident difference in survival between age groups (Figure 4) in this large national database. Patients 80-89 years of age had 5-year OS of 45%, while patients older than 90 were alive after 5 years in only 25% of cases. Patients younger than 60 years old had 5-year OS between 80% and 92%, with the very young group (30-39 years old) having excellent survival of 92%. These survival numbers are comparable with the data provided in our study (33% for patients older than 80 and 78% for patients younger than 63) confirming that patients treated in our hospital can expect survival similar to that achieved at national level. In our study data showed that patients were 5% more likely to die during the follow-up period for each year of age when entering study (with confidence interval between 3% and 7%).

Our study did not confirm potential survival impact of any other risk factors

analyzed. In accordance with historical experience, size of the tumor did not impact survival, supporting practice not to include this factor into colon cancer staging schema. Number of evaluated lymph nodes was previously shown to be important prognostic factor in colon cancer (17). Common explanation was that increased number of lymph nodes give us more reliable staging (Stage II vs. III). Herein we observed the same trend (border line statistical significance) in patients with single stage II colon cancer. Possible explanation for these findings is that elimination of larger number of lymph nodes reduces potentially residual burden of disease.

There is still controversy in the literature whether the number of lymph nodes resected and evaluated impacts patient outcomes (17-26). When lymph node (LN) number is used as categorical variable (> or < than 12 LN removed) was entered into the Cox model, results were consistent with those seen using LN count as a continuous variable (HR=0.98, CI=0.62-1.56, p=0.07). Therefore, our study does not support 12 LN benchmark. We recommend re-evaluation of this specific benchmark.

In our study there was no association between the survival and the sex of the patient. The stage of the disease 2A vs. 2B did not reach statistical significance, but both the widespread confidence interval (0.76-2.35) and the hazard ratio of 1.34 probably indicates that the sample size for analysis was too small and may be the stage of the tumor in itself is not a reliable parameter for the survival analysis.

We did not have available advanced genetic test results such as Microsatellite instability or Colo print (27-32). These tests identify important markers of tumor biology and can serve as drivers of treatment decisions. This represents another limitation of this study. More data is still needed to identify markers capable of establishing which stage 2 patients will suffer from recurrence and which could potentially benefit from adjuvant treatment.

Conclusion

Our study despite its limitations of retrospective data collection and the small number of patients did show that adjuvant chemotherapy could potentially be beneficial for the survival of patients with Stage II colon cancer. In addition, the number of resected lymph nodes (>12) seems to represent a distinct survival factor not only in Stage III, but also possibly in Stage II disease. However, the most important factor for survival is still age of the patient. Finally, survival of patients with Stage II colon cancer at Sparrow Hospital parallels national statistics for the United States of America.

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Conflict of interest: The authors declare that they have no conflict of interest.

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The relationship of Bradykinin B_2 receptor gene variation with obesity, hypertension and lipid variables in obese patients

Nur Bakir[1], Hasan Mert Bozkuş[1], Meliha Koldemir Gündüz[1], Penbe Çağatay[2], Mustafa Taşkın[3], Belgin Süsleyici Duman[1]

[1]Marmara University, Faculty of Science and Arts, Department of Biology İstanbul, Turkey
[2]Istanbul University Istanbul Medical Faculty, Department of Biostatistic and Medical Informatics, İstanbul, Turkey
[3]Istanbul University Cerrahpasa Medical Faculty, Department of General Surgery Istanbul

Corresponding author:
Belgin Süsleyici Duman
Marmara University
Faculty of Science and Arts
Department of Biology
Göztepe-Istanbul, Turkey
belgin.susleyici@marmara.edu.tr
Tel.: + 9 0216 346 45 53
Fax.: + 9 0216 347 87 83

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Objective. This study examined the association of C-58T genotypes with obesity/hypertension related parameters and serum lipids in obese (n=108) and non-obese (n=80) patients. **Materials and methods.** Bradykinin receptor (B_2R) C-58T genotypes were determined by PCR–RFLP. **Results**. B_2R gene C-58T frequencies for T/T (homozygous wild type), T/C (heterozygous) and C/C (homozygous polymorphic) genotypes for obese and non-obese patients were respectively: 36.1%, 37.5%; 45.4%, 52.5% and 18.5%, 10%. Obese patients using diuretic medication had lower C/C genotype frequency compared to T/T and T/C genotypes. Total cholesterol (T-Chol) (p=0.035) levels were found to be associated with B_2R C-58T polymorphism, where the T/T genotype had higher total cholesterol levels compared to the T/C genotype in obese patients. Non-obese patients using oral antidiabetic medication had higher C/C genotype frequency than that of T/T and T/C genotypes. Waist circumference (p=0.016) and diastolic blood pressure (p=0.01) levels were elevated in the non-obese subjects with the C/C genotype compared to T/C and T/T. **Conclusion.** Although B_2R C-58T gene polymorphism was not found to be effective on obesity with logistic regression analysis in the whole study population in obese subjects, the T-Chol decreasing effect of the B_2R gene C allele and the higher waist circumference measurements in the non-obese subjects may indicate there may be a link between B_2R gene C-58T polymorphism and obesity in study populations of higher numbers.

Key words: B_2R C-58T polymorphism, PCR, Obesity, Serum lipids, Oral antidiabetic.

Introduction

Obesity is a multifactorial disease influenced by genetic and environmental factors (1, 2). The kallikrein-kinin system (KKS) has important regulatory roles in peripheral glucose utilization (3-5), insulin action, blood pressure and sodium regulation in the renal tubulus (6-9). It is hypothesized that the Renal-Bradykinin-System plays an important role in the development of hypertension (10). Infusion of bradykinin in the renal artery mediates the release of prostoglandins and nitric oxide (NO), following increased blood flow to the kidney, thus leading to di-

uresis and natriuresis (11, 12). Bradykinin and kallidin (Lys-bradykinin) are enzymatically cut and released via kallikreins during inflammation and related states (13-16). Moreover, the kinins have attendant increasing insulin sensitivity obtained with angiotensin converting enzyme (ACE) inhibitors, in both animal models and in humans with an insulin resistant condition (17, 18).

A study performed on different tissues of B_2R gene knockout mice, corresponds to a state similar to insulin resistance (19). B_2R acts by potentiating insulin-induction and bradykinin enhanced insulin- stimulated GLUT4 translocation from intracellular fraction, insulin-stimulated tyrosine phosphorylation of the insulin receptor and insulin receptor substrate-1, and B_2R enhances dephosphorylation of the insulin receptor (20). The bradykinin receptor has two subtypes, namely; bradykinin B1 (B_1R) and bradykinin B2 (B_2R) (9, 21-24). The bradykinin subtypes are categorized under G protein coupled receptor superfamily. B_2R is known to play predominant role in the KKS (25). B_2R is constitutively expressed in most tissues (14). B_2R exerts a protective role in hypertension and cardiovascular disease (25). Human B_2R has been proved to be candidate gene for essential hypertension and cardiovascular disease, whereas its exact role in obesity and type 2 diabetes mellitus (T2DM) still remains to be elucidated. Human B_2R genomic structure has been characterized (25). Four polymorphisms located in each of the 3 exons and 1 polymorphism located in the promoter region have been identified within the B_2R gene (26, 27). C-58T polymorphism in the B_2R gene is known to have contradictory effects against hypertension in different races, with a protective effect in Asians and Afro-Americans, but not in Caucasians (28).

The aim of the present study was to determine and compare the genotypic frequencies of B_2R gene C-58T polymorphism in obese and non-obese patients. The associations of C-58T polymorphism with obesity related phenotypes, blood pressure levels and medication were also studied.

Materials and methods

Study subjects

The study was performed between April 2012 and February 2014. Blood samples were collected from Istanbul University Cerrahpasa Medical Faculty, Department of General Surgery (Istanbul, Turkey) from obese and non-obese patients. A total of 108 obese (BMI ≥25) and 80 non-obese control individuals were included in the study. Subjects with secondary hypertension (renal artery stenosis, glomerulonephritis), diabetic nephropathy (Kimmelstiel-Wilson syndrome), hypertension with endocrinopathies (phoechromocytoma, Cushing syndrome, hyper and hypothyroidism), patients with pseudohypertension, neoplasia and those who were taking oral contraceptives and illicit drugs were not included in the study. All disease diagnoses were made by an expert endocrinologist from Istanbul University Cerrahpasa Medical Faculty, and medication usage information was taken from the hospital files. Height was measured in meters with a stadiometer, by measurement of the maximum distance from the floor to the highest point on the head, when the subject was facing directly ahead. The individual's shoes were removed, their feet were together, and arms by their sides. Heels, buttocks and upper back were also allowed to be in contact with the wall during height measurement. Weight measurement was performed using a calibrated scale while the individual was standing with minimal movement, with hands by their sides. Shoes and excess clothing were removed during weight measurement.

Obesity, T2DM and hypertension were diagnosed according to the International Diabetes Federation (IDF) guidelines (29). Body mass index is defined as the individual's body mass divided by the square of their height (kg/m^2). Body fat quantification:, first lean body mass (LBM) was calculated by the formula given by Hume (30): for males, LBM (kg): 0.32810 X weight (in kilograms) +0.33929 X height (in cm) -29.5336; for females (kg): 0.29569 X weight (in kg) +0.41893 height (in centimeters) -43.2933. Body fat was calculated by subtracting the lean body mass from the present body weight. For evaluation of arterial blood pressure, the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure guidelines were used (31). Metabolic syndrome (MS) patients met all the criteria defined by the American Heart Association's National Heart, Lung, and Blood Institute (NHLBI) (32). The MS criterion was a cluster of three or more of the following abnormalities: waist circumference >102 cm in men and >88 cm in women, serum triglycerides ≥1.7 mmol/L; high-density lipoprotein cholesterol (HDL-Chol) <1.03mmol/L in males and <1.29 mmol/L in females or specific treatment for this lipid abnormality (fibrates and nicotinic acid); blood pressure ≥130 / ≥85 mmHg or fasting serum glucose ≥5.6 mmol/L or drug treatment for hypertension or type 2 diabetes, respectively (32).

Biochemical measurements

Serum glucose was detected by the enzymatic reference method with glucose oxidase. HDL-Cholesterol and low-density lipoprotein-cholesterol (LDL-Chol) were directly determined by enzymatic colorimetric assay; serum total cholesterol was measured using the enzymatic, colorimetric method by cholesterol esterase; triglycerides were determined by the enzymatic colorimetric method (GPO/PAP) with cholesterol phosphate oxidase and 4-aminophenazone on an opeRA analyzer.

DNA extraction and genotyping

Genomic DNA was extracted from peripheral blood leukocytes using a salting out method (33). DNA concentration was measured spectrophotometrically. Absorbance ratios at 260nm and 280nm were used to assess the purity of DNA. Ratios over 1.8 were subjected to PCR analysis. Purified DNA (concentration of 50 ng) was stored at -20°C. The B_2R gene C-58T polymorphism was determined by the polymerase chain reaction (PCR) method, followed by restriction fragment length polymorphism (RFLP). The B_2R polymorphism studied was characterized by substitution of a thymine for cytosine at nucleotide position -58 in the promoter region (28). Since C-58T substitution does not change the recognition sequence for Mae III, a partial recognition site for Mae III was added as a single mismatched base in the sense primer for PCR amplification. The Mae III site was then completed in the presence of the -58C allele. The PCR primers were chosen to specifically target the human bradykinin gene covering B_2R polymorphism in the promoter/exon1 region. The PCR conditions were 30–50 ng genomic DNA, 0.2 units of Taq Polymerase (Fermentas), 0.5µl of 100 mol/l dNTPs, and 0.15 µl of 50 µmol/l primers in a 25µl reaction. The PCR cycling conditions were: 95°C 7 min; 35 x (94°C 20 sec, 55°C 20 sec, 72°C 20 sec), 72°C 10 min (28). The PCR products were restriction digested for 4 hr at 37°C. The C-58T primer sequences were as follows: left primer, 5'-GCCCAGGAGGCTGATGACGTCA-3'; right primer, 5'-TCACCAACCCTCCGGACCC-3'. Digestion was overnight with 5 units of Mae III (*Fermentas*). The PCR products were 110 bp

in length, producing 92 bp and 18 bp fragments after Mae III digestion. The digested products were evaluated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) for genotype analysis.

Ethics statement

The study was approved by the local Ethics Committee of Marmara University. All the subjects who contributed to the study gave informed consent prior to participating in the study.

Statistical analysis

Statistical analyses were performed using the SPSS 17.0 software program. Data were expressed as Median (Min-Max) IQR for numeric data. Data distribution testing was performed using the Shapiro-Wilks test. In the case of normal data distribution, one-way ANOVA was used for genotype comparisons (Table 4), followed by the Bonferroni test for statistically significant results for pairwise comparison. In the case of discrete data (Table 5) the Kruskal-Wallis test was used for comparison of genotypes, and afterwards for statistically significant parameters, the Bonferronni corrected Mann Whitney- U test was used for pairwise comparison. For the Bonferronni corrected Mann Whitney-U test, the limit for statistical significance was p=0.016. The categorical variables were expressed as a sample number (%). For categorical variables, c2 testing was used to assess differences in proportions (or Fisher's exact test when cell frequencies were small). The general significance level was $p<0.05$.

Results

The B_2R gene C-58T genotype frequencies in obese and non-obese study groups are presented in Table 1. The B_2R gene C-58T polymorphism frequencies for wild type homozygous (T/T), heterozygous (T/C) and homozygous polymorphic (C/C) genotypes were respectively: 36.1%, 45.4%, 18.5%, in the obese group, and 37.5%; 52.5% 10% in the non-obese group. The B_2R gene C-58T genotype frequencies did not differ significantly between the study groups (χ^2=2.749, p=0.253) and genotype frequency distributions did not obey the Hardy-Weinberg equilibrium (Table 1).

The disease data and the characteristics of the study population is given respectively in Table 2 and Table 3.

LDL-cholesterol, TG, T-cholesterol, glucose, systolic blood pressure, diastolic blood pressure, waist circumference, BMI, fat mass were significantly higher in obese compared to non-obese patients (Table 3).

The associations of B_2R gene C-58T genotypes in the obese group, with the analyzed biochemical and clinical parameters are presented in Table 4. The B_2R gene C-58T genotypes were not found to be associated with the analyzed phenotypes such as: weight, height, waist circumference, body mass in-

Table 1 Bradykinin C-58T polymorphism genotype frequencies in obese and non-obese subjects

Subjects	Bradykinin C-58T genotypes		
	Homozygous wild type n (%)	Heterozygous n (%)	Homozygous polymorphic n (%)
Obese	39 (36.1)	49 (45.4)	20 (18.5)
Non-Obese	30 (37.5)	42 (52.5)	8 (10)

There were no significant differences between the groups (χ^2=2.749, p=0.253).

Table 2 Disease data of the study population

Diseases	Subjects	
	Obese, n (%)	Non-Obese, n (%)
Hypertension	51 (47.2)	12 (15)
Type 2 Diabetes Mellitus	54 (50)	12 (15)
Dyslipidemia	26 (24.1)	13 (16.2)

Table 3 Characteristics of the study population

Characteristics	Subjects						p
	Obese (n=108)			Non-Obese (n=80)			
	Median	Min-Max	IQR	Median	Min-Max	IQR	
Age (years)	61.0	44.0-82.0	14.0	56.0	25.0-90.0	13.5	0.031
Weight (kg)	80.0	50.0-120.0	17.0	68.0	50.0-108.0	16.0	0.001
Height (m)	1.6	1.4-1.8	0.1	1.6	1.5-1.9	0.1	0.004
LBM (kg)	47.0	34.3-69.2	9.9	47.1	34.3-66.6	7.9	0.723
FM (kg)	30.3	15.7-50.8	9.4	20.0	10.2-44.8	12.1	0.0001
BMI (kg/m^2)	31.1	22.2-42.3	5.7	24.6	20.0-42.9	6.8	0.0001
Waist (cm)	101.5	72.0-125.0	12.7	86.0	67.0-130.0	28.2	0.0001
T-Chol (mmol/l)	5.5	3.2-7.6	75.0	4.6	1.3-7.9	53.9	0.040
TG (mmol/l)	1.5	0.6-4.3	65.1	1.2	0.6-3.4	42.5	0.018
HDL-Chol (mmol/l)	1.2	0.6-2.0	18.3	1.2	0.6-2.1	18.6	0.518
LDL-Chol (mmol/l)	3.1	0.6-5.7	81.0	2.3	0.7-9.5	72.8	0.007
Glucose (mmol/l)	7.4	4.3-21.2	116.0	4.5	2.2-16.5	55.2	0.0001
SBP (mmHg)	150.0	120.0-220.0	20.0	125.0	100.0-180.0	25.0	0.0001
HbA1c (%)	8.2	4.9-13.8	4.6	6.2	4.9-11.3	2.4	0.181
DBP (mm Hg)	90.0	65.0-110.0	10.0	75.0	60.0-120.0	10.0	0.0001

BMI=Body mass index; LBM=Lean body mass; FM=Fat mass; T-Chol=Total cholesterol; HDL-Chol=High-density lipoprotein; LDL-Chol=Low-density lipoprotein; TG=Triglyceride; SBP=Systolic blood presure; DBP=Diastolic blood presure.

Table 4 Association of bradykinin C-58T genotypes with various phenotypes in the obese group

Characteristics	Bradykinin genotypes									p
	Homozygous wild type (n=39)			Heterozygous (n=49)			Homozygous polymorphic (n=20)			
	Median	Min-Max	IQR	Median	Min-Max	IQR	Median	Min-Max	IQR	
Weight (kg)	80.0	60.0-105.0	19.0	80.0	60.0-110.0	19.0	79.0	(65.0-120.0)	17.0	0.903
Height (m)	1.6	1.4-1.8	0.1	1.6	1.4-1.8	0.2	1.6	(1.5-1.8)	0.1	0.466
Waist (cm)	102.0	74.0-130.0	18.0	102.0	71.0-125.0	19.0	100.0	(72.0-120.0)	16.0	0.814
BMI(kg/m^2)	29.5	25.1-42.9	6.8	30.0	22.0-42.3	6.4	30.4	(25.4-39.2)	5.6	0.953
LBM (kg)	48.8	25.8-61.1	9.6	47.0	38.6-69.3	11.0	49.5	(40.2-69.2)	11.9	0.415
FM (kg)	29.0	20.6-51.1	13.6	30.3	13.9-48.9	9.9	29.7	(21.6-50.8)	11.0	0.923
T-Chol (mmol/l)	5.8	3.8-8.8	2.1	4.6	3.2-7.1	1.7	5.4	(3.7-7.0)	1.9	0.035
TG (mmol/l)	1.5	0.8-3.4	1.2	1.4	0.6-4.3	0.8	1.4	(0.6-2.6)	0.9	0.514
HDL-Chol (mmol/l)	1.1	0.9-2.1	0.6	1.2	0.6-1.9	0.4	1.1	(0.6-1.6)	0.6	0.521
LDL-Chol (mmol/l)	2.1	0.8-7.2	2.3	2.7	0.6-4.6	2.0	3.3	2.1-4.7	1.5	0.229
Glucose (mmol/l)	4.5	3.0-16.6	4.5	6.1	3.2-9.6	5.3	5.6	4-2.17.0	4.0	0.194
SBP (mmHg)	150.0	100.0-180.0	38.0	140.0	110.0-220.0	34.0	140.0	(120.0-170.0)	30.0	0.433
DBP (mmHg)	85.0	60.0-120.0	10.0	82.5	65.0-110.0	18.0	90.0	(70.0-100.0)	10.0	0.867

IQR=Inter quartal range; BMI=Body mass index; LBM=Lean body mass; FM=Fat mass; T-Chol=Total cholesterol; HDL-Chol=High-density lipoprotein; LDL-Chol=Low-density lipoprotein; TG=Triglyceride; SBP=Systolic blood presure; DBP=Diastolic blood presure.

dex (BMI), lean body mass (LBM), fat mass (FM), triglycerides (TG), high density lipoprotein-cholesterol (HDL-chol), low density lipoprotein-cholesterol (LDL-chol), systolic blood pressure (SBP), diastolic blood pressure (DBP) in obese patients (Table 4).

B_2R gene C-58T polymorphism was found to be associated with T-Chol (p=0.035) in

the obese patients (Table 4). The paired comparison of bradykinin C-58T genotypes for T-chol levels in the obese group showed that higher T-Chol levels in the T/T genotype existed in comparison to the T/C genotype (p=0.01) by the Bonferronni test (data not included, data normally distributed). The differences for T-Chol in the obese group and systolic blood pressure and waist measurement in the non-obese group between bradykinin C-58T genotypes occur mostly between the CC genotype and the others (TT and TC). Especially in the non-obese group the small number of CC genotype carriers is a limitation and may influence the magnitude of the significant association detected.

The asssociations of B_2R gene C-58T genotypes in the analyzed biochemical and clinical parameters in the non-obese group are presented in Table 5. B_2R gene C-58T polymorphism was not found to have any significant relation to serum lipids in the non-obese study group by the Kruskal Wallis test, since the data were discrete. The waist circumference (p=0.016) and diastolic blood pressure (DBP) (p=0.010) measurements were significantly higher in the C/C genotype carrying non-obese patients in comparison to T/T and T/C genotype carriers (Table 5). The paired comparisons of B2R gene C-58T genotypes for SBP levels in the non-obese group showed them to be significantly higher in the C/C genotype carrying non-obese patients than those of T/T (p=0.002) and T/C genotype (p=0.003) using the Bonferronni corrected Mann-Whitney U test (data not given). The paired comparison of bradykinin C-58T genotypes for DBP levels showed them to be significantly higher in the C/C genotype carrying non-obese patients in comparison to those with the T/C genotype with Bonferronni corrected Mann-Whitney U test (p=0.002) (data not included). The paired comparison of bradykinin C-58T genotypes for the waist circumference measurements in non-obese group showed them to be higher in the C/C genotype carrying non-obese patients in comparison to T/T (p=0.011) and T/C genotypes (p=0.009) using the Bonferronni corrected Mann-Whitney U test (data not in-

Table 5 Association of bradykinin C-58T genotypes with various phenotypes in the non-obese group

Characteristics	Bradykinin genotypes									p
	Homozygous wild type (n=39)			Heterozygous (n=49)			Homozygous polymorphic (n=20)			
	Median	Min-Max	IQR	Median	Min-Max	IQR	Median	Min-Max	IQR	
Weight (kg)	65.0	45.0 -80.0	8.0	62.5	41.0-86.0	15.0	64.0	50.0-67.0	5.0	0.495
Height (m)	1.7	1.5-1.8	0.1	1.6	1.5-1.9	0.2	1.6	1.5-1.7	0.1	0.291
Waist (cm)	72.0	67.0-100.0	15.0	74.5	67.0-94.0	11.0	87.0	78.0-106.0	12.0	0.016
BMI (kg/m^2)	22.8	18.7-45.0	1.9	22.7	17.7-45	2.3	23.6	22.2-25.0	2.0	0.356
LBM (kg)	47.4	37.8-58.5	5.1	44.6	32.5-57.8	12.3	46.4	34.3-48.1	8.7	0.410
FM (kg)	17.3	7.2-25.0	4.9	17.3	7.5-31.4	5.0	16.9	15.2-20.6	3.2	0.999
T-Chol (mmol/l)	4.8	3.0-5.9	1.7	4.5	1.3-8.0	1.3	ISN	-	-	
TG (mmol/l)	1.1	0.8-1.4	0.5	1.1	0.9-3.0	0.3	ISN	-	-	
HDL-Chol (mmol/l)	1.3	1.0-1.7	0.3	1.3	0.7-1.8	0.5	1.3	1.1-1.7	0.5	0.885
LDL-Chol (mmol/l)	1.1	0.7-4.1	2.4	1.8	0.8-9.5	1.7	3.2	1.4-3.4	1.1	0.216
SBP (mmHg)	120.0	100.0-140.0	13.0	120.0	100.0-160.0	15.0	145.0	130.0-170.0	25.0	0.007
DBP (mmHg)	70.0	60.0-90.0	15.0	70.0	60.0-90.0	10.0	85.0	80.0-100.0	13.0	0.010

IQR=Inter quartal range; BMI=Body mass index; LBM=Lean body mass; FM=Fat mass; T-Chol= Total cholesterol; HDL-Chol=High-density lipoprotein; LDL-Chol=Low-density lipoprotein; TG=Triglyceride; SBP=Systolic blood presure; DBP=Diastolic blood presure; ISN=Inadequate sample number.

cluded). The mean ± SE; median (min-max) values are not given for T-chol and TG due to the absence of the data (Table 5).

The frequency of Bradykinin C-58T genotypes for different medications in obese patients are represented in Table 6. Obese patients using diuretic medication were found to have T/T genotype in higher freqency than those with T/C and C/C genotypes (Table 6). There was no signifcant difference between genotype groups for any other medication used by the obese patients (Table 6).

The frequencies of Bradykinin C-58T genotypes for different medications in

Table 6 The frequencies of Bradykinin C-58T genotypes for different medications in obese patients.

Medication	Bradykinin C-58T Genotypes			
	T/T	T/C	C/C	p
	n (%)	n (%)	n (%)	
Diuretic	13 (61.9)	14 (38.9)	3 (20)	0.038
ACE	4 (20)	10 (30.3)	4 (26.7)	0.720
BB	12 (57.1)	18 (50)	8 (53.3)	0.872
Nitrit	2 (9.5)	7 (19.4)	0 (0)	0.180
ASA	9 (42.9)	18 (50)	7 (46.7)	0.872
ARB	1 (4.8)	3 (8.3)	2 (13.3)	0.730
CCB	2 (9.5)	2 (5.6)	4 (26.7)	0.110
Oral antidiabetic	10 (47.6)	20 (55.6)	9 (56.3)	0.818
Sulphonylurea	1 (4.8)	5 (13.9)	5 (33.3)	0.073
Glinide	5 (23.8)	14 (38.9)	6 (40)	0.458
Metformin	8 (38.1)	13 (36.1)	1 (6.7)	0.067
Insulin	4 (19)	10 (27.8)	17 (23.6)	0.076
Statin	8 (38.1)	16 (44.4)	3 (20)	0.259

T/T= Homozygous wild type; T/C= Heterozygous; C/C= Homozygous polymorphic; ACE=Angiotensin converting enzyme inhibitor; BB=Beta blocker; ASA=Acetyl salicylic acid; ARB=Angiotensin II receptor blocker; CCB=Calcium Channel Blocker.

Table 7 The frequencies of Bradykinin C-58T genotypes for different medications in non-obese patients

Medication	B2R C-58T genotypes			p
	T/T n (%)	T/C n (%)	C/C n (%)	
Beta blocker	1 (7.1)	4 (16)	1 (14.3)	0.840
Oral antidiabetic	3 (21.4)	5 (20)	3 (42.9)	0.045
Statin	1 (7.1)	1 (4)	3 (42.9)	0.032

T/T=Homozygous wild type; T/C=Heterozygous; C/C=Homozygous polymorphic.

Table 8 Identification of risk factors for their association with obesity by multiple logistic regression analysis

Risk factor	All Subjects			
	B	SE	OR	p
B2R C-58T T/T Genotype	-	-	-	0.226
B2R C-58T T/C Genotype	-1.098	0.647	0.333	0.089
B2R C-58T C/C Genotype	-0.933	0.841	0.394	0.268
Dyslipidemia	-1.416	0.738	0.243	0.055
Type 2 Diabetes Mellitus	0.653	0.601	1.920	0.278
Hypertension	0.415	0.629	1.514	0.510

B=indicates estimated coefficient; SE=standard error; OR=adjusted odds ratio.

non-obese patients are given in Table 7. Non-obese subjects using oral antidiabetics (p=0.045) and statin (p=0.032) were found to have the C/C genotype in higher frequencies than T/T and T/C genotypes (Table 7).

Risk factors associated with obesity such as: T2DM, dyslipidemia, hypertension and bradykinin C-58T polymorphism were evaluated using logistic regression analysis (Table 8). The bradykinin C-58T genotypes were not found to be independent progressive or regressive factors related to obesity (Table 8).

Discussion

According to our knowledge, this is the first study evaluating the relationship between B2R gene C-58T variation and obesity in Turkish subjects. Fallo et al. (9) reported B_2R C-58T polymorphism with 21.7% C/C, 51.1% C/T and 27.2% T/T genotype frequency distributions in obese patients. Other studies evaluating the effects of the same polymorphism have mostly been performed in essential hypertension patients. In detail, Mulatero et al. (28) found the C/C, C/T and T/T genotype frequencies respectively to be: 32.3%, 49.1%, 18.6% in hypertensive primary aldosteronism patients. A study performed on 200 Japanese individuals (100 hypertensive, 100 normotensive) reported B_2R C-58T genotype frequencies, where Mukae et al. (34) found the hypertensive and normotensive frequencies to be respectively: 28% and 18% for C/C, 59% and 57% for C/T, 13% and 25% for T/T. Fu et al. (35) analyzed 275 hypertensive and 441 normotensive patients for the effects of B_2R C-58T variation on essential hypertension. The hypertensive and normotensive genotype distributions were found to be respectively: 24% and 22% for C/C, 51% and 52% for C/T, 25% and 26% for T/T (35). Fu et al. (35) were not able to find any association between B_2R C-58T variation and essential hypertension. C-58T polymorphism is located at position -58 of the B_2R gene promoter.

The presence of -58C allele results in a decrease in gene transcription (27). B_2R is a candidate gene in the pathogenesis of insulin resistance and is often related to other diseases in metabolic syndrome (36, 37). C-58T polymorphism has been found to be related to bradykinin activity as a vasodilator in a limited number of studies (24, 38). In our study group, the frequencies of hypertensive patients were respectively 46% and 15% within the obese and non-obese groups. The B_2R gene C-58T frequencies observed in our study were similar to the results of Fallo et al. (9) and Fu et al. (35), where the polymorphic genotype frequencies were higher in obese versus non-obese subjects (9, 35). Despite a trend in our non-obese patients towards increased diastolic ($p<0.01$) and systolic ($p>0.05$) blood pressure values across genotypes, with the highest values in C/C and lowest in T/T, the lack of significant differences in obese patients does not allow the confirmation of our data. Insulin resistance is a predominant factor leading to T2DM, dyslipidemia and hypertension (39). As previously mentioned, insulin resistance may not necessarily be associated with an increase in LDL-cholesterol levels, but rather with a combination of elevated levels of other serum lipids (32, 40, 41). A close relationship between insulin resistance and hypertension has also been established in some studies (39, 41, 42, 43). Approximately half of all patients with essential hypertension are known to be insulin-resistant (44). Barros et al. (45) showed that genetically obese mice (ob/ob) lacking the B_2R gene (obB_2KO) showed increased fasting glycemia, hyperinsulinemia and impaired glucose tolerance compared to ob/ob control mice (obWT) which indicates

the presence of insulin resistance and impaired glucose homeostasis (45).

Researchers have shown that mutant mice lacking B2Rs display a moderate rise in basal blood pressure, but under a heavy sodium diet they showed heavy hypertension and end-organ damage (46, 47). A recent meta-analysis of B_2R gene C-58T polymorphism with hypertension suggested that the T allele exhibits a protective effect on hypertension in Asians and Afro-Americans, but not in Caucasians. Mulatero et al. (28) analyzed the effects of B_2R gene C-58T genotypes on BMI, and found insignificantly lower levels in T/T carriers, than those with variant and heterozygous genotypes (28). In our study, we detected non-significantly higher BMI measurements in those with T/T genotypes only in the non-obese group. Additionally we also detected lower measurements of waist circumference in non-obese patients with the T/T genotype of the B_2R gene. Two study groups investigated the B_2R gene C-58T variant C allele that increased both systolic and diastolic blood pressures in hypertensive patients in comparison to the wild type allele (28, 34). We observed that C/C genotype carriers have higher diastolic blood pressure levels, and the decreasing effect of T/T genotypes over diastolic blood pressure in non-obese groups, in accordance to the results of Mulatero et al. (28), and Mukae et al. (34), which was reported in hypertension study groups.

The higher frequency of B_2R gene C-58T T/T genotype frequency in obese diuretic users may be due to the relatively high frequency (46%) of hypertension in the obese group. Additionally, polymorphic C/C genotype frequency was observed to be higher in the non-obese patients using oral antidiabetics compared to the T/T and C/T genotypes. None of the risk factors such as: hypertension, type 2 diabetes, dyslipidemia and B_2R gene C-58T genotypes were found as independent risk factors for obesity when tested by logistic regression analysis. Among the analyzed serum lipids, in the obese group only T-Chol levels were found to be associated with B_2R C-58T polymorphism, where T/T genotype patients had higher T-Chol measurements than those of the T/C genotype.

The relatively small number of the study size, together with the low number of CC genotype carriers in the non-obese group limit us by rather low statistical power to determine any association of C-58T polymorphism with obesity or to detect any significant difference or interactions between other parameters.

Conclusion

In conclusion, while our results need to be confirmed in a more representative, large scale population, B_2R C-58T gene polymorphism was not found to be effective on obesity with logistic regression analysis in the whole study population. In the obese subjects, the T-Chol decreasing effect of the B_2R gene C allele and the higher waist circumference measurements in the non-obese subjects may indicate there may be a link between B_2R gene C-58T polymorphism and obesity in study populations with higher numbers.

Authors' contributions: Conception and design: BSD, MT; Acquisition, analysis and interpretation of data: NB, HMB, MKG, PÇ, MT, BSD; Drafting the article: NB, MKG, BSD; Revising it critically for important intellectual content: BSD, MT.

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Original professional article

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Only a minority of patients in the urological emergency unit need urgent urology care

Žana Saratlija Novaković, Davor Librenjak

Department of Urology, University Hospital Split, Split, Croatia

Corresponding author:
Žana Saratlija Novaković
Department of Urology
University Hospital Split
Šoltanska 1, 21000 Split
Croatia
saratlijanovakovic.zana@gmail.com
Tel.: + 385 21 557 775
Fax.: + 385 21 464 554

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Objective. To present patients who were examined, monitored and admitted at the urological emergency unit (UEU) at the University Hospital, Split during the summer and winter of 2010 and to establish who of them were really in need of immediate urological care. **Methods.** A retrospective study of patients and diagnoses of patients examined at the UEU was undertaken during two winter and two summer months 2010. We compared the total number of patients, the number of patients with urological issues, patients with urological emergencies, patients with non-urological issues, patients who were briefly monitored at the UEU, and patients admitted to the urology department, within these two periods. Descriptive statistic and chi squared tests were used. **Results.** During the winter period 465 patients were examined at the UEU and during the summer 733 patients. During the summer period there were statistically more urological issues (χ^2=12.3; p=0.005) and urological emergencies (χ^2=4.14; p=0.042) while in the winter period there were more non-urological issues and more patients were monitored at the UEU (χ^2=33.9; p<0.001). The most common diagnoses are: renal colic and urine retention, in both periods. Only 8% of patients in both the winter and summer periods were admitted to hospital after examination at the UEU, which represents the actual number of patients who needed immediate urological care. **Conclusion.** Of all the patients examined at the UEU, only a fraction constituted real, life-threatening urological emergencies. Primary care physicians and general emergency departments should be more educated in urological emergencies so that they can resolve more non-emergency patients themselves.

Key words: Emergency urology, Renal colic, Urine retention.

Introduction

The urology emergency unit (UEU) should be managing patients with appropriate diagnoses. Before coming to the UEU, every patient should be examined by a primary care physician or emergency medicine physician. These physicians should provide immediate care to patients, such as catheterization of the urinary bladder, or give analgesic therapy for renal colic (1, 2). Only a fraction of urology patients have life threatening conditions, which require immediate urologic intervention (2). Some urological emergencies have seasonal variations, and in different parts of the world different physicians

or other medical staff deal with emergency urological issues (3-5).

The overall number of emergency admissions to hospital has increased over recent years, exerting a significant strain on the ability of many hospitals to undertake elective work. Lower thresholds for referral to hospital and the greater tendency of patients to present themselves to emergency departments with 'surgical' problems are likely to be significant factors in the increase of the burden on hospital departments (6).

Therefore we decided to investigate how many patients at UEU really do need urological intervention, how many of them need hospital admission or are monitored for a few hours at the UEU. We were also interested in differences between the winter and summer periods in the structure of UEU patients according to their discharge diagnosis.

Methods

A retrospective study of urological emergency department admissions was conducted. The number of patients and diagnoses of patients examined at the UEU were analyzed during two winter months (January-February 2010) and two summer months (July-August 2010). The working hours of this department are 4 pm to 8 am. Diagnoses upon discharge were noted for each patient examined at the UEU within these periods. Patients with polytrauma, patients examined during regular the Urology Department working hours of 8 am – 4 pm, and patients examined in other hospital departments were not included in this analysis. The UEU is located in the University Hospital. A urologist works in this unit with or without a resident.

We collected patient data, analyzed how many of them had urological issues, which of them were really an emergency, how many patients were monitored at the UEU, and how many of them were hospitalized in our department for further evaluation and treatment. After that we compared the data between the two months in the winter and summer periods.

Statistical analyses

Data were analyzed using the program Statistica 7.0 (StatSoft Inc, Tulsa, OK, USA). Descriptive statistics were used where appropriate. Additionally, we used the chi squared test. The significance level was set at $p<0.05$.

Results

At the UEU of the University Hospital Split, 465 patients were examined during January and February 2010 and 733 patients during July and August of the same year (Table 1). About 30% of patients were not examined in an emergency medicine unit or by a primary care physician before presenting at the UEU. We also analyzed the number of urological diagnosis, non-urological diagnosis and urological emergencies throughout these months (Table 1).

Significantly more patients with urological issues presented at the UEU during the summer period (95% vs. 89%) than during the winter period ($\chi^2=12.3$; $p=0.005$). Also, during the summer period, there were significantly more urological emergencies (76% vs. 70%) than during the winter period ($\chi^2=4.14$; $p=0.042$). Patients came to the UEU without evident urological issues during the winter period 2.2 times more frequently (11% vs. 5%) than during the summer period.

Some patients were monitored in the UEU for a few hours after examination. Short-term monitoring was indicated in these patients because they had received analgesics, or they were waiting for the results of diagnostics. In the winter period there

Table 1 Distribution of patients at the urology emergency unit

Patients	Time of the year, 2010	
	January and February	July and August
Total (n)	465	734
Urological issues, n (%)	415 (89.3)	695 (94.7)
Urological emergencies, n (%)	291 (70.1)	526 (75.7)
Non-urological issues, n (%)	50 (10.8)	38 (5.2)

were 174 (37%) monitored patients, and 161 (22%) such patients in the summer period. During the winter period we monitored 1.7 times more patients at the UEU than during the summer (χ^2=33,9; p<0.001), (Figure 1).

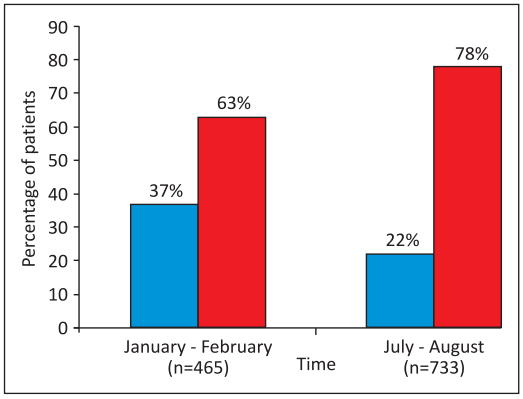

Figure 1 The percentage of patients monitored in the UEU after initial examination. The blue bars denote the percentage of monitored patients, and the red bars indicate patients who were discharged from the UEU after examination.

The most common urological emergencies treated at the UEU in the analyzed periods were renal colic, urine retention, hematuria and acute prostatitis (Table 2).

There was no significant difference between the number of patients according to their final diagnosis and the time of year when they were examined (χ^2=4.18; p=0.309), although the absolute values of patients presenting with renal colic, urine retention, hematuria, acute prostatitis and obstructive uropathies were higher during the summer period (Table 2).

Among all the patients only 57/733 (7.8%) were admitted to the urology department for further evaluation and treatment during the summer period, and 38/465 (8.2%) during the winter period. There was no difference in frequencies in diagnoses between these two groups (χ^2=0.06; p=0.799).

Table 2 Urological emergencies treated at the urology emergency unit

Urologic emergencies	Number of patients during the specified time of year 2010	
	January and February 291/465	July and August 526/734
Renal colic	133	202
Urine retention	66	127
Hematuria	29	55
Acute prostatitis	27	60
Obstructive uropathies	16	34
Complicated uroinfections	6	10
Minor genital lesions	4	15
Renal trauma	4	15
Testis torsion	4	5
Paraphimosis	2	3

Discussion

The daily work of the urological profession in our hospital certainly includes many "urgent" patients encountered in the urological emergency unit. Most patients present with two main conditions: renal colic and urinary retention. Uncomplicated renal colic (90%) and most cases of retention should be resolved by emergency physicians or primary care physicians (2).

Our results show that this is not the case in our setting. Renal colic and urinary retention were the two most common diagnoses with which patients presented at our UEU, which is located in a university hospital. The frequencies of diagnoses in our study were comparable to the results of previous studies in urological emergency care units (6, 7). In our study, only 8% of patients in both the winter and summer periods were admitted to hospital after examination at the UEU. A case study at a French university teaching hospital showed that 15.6% of patients at their UEU were admitted to the hospital. Diagnoses encountered at the French UEU did not include a high number of renal colics, as in our case, which means perhaps that the patients seen at their UEU were highly selected before being referred to the UEU (8).

We very rarely encountered patients at the UEU who had previously received any analgesics for renal colic or patients in whom somebody had attempted catheterization for urinary retention. This makes us wonder what went wrong with the previous steps in patient care – i.e. the primary care physician and general emergency department. The question for our health care system is how can we promptly help our patients and save costly resources.

A significant number of patients were referred to the UEU without having evident urological symptoms. Our hospital is in a tourist area so we noticed a larger number of emergency patients during the summer period, but during the winter period we diagnosed more patients at our UEU without urological issues. We monitored more patients during the winter period at the UEU, for which we do not have a plausible explanation. The number of patients examined at the UEU and then admitted to our department for further treatment did not differ between the summer and winter. Office based physicians increasingly rely on specialised emergency departments to evaluate complex patients with potentially serious problems, rather than managing these patients themselves.

Strategies are needed to contain emergency-department attendance. Quality of care in general practice might influence the use of emergency departments, including management of patients with chronic conditions and access to consultations. A study by Baker et al. showed that satisfaction with telephone access to primary physicians may predict rates of attendance at emergency departments. Consideration should be given to improving access to some general practices to reduce the use of emergency care resources (9).

In this setting, interventions, such as education and implementing protocols for primary care physicians, which would prevent unnecessary specialist referrals, would be beneficial.

Conclusion

Indeed, only a minority of patients at the UEU need urgent urology care, suggesting the need for changes in organization when treating such patients. Seasonal variations between summer and winter periods due to the overall number of patients, urological emergencies and non-emergencies were as we expected.

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Original professional article

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Congenital cardiac anomalies in myelomeningocele patients

Iman Moeini Naghani[1], Taraneh Hashemi Zonouz[1], Shima Shahjouei[1], Amir Azar Homayoun[2], Farideh Nejat[1], Mostafa El Khashab[3]

[1]Department of Neurosurgery, Children's Hospital Medical Center, Tehran University of Medical Science, Tehran, Iran
[2]Department of Neurosurgery, Sina Hospital, Tehran University of Medical Science, Tehran, Iran
[3]Department of Neurosurgery, Hackensack University Medical Center, New Jersey, US

Corresponding author:
Farideh Nejat
Mailbox: Tehran, F. Nejat, 14155-7854
nejat@sina.tums.ac.ir
Tel: +98 21 66420098
Fax.: + 98 21 66930024

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Objective. Myelomeningocele may be isolated but more frequently is associated with other anomalies. Congenital heart disease occurs with different incidence rate in myelomeningocele which is observed more frequently with skeletal malformations. **Methods**. This study was undertaken in the Children's Hospital Medical Center between 2010 to 2012 to evaluate 75 myelomeningocele patients for cardiac anomalies, with electrocardiography and echocardiography in addition to clinical examination of the cardiopulmonary system. Demographic information, myelomeningocele location and characteristics, orthopedic deformities, neurological deficits and radiographic findings were studied besides cardiologic assessments. **Results**. The ages of the patients ranged from 1 day to 4 years. The myelomeningocele locations were lumbosacral, lumbar and sacral area in most cases. Physical examination of the heart was abnormal in 6 children, but echocardiography revealed cardiac anomalies in only two children. Both children were female patients with severe scoliosis, multiple rib deficiencies and associated vertebral anomalies. **Conclusion**. Congenital heart defects are not very common in MMC patients. Female patients with suspicious clinical examinations for cardiac anomalies and associated rib and vertebral anomalies are advised to be investigated by echocardiography to rule out associated cardiac anomalies.

Keywords: Cardiac abnormality, Myelomeningocele, Screening, Echocardiography.

Introduction

Myelomeningocele (MMC) is a common type of spina bifida, which is frequently associated with hydrocephalus and Chiari-malformation. Sometimes other anomalies, such as anorectal malformations, skeletal abnormalities involving the spinal column or ribs, genitourinary anomalies and congenital heart diseases, are found with MMC (1). Genetic factors, folate deficiency, radiation and gestational medication exposures are suggested etiological factors (2). The clinical features and outcome of MMC patients depend on the neurological impairment and the severity of concomitant malformations (3, 4). Most gross anomalies in MMC patients are detected by prenatal ultrasound evaluation, at birth or soon after that, but anomalies involving the internal organs, such as kidneys, gasterointestinal tract and, rarely, the heart, might be undetected (5).

Congenital heart disease is one of the leading causes of morbidity in childhood. Atrial septum defect (ASD), ventricular

septum defect (VSD) and tetralogy of Fallot (TOF) are the three most common causes of cardiac defects found in infants with MMC. Apart from ASD, most congenital cardiac anomalies are detected in early childhood (6). Congenital heart defects in MMC patients have been observed more frequently in association with skeletal malformations, including scoliosis, hemivertebrae, multiple rib anomalies and chest wall asymmetry (7-9).

Here we report the cardiological screening of our MMC patients over a short period of time and present the incidence and type of congenital heart defects.

Methods

This study was undertaken at the Children's Hospital Medical Center, Tehran, between March 2010 and April 2012. As a routine protocol, all patients admitted to the neurosurgical department with diagnosis of MMC are investigated for accompanying intracranial and urogenital anomalies, such as hydrocephalus and neurogenic bladder. According to the findings of the physical examination, further paraclinical assessments might be ordered. In this study, we added a more careful evaluation of the cardiopulmonary system, including electrocardiography (ECG) and echocardiography. Demographic information, MMC location and characteristics, prenatal diagnosis, orthopedic deformities, neurological status and radiographic findings (brain CT or MRI to diagnose ventriculomegaly and Chiari malformation) were recorded beside cardiologic assessments. All patients were enrolled in this study after obtaining informed consent.

Results

Seventy-five patients (41 boys and 34 girls) were consecutively enrolled in the study. The age at admission ranged from 1 day to 4 years (mean age 7 months). The children were the products of uneventful pregnancies from healthy parents. All except for five patients were full-term babies. Preterm babies were born at 27 to 33 weeks of gestational age.

Unfortunately MMCs were diagnosed in only 21 children during prenatal ultrasound evaluations, where all of them were discovered between 22 and 38 weeks of pregnancy. None of the patients with cardiac anomalies were diagnosed during prenatal ultrasound study. The MMC defects were located in the lumbosacral area in 30 (40%), lumbar in 16 (21%), sacral in 14 (18.7%), thoracolumbar in 7 (9.3%), cervical in 5 (6.7%) and the thoracic area in 3 (4) patients. All MMCs were cystic lesions except for 7 instances that were rachischisis. Cerebrospinal fluid (CSF) leakage was evident at the time of admission in 10 patients. Normal motor function was noted in 18 patients. Paraplegia was found in 20 patients and distal leg weakness with intact proximal force was observed in 37 patients, in whom 2 patients had hand weakness due to symptomatic Chiari type II.

Brain CT scan or MRI was performed in all patients, which confirmed ventriculomegaly in 70 patients and pneumocephalus in 2 patients, who had CSF leakage. Symptomatic hydrocephalus was managed with ventriculoperitoneal shunting in 30 children and endoscopic third ventriculostomy in 5 patients. Repeated attacks of stridor and apnea were observed in one patient who was treated for hydrocephalus and then cervical decompression because of persistence after shunting. According to urological evaluation (radiological, ultrasound and electrophysiological), neurogenic bladder was diagnosed in 64 patients at the time of admission. Anal folds were absent in 43 patients at the time of surgery.

Orthopedic deformities observed at admission time were: clubfoot in 15 patients, hip dislocation in 12 patients, and sever flexion deformity of the hip in 5 patients.

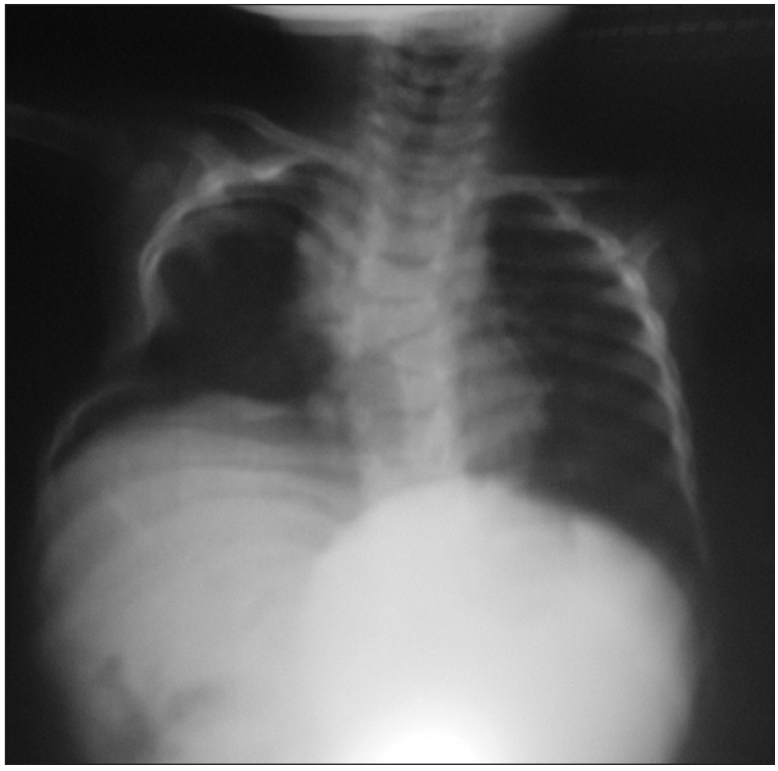

Figure 1 Plain X-ray of patient before surgery shows rib and vertebral anomalies. Notice the soft tissue shadow of the myelomeningocele sac.

Table 1 Associated anomalies in seventy-five patients with myelomeningcele

Associated anomalies	Number
Chiari malformation	37
Ventriculomegaly	70
Limb deformity	
Clubfoot	15
Hip dislocation	12
Neurogenic bladder	64
Rib anomalies	5
Cardiac anomalies	2

Five children had rib anomalies, including absence of one to four ribs found on chest X-ray (Table 1).

Cardiac physical examination was abnormal in 6 children, including abnormal sounds and murmur (systolic Thrill, cyanosis, S3, and harsh systolic murmur) but echocardiography revealed cardiac anomalies in only two of them. One was a 3-month old girl with VSD and ASD. She was admitted for management of thoracolumbar MMC and tandem split cord malformations (SCM) type one. She also had scoliosis and multiple rib deficiency. Tetralogy of Fallot was identified in the second patient (Figure 1). She was a 4-month old girl with repeated attacks of cyanosis during crying. She had lumbar MMC and multiple hemivertebra causing scoliosis. A single kidney was noticed in this patient. Both of these children had uneventful neurosurgical operations and are being followed up for management of their cardiac problems.

Discussion

MMC develops between the 18th and 25th days of gestation, with cord and meninges protrusion through the spinal defect. Association with other congenital anomalies is not uncommon and could happen in the same embryological period or as a conse-

quence of the underlying disease (1). Genetic and environmental factors have been proposed as the etiology of multiple anomalies in MMC children (10).

Cardiac anomalies associated with MMC have been reported, with diverse incidence rates from several studies ranging between 1.5 to 37% (1, 11). We found an incidence of less than 3% in this study. The different incidence rates may be related to the diverse methods used for those studies, including the age or gender of the patients. Some cardiac anomalies may be resolved spontaneously as the children grow up, consequently, the incidence of these anomalies could be under-detected when evaluating after the neonatal or infancy period. The degree of detailed cardiological investigations is another factor affecting the chance of detection of some equivocal or small size anomalies.

In concordance with the result of previous studies (9, 11), we found that congenital heart anomalies are more frequent in female children with MMC. Cardiac abnormalities have not been reported to occur more often with any specific location of the MMC defect (11). Our study was unable to show any significant relationship between MMC location and heart anomalies either. In this study, skeletal abnormalities involving the ribs and vertebral column (associated with spinal curvature deformity) were found in both MMC children with congenital heart anomalies, while other authors did not mention this association (1, 9-11).The detection of cardiac anomalies prior to surgery is important to support the child by giving special care during anesthesia and the operation, and prevent perioperative life threatening complications, such as air emboli or endocarditis.

Clinical examination are able to detect most cardiac anomalies, but some of them may only be detected with echocardiography or ECG. This study could not show a high incidence of cardiac anomalies in MMC patients, and congenital heart defects were found only in girl infants with gross ribs and vertebral column structural and curvature anomalies.

According to these findings, we do not advise echocardiography routinely in all patients with postnatal diagnosis of MMC. However, any clinical findings of cardiac problem, female sex and associated rib and vertebral anomalies should be considered as risk factors for cardiac anomalies in MMC patients, which necessitate further cardiac investigation by echocardiography, to rule out associated heart anomalies. On the other hand, regarding the importance of prenatal diagnosis of MMC and the associated anomalies, we emphasize routine prenatal ultrasound in any pregnancy to detect these anomalies. In suspicious fetuses for MMC, precise ultrasound evaluation and fetal echocardiography are highly recommended.

Conclusion

Cardiac abnormalities are not common anomalies in MMC patients. Female patients with suspicious clinical examinations for cardiac anomalies and associated rib and vertebral anomalies should be investigated by echocardiography to rule out associated cardiac anomalies.

Authors' contributions: Conception and design: FN and IMN; Acquisition, analysis and interpretation of data: FN, SS, THZ, IMN and MEK; Drafting the article IMN, THZ and SS; Revising it critically for important intellectual content: SS, MEK and FN.

Conflict of interest: The authors declare that they have no conflict of interest.

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Case report

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Mitral valve replacement in a patient with infective endocarditis and aneurysm of the cerebral artery: A case report

Senka Mesihović-Dinarević[1], Mirza Halimić[1], Zijo Begić[1], Almira Kadić[1], Mirsad Kacila[2], Edin Omerbašić[2], Nusreta Hadžimuratović[2], Eldin Burazerović[3]

[1]Paediatric clinic, University Clinical Centre Sarajevo
[2]Clinic and Department of Cardiosurgery University Clinical Centre Sarajevo
[3]Clinic and Department of Neurosurgery University Clinical Centre Sarajevo

Corresponding author:
Mirza Halimić
Paediatric clinic
University Clinic Centre Sarajevo
71000 Sarajevo
Bosnia and Herzegovina
halimicm@gmail.com
Tel.: + 387 33 566 450
Fax.: + 387 33 566 525

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Objective. Endocarditis can have profound and devastating neurological consequences, with the vast majority of these complications in patients with left-sided valvular disease. The approach to the acute management of stroke in children with infective endocarditis is limited by the inadequacy of published data on their clinical course and outcome. **Case report.** This case report presents a 12 year old girl with diagnosed endocarditis, complicated with intracranial hemorrhage, due to the rupture of an aneurysm of the peripheral branch medial cerebral artery and gradient therapeutic approach, with an excellent final result. **Conclusion.** Congestive heart failure resulting from valvular insufficiency required mitral valve replacement, after cerebral aneurysm clipping.

Key words: Endocarditis, Intracranial hemorrhage, Aneurysm, Treatment.

Introduction

Approximately 15-40% of patients with infective endocarditis (IE) eventually require surgery (1). The overall prevalence of hemorrhage in central nervous system involvement of infective endocarditis is 3-7%. Aneurysms of arteries supplying the brain account for approximately 15% of the aneurysms occurring in infective endocarditis. The term "mycotic aneurysm" describes a mushroom-shaped aneurysm associated with IE (1-3).

Timing of surgery in patients with infective endocarditis and embolic stroke remains controversial, but a report has suggested that surgery can be performed relatively safely within 3 days of the stroke if heart failure is severe; otherwise, a delay of 2-4 weeks is preferable. In patients with associated hemorrhage, a delay of at least 4-6 weeks is preferred (3-5).

The aim of this report is to confirm the validity of a gradient therapeutic approach in a patient with infective endocarditis and intracerebral hematoma, according to the latest European Society of Cardiology (ESC) clinical practice guidelines.

Case report

A 12 year old girl with a fever for the two months prior to hospitalization on peroral antibiotic therapy, with prostration, anxiety, screaming, confusion, tachycardia, hallucination and a loud holosystolic murmur on the lower left sternal border, was admitted to the Clinic for Infectious Diseases of the University Clinical Centre Sarajevo, and after initial examination transferred to the Paediatric Clinic. The neurological exam was significant for left leg weakness and diffuse hyper-reflexia of all four extremities.

In the blood culture Streptococcus pneumoniae was detected. Blood studies showed anaemia, a mildly elevated erythrocyte sedimentation rate, as well as C reactive protein with a normal coagulation panel. Transthoracal ultrasound showed vegetation (19x11mm) over the anterior mitral leaflet (Figure 1), with mitral regurgitation, confirmed by CW Doppler. The child was treated with ceftriaxone.

A CT-Scan of the head was interpreted as a left-sided parietal intracerebral hematoma, diameter 30 mm, with compressive oedema on the posterior horn of the lateral ventricle (Figure 2). For technical reasons, digital subtraction angiography (DSA) could not be performed. MRI and MRA brain scan confirmed aneurysm of the peripheral branch of the medial cerebral artery.

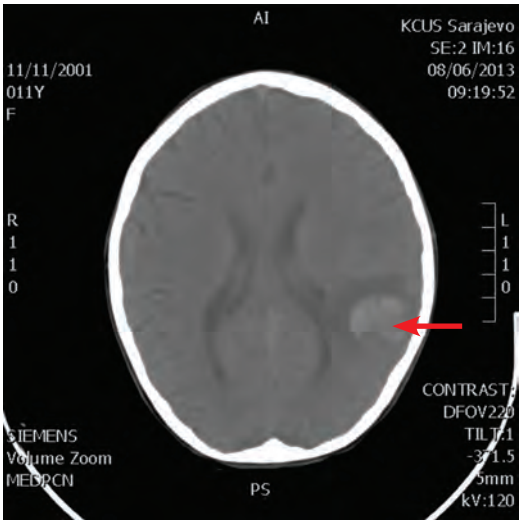

Figure 2 CT scan of the intracranial haemorrhagia (arrow).

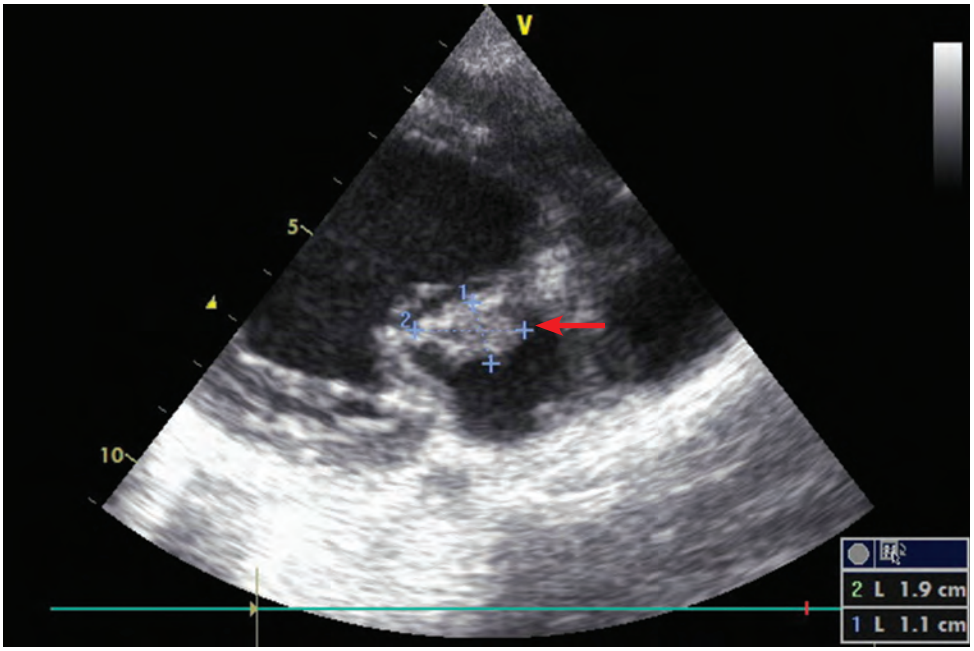

Figure 1 Transthoracic echocardiogram showing vegetation (19x11 mm) over the anterior leaflet of mitral valve (arrow).

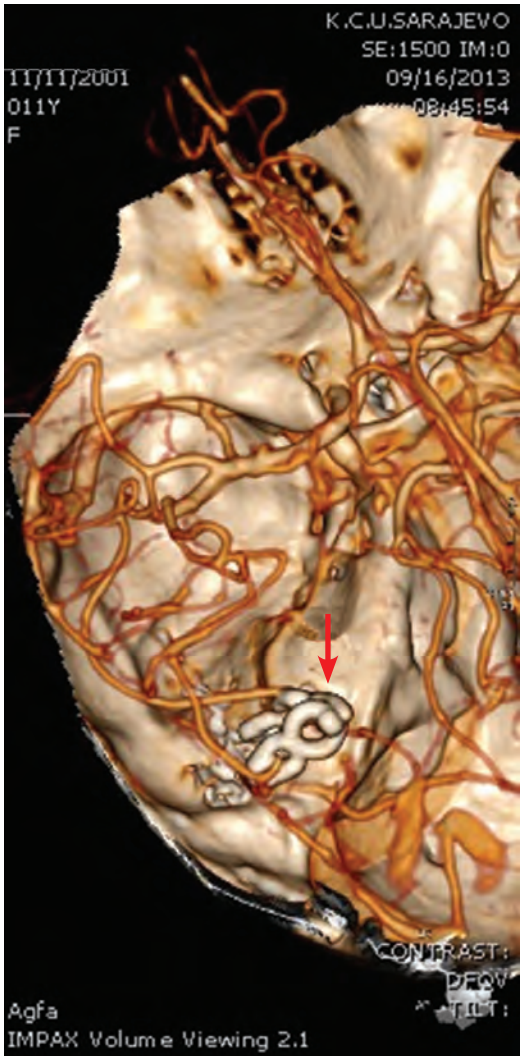

Figure 3 CT angiography scan after cerebral aneurysm clipping (arrow).

Transesophageal echocardiogram (TEE) successfully visualized two mobile vegetations on the atrial side of the mitral valve (anterior leaflet-20x11mm and posterior leaflet 18x8mm), with severe mitral and mild tricuspid regurgitation. On control transthoracal and transesophageal echocardiography, after 6 weeks of parenteral antibiotic treatment, significant reduction of the visible vegetation was shown (diameter 6x3mm and 4x3mm), with partial prolapse of the A2/P2 segment of the mitral valve, dilatation of the mitral annulus and severe mitral regurgitation (EROA 0.40 cm^2, regurgitant volume 62 ml/beat).

After interdisciplinary paediatric, cardio- and neurosurgical consultation, the decision was made for primary cerebral aneurysm clipping. An interventional radiological approach was not feasible. Surgical treatment was by open craniotomy, multiple clip occlusions and clip reconstruction of the parent vessel, without bypass, as these aneurysms, due to inherent structural characteristics, mainly is not amenable to direct clipping. The aneurysm had poor consistency, was degenerated and had friable walls. Control CT angiography scan showed absence of the aneurysm after clipping (Figure 3). The aneurism had poor consistency, was degenerated and had friable walls. The second postoperative day showed no neurological deficit. Two months after brain surgery the girl was hospitalized again with signs of congestive heart failure, pleural effusion and thoracic drainage. Blood cultures were negative. Transthoracal ultrasound showed a dysmorphic mitral valve, with severe regurgitation, but without proven abscess of the subvalvular apparatus, perforation or chordae rupture. It was not possible to repair the mitral valve and a second relapse required replacement of the mitral valve, with a biological valve prosthesis implantation in the mitral position. Echocardiography showed a small pericardial effusion and reduced cardiac output, FS 16%, EF 37%. The child was discharged with anticongestive and anticoagulant therapy, then on further follow up (three months later) she presented with gradually increasing cardiac output, up to the normal range (LVFS 28-32%, EF 67%).

Discussion

Cerebrovascular complications include ischemic or haemorrhagic stroke, transient ischemic attack, silent cerebral embolism, symptomatic or asymptomatic mycotic an-

eurysm, cerebral abscess, meningitis, toxic encephalopathy and seizure (1-8).

In most cases, the neurologic sequelae are present before the initiation of antimicrobial therapy (76%), as in this reported case. One series showed that in patients with infective endocarditis who had CNS involvement, four fifths had ischemic strokes and one fifth had haemorrhages, which is a distribution similar to that of strokes in general.

Aneurysms of arteries supplying the brain account for approximately 15% of aneurysms occurring in infective endocarditis. Mycotic aneurysms involve the medial cerebral artery territory four times more often than they do the anterior or posterior cerebral arteries. They occur at secondary branches and bifurcations, usually in the lateral fissure near the trifurcation of the medial cerebral artery. When aneurysms are formed, the most likely mechanism is bacterially induced weakening (9).

The onset of valve dysfunction or moderate-to-severe congestive heart failure should lead to an evaluation for immediate valve replacement, which was the case of surgical management in our patient. Cardiac surgery is not contraindicated after an ischemic stroke, a silent cerebral embolism or transient ischemic attack (10).

In patients with neurological complications, the safety of cardiopulmonary bypass has been controversially debated for years. Anticoagulation during cardiac surgery may increase the risk of haemorrhagic transformation of an asymptomatic ischemic stroke. In this case, cardiac surgery should be delayed for 2–3 weeks. If there is a ruptured mycotic aneurysm, it must be resected, clipped or embolised before cardiac surgery (11).

These recommendations for surgery have been made by "The Task Force on the Prevention, Diagnosis, and Treatment of Infective Endocarditis of the European Society of Cardiology" (12). From studies published during the mid-1990s (4, 5, 13, 14), an interval of at least 2 weeks between an embolic event and cardiac surgery was recommended. In this reported case, this interval was longer.

Operative mortality is variable but has been reported as 7.6%, with risk factors for death being cardiogenic shock, insidious illness and they increase with age (5). The 9-year survival rate has been reported to be 71%. Risk factors for death include preoperative neurologic complications (5, 15). The risk of neurologic deterioration after valve replacement for infective endocarditis is 20% in the first 72 hours, 20-50% 4-14 days postoperatively, less than 10% beyond 14 days postoperatively, and less than 1% after 4 weeks (15). In our reported case there was no deterioration during the follow up period after four weeks.

Conclusion

A multidisciplinary approach in patients with this complex cardio-neurological illness is necessary, concerning the timing of decision making relation to valve surgery for the sake of outcome improvement.

Authors' contributions: Conception and design: MH and SM; Acquisition, analysis and interpretation of data: MH and SM; Drafting the article MH and SM; Revising it critically for important intellectual content: SM, ZB, AK, MK, EO, NH, EB.

Conflict of interest: The authors declare that they have no conflict of interest.

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Double superior vena cava: Two cases in Thai cadavers

Sitthichai Iamsaard, Pipatphong Kanla, Channarong Arunyanart

Department of Anatomy, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand

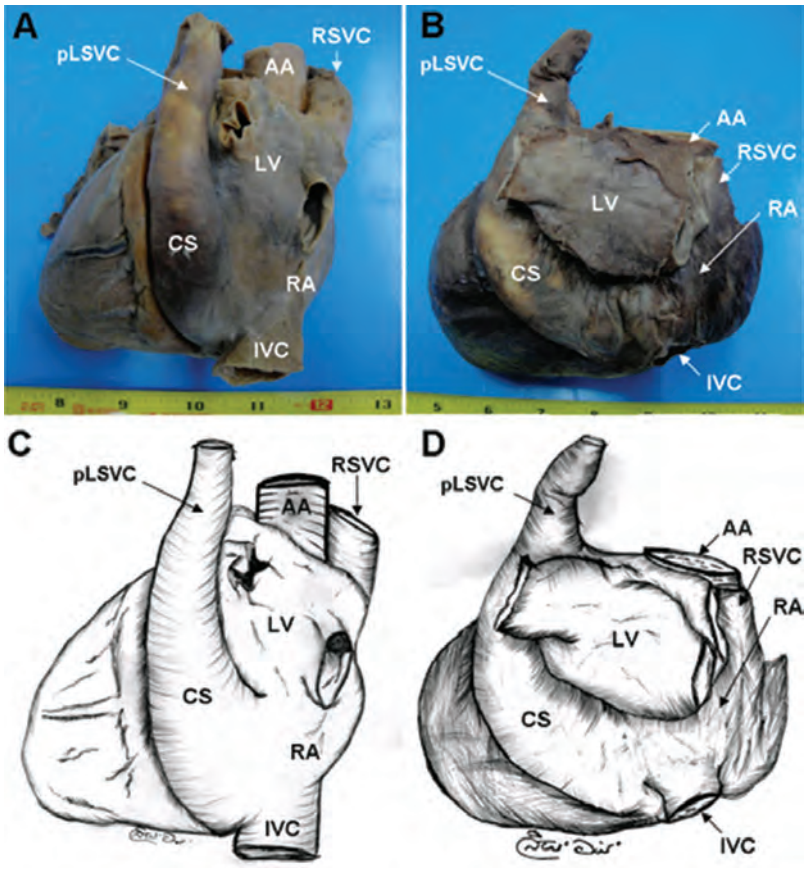

Figure 1 Photographs (A; case 1 and B; case 2) and illustrations of Figure 1A (C) and Figure 1B (D) showing double superior vena cava: right connected to right atrium and left to the coronary sinus. pLSVC: persistent left superior vena cava; RSVC; right superior vena cava; CS: coronary sinus; LV: left ventricle; RA: right atrium; AA ascending aorta; IVC: inferior vena cava. In both cases, the venous drainage pattern was similar as shown in Figure 2. It was investigated that the right or left brachiocephalic vein drained into its own superior vena cava, i.e. RSVC or pLSVC (Figure 2). Additionally, RSVC joined with an azygos vein and drained into the right atrium directly. No defects of inferior vena cava and/or venous connections between RSVC and pLSVC were observed in either case.

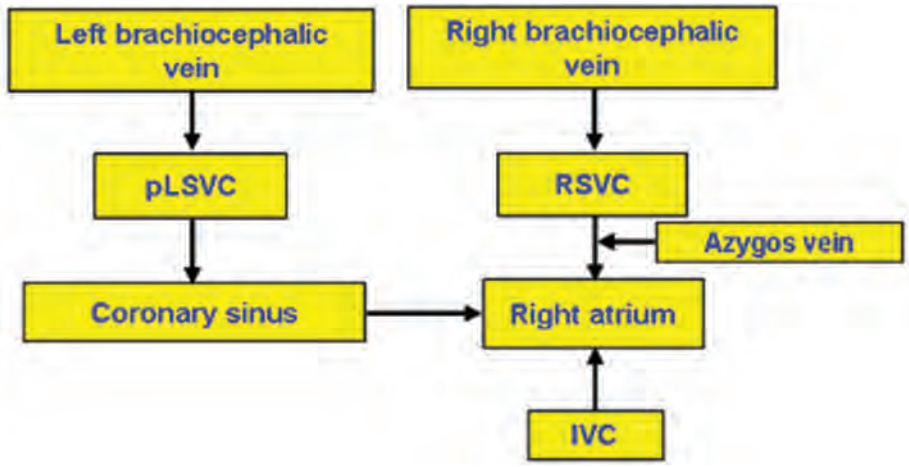

Figure 2 Flowchart showing the venous drainage pattern of double superior vena cava (posterior view) in the present report (pLSVC: persistent left superior vena cava; RSVC: right superior vena cava; IVC: inferior vena cava). Note: right or left brachiocephalic veins drain into individual-sided SVC and pLSVC drains into right atrium via coronary sinus while RSVC including azygos vein is connected to the right atrium directly. As previously described (3), the two cases demonstrated in this report are of Type I-R. To our knowledge, this is the first report demonstrating the DSVC in the Thai population (0.42%; 2 found cases from 480).

The coexistence of persistent left superior vena cava (pLSVC) and right superior vena cava (RSVC) is also called "double superior vena cava (DSVC)". Although some cases of DSVC have been reported in many populations (1-3), the DSVC in Thais is very rare. We report two cases of DSVC observed in 480 Thai cadavers. We found two cases of DSVC: in a 18 year-old cadaver, death by driving (Figure 1A) and in a 75 year-old cadaver, death by heart failure (Figure 1B).

Key words: Double superior vena cava, Persistent left superior vena cava, Right superior vena cava, Coronary sinus, Right atrium.

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Authors' contributions: Conception and design: SI; Acquisition, analysis and interpretation of data's: SI, PK, CA; Drafting the article: SI; Revising it critically for important intellectual content: SI.

Conflict of interest: The authors declare that they have no conflict of interest.

Corresponding author:
Sitthichai Iamsaard
Department of Anatomy, Faculty of Medicine
Khon Kaen University
123 Mitraparp Road, Amphoe Muang
Khon Kaen 40002, Thailand
Iamsaard_sitt@yahoo.com
Tel.: + 66 4336 3212; Fax.: + 66 4336 3212

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A call for greater power in an era of publishing negative results

Anna L Oberhofer[1], Robert P Lennon[2]

[1]1Department of Family Medicine, Naval Hospital Jacksonville, Jacksonville FL, USA, [2]Department of Family Medicine Naval Hospital Okinawa, Okinawa, Japan

Corresponding author:
Robert P Lennon
ATTN Bush Clinic
Naval Hospital Okinawa
PSC 482, FPO AP 96362
Robert.lennon@med.navy.mil
Tel.: + 080 4881 0766

Historically, medical journals published only positive results from experimentation. With the growth of electronic media, many journals are now able to publish negative results as well. Because most medical experiments are designed to have different thresholds of significance for positive and negative results, this can lead readers to misunderstand the level of significance of a published negative result. We propose a technical shift – setting α equal to β – to avoid this potential for misunderstanding.

Key words: Statistics, Beta statistic, Power negative results.

To the Editor,

When searching for truths in nature, we risk committing two types of error. A Type I error is rejecting a hypothesis that should have been accepted. A Type II error is accepting a hypothesis that should have been rejected (1, 2). Alpha is the probability of making a Type I error while beta is the probability of making a Type II error. The medical community generally accepts as true those results with a five percent or less chance of being random – our threshold for claiming statistical significance. That is, after the probability of the null hypothesis is calculated, we accept as "true" results in which $\alpha \leq 0.05$. We accept an error rate for significant negative outcomes of 20%. That is, $\beta \leq 0.2$. The statement, "an observed difference if $\alpha > 0.05$ fails to show significance" means that we are unable to assert that there is a 95% chance that an observed difference is due to intervention and not chance. We are tempted to publish a negative result indicating that the intervention does not work. However, although the observed difference is statistically a lack of difference, the appropriate evaluation of the negative relies on the β statistic. The lack of difference if $\beta \leq 0.2$ means that one can only assert that there is an 80% chance that the lack of difference is not due to chance.

A more rigorous threshold of acceptance of a positive value than a rejection of a negative value was acceptable when only studies with positive results were published. In an age of publishing negative results, this is problematic. To reject a hypothesis that fails to meet 95% certainty in the positive is to accept the opposite hypothesis with only 80% certainty – a historically unacceptable level of surety. To avoid rejecting true hypotheses using current conventions, the negative result must be interpreted differently than the positive result. There is no compelling reason for this.

One could evaluate both positive and negative results to 95% certainty by setting both α and β to ≤ 0.05. Only then is one's re-

jection of the "not-different" (which ought to be measured by the β statistic) as sure as one's acceptance of the different (which is measured by the α statistic). This will increase the difficulty of performing quality studies; as β is lowered from 20% to 5%, power decreases. This may be offset by increasing enrollment or identifying interventions with greater effect size, but may limit researchers' ability to identify significant differences in situations in which attaining such power is not feasible. However, this will also increase the value of our studies. When α equals β, we are equally sure of our results regardless of outcome, and we can accept negative results with the confidence with which we accept positive results.

The views presented are those of the authors and do not necessarily reflect the official policy or position of the Department of the Navy, Department of Defense, or the United States Government.

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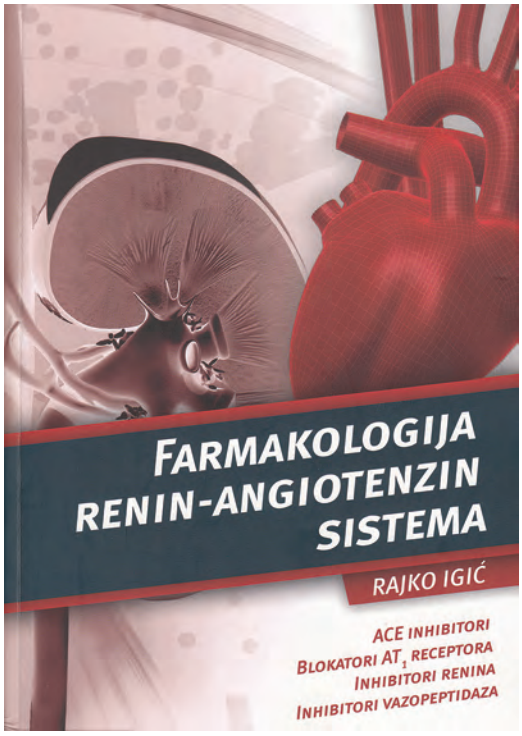
Farmakologija renin-angiotenzin sistema [Pharmacology of the Renin-Angiotensin System]

Author: Rajko Igić. Publisher: Medicinski fakultet, Banja Luka, 2014, ix + 122 pages, illustrated. ISBN: 978-99938-42-781. [In Serbian.]

Enver Zerem, Corresponding Member of ANUBiH
Professor of Medicine, University of Tuzla, Tuzla, Bosnia and Herzegovina



The renin-angiotensin system (RAS) and the kallikrein-kinin system (KKS) were discovered more than a century ago. It is interesting that both systems were defined after initial observations from experiments on the urinary system. Renin was discovered when extracts of rabbit kidney caused a hypertensive effect, while hypotension due to intravenous injection of urine led to the discovery of kallikrein. Kallikrein got its name from the Greek *kallikréas* (pancreas), where the highest concentration was found. Renin was rediscovered several decades later when it was found that occlusion of the renal artery in dogs caused hypertension. Soon it was revealed that renin releases an inactive decapeptide, angiotensin (Ang) I, from a substrate, angiotensinogen, and this peptide is further cleaved by angiotensin converting enzyme (ACE) to the strongly hypertensive octapeptride Ang II.

The KKS participates in various vascular mechanisms releasing two peptides: a nonapeptide, bradykinin (BK), and a decapeptide, Lys-bradykinin (kallidin). Bradykinin causes hypotension, cough, and relaxation/contarctions of smooth muscles. It is 10 times stronger than histamine on a molar basis. ACE has a dual function; it activates Ang I and inactivates BK. The antihypertensive ACE inhibitors decrease the formation of Ang II and increase the level of BK. These effects have therapeutic importance, but they also contribute to side effects of the ACE inhibitors. The first orally active ACE inhibitor, captopril, was discovered in 1977 by the researchers of the Squibb Company. Today 16 ACE inhibitors are in clinical use.

In the book "Pharmacology of the Renin-Angiotensin System", Professor Rajko Igić has integrated his extensive research experience, focusing on biochemical, pharmacological, clinical, toxicological and epidemiological aspects of the RAS blockade. In addition, the book also gives an overview of current pharmacological, immunological

and genetic research in the field of RAS. The book is divided into eleven chapters, organised in methodical and logical order.

The introductory chapters of the book give a short history of the RAS and KKS. The components of the RAS and their physiological and pharmacological importance are described in chapters on prorenin and renin, (pro)renin receptor, angiotensinogen, ACE, Ang I, Ang II, genetic polymorphysms of ACE, sarcoidosis and ACE, chymase, ACE2, angiotensin receptors, and angiotensinases. The synthesis and release of prorenin/renin, the essential steps for the RAS activation, are described in detail. The release is controlled by three mechanisms: sympathetic nerve stimulation (fast release), renal baroreceptors (long lasting release), and influence of ions via macula densa (chronic release). Pharmacological and clinical data for ACE inhibitors, Ang receptor blockers, and renin inhibitors are presented in separate chapters. Another chapter is devoted to vasopeptidase inhibitors, including omapatrilat, ilepatril, bonsetan, and inhibitors of endothelin-1 converting enzyme (ECE-1). The final chapter discusses future research on the RAS.

The appendix describes arterial hypertension, heart failure, myocardial infarction, and sleep apnea in short chapters prepared for non-physicians, such as pharmacists, medical biochemists, and biomedical students. Perhaps the appendix should include a chapter on nephrology, as well.

Each chapter is concise, giving clear learning outcomes, enabling the reader to consolidate their gained knowledge. The simple, original illustrations help the reader to follow the complex relationships of the RAS and KKS and vasopeptidases. The book is well referenced and guides the enthusiast towards further reading. It includes 92 references; 15 appear in the appendices as footnotes. A short biographical note about the author includes 56 references to his publications in various journals.

This well-written book is aimed at clinicians, students, and biomedical investigators who study or treat cardiovascular diseases and who may use it as an excellent guide. The book is also useful to those who wish to review the pharmacology and therapeutics of this complex subject.

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Medicine University of Zurich, Zurich, Switzerland; Department of Cardiology, Clinic Thorax Institute, Hospital Clinic de Barcelona, Barcelona, Spain; SFL Egypt Champion, Faculty of Medicine, Alexandria University, Alexandria, Egypt; National Scientific Center 'the MD Strazhesko Institute of Cardiology' Academy of Medical Science of Ukraine, Kiev, Ukraine; Cardiac Centre, University Hospital of J.A. Reiman, Prešov, Slovakia; BH Heart Center Tuzla, Tuzla, Bosnia and Herzegovina; Department of Health Economics, Medical School Hamburg, Hamburg, Germany; Cardiocenter, Third Faculty of Medicine, Charles University Prague, Prague, Czech Republic.

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Department of Medical Sciences, The Academy of Sciences and Arts of Bosnia and Herzegovina, Sarajevo, Bosnia and Herzegovina; Medical School of Tuzla, University of Tuzla, Tuzla, Bosnia and Herzegovina; Department of Gastroenterology, Clinical Center Banjaluka, Banjaluka, Bosnia and Herzegovina.

Zvorničanin J, Zvorničanin E. Significance of Ultrasonography in Evaluation of Vitreoretinal Conditions. Semin Ophthalmol. 2014 Jun 25:1. [Epub ahead of print]

Eye Clinic, University Clinical Center Tuzla, Tuzla, Bosnia and Herzegovina.

by Nerma Tanović

Instructions to authors

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Acknowledge. Anyone who contributed towards the study by making substantial contributions to conception, design, acquisition of data, or analysis and interpretation of data, or who was involved in drafting the manuscript or revising it critically for important intellectual content, but who does not meet the criteria for authorship. List the source(s) of funding for the study and for the manuscript preparation in the acknowledgements section.

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Sample references

Articles in journals

Standard journal article (*List the first six authors followed by et al.*):

Halpern SD, Ubel PA, Caplan AL. Solid-organ transplantation in HIV-infected patients. N Engl J Med. 2002;347(4):284-7.

More than six authors:

Rose ME, Huerbin MB, Melick J, Marion DW, Palmer AM, Schiding JK, et al. Regulation of interstitial excitatory amino acid concentrations after cortical contusion injury. Brain Res. 2002;935(1-2):40-6.

Organization as author:

Diabetes Prevention Program Research Group. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. Hypertension. 2002;40(5):679-86.

No author given:

21st century heart solution may have a sting in the tail. BMJ. 2002;325(7357):184.

Volume with supplement:

Geraud G, Spierings EL, Keywood C. Tolerability and safety of frovatriptan with short- and long-term use for treatment of migraine and in comparison with sumatriptan. Headache. 2002;42(Suppl 2):S93-9.

Issue with supplement:

Glauser TA. Integrating clinical trial data into clinical practice. Neurology. 2002;58(12 Suppl 7):S6-12.

Issue with no volume:

Banit DM, Kaufer H, Hartford JM. Intraoperative frozen section analysis in revision total joint arthroplasty. Clin Orthop. 2002;(401):230-8.

Letters or abstracts:

Tor M, Turker H. International approaches to the prescription of long-term oxygen therapy [letter]. Eur Respir J. 2002;20(1):242.;
Lofwall MR, Strain EC, Brooner RK, Kindbom KA, Bigelow GE. Characteristics of older methadone maintenance (MM) patients [abstract]. Drug Alcohol Depend. 2002;66 Suppl 1:S105.

Article republished with corrections:

Mansharamani M, Chilton BS. The reproductive importance of P-type ATPases. Mol Cell Endocrinol. 2002;188(1-2):22-5. Corrected and republished from: Mol Cell Endocrinol. 2001;183(1-2):123-6.

Article with published erratum:

Malinowski JM, Bolesta S. Rosiglitazone in the treatment of type 2 diabetes mellitus: a critical review. Clin Ther. 2000;22(10):1151-68; discussion 1149-50. Erratum in: Clin Ther. 2001;23(2):309.

Article published electronically ahead of the print version:

Yu WM, Hawley TS, Hawley RG, Qu CK. Immortalization of yolk sacderived precursor cells. Blood. 2002 Nov 15;100(10):3828-31. Epub 2002 Jul 5.

Books and other monographs

Personal author(s):

Murray PR, Rosenthal KS, Kobayashi GS, Pfaller MA. Medical microbiology. 4th ed. St. Louis: Mosby; 2002.

Editor(s), compiler(s) as author:

Gilstrap LC 3rd, Cunningham FG, VanDorsten JP, editors. Operative obstetrics. 2nd ed. New York: McGraw-Hill; 2002.

Organization(s) as author:

Royal Adelaide Hospital; University of Adelaide, Department of Clinical Nursing. Compendium of nursing research and practice development, 1999-2000. Adelaide (Australia): Adelaide University; 2001.

Chapter in a book:

Meltzer PS, Kallioniemi A, Trent JM. Chromosome alterations in human solid tumors. In: Vogelstein B, Kinzler KW, editors. The genetic basis of human cancer. New York: McGraw-Hill; 2002. p. 93-113.

Conference paper:

Christensen S, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer; 2002. p. 182-91.

Dissertation:

Borkowski MM. Infant sleep and feeding: a telephone survey of Hispanic Americans [dissertation]. Mount Pleasant (MI): Central Michigan University; 2002.

Other published material

Newspaper article:

Tynan T. Medical improvements lower homicide rate: study sees drop in assault rate. The Washington Post. 2002 Aug 12;Sect. A:2 (col. 4).

Dictionary and similar references:

Dorland's illustrated medical dictionary. 29th ed. Philadelphia: W.B. Saunders; 2000. Filamin; p. 675.

Electronic material

CD-ROM:

Anderson SC, Poulsen KB. Anderson's electronic atlas of hematology [CD-ROM]. Philadelphia: Lippincott Williams & Wilkins; 2002.

Audiovisual material:

Chason KW, Sallustio S. Hospital preparedness for bioterrorism [videocassette]. Secaucus (NJ): Network for Continuing Medical Education; 2002.

Journal article on the Internet:

Abood S. Quality improvement initiative in nursing homes: the ANA acts in an advisory role. Am J Nurs [serial on the Internet]. 2002 Jun [cited 2002 Aug 12];102(6):[about 3 p.]. Available from: http://www.nursingworld.org/AJN/2002/june/Wawatch.htm.

Monograph on the Internet:

Foley KM, Gelband H, editors. Improving palliative care for cancer [monograph on the Internet]. Washington: National Academy Press; 2001 [cited 2002 Jul 9]. Available from: http://www.nap. edu/books/0309074029/html/.

Homepage/Web site:

Cancer-Pain.org [homepage on the Internet]. New York: Association of Cancer Online Resources, Inc.; c2000-01 [updated 2002 May 16; cited 2002 Jul 9]. Available from: http://www.cancer-pain.org/.

Part of a homepage/Web site:

American Medical Association [homepage on the Internet]. Chicago: The Association; c1995-2002 [updated 2001 Aug 23; cited 2002 Aug 12]. AMA Office of Group Practice Liaison; [about 2 screens]. Available from: http://www.ama-assn.org/ama/pub/category/1736.html.

Database on the Internet:

Who's Certified [database on the Internet]. Evanston (IL): The American Board of Medical Specialists. c2000 – [cited 2001 Mar 8]. Available from: http://www.abms.org/newsearch.as.


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