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ASSOCIATION OF MEDICAL DOCTORS OF THE REPUBLIC OF SRPSKA,
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53

Q3

Original Articles

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Prevalence and Risk Factors of Acne Among Adolescents in Kosovska Mitrovica: a Cross-Sectional Study

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ORIGINAL ARTICLE

Metabolic Disorders in Patients With Impaired Glucose Tolerance, With or Without Underlying Ischaemic Heart Disease

Milena Brkić,[1] Danijel Đekić,[1, 2] Jelena Jovanić,[1, 3] Goran Topić,[1, 4] Aleksandra Grbić,[1, 5] Tatjana Šutilović[6]

Abstract

Background/Aim: The evidence showed that in the development of diabetes mellitus type 2 (DMT2) and coronary heart disease (CHD) significant role is played by metabolic risk factors: insulin resistance (IR), dyslipidaemia and obesity. Beside metabolic factors, increase in inflammatory markers such as fibrinogen and hs-C reactive protein (hsCRP) plays a role in developing CHD. Metabolic disorders are thought to also be present in patients with impaired glucose tolerance (IGT) and could contribute to development of CHD in these individuals. Aim of this study was to investigate the behaviour of metabolic parameters and chronic inflammation markers in patients with IGT on glucose tolerance test and associated CHD.

Methods: The trial included 4 groups of 30 subjects: a) IGT with CHD, b) IGT without CHD, c) CHD without IGT and d) control group without CHD and with normal glucose tolerance (NGT). Within each group glucoregulation parameters were measured (fasting glucose and Hb1Ac). Oral glucose tolerance test (OGTT) with 75 g glucose load was performed and IR parameters calculated (using HOMA-IR, Matsuda index, Quicki index, HOMA1- %B), lipid profile was done, waist/hip ratio was measured, as well as fibrinogen and hsCRP. CHD diagnosis was determined by typical signs of previous myocardial infarction on ECG, echocardiogram and/or ergometry (Bruce protocol).

Results: Subjects with IGT, but no CHD and those with both IGT and CHD had statistically significantly higher triglyceride and cholesterol levels and manifest IR with decreased insulin sensitivity compared to subjects with CHD, but no IGT and control group. Group with both IGT and CHD was found to have significantly higher fibrinogen and hsCRP concentrations.

Conclusion: IR and hyperlipidaemia, together with chronic inflammation mediators, are potential predictors of the development of glucose tolerance disorders; hence interventional treatment during IGT period or during hyperinsulinaemia could give patients better opportunity to prevent or postpone onset or development of diabetes and its complications.

Key words: Impaired glucose tolerance; Insulin resistance; Coronary heart disease; Chronic inflammation mediators.

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Introduction

The best evidence on increased risk of developing CVD was obtained in DECODE study[1] which showed that impaired glucose tolerance (IGT) was significant predictor of coronary heart dis-

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ease (CHD) mortality. Numerous studies have confirmed that the risk of CHD is significantly higher in diabetics compared to individuals without glucoregulation disorders.[2-7] In manifestation of CHD in patients with diabetes mellitus type 2 (DMT2) indisputable role is played by inflammation mediators, as well as metabolic risk factors complementary to them. Hyperinsulinaemia can have direct atherogenic effect on the blood vessel wall,[7-9] but its atherogenic effect can also be realised indirectly through lipid disorders,[7, 10-12] fibrinolysis[13, 14] and obesity.[15]

Within these studies there is a particular interest in elucidating the effect of insulin levels on severity and progression of CHD as a late DMT2 complication. Decrease in insulin sensitivity is an early sign of susceptibility to diabetes type 2 and it usually manifests as elevated fasting insulin levels.[2] Insulin is a key glucose metabolism regulator that promotes glucose uptake into peripheral tissues and inhibits glucose production in the liver. Insufficient insulin action results in increased levels of fasting glucose and ultimately in overt DMT2.[16-18] Insulin resistance (IR) is also associated with development of cardiometabolic complications, a risk that arises even before development of DMT2.[19, 20] Development of CHD was observed in individuals with IGT without overt clinical or subjective DMT2 signs, therefore current and future research faces a challenge of identifying and preventing potential risk factors for development of CHD in those individuals. Oral glucose tolerance test (OGTT) assesses an individual's ability to clear circulating glucose after a 75 g oral glucose load, after an overnight fast. OGTT induces transition from fasting to feeding and subsequent changes in various metabolic nutrients happen while the body adjusts in order to achieve glucose homeostasis.[21] WHO recommends OGTT as a diagnostic test in the range of fasting plasma glucose between 5.5 and 11.1 mmol/L. Majority of studies conducted in Europe, which compared the ADA and WHO diagnostic criteria, have found that of individuals with DM diagnosed using ADA criteria only 46 % had 2 h glucose concentration higher than 11.1 mmol/L, which indicates that WHO 2 h glucose criterion is more reliable diagnostic test for DM diagnosis.[22]

Aim of this study was to investigate characteristics of metabolic parameters and chronic inflammation markers in individuals with IGT, with and without CHD.

Methods

The study included 120 individuals, ages 45 to 70, divided in 4 groups of 30 subjects: 1st group IGT with CHD, 2nd group IGT without CHD, 3rd group CHD without IGT and 4th group healthy subjects (control group without CHD and with normal glucose tolerance – NGT). The study was conducted at the Department of Endocrinology with General Internal Medicine and at the Clinic of Cardiovascular Diseases of University Clinical Centre in Banja Luka. Patient sample was available to researchers and at the same time it was statistically representative in order for the study to achieve sufficient power. All subjects were informed about the scheduled study and their informed consent was obtained. In accordance with ethical principles inclusion criteria were based on risk factors for impaired glucose metabolism (body mass index ≥ 25, DMT2 in first-degree relatives, middle or older age, history of lipid profile disorder, fasting glucose from 5.0 to 6.5 mmol/L) and on the evidence of CHD, including signs of ischaemia or previous myocardial infarction (IM) on ECG, echocardiogram and/or ergometry.

Exclusion criteria included existing diagnosis of diabetes mellitus, chronic kidney disease requiring dialysis, pregnancy, use of medication or presence of an existing condition that could affect glucoregulation, as well refusal to participate in the study. In each subject following parameters were measured:

- Glucoregulation (fasting glucose, HbA1c);
- OGGT was performed with 75 g oral glucose load after a 12 h fast and glucose and insulin levels were measured at 0 (initial fasting levels, right before taking glucose), 30, 60, 90 and 120 min;
- Parameters of IR were calculated using Homeostatic Model Assessment of Insulin Resistance (HOMA-IR), Matsuda index, Quantitative Insulin Sensitivity Index (QUICKI index), insulinogenic index, HOMA1- %B, lipid profile, waist-to-hip ratio, fibrinogen and CRP;
- Blood pressure and anthropometric measures were taken (waist-to-hip ratio (W/H), Body Mass Index (BMI)).

Apart from OGTT, all the other metabolic parameters were included in the routine patient evaluation during hospitalisation. IGT was determined based on glucose levels between 7.8 and 11.1 mmol/L after 75 g oral glucose load during

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OGTT or based on fasting glucose levels of ≥ 6.1 mmol/L and < 7.0 mmol/L. Fasting serum glucose levels were measured using enzyme glucose oxidase method (Biosen C line analyser and Chronolab photometer, CHL-1310). HbA1c levels were measured from haemolysed whole blood samples processed on COBAS INTEGRA 400 plus analyser using turbidimetric inhibition immunoassay (TINIA). Insulin was measured by radioimmunoassay method using INEP RIA INSULIN (PEG) kit. Intra assay coefficient of variation (CVI) was 3.84 %. HOMA index was calculated using this formula: HOMA-IR = (FPI x FPG) / 22.5 (FPI = Fasting Plasma Insulin, FPG = Fasting Plasma Glucose).

Insulin secretion capacity was estimated using insulinogenic index, based on the increase in insulin and glucose levels at 30 min using the following formula: ▲I30/▲G30, where ▲I30 is the difference in insulin levels (mmol/L) at 30 and at 0 min and ▲G30 is the difference in glucose levels (mmol/L) at 30 and at 0 min of the test. QUICKI = 1/[log(I0) + log(G0)], where I0 represents fasting insulin level and G0 fasting glucose level. Matsuda index ISI (Matsuda) = 10000/√ (glucose concentration 0' × insulin concentration 0' × glucose concentration OGTTx × insulin concentration OGTTx), where glucose concentration 0' represents glucose concentration at 0 min (mg/dL), insulin concentration 0' - insulin concentration at 0 min (mIU/L), glucose concentration OGTTx – mean glucose concentration during OGTT (mg/L) and insulin concentration OGTTx – mean insulin concentration during OGGT (mIU/L). Beta cell basal insulin secretion indicator HOMA1- %B = (20 × FPI)/(FPG - 3.5).

For total cholesterol, triglycerides, HDL cholesterol and LDL cholesterol measurement chromatography was used on Roche/Hitachi 917 analyser. CHD diagnosis was made by cardiologist and based on typical findings of previous myocardial infarction on ECG, echocardiogram and/or Bruce protocol ergometry. Statistical data analysis was performed using the Statistica StatSoft program. Results were expressed as mean ± standard deviation. For statistical analysis, Student t-test, Chi-square test, correlation coefficient, uni- and multivariate logistic regression, one-factor and two-factor analyses of variance or Kruskal-Wallis test were used.

Results

Clinical characteristics of patients and biochemical, ie, metabolic parameters are shown in Table 1 and 2.

OGTT was performed on all the subjects and highly statistically relevant difference ($p < 0.01$) was always observed across the groups (Figure 1). At 0 min glucose concentration was approximately the same in the first two groups and was significantly higher compared to the last two groups, whereas in groups 3 and 4 there was no significant difference. At 30 min the highest glucose level (up to 13 mmol/L) was observed in group 2, then in group 1, while in groups 3 and 4 levels were almost the same. Trend towards increase in glucose level at 60 and 90 min was observed in group 1, while decrease in glucose concentration at 120 min compared to at 30 min was observed across all groups. Glucose levels did not return to normal values in the first two groups (9.0 mmol/L in the 1st group and 8.30 mmol/L in the 2nd group), while in the 3rd and 4th group glucose levels did return to values within reference range.

Aside from glucose levels OGTT insulin levels were also measured and highly statistically significant difference among groups ($p < 0.01$) was observed at 0 min and at 120 min, while at 30, 60 and 90 min there was no statistically significant difference ($p > 0.05$) (Figure 2). At 0 min the highest concentration was reported in IGT with CHD group. In the IGT without CHD group insulin concentration was 6 times higher at 30 min compared to fasting level and only at 120 min there was a slight decrease in serum insulin level. In contrast to the control group and the CHD without IGT group where significant steep decline in insulin concentration was observed after the initial increase, it was noted that in IGT with CHD group no decrease was observed, on the contrary, further increase in serum insulin concentration was observed compared to fasting insulin levels.

HOMA-IR index was calculated to determine IR and it showed higher values in subjects with IGT both with and without CHD compared to subjects with normal glucose tolerance and those with CHD, but no IGT, in the statistically significant range (Figure 3).

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Table 1: Clinical characteristics of patients

Caracteristics (n = 120)	IGT with IHD (n = 30)	IGT without IHD (n = 30)	IHD without IGT (n = 30)	Healthy (n = 30)
Gender (M/W)	20/10	18/12	17/13	14/16
Age	68.50 ± 7.6	57.50 ± 9.76	58.00 ± 10.91	47.00 ± 6.90
BMI (kg/m²)	26.60 ± 4.52	28.69 ± 4.24	25.06 ± 4.12	23.03 ± 4.68
W/H	0.92 ± 0.09	0.97 ± 0.09	0.91 ± 0.10	0.82 ± 0.13
SBP (mm Hg)	145 ± 17.98	140 ± 16.05	145 ± 18.72	120 ± 13.29
DBP (mm Hg)	90 ± 10.31	87.50 ± 10.08	90.00 ± 9.35	80.00 ±7.03
Tobacco (yes/no)	20/10	13/17	21/9	8/22

IGT: impaired glucose tolerance; IHD: ischaemic heart disease; BMI: body mass index: W/H: waist to hip ratio; SPB: systolic blood pressure; DBP: diastolic blood pressure;

Table 2: Patients' metabolic parameters

Caracteristics (n = 120)	IGT with IHD (n = 30)	IGT without IHD (n = 30)	IHD without IGT (n = 30)	Healthy (n = 30)
Fasting glucose (mmol/L)	6.30 ± 0.58	5.90 ± 0.59	5.00 ± 0.80	4.45 ± 0.52
HgA1C (%)	5.00 ± 0.89	5.40 ± 0.88	4.00 ± 0.73	3.50 ± 0.91
Glucose 0 min (mmol/L)	6.30 ± 0.86	6.15 ± 0.90	4.70 ± 0.83	4.85 ± 0.91
Glucose in 120 min (mmol/L)	9.00 ± 1.65	8.30 ± 1.65	4.20 ± 1.28	4.05 ± 1.20
Insulin 0 min (pmol/L)	13.78 ± 8.75	8.75 ± 24.91	12.96 ± 11.80	1.29 ± 6.33
Insulin in 120 min (pmol/L)	86.97 ± 57.74	57.74 ± 144	29.37 ± 31.82	28.94 ± 37.00
HOMA indeks	4.22 ± 2.46	7.02 ± 6.36	3.20 ± 2.66	2.72 ± 1.37
HOMA1 - % B	268.57 ± 355.00	283.81 ± 446.21	197.56 ± 420.00	110.32 ± 106.98
QUICKI indeks	0.31	0.32	0.34	0.34
Matsuda index	3.21	3.29	4.88	5.40
Holesterol (mmol/L)	5.20 ± 1.38	5.78 ± 2.05	5.75 ± 1.24	5.50 ± 0.87

IGT: impaired glucose tolerance; IHD: ischaemic heart disease;

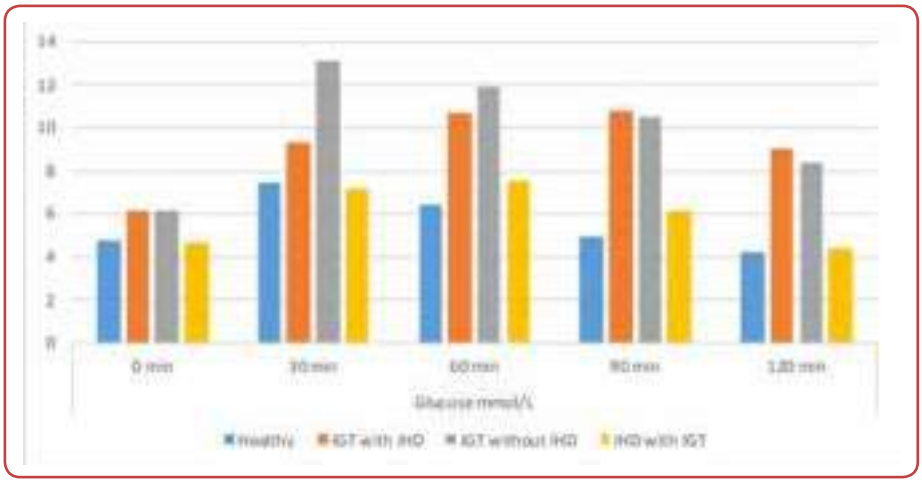

Figure 1: Glucose concentration during OGTT across study groups

IGT: impaired glucose tolerance; IHD: ischaemic heart disease; OGTT: oral glucose tolerance test;

Analysis of QUICKI index values showed that mean values across groups of patients of different status were quite similar. However, ANOVA results suggest that there was a statistically significant difference between values ($F(3) = 3.50$, $p < 0.05$). Review of the results of LSD, post hoc test, revealed that QUICKI index values in the IGT without CHD group were significantly lower on average compared to control and the CHD without IGT group. It was observed that MATSUDA index values were on average the highest in the control group, slightly lower in the CHD without IGT group and the lowest in the IGT without and IGT with CHD groups and statistically significant difference ($F(3) = 2.74$, $p < 0.05$) was recorded between the mean values.

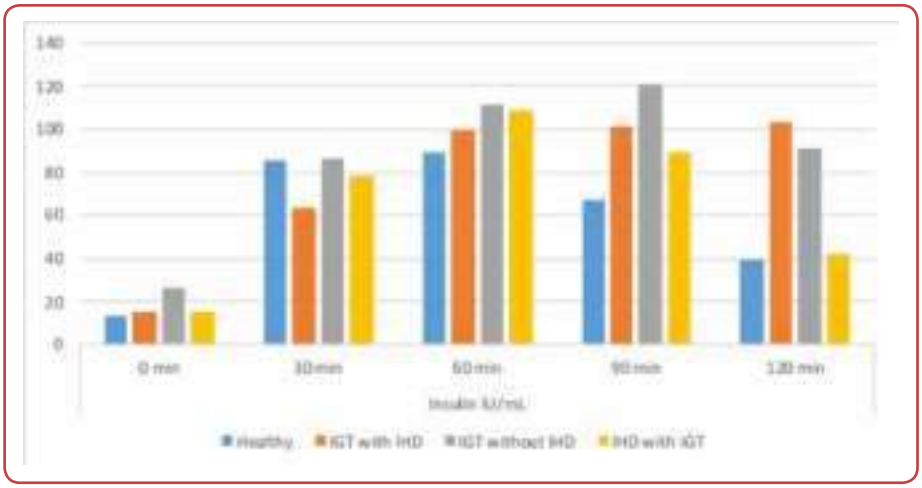

Figure 2: Insulin concentration during OGTT across study groups

IGT: impaired glucose tolerance; IHD: ischaemic heart disease; OGTT: oral glucose tolerance test;

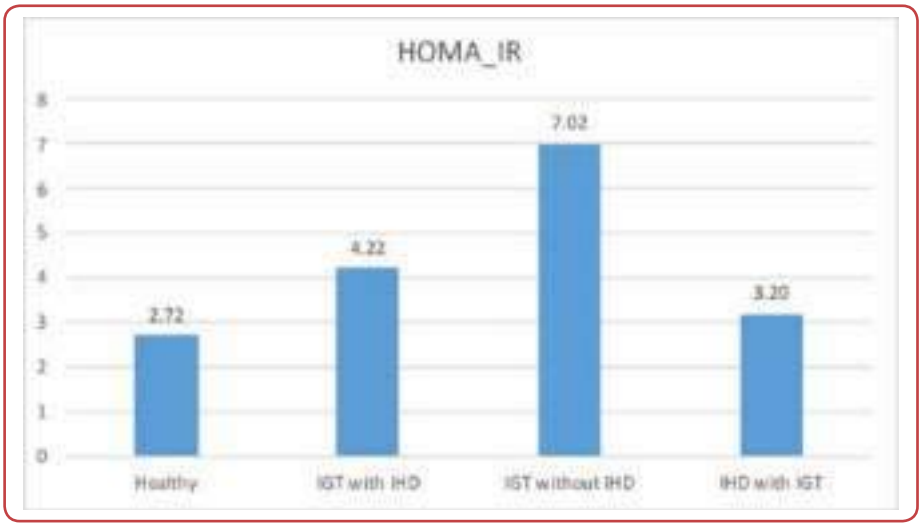

Figure 3: HOMA index of insulin resistance across study groups

IGT: impaired glucose tolerance; IHD: ischaemic heart disease;

Table 3: Levels of hsCRP across study groups

Category	N	M	SD	F	Df	P
IGT with CHD	30	4.82	1.47	133.62	3	.000**
IGT without CHD	30	0.91	0.42			
CHD without IGT	30	1.95	0.88			
Healthy	30	0.68	0.37			

***statistically significant at the p < 0.01 level*

hsCRP: high sensitive C-reactive protein; IGT: impaired glucose tolerance; IHD: ischaemic heart disease; N: number of patients; M: mean; SD: standard deviation; F: f-value, analysis of variance; Df: degrees of freedom; p: p-value;

Table 4: Levels of fibrinogen across study groups

Category	N	M	SD	F	Df	P
IGT with CHD	30	4.93	1.31	47.72	3	.000**
IGT without CHD	30	3.13	0.99			
CHD without IGT	30	3.83	0.96			
Healthy	30	1.85	0.76			

***statistically significant at the p < 0.01 level*

IGT: impaired glucose tolerance; IHD: ischaemic heart disease; N: number of patients; M: mean; SD: standard deviation; F: f-value, analysis of variance; Df: degrees of freedom; p: p-value;

Analysis of the lipid profiles across the groups showed the highest total cholesterol and LDL cholesterol in the IGT with and without CHD groups, although there was no statistically significant difference ($p > 0.05$) between the groups. The highest triglyceride levels were recorded in the CHD without IGT group and the highest mean HDL cholesterol levels in the control group. In subjects with marked postprandial hyperglycaemia, elevated levels of triglycerides and cholesterol and significant hyperinsulinaemia were also observed. In subjects with the highest IR HDL cholesterol levels were the lowest. Triglyceride levels were not the highest in the IGT groups with the highest IR, but in the CHD without IGT groups with normal peripheral sensitivity to insulin, therefore no cause-and-effect relationship with IR was observed in those subjects.

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The habit of smoking as the risk factor for CHD was noted in the highest percentage of subjects in the CHD groups with or without IGT, whereas in the group of healthy subjects the habit of smoking was observed in low percentage.

Statistical significance of the differences between CRP and fibrinogen levels across all four study groups was investigated using ANOVA, as is shown in Tables 3 and 4. It was noted that the mean values of CRP and fibrinogen in the IGT with CHD group were markedly higher than in the other groups and were, on average, the lowest in the healthy group.

Discussion

Several researchers suggest that IR is present even before blood glucose abnormalities are detected in patients with diabetes[23, 24] and that hyperinsulinaemia develops before pathophysiological abnormalities associated with IGT. Hyperinsulinaemia is harmful in individuals with normal and abnormal glucose tolerance. Helsinki Policemen Study,[25] Busselton Study,[26] Wisconsin Epidemiologic Study[27] and RISC study[28] have shown that high plasma insulin levels, fasting or after OGTT, are associated with increased risk of major cardiovascular events independent of the other conventional cardiovascular risk factors (including blood glucose, cholesterol, triglycerides, blood pressure, obesity, smoking and physical activity). Study results show that patients in prediabetes state (IGT, IFG – impaired fasting glucose) are at higher risk of CHD. Data from routine preventive exams show that blood glucose concentration, even levels below DM threshold, are associated with coronary artery disease.[29-31] IFG category was introduced to mark the range between the upper threshold of normal FPG and the lower threshold of diabetes FPG. IFG represents an intermediate state of abnormal glucose regulation and is a risk factor for future development of diabetes and CHD.[32]

In 2003 ADA recommended changing the lower threshold for IFG diagnosis from 6.1 to 5.6 mmol/L.[33] As Dankner et al[24] found basal hyperinsulinaemia in normoglycaemic adults is an independent risk factor for metabolic worsening of dysglycaemia in adulthood and may help identify seemingly healthy individuals at increased risk of diabetes. A large number of studies have confirmed the correlation between this metabolic state, decreased insulin sensitivity and resulting IR and development of CVD and the significance of OGTT as a diagnostic method for recruiting these individuals from the general population, in order to timely detect and prevent DMT2.[34-36]

Study results presented in this paper are comparable to other studies. In individuals with IGT, with or without associated CHD, IR index was significantly higher. In healthy controls HOMA index was slightly higher compared to individuals with CHD, which could be explained by BMI in the severe obesity range in a couple of individuals who had normal blood glucose and plasma lipoprotein concentrations and normal blood pressure readings. Individuals with IGT, with or without associated CHD, have impaired insulin sensitivity, significant impairment of beta cell function, manifest hyperinsulinism and the process of pancreatic beta cell apoptosis has probably started. Increased postprandial glucose levels are accompanied by increase in insulin secretion in pancreatic beta cells, but insulin secretion in groups 2 and 1 does not follow the increase in glucose concentrations, that is, the 1st phase of insulin secretion is delayed compared to the last two groups, particularly to the group of healthy individuals where insulin secretion significantly increases and follows the increase in postprandial hyperglycaemia in linear fashion.

Low QUICKI values are a good predictor of diabetes development in adults.[37, 38] In this study this predictive factor was noted in subjects with IGT without and with CHD. In clinical practice, all kinds of simple insulin sensitivity indices are used for the assessment of IR, which has been done in this study as well. Matsuda index is dependent on the accuracy of the insulin measurement method, cannot be used in individuals with extensive damage to beta cells and is more suitable for a large group epidemiologic research on IR, which is a limitation of this study.

A study conducted on large number of subjects with NGT, IGT and DM that investigated direct insulin sensitivity and its correlation with fasting insulin levels and insulin levels post glucose load, well known risk factors for CHD, has found that there is a very significant independent association between insulin sensitivity and CHD and between CHD and fasting insulin and has confirmed that hypertension, dyslipidaemia and hypergly-

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caemia play a role in these relationships, unlike some studies that have denied indirect relationship between these dysmetabolic states.[39, 40] This study has also shown that IGT together with IR and other metabolic disorders could contribute to the development of CHD to a certain extent.

De Fonzo[41] suggests that high CHD risk in diabetics is caused by IR rather than by hyperglycaemia and that CHD rates double if obesity, dyslipidaemia and hypertension are also present in those patients. He believes that elevated insulin levels lead to an alternative pathway of insulin signalling, activating mitogen activated protein kinase (MAPK), whose activity is central to mitogen-proatherosclerotic pathway.

Diabetes impairs utilisation of lipids and lipoproteins causing diabetes induced atherogenic dyslipidaemia, which is one of the most important risk factors for atherosclerosis in people with DM.[42, 43] Atherosclerosis is one of the main causes of the development of CVD.[44] Diabetic dyslipidaemia is characterised by increased levels of serum low-density lipoproteins (LDL) and triglycerides (TG) and decreased levels of high-density lipoproteins (HDL).[45]

Also, recently conducted prospective studies have shown that patients with DMT2 who developed CHD had higher levels of very low-density cholesterol (VLDL-h) with lower levels of high-density lipoprotein cholesterol 2 (HDL2) compared to individuals who did not develop CHD.[46] This association is noted in both type 2 diabetics and nondiabetics. The most significant predictive risk factor for CHD is increased levels of total and VLDL TG which manifest their atherogenic effect by inducing a decrease in HDL cholesterol and an increase in small, dense LDL particles, as well as an increase in production of apolipoprotein B. Research has shown that combined changes in lipid profile, ie small, dense LDL-h particles and prediabetes have a strong effect on the development of CHD.[47]

This study has found that total cholesterol levels were the highest in subjects with IGT, whereas total triglyceride levels were the highest in subjects with CHD. HDL cholesterol levels were the lowest in subjects with IGT and CHD, in accordance with previously mentioned studies.

The role of immune system is so important that atherosclerosis is nowadays considered primarily an inflammatory phenomenon.[48] Inflammatory model is useful in interpreting a large number of epidemiological data and it explains why serum levels of proteins, such as ceruloplasmin, fibrinogen and albumin are associated with cardiovascular risk.[49] Meta analysis of 14 prospective long-term studies has found that, after adjusting for age, smoking, cardiovascular risk factor CRP is strongly associated with CHD.[50, 51] Apart from pro-atherosclerotic effect of CRP, primarily in the early stages of atherogenesis, researchers have recently discovered that CRP could also play a role in the later stages of atherosclerosis. In 2003 the American Heart Association (AHA) and the Centre for Disease Control (CDC) recommended that inflammation markers be used together with other markers for cardiovascular risk assessment.[52] In accordance with that, the present study has shown that chronic inflammation factors are higher in IGT with CHD group and together with IR, they could be potential risk factors for the development of CHD in IGT. The study conducted in 2016 has found no association between serum fibrinogen and glucose impairment.[53]

Elevated hsCRP levels were associated with prediabetes in correlation with age, obesity and abnormal lipid profile, which was proven in Aishwarya Ghule study in 2021[54] and is comparable to this study where the levels were the highest in IGT with CHD group where atherosclerosis had, probably, already started developing.

Up until a few years ago, it was believed that the CHD typically affected men and most medical papers had adopted the idea that women could suffer more from breast cancer than from CVD. In 2004 the World Health Organization reported total CVD mortality in Europe: 55 % in women and 43 % in men. CHD, stroke and other CVD represent 23 %, 18 % and 15 % of cases in women and 21 %, 11 % and 11 % of cases in men.[55] After ages 45 for men and 55 for women CHD risk increases in similar fashion in both groups. It is presumed that oestrogen has cardioprotective effect in premenopausal women (usually under the age of 55). It is of utmost concern that CHD mortality rates in young women aged 35 to 45 continue to rise.[56-58] Older age, hypertension, total and LDL cholesterol have the biggest effect on men, whereas menopause, systolic hypertension, smoking, diabetes, triglycerides and HDL cholesterol levels mostly have an effect on women. Failure to recognise early symptoms and to adequately assess CHD in young women, despite well

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known risk factors, could contribute to this disturbing trend.[57] By middle age more than 80 % of women have 1 or more traditional risk factors for heart attack.[59] Diabetic women are more than six times more likely to die from CHD compared to women without diabetes.[60] There is evidence that the loss of oestrogen at any age contributes to endothelial dysfunction. In the Women's Health Study, a global risk prediction model, which has included hsCRP, improved prediction of cardiovascular risk in women.[61] Also, hsCRP was found to be a stronger cardiovascular event predictor in women than LDL cholesterol. After menopause, the lack of oestrogen leads to structural and functional changes in the cardiovascular system: endothelial dysfunction, imbalance of autonomic activity towards increased adrenergic activity, visceral obesity, increased systemic inflammation. All these factors contribute to the development of systemic hypertension, IGT, abnormal lipid profile and IR.

CHD group included mostly smokers and the difference between sexes was minimal in favour of men, in accordance with most previous studies. In individuals aged 45 to 54, 38.2 % of cardiovascular mortality in men and 33.7 % in women could be attributed to smoking.[62] Although the greatest risk burden is seen in middle age, smoking is a strong independent risk factor for cardiovascular events and mortality even in older age, increasing cardiovascular mortality for more than five years in smokers older than 60.[63] In all age groups, women smokers are at significantly higher risk of CHD events (fatal and nonfatal), compared to non-smoking women.[64] Smoking is more harmful to women than to men, with relative risk of cardiovascular events of 3.6 in women and 2.4 in men. While cardiovascular risk remains similar in men regardless of daily cigarette consumption, that risk increases in women with the number of cigarettes smoked per day ranging from relative risk of 2.3 for 1-9 cigarettes per day to 5.9 for more than 20 cigarettes per day.[65]

Several limitations of this report that cannot be considered definitive are to be acknowledged. Firstly, limited size of statistically representative sample.
In fact, data gathered here will be used as the basis for larger, more definitive study that will include remaining risk factors such as stress, concentrations of counterregulatory hormones that affect glucoregulation, menopause and the like.

Secondly, the limitation lies in inhomogeneous patient distribution among groups. The goal was to recruit representative sample of patients with IGT and CHD. Thus, the control group included significantly more women, fewer smokers and younger subjects compared to the other groups. Without a clear insight into the lifestyle, daily stress and regularity of menstrual cycles, considering that perimenopausal women were included in the study, the effect of oestrogen cardioprotection could not be asserted with certainty. The remaining three groups were homogenous regarding investigated parameters, sex, age and smoking habits. Limited sex specific data on the CHD risk associated with IGT, that were analysed in this study, did not support any significant difference between sexes. As a guideline, the results of the 2001 DECODE study were used that had determined that relative risks of CHD among participants with 2-hour glucose abnormalities corresponding to IGT, were very similar in men and women.[66] More studies are needed to better elucidate sex specific risks that can be attributed to IFG and IGT. This study did not address the question whether the risk of developing CHD was limited to individuals with IGT who developed diabetes or if it was still increased in individuals with IGT even if they never developed diabetes. Potential confounding factor such as physical activity, stress that could have significant effect on the sensitivity of peripheral tissues to insulin, is one of the limitations of this study. An economic analysis that would include diabetes prevention and CVD could provide additional useful information that would help guide future discussions of the need for screening for IGT in general population or in specific high-risk population groups.

Conclusion

Metabolic disorders, together with inflammation mediators, are possible predictors of the development of IGT. Decreased sensitivity of peripheral tissues to insulin and the resulting hyperinsulinism, increased concentrations of triglycerides and LDL cholesterol and decreased HDL cholesterol, as well as an increase in hsCRP and fibrinogen concentrations in patients, have all potentiated development of glucose tolerance disorders and potential

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effect on accelerated development of atherosclerosis in the vascular system of the heart of those patients. In practice, this requires the earliest possible detection of patients with hyperinsulinaemia and normal glucose tolerance in order to start a timely intervention to reduce glucotoxicity, lipotoxicity and IR, thus preventing the development of DMT2 and associated complications.

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None.

Conflict of interest

None.

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ORIGINAL ARTICLE

Prevalence and Risk Factors of Acne Among Adolescents in Kosovska Mitrovica: a Cross-Sectional Study

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Abstract

Background/Aim: Acne can occur in people of all ages, but mostly affects the population at puberty. Given the high prevalence and large impact that acne has on young people, the aim of this study was to assess adolescents' knowledge about factors that improve or worsen the clinical picture of acne, as well as to evaluate the sources used to obtain information on acne.
Methods: This cross-sectional study was conducted on a sample of 460 high school pupils from the Medical School and Gymnasium in Kosovska Mitrovica. A self-administrated questionnaire was used. Univariate and multivariate logistic regressions were used to model the association between gender (males/females) or presence of acne (no/yes) and potential exacerbating and ameliorating factors, as well as sources of information.
Results: 36.7 % of the respondents were male and 63.3 % were female. 48.9 % of high school pupils confirmed that they had acne. The main factors that worsen the condition of acne, were irregular face washing (88.7 %), hormones (87.0 %), fatty foods (80.9 %) and sweets (79.3 %). The majority of respondents believed that the intake of more water (83.9 %), cosmetic treatment (77.8 %), dietary changes (75.9 %), holiday (54.1 %) and sunbathing (39.3 %) affect improving acne. Taking more water (OR = 1.77; 95 % CI = 1.01-3.11) as a factor in improving acne was significantly more common in girls, while boys more often believed that sunbathing (OR = 0.62; 95 % CI = 0.41-0.94) and weight loss (OR = 0.53; 95 % CI = 0.32-0.88) affect the improvement of acne. The most important sources of information about acne were the Internet (73.0 %) followed by parents (62.6 %), friends (54.1 %), and a doctor (42.8 %).
Conclusion: Acne was more common in women and those with a positive family history. The presence of misconceptions among young people regarding the factors that improve or worsen the condition of acne indicates the need for additional education.

Key words: Acne vulgaris; Prevalence; Risk factors; Adolescent; Serbia.

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Introduction

Acne vulgaris is recognised as a common multifactorial chronic skin disease. The condition leads to negative psychosocial consequences in patients, increases the risk of developing depression and anxiety and impacts the quality of life overall.[1-3] In addition, sensitive period of life, such as adolescence and young adulthood, contributes to creating psychological trauma, inferiority complex, insecurities and psychological suffering.[4] Acne is the 8th most prevalent disease and impacts 9.4 % of the world population.[5] Acne can affect all age groups, but it is most commonly found in adolescents, up to 95 %.[6] In European population aged 15-27, average prevalence reaches 57.8 %, with the highest prevalence (65.8 %) in group aged 15-17.[7] According to results of the study performed

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in Serbia in 2019[8] acne was self-reported in 57.7 % of surveyed pupils. On time information about acne is a precondition for planning, program tailoring and treatment of this disease. The actual cause of acne is unknown, but there are factors contributing to acne development, such as genetic, lifestyle and environmental factors.[9, 10] Identification of factors that cause acne is beneficial in treatment of the disease.[11] Patients with acne often have poor knowledge about their condition and turn to various sources of information and in most cases came across myths and misconceptions.[12] Most patients wait sometimes more than a year before turning to a doctor for help and during that period they look for information about acne treatment mainly from the media, the Internet and parents and friends, which is inadequate and also represents a lack of education.[13, 14]

The aim of this study was to determine the prevalence of acne among adolescents, as well as, to assess their information and knowledge about acne exacerbating and ameliorating factors. Also, differences between male and female pupils were analysed.

Methods

This cross-sectional study was conducted on a sample of 460 high school pupils (first to fourth graders) from the Medical School and Gymnasium in Kosovska Mitrovica. The data were collected in April – June period of 2021. Researchers obtained approval from the principals of both schools to conduct the study and the data were collected in the break between classes. Participation was voluntary and anonymous. It was explained to the high school pupils that their anonymity is guaranteed, that their identity is impossible to reveal and that the data will be available only to the researchers, exclusively for scientific purposes.

Data collection

All pupils were asked to fill in a self-administered questionnaire which included questions on age, sex, presence of acne, seeking medical help, perceived acne aggravating or triggering factors (15 questions), perceived acne ameliorating factors (8 questions) and sources of information about acne (7 questions). All questions, except those on age and sex, were dichotomous with: “yes” and “no” answers. It took pupils 6 minutes in average to complete the questionnaire.

Statistical analysis

Categorical variables were presented as counts and percentages while continuous variable age was expressed as mean ± standard deviation. Comparisons between girls and boys were evaluated using Chi-square test. Univariate and multivariate logistic regression was used to model the association between gender (males/females) or presence of acne (no/yes) and potential exacerbating and ameliorating factors, as well as sources of information. Univariate analyses were performed first, followed by multivariate analyses adjusted for gender or the presence of acne where appropriate. Results were presented as odds ratios (OR) with 95 % confidence intervals (CI). Statistical analysis was performed with the Statistical Package for the Social Sciences, SPSS version 19.0 (SPSS Inc., Chicago, IL, USA). The level of statistical significance in all tests was $p < 0.05$.

Results

A total of 460 pupils were included in the current study, 36.7 % boys and 63.3 % girls. The mean age of pupils was 16.55 ± 1.05 (range 15–19) and 31.1 % were 17 years old. Acne was self-reported in 225 (48.9 %) pupils, out of which 88 (39.1 %) were boys and 137 (60.9 %) girls. 60 % of respondents with acne also had positive family history.

Self-perceived factors which can cause or aggravate acne in surveyed pupils are presented in Tables 1 and 2.

The main factors that aggravate acne, reported by pupils were inadequate face wash (88.7 %), hormones (87.0 %), greasy food (80.9 %) and sweets (79.3 %). Most respondents also belived that stress (78.0 %), makeup (73.0 %) and sweating (64.1 %) can aggravate acne (Table 1). Girls more frequently than boys reported that stress (OR = 2.95; 95 % CI = 1.76-4.96), hormones (OR = 2.12; 95 % CI = 1.15-3.90) and sweating (OR = 1.64; 95 % CI = 1.06-2.59) can worsen acne, while boys more frequently believed that exercise (OR = 0.37; 95 % CI = 0.21-0.67) can worsen the condition of acne (Table 1).

Table 1: Self-perceived acne risk or aggravating factors among pupils according to sex

Factor	Total N (%) (N = 460)	Sex N (%)		Univariate logistic regression analysis			Multivariate logistic regression analysis*		
		Boys (169)	Girls (291)	OR	95 % CI	*P*	OR	95 % CI	*P*
Hormones	400 (87.0)	134 (79.3)	266 (91.4)	2.779	1.598 - 4.834	0.000	2.122	1.153 - 3.904	0.016
Stress	359 (78.0)	109 (64.5)	250 (85.9)	3.356	2.126 - 5.298	0.000	2.952	1.758 - 4.955	0.000
Greasy food	372 (80.9)	136 (80.5)	236 (81.1)	1.041	0.644 - 1.683	0.869	0.737	0.419 - 1.295	0.288
Sweets	365 (79.3)	125 (74.0)	240 (82.5)	1.656	1.048 - 2.618	0.031	1.525	0.989 - 2.591	0.119
Dairy	162 (35.2)	54 (32.0)	108 (37.1)	1.257	0.841 - 1.878	0.264	1.227	0.772 - 1.951	0.387
Sweating	295 (64.1)	96 (56.8)	199 (68.4)	1.645	1.111 - 2.434	0.013	1.654	1.057 - 2.587	0.028
Exercise	69 (15.0)	35 (20.7)	34 (11.7)	0.507	0.302 - 0.849	0.010	0.374	0.208 - 0.673	0.001
Sun exposure	71 (15.4)	21 (12.4)	50 (17.2)	1.462	0.844 - 2.532	0.175	1.356	0.735 - 2.501	0.329
Less sleep hours	198 (43.0)	67 (39.6)	131 (45.0)	1.246	0.848 - 1.832	0.262	0.918	0.575 - 1.467	0.720
Inadequate face wash	408 (88.7)	143 (84.6)	265 (91.1)	1.853	1.037 - 3.311	0.037	1.052	0.529 - 2.094	0.884
Smoking	179 (38.9)	61 (36.1)	118 (40.5)	1.208	0.816 - 1.786	0.345	0.862	0.503 - 1.476	0.587
Alcohol	152 (33.0)	50 (29.6)	102 (35.1)	1.264	0.853 - 1.933	0.230	1.005	0.571 - 1.768	0.987
Coffee	80 (17.4)	28 (16.6)	52 (17.9)	1.096	0.662 - 1.814	0.723	1.020	0.555 - 1.875	0.948
Cosmetics/makeup	336 (73.0)	110 (65.1)	226 (77.7)	1.865	1.226 - 2.837	0.004	1.396	0.867 - 2.246	0.170

**Adjusted for the presence of acne; OR: odds ratio; CI: confidence interval*

Table 2: Self-perceived acne risk or aggravating factors among pupils according to the presence of acne

Factor	Total N (%) (N = 460)	Acne presence N (%)		Univariate logistic regression analysis			Multivariate logistic regression analysis*		
		No (N = 235)	Yes (N = 225)	OR	95 % CI	*P*	OR	95 % CI	*P*
Hormones	400 (87.0)	205 (87.2)	195 (86.7)	0.951	0.553 - 1.637	0.857	0.867	0.484 - 1.552	0.630
Stress	359 (78.0)	185 (78.7)	174 (77.3)	0.922	0.593 - 1.434	0.719	0.813	0.492 - 1.342	0.418
Greasy food	372 (80.9)	185 (78.7)	187 (83.1)	1.330	0.833 - 2.124	0.233	1.120	0.671 - 1.871	0.664
Sweets	365 (79.3)	179 (76.2)	186 (82.7)	1.492	0.944 - 2.357	0.086	1.294	0.785 - 2.133	0.312
Dairy	162 (35.2)	75 (31.9)	87 (38.7)	1.345	0.916 - 1.974	0.130	1.207	0.793 - 1.837	0.379
Sweating	295 (64.1)	152 (64.7)	143 (63.6)	0.952	0.650 - 1.394	0.801	0.873	0.576 - 1.325	0.524
Exercise	69 (15.0)	33 (14.0)	36 (16.0)	1.166	0.699 - 1.946	0.557	1.093	0.624 - 1.915	0.756
Sun exposure	71 (15.4)	35 (14.9)	36 (16.0)	1.088	0.656 - 1.805	0.743	1.019	0.596 - 1.743	0.944
Less sleep hours	198 (43.0)	93 (39.6)	105 (46.7)	1.336	0.923 - 1.934	0.125	1.327	0.867 - 2.030	0.193
Inadequate face wash	408 (88.7)	203 (86.4)	205 (91.1)	1.616	0.894 - 2.919	0.112	1.677	0.872 - 3.224	0.121
Smoking	179 (38.9)	92 (39.1)	87 (38.7)	0.980	0.673 - 1.426	0.916	0.788	0.481 - 1.289	0.342
Alcohol	152 (33.0)	74 (31.5)	78 (34.7)	1.154	0.783 - 1.703	0.469	1.182	0.702 - 1.991	0.529
Coffee	80 (17.4)	39 (16.6)	41 (18.2)	1.120	0.691 - 1.814	0.646	0.961	0.551 - 1.676	0.888
Cosmetics/makeup	336 (73.0)	166 (70.6)	170 (75.6)	1.285	0.849 - 1.943	0.235	1.254	0.799 - 1.968	0.325

**Adjusted for sex; OR: odds ratio; CI: confidence intervals*

There was no statistically significant difference between pupils with and without acne in the perceived factors that aggravate acne (Table 2).

Self-perceived factors which ameliorate acne in surveyed pupils are presented in Tables 3 and 4.

The majority of respondents believed that increased water consumption (83.9 %) and cosmetic treatment (77.8 %) can improve the condition of acne (Table 3). The other most prevalent acne ameliorating factors were a diet change (75.9 %), school holidays (54.1 %) and sun exposure (39.3 %). Girls more frequently than boys reported cosmetic treatment (OR = 2.22; 95 % CI = 1.41-3.50) and increased water consumption (OR = 1.77; 95 % CI = 1.01-3.11) as acne ameliorating factors, while boys more frequently believed in the benefit of sun exposure (OR = 0.62; 95 % CI = 0.41-0.94) and weight loss (OR = 0.53; 95 % CI = 0.32-0.88) (Table 3). There was no statistically significant difference between pupils with and without acne in the perceived factors that ameliorate acne (Table 4).

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Table 3: Self-perceived acne ameliorating factors among pupils according to sex

Factor	Total N (%) (N = 460)	Sex N (%)		Univariate logistic regression analysis			Multivariate logistic regression analysis*		
		Boys (169)	Girls (291)	OR	95 % CI	*P*	OR	95 % CI	*P*
Cosmetic treatment	349 (75.9)	110 (65.1)	239 (82.1)	2.465	1.594 - 3.812	0.000	2.219	1.406 - 3.501	0.001
Diet change†	358 (77.8)	124 (73.4)	234 (80.4)	1.490	0.952 - 2.330	0.081	1.312	0.809 - 2.127	0.272
Gaining weight	25 (5.4)	17 (10.1)	8 (2.7)	0.253	0.107 - 0.599	0.002	0.389	0.145 - 1.046	0.061
Losing weight	88 (19.1)	41 (24.3)	47 (16.2)	0.601	0.376 - 0.962	0.034	0.533	0.321 - 0.884	0.015
Water hydrate	386 (83.9)	131 (77.5)	255 (87.6)	2.055	1.244 - 3.395	0.005	1.774	1.012 - 3.109	0.045
Sun exposure	181 (39.3)	78 (46.2)	103 (35.4)	0.639	0.434 - 0.941	0.023	0.620	0.411 - 0.936	0.023
Smoking	20 (4.3)	13 (7.7)	7 (2.4)	0.296	0.116 - 0.757	0.011	0.735	0.238 - 2.270	0.593
School holidays	249 (54.1)	82 (48.5)	167 (57.4)	1.429	0.976 - 2.091	0.066	1.355	0.896 - 2.049	0.150

**Adjusted for the presence of acne; †toward healthier food choices; OR: odds ratio; CI: confidence interval*

Table 4: Self-perceived acne ameliorating factors among pupils according to the presence of acne

Factor	Total N (%) (N = 460)	Acne presence N (%)		Univariate logistic regression analysis			Multivariate logistic regression analysis*		
		No (N = 235)	Yes (N = 225)	OR	95 % CI	*P*	OR	95 % CI	*P*
Cosmetic treatment	349 (75.9)	182 (77.4)	167 (74.2)	0.838	0.547 - 1.286	0.419	0.811	0.518 - 1.269	0.359
Diet change†	358 (77.8)	178 (75.7)	180 (80.0)	1.281	0.823 - 1.994	0.273	1.241	0.782 - 1.970	0.360
Gaining weight	25 (5.4)	18 (7.7)	7 (3.1)	0.387	0.158 - 0.946	0.037	0.445	0.166 - 1.190	0.107
Losing weight	88 (19.1)	47 (20.0)	41 (18.2)	0.891	0.560 - 1.420	0.628	0.906	0.556 - 1.476	0.693
Water hydrate	386 (83.9)	197 (83.8)	189 (84.0)	1.013	0.616 - 1.666	0.960	0.893	0.518 - 1.540	0.685
Sun exposure	181 (39.3)	86 (36.6)	95 (42.2)	1.266	0.870 - 1.842	0.217	1.302	0.884 - 1.919	0.182
Smoking	20 (4.3)	15 (6.4)	5 (2.2)	0.333	0.119 - 0.933	0.036	0.396	0.126 - 1.247	0.114
School holidays	249 (54.1)	133 (56.6)	116 (51.6)	0.816	0.565 - 1.178	0.278	0.788	0.536 - 1.159	0.226

**Adjusted for sex; †toward healthier food choices; OR: odds ratio; CI: confidence interval*

Table 5: Source of information about acne among pupils according to sex;

Variable	Total N (%) (N = 460)	Sex N (%)		Univariate logistic regression analysis			Multivariate logistic regression analysis*		
		Boys (169)	Girls (291)	OR	95 % CI	*P*	OR	95 % CI	*P*
Parents	288 (62.6)	109 (64.5)	179 (61.5)	0.880	0.593 - 1.304	0.524	0.714	0.467 - 1.093	0.121
Doctor	197 (42.8)	71 (42.0)	126 (43.3)	1.054	0.718 - 1.547	0.788	1.132	0.734 - 1.745	0.576
Pharmacist	110 (23.9)	34 (20.1)	76 (26.1)	1.404	0.888 - 2.219	0.147	1.331	0.790 - 2.242	0.282
Friends	249 (54.1)	75 (44.4)	174 (59.8)	1.864	1.270 - 2.735	0.001	1.636	1.075 - 2.492	0.022
Internet	336 (73.0)	104 (61.5)	232 (79.7)	2.458	1.612 - 3.746	0.000	2.460	1.539 - 3.934	0.000
TV	188 (40.9)	67 (39.6)	121 (41.6)	1.084	0.736 - 1.595	0.684	0.559	0.354 - 0.883	0.013
Magazines	118 (25.7)	25 (14.8)	93 (32.0)	2.705	1.656 - 4.420	0.000	2.515	1.458 - 4.337	0.001

**Adjusted for the presence of acne; OR: odds ratio; CI: confidence interval*

Table 6: Source of information about acne among pupils according to the presence of acne

Variable	Total N (%) (N = 460)	Acne presence N (%)		Univariate logistic regression analysis			Multivariate logistic regression analysis*		
		No (N = 235)	Yes (N = 225)	OR	95 % CI	*P*	OR	95 % CI	*P*
Parents	288 (62.6)	138 (58.7)	150 (66.7)	1.406	0.962 - 2.055	0.079	1.290	0.867 - 1.918	0.209
Doctor	197 (42.8)	88 (37.4)	109 (48.4)	1.570	1.082 - 2.276	0.017	1.416	0.945 - 2.123	0.092
Pharmacist	110 (23.9)	49 (20.9)	61 (27.1)	1.412	0.918 - 2.172	0.116	1.385	0.859 - 2.234	0.181
Friends	249 (54.1)	122 (51.9)	127 (56.4)	1.200	0.831 - 1.733	0.330	1.250	0.836 - 1.870	0.276
Internet	336 (73.0)	166 (70.6)	170 (75.6)	1.285	0.849 - 1.943	0.235	1.545	0.975 - 2.449	0.064
TV	188 (40.9)	101 (43.0)	87 (38.7)	0.836	0.576 - 1.214	0.347	0.787	0.512 - 1.209	0.275
Magazines	118 (25.7)	70 (29.8)	48 (21.3)	0.639	0.418 - 0.977	0.039	0.598	0.369 - 0.970	0.037

**Adjusted for sex; OR: odds ratio; CI: confidence interval*

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In the present study the most frequent source of information about acne was the Internet (73.0 %), followed by parents (62.6 %), friends (54.1 %) and a doctor (42.8 %) (Table 5 and 6). Girls more frequently than boys reported magazine (OR = 2.52; 95 % CI = 1.46-4.34), the Internet (OR = 2.46; 95 % CI = 1.54-3.93) and friends (OR = 1.64; 95 % CI = 1.08-2.49), while boys more frequently reported TV (OR = 0.56; 95 % CI = 0.35-0.88) as a source of information (Table 5). The magazines (OR = 0.60; 95 % CI = 0.37-0.97) were more frequently reported by pupils without acne compared to those with acne (Table 6).

Discussion

In this study, 48.9 % pupils reported acne. Overall prevalence is in accordance with the results from numerous studies conducted worldwide.[15-17] Acne prevalence is a bit higher in some European countries. [18-20] The highest prevalence is noted in Brazilian study where 96 % of pupils aged 10-17 reported acne.[21] According to the results of the present study, acne was more commonly found in females compared to males (60.9 % vs 39.1 %), which correlates with other studies' results.[22-24] There are also studies where the percentage of male and female respondents suffering from acne is very similar,[7, 25] as well as studies where the prevalence of acne among male adolescents is significantly higher compared to female adolescents.[26-28]

Surveyed participants considered inadequate face washing, hormones, greasy food, increased sweets consumption, stress, makeup and sweating as leading acne aggravating factors, which is in accordance with findings from other studies that examined the knowledge about acne among adolescents.[29, 30] The study results confirmed that girls more frequently report stress, hormones and sweating while boys more often report exercising as factors that can worsen acne, which is consistent with the results from a Serbian study.[8] Over one-half of Serbian boys believed sweating and exercising worsen acne whereas girls reported stress, sweets consumption, greasy food, sun exposure and sleep deprivation as aggravating factors.[8] In Montenegrin study[16] girls more often reported genetics, stress, sweets consumption, improper face washing and makeup as acne aggravating factors. In a study by Rigopoulos et al,[29] girls more frequently considered diet change as an acne contributing factor.

The relationship between diet and acne has been observed to a large extent and lower acne prevalence was noted in underdeveloped countries where low calorie diet is present compared to developed countries with the highest prevalence where the diet is characterised by high glycaemic load and high calories.[11]

Greasy food can also worsen acne condition by releasing fatty acids from triglycerides which triggers acne development.[31] Higher BMI is associated with high glycaemic overload which increases glucose and insulin concentration and induces Insulin growth factor IGF-1 synthesis.[32-34] Several studies showed that high chocolate intake is an important aggravating factor for acne,[7, 35] but there is also a study that did not discover any association.[36] According to a study conducted by Vongraviopapa and Asamocnonda daily intake of 25 grams of chocolate contributes to acne presentation.[37] Meta-analysis of observational studies[35] confirmed a relationship between dairy consumption and acne. Regardless of type and quantity of dairy products or milk, there is a significantly high risk for appearance of acne because of hormone or sugar presence in a milk.[38]

Stress is certainly one of the risk factors that also affects the worsening of already existing acne.[39] A study performed among adolescents in Northeast China,[17] reported depression and stress as the main risk factors. Halvorsen et al[40] in their study which included 3,777 adolescents found an association between increased stress and the severity of acne. Chiu et al,[41] as well as respondents from India,[42] reported worsening of acne condition during exam period. Stress induces the local expression of neuropeptides that may represent a pathogenic step for the development of acne.[43]

In this study the majority of subjects (87 %) reported hormones as aggravating factors for acne and girls blamed hormones twice as more compared to boys. Several studies confirmed hormones (androgens) as a key role in the pathogenesis of acne.[43-45] Increased androgen activity causes sebum build-up inside the pilosebaceous unit which represents a suitable environment for acne development.[46]

Sweating potentially increases risk of acne.[47] Nakano et al[48] discovered changes in sweat content in acne patients, that is, detected decreased dermcidin expression, peptide active against *Propionibacterium acnes*, which provides undisturbed growth of the bacteria.

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There is still a lack of evidence regarding improper facial hygiene as an acne aggravating factor.[49, 50] In this study, 73 % of the respondents believed that makeup triggers acne, with similar results obtained in other studies.[51-53] Even 90 % of respondents used some kind of makeup product, according to research from Sri Lanka.[52] Levin[54] noted that inadequate skin care can modify the protective role of the skin, leading to sebum production changes and microbiome balance disturbances.

Regarding the results of this study, sun exposure as a risk factor that worsens the condition of acne was mentioned by only 15.4 % of respondents, which is consistent with the results of the Serbian study (13.4 %),[8] as well as the Montenegrin study,[16] where that percentage was slightly higher and equals 20.8 %. In research performed by Engel et al,[55] sun exposure was associated with worsening condition of acne. In one Indian study[56] 26 % of participants confirmed it while in Khunger and Kumara's research,[57] 33.2 % of patients declared worsening of the acne condition after sun exposure. Korean study[58] discovered that UVB radiation leads to inflammatory cytokines expression in sebocytes, which was also confirmed by Suh et al.[59] A hot environment can stimulate sweat and sebum production, increase hydration level and transepidermal water loss and lower skin pH.[60]

There is no clear evidence of the relationship between smoking and acne prevalence. Particular studies showed a connection between smoking status and severe acne conditions, as well as significantly higher prevalence in smokers compared to non-smokers.[61, 62] However, some studies found lower acne prevalence in smokers.[63, 64] A French study performed by Wolkenstein et al[64] conducted on adolescents and young adults showed that smoking more than 10 cigarettes a day was highly associated with having no acne.

Inadequate sleep duration as a risk factor was reported by 43 % of participants in this study, whereas 47 % of Montenegrin respondents confirmed it.[16] The lack of sleep triggered acne in 10 % of Serbian pupils[8] while Al-Hoqaila discovered that 40.2 % of men had acne exacerbation as a result of improper sleep.[65]

The majority of respondents in the presented study (77.8 %) stated that a change in diet is one of the factors that potentially affects the condition of acne. This was also proven in the study by Claudel et al,[66] where a diet consisting of vegetables and fish has a potentially beneficial effect. Omega-3 fatty acids found in fish and high fibre content in fruits and vegetables can reduce acne risk by decreasing IGF-1 levels.[67]

Concerning the results of this study, taking more water was recognised by the respondents as an important factor that leads to the improvement of the acne condition and approximately 84 % of them shared this opinion. Girls had this attitude significantly more often than boys. The results are in line with the results of the Montenegrin study [16] in which 77.6 % of respondents believed that drinking more water affects acne remission.

A total of 76 % of participants in this study declared cosmetic treatment as effective for the improvement of skin condition and girls believed in the benefits of the treatment two times more than boys. The results are consistent with a study from Montenegro[16] with 80.4 %, whereas Chilicka et al[68] observed improved life quality in patients with acne who received cosmetic treatment.

In this research, 54 % of respondents believed being out of school leads to acne improvement, while 62 % of Montenegrin participants shared that opinion.[16] Other authors considered school holidays as ameliorating factor by minimising anxiety and incorporating healthier habits.[8, 16]

Although most of the studies confirmed sun tanning as an acne triggering factor because of its carcinogenic effect,[69] this results showed that 39.3 % of respondents believe it improves the condition, which is in accordance with other studies.[8, 16] In addition, some studies showed that boys significantly more frequently declare sun tanning as a factor for acne improvement.[8, 10, 16]

In the present study 19 % of respondents stated that losing weight leads to acne improvement, compared to 28.8 % in a Montenegrin study.[16] Di Landro et al[36] discovered that people with lower BMI have a significantly lower risk of developing acne.

Regarding sources of information about acne, the Internet (73.0 %), parents (62.6 %), friends (54.1 %) and medical professionals (42.8 %) were considered the most important ones. Girls mostly learned facts about the acne condition from magazines and the Internet, while boys acquired knowledge mostly from the television. Saudi Ara-

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bic boys[70] stated that the most popular sources are mass media (47.7 %), friends (45.6 %) and magazines (28 %), whereas Turkish students[71] considered physicians (35 %), Internet (28.3 %), friends (19.1 %) and pharmacists (14 %) as valuable sources. Majority of pupils in Lithuania,[72] aged 7-19 learned about the disease thanks to parents (76.3 %), magazines (35.5 %) and friends (29.3 %). The results of this study showed that a doctor was consulted in 42.8 % of cases, which is consistent with the results of studies by Al-Natour,[70] Delarue et al[73] and a Greek study,[29] where one third of respondents consulted a doctor. The most popular source of information in the study by Djurović et al[16] was the Internet and parents, while in the study by Yorulmaz and Yalcin,[12] the most frequently used source of information were dermatologists, the Internet and social media.

According to our knowledge, this is the first study on the prevalence of acne and the awareness of young people about acne and its risk factors in Kosovo and Metohija. The advantage of the study is reflected in the fact that the research was conducted on a representative sample of high school pupils from Kosovska Mitrovica, as well as the fact that the response rate was high.

However, certain limitations should be mentioned. The survey did not include high school pupils from other cities of Kosovo and Metohija, so the results cannot be generalised. Then, the limitation also refers to the objectivity of the answers obtained through the survey because it includes questions on sensitive topics and there is a possibility that the answers obtained do not fully reflect the attitudes of the respondents. One of the limitations could be the small age range (15-19) of the pupils surveyed in this study.

Despite all the shortcomings of this study, the obtained results can serve as an indicator of the level of awareness of high school pupils in Kosovska Mitrovica about acne and indicate the need for their education about this skin disease.

Conclusion

Acne was more common in women and those with a positive family history. The presence of misconceptions among young people regarding the factors that improve or worsen the condition of acne indicates the need for additional education.

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Conflict of interest

None.

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ORIGINAL ARTICLE

The Effects of Subchronic Intake of Magnesium Hydrocarbonate-Rich Mineral Water on Cardiometabolic Markers and Electrolytes in Rats With Streptozotocin-Induced Diabetes

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Abstract

Background/Aim: Hypomagnesaemia is one of the most detected electrolyte abnormalities in diabetics. Modulation of numerous cardiovascular pathophysiological processes is a potential goal for anti-diabetic therapy. Magnesium supplementation prevents subclinical tissue magnesium deficiency, thus delaying the onset of metabolic imbalance in diabetes, but long-term effects of magnesium supplementation in chronic diabetes and numerous pathophysiological processes remain unknown. Aim of this study was to determine the effects of subchronic intake of magnesium hydrocarbonate-rich mineral water on cardiometabolic markers and electrolytes in rats with streptozotocin-induced diabetes.

Methods: A total of 28 Wistar, male rats, body weight 160 g at start, were divided into four groups of 7 each: two controls, group that drank tap water and received a single ip injection of saline (0.9 % NaCl) (TW-C), group that drank mineral water rich in magnesium hydrocarbonate and received a single ip injection of saline (0.9 % NaCl) (MW-C); and two experimental groups with streptozotocin-induced diabetes, group that drank tap water and received a single ip injection of streptozotocin (100 mg/kg) in saline (0.9 % NaCl, 1 mL) (TW-DM), group that drank mineral water rich in magnesium hydrocarbonate and received a single ip injection of streptozotocin (100 mg/kg) in saline (0.9 % NaCl, 1 mL) (MW-DM).

Results: Regarding the biochemical parameters, a decrease was observed in the MW-C group for vitamin B_{12} and proteins, while triglycerides were higher compared to the TW-C group. By comparing the haemostatic biomarkers between TW-C and MW-C groups, a statistically significant decrease was found for fibrinogen, while the electrolyte analysis showed an increase in phosphates for the MW-C group. Biochemical value comparison between TW-DM and MW-DM groups showed that magnesium hydrocarbonate usage in diabetic rats did not significantly reduce glycaemia although the average glycaemic values were lower in the group treated with magnesium hydrocarbonate. Regarding the electrolyte values, a statistically significant decrease was observed for sodium, potassium and phosphate in the MW-DM group. The MW-DM group also showed a significant increase in iron value compared to TW-DM group.

Conclusion: Subchronic intake of magnesium hydrocarbonate-rich mineral water, as a form of magnesium supplementation, did not cause a significant improvement in glycaemia or normalisation of diabetes-induced dyslipidaemia. This study showed the reduction of fibrinogen value, thus indicating the possibility of usage of this form of magnesium supplementation in different pro-thrombogenic conditions.

Key words: Magnesium; Streptozotocin-induced diabetes; Cardiometabolic markers; Electrolytes; Rat.

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Introduction

Diabetes mellitus (DM) is one of the most important public health problems globally, with ever increasing incidence and prevalence.[1] Besides hyperglycaemia, which is the most frequent sign, diabetes is associated with impaired effects and secretion of insulin, metabolism of carbohydrates, lipids, as well as an increased risk for the development of various micro- and macrovascular complications.[2] Cardiovascular complications are one of the most important causes of death in patients with diabetes.[3] These complications represent the result of the pathological process of remodelling of the heart and blood vessels, which is directly induced by metabolic disorders that occur with diabetes, such as hyperglycaemia, dyslipidaemia, acid-base disturbances and the electrolyte levels.[4-6] The ensuing diabetic cardiomyopathy and coronary artery disease predisposes the myocardium for the development of the contractile dysfunction, ischaemic heart disease or various forms of the disturbance of the heart rhythm. Additionally, micro- and macrovascular angiopathy, as key pathoanatomic substrates in diabetes, induce lesions in other target tissues, such as brain, kidney or eyes.[7] Because of the consequences that it leaves on the cardiovascular system, diabetes should be viewed not just classically, as a metabolic disease, but also as a cardiovascular disease, because of which the modulation of the pathological process of remodelling of the cardiovascular system, the mechanism of which is not known yet, could be one of the main goals of the anti-diabetic therapy.[8]

Hypomagnesaemia is one of the most frequent electrolytic impairment seen in diabetic patients, especially in those ones with poorly regulated diabetes.[9-10] The decreased body concentration of magnesium (Mg^{2+}) is closely related to atherosclerosis, coronary artery disease and cardiac dysrhythmias.[11-13] However, although it is shown that use of magnesium in rats with experimentally induced diabetes results in modulation of insulin receptors and improvement of the metabolic balance, the role of magnesium in the process of cardiovascular remodelling remains unclear.[14] A problem of the revealing the concrete role that magnesium has in target tissues in the pathogenesis of diabetes is in the fact that tissue magnesium deficits are practically non-detectable, since Mg^{2+} is predominantly intracellular cation, bound to cellular components that does not readily pass into the extracellular fluid.[15] In addition to this, clinical hypomagnesaemia reflects the decrease in the concentration of ionised serum Mg^{2+} that cannot be used for estimation of the magnitude of its tissue deficit, since blood magnesium accounts for only 0.3 % of the total body magnesium.[16-21] Use of Mg^{2+} in a form of supplements can prevent the occurrence of subclinical deficits of intracellular magnesium and potentially postpone the occurrence of metabolic disbalance caused by conditions stressful for the organism, such as diabetes. It has been previously shown that the administration of magnesium as a supplement in rats in the early phase of diabetes leads to improved compliance of heart ventricles, as well as to the normalisation of the autonomous system of the myocardium, while the long-term effects of magnesium supplementation and their mechanisms remain unknown.[22]

The aim of this study was to ascertain the effect of subchronic intake of magnesium hydrocarbonate -rich mineral water, as a form of magnesium supplementation, on cardiometabolic markers and electrolyte levels in rats with streptozotocin-induced diabetes.

Methods

This research was done as a part of the project of the Ministry of Education, Science and Technological Development of the Republic of Serbia (No 200110/00402). Ethics consent was obtained from the Ministry of Agriculture, Forestry and Water Management of the Republic of Serbia and based on the ethics approval issued by the Ethics Committee for the Protection of Welfare of Experimental Animals of the Faculty of Medicine, University of Belgrade. In this 6-week-long experimental study 25-30 days old and 160 g of weight male Wistar rats obtained from the vivarium of the Military Medical Academy in Belgrade were used. Rats were kept individually in plexiglas cages with constant ambient conditions (temperature 21 ± 2 ºC; humidity 55 ± 5 %; light-dark cycle of 12 h with the start of the light cycle at 07:30 h) and their daily food and water consumptions were registered. The animals were allowed access to food and water *ad libitum*. Diabetes mellitus was induced with a single injection of streptozotocin ($C_8H_{15}N_3O_7$, *Sigma-Aldrich, Darmstadt, Germany*) 100 mg/

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kg) dissolved in physiological saline (0.9 % NaCl, 1 mL). Samples of blood from the tail vein were drawn 72 h after the streptozotocin injection and after that on a weekly basis. Rats were allowed to drink either the standard tap water or mineral water rich in magnesium hydrocarbonate (oligomineral, magnesium hydrocarbonate, natural spring mineral water "Mg Mivela" produced by *Nova Sloga d.o.o. Trstenik, Serbia*; with mineral composition (mg/L), cations: Mg^{2+} 343, Na^{+} 138, Ca^{2+} 21.670, K^{+} 9.510, anions: HCO_3^- 2109.400, Cl^- 13.400, SO_4^{2-} < 1.000, F^- 0.205, according to the manufacturer's declaration on the purchased products).

A total of 28 animals were divided into four groups of 7 each:

1. group that drank tap water and received a single ip injection of saline (0.9 % NaCl) (TW-C),
2. group that drank mineral water rich in magnesium hydrocarbonate and received a single ip injection of saline (0.9 % NaCl) (MW-C),
3. group that drank tap water and received a single ip injection of streptozotocin (100 mg/kg) in saline (0.9 % NaCl, 1 mL) (TW-DM),
4. group that that drank mineral water rich in magnesium hydrocarbonate and received a single ip injection of streptozotocin (100 mg/kg) in saline (0.9 % NaCl, 1 mL) (MW-DM).

After completion of a 6-week-long treatment, animals were sacrificed and their blood was collected for analyses of biochemical parameters in plasma and serum, including biomarkers of cardiac and neural injury: aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), creatine kinase (CK), high-sensitivity troponin T (hs-TnT), homocysteine (Hcy) and vitamin B_{12}; haemostatic biomarkers: fibrinogen, von Willebrand factor (vWF), vWF %; lipid profile: total cholesterol, low density lipoprotein (LDL) cholesterol, high density lipoprotein (HDL) cholesterol, triglycerides; pancreato-hepatorenal biomarkers: glucose, urea, creatinine, uric acid, proteins, albumin, alkaline phosphatase (ALP), amylase; as well as the ionogram (Na^{+}, K^{+}, Ca^{2+}, Mg^{2+}, Fe^{2+}, HCO_3^-, Cl^-, PO_4^{3-}).

Analyses were performed by commercial kits (*Siemens Healthcare Diagnostics Ltd., Frimley, Camberley, UK*) on automated analyser (*Dimension Xpand, Siemens, Erlangen, Germany*), on atomic spectrophotometer and using spectrophotometric method. Concentration of glucose in blood drawn from tail vein was determined by use of ACCU-CHEK analyser (*Roche Diabetes Care, Inc, Indianapolis, USA*).

Statistical analysis

Statistical analysis was performed by using the GraphPad Prism 5 program. Normality of the data distribution was checked with Shapiro-Wilk test, since there were less than 50 units of observance within the groups, with values of the test $p > 0.05$ indicating the normal distribution of data. All the parameters in the present study were numeric and continual and, provided their distribution was normal, t-test for independent samples was used. In case that the data did not show normal distribution, Mann-Whitney test was used. Values of $p < 0.05$ were considered statistically significant.

Results

Comparison of the parameter values between the groups of rats subchronically treated with tap water (TW) or water rich in magnesium hydrocarbonate (MW)

Among the monitored biochemical parameters, values of the biomarkers of cardiac and neural injury (AST, ALT, LDH, CK, hs-TnT, Hcy) were not significantly decreased in the group of animals subchronically exposed to water rich in magnesium hydrocarbonate (Table 1). A significant decrease in the vitamin B_{12} levels was found (MW-C: 297.0 (288.0 – 359.0); TW-C: 400.0 (382.0 – 426.0)) ($p < 0.05$). Among the investigated haemostatic biomarkers, a significant decrease in fibrinogen levels was found in rats with subchronic intake of magnesium hydrocarbonate -rich mineral water (MW-C: 2.004 ± 0.063), in comparison with the control group who drank tap water (TW-C: 2.173 ± 0.177) (Table 1). Increase in the levels of triglycerides (MW-C: 1.110 ± 0.403; TW-C: 1.101 ±0.145) ($p < 0.05$) was detected in MW-C group compared to TW-C group (Table 1), while there was no significant difference between the lipid profiles (LDL and HDL cholesterol) of the two groups ($p > 0.05$). Decrease of protein levels (MW-C: 60.00 (57.00 – 60.00); TW-C: 61.00 (60.00 – 61.00)) ($p < 0.05$) (Table 1) was found in the MW-C group in comparison with the TW-C group of animals. After studying the ionograms

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between the groups, an increase in the phosphate levels was found in the MW-C group (MW-C: 2.336 ± 0.199), as compared to the control group of rats that drank tap water (TW-C: 2.166 ± 0.056) (Table 2).

Table 1: Values of biochemical parameters in the control group of rats subchronically exposed to tap water (TW-C) or water rich in magnesium hydrocarbonate (MW-C)

Parameter	Groups		*p* value
	TW-C	MW-C	
Biomarkers of cardiac and neural injury			
AST [a]	172.286 ± 27.103	187.857 ± 46.323	0.386
ALT [a]	54.714 ± 6.184	57.429 ± 7.955	0.901
LDH [b]	4182 (3946 – 5807)	5371 (3332 – 6013)	0.482
CK [a]	6447.143 ± 1592.075	6642.333 ± 1731.076	0.932
hsTnT[a]	19.143 ± 9.547	21.500 ± 15.859	0.373
Hcy [a]	13.724 ± 3.597	14.200 ± 2.433	0.586
Vitamin B12 [b]	400.0 (382.0 – 426.0)	297.0 (288.0 – 359.0)	0.018 *
Haemostatic biomarkers			
Fibrinogen[a]	2.173 ± 0.177	2.004 ± 0.063	0.012 *
vWF[a]	0.324 ± 0.027	0.299 ± 0.031	0.600
vWF %[a]	200.086 ± 31.695	172.943 ± 34.829	0.661
Lipid profile			
Total cholesterol [a]	1.654 ± 0.237	1.479 ± 0.167	0.451
LDL cholesterol [a]	0.411 ± 0.156	0.357 ± 0.148	0.918
HDL cholesterol [b]	0.720 (0.690 – 0.750)	0.650 (0.610 – 0.720)	0.063
Triglycerides [a]	1.101 ± 0.145	1.110 ± 0.403	0.017 *
Pancreato-hepatorenal biomarkers			
Glucose [a]	6.600 ± 0.258	6.457 ± 0.597	0.057
Urea [a]	7.600 ± 0.686	7.200 ± 0.978	0.194
Creatinine [b]	47.00 (47.00 – 48.00)	47.00 (44.00 – 47.00)	0.246
Uric acid [b]	70.00 (62.50 – 70.00)	70.00 (60.00 – 100.00)	0.643
Proteins [b]	61.00 (60.00 – 61.00)	60.00 (57.00 – 60.00)	0.019 *
Albumin [b]	29.00 (28.00 – 30.00)	29.00 (29.00 – 30.00)	0.945
ALP [a]	299.000 ± 29.586	278.143 ± 43.071	0.239
Amylase [a]	1739.286 ± 219.445	1625.000 ± 275.451	0.696

a – Values are presented as mean ± SD. p < 0.05, t test;*
*b – Values are presented as median (25% – 75% percentile). * p < 0.05, Mann-Whitney test;*
AST: aspartate aminotransferase; ALT: alanine aminotransferase; LDH: lactate dehydrogenase; CK: creatine kinase; hsTnT: high-sensitivity troponin T; Hcy: homocysteine; vWF: von Willebrand factor; LDL: low density lipoprotein; HDL: high density lipoprotein (HDL); ALP: alkaline phosphatase

Table 2: Serum ion concentrations in the control groups of rats with subchronic intake of tap water (TW-C) or mineral water rich in magnesium hydrocarbonate (MW-C)

Parameter	Groups		*p* value
	TW-C	MW-C	
Na^+	140.140 ± 1.069	140.28 ± 1.380	0.351
K^+	5.350 ± 0.423	5.583 ± 0.542	0.789
Ca^{2+}	2.461 ± 0.046	2.470 ± 0.073	0.220
Mg^{2+}	0.896 ± 0.036	0.951 ± 0.055	0.246
Fe^{2+}	34.614 ± 5.189	45.314 ± 4.167	0.774 *
HCO_3^-	23.286 ± 1.604	22.714 ± 1.496	0.833
Cl^-	101.000 ± 1.633	101.000 ± 0.817	0.230
PO_4^{3-}	2.166 ± 0.056	2.336 ± 0.199	0.023

*Results are presented as mean ± SD. * p < 0.05, t test;*
Na^+: sodium; K^+: potassium; Ca^{2+}: calcium; Mg^{2+}: magnesium; Fe^{2+}: iron; HCO_3^-: bicarbonate; Cl^-: chloride; PO_4^{3-}: phosphate

Comparison of parameters between the experimental groups of rats with streptozotocin-induced diabetes mellitus subchronically exposed to tap water (TW-DM) or magnesium hydrocarbonate-rich water (MW-DM)

Comparison of the values of the biochemical parameters between groups, including biomarkers of cardiac and neural injury, haemostatic biomarkers and lipid profile, did not reveal any significant difference (p > 0.05) (Table 3).

Table 3: Values of biochemical parameters in experimental group of rats with streptozotocin-induced diabetes mellitus subchronically treated with tap water (TW-DM) of water rich in magnesium hydrocarbonate (MW-DM)

Parameter	Groups		*p* value
	TW-DM	MW-DM	
Biomarkers of cardiac and neural injury			
AST [a]	213.667 ± 29.092	223.000 ± 42.426	0.453
ALT [a]	118.333 ± 60.451	102.867 ± 47.894	0.566
LDH [b]	3484 (2589 – 4453)	4415 (2187 – 6305)	0.724
CK [a]	5985.667 ± 3266.870	3939.333 ± 1133.324	0.167
hsTnT[a]	39.000 ± 36.497	22.000 ± 20.753	0.209
Hcy [a]	5.653 ± 1.483	9.223 ± 5.735	0.075
Vitamin B12 [b]	511.0 (388.5 – 698.0)	417.0 (300.0 – 612.8)	0.386
Haemostatic biomarkers			
Fibrinogen[a]	2.780 ± 0.726	1.120 ± 1.160	0.282
vWF %[a]	278.300 ± 95.529	278.367 ± 54.658	0.355
Lipid profile			
Total cholesterol [a]	7.770 ± 7.632	7.375 ± 6.966	0.153
LDL cholesterol [a]	0.550 ± 0.673	0.760 ± 0.905	0.548
HDL cholesterol [b]	0.860 (0.570 – 0.990)	0.970 (0.370 – 1.10)	0.827
Triglycerides [a]	3.357 ± 4.016	3.260 ± 2.483	0.287
Pancreato-hepatorenal biomarkers			
Glucose[a]	30.813 ± 17.580	13.973 ± 5.337	0.102
Urea [a]	9.267 ± 1.222	11.133 ± 4.881	0.153
Creatinine [b]	62.00 (46.00 – 63.00)	54.00 (47.00 – 76.00)	0.827
Uric acid [b]	70.00 (70.00 – 70.00)	120.00 (100.00 – 140.00)	0.221
Proteins [b]	62.00 (56.00 – 66.00)	61.00 (56.00 – 134.00)	1.000
Albumin [b]	22.00 (20.00 – 27.00)	27.00 (22.00 – 30.00)	0.261
ALP [a]	1021.750 ± 503.407	1138.000 ± 680.090	0.634
Amylase [a]	1446.500 ± 332.591	1079.333 ± 355.213	0.935

a – Values are presented as mean ± SD;
b – Values are presented as median (25% – 75% percentile);
AST: aspartate aminotransferase; ALT: alanine aminotransferase; LDH: lactate dehydrogenase; CK: creatine kinase; hsTnT: high-sensitivity troponin T; Hcy: homocysteine; vWF: von Willebrand factor; LDL: low density lipoprotein; HDL: high density lipoprotein (HDL); ALP: alkaline phosphatase

In vivo treatment of rats with magnesium hydrocarbonate did not induce a significant decrease in serum glycaemia (p > 0.05), although the mean glucose concentrations in rats with diabetes tended to be lower in rats treated with magnesium hydrocarbonate (MW-DM: 13.973 ± 5.337) in comparison with the diabetic rats that drank tap water (TW-DM: 30.813 ± 17.580).

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Table 4: Serum ion concentrations in the experimental group of rats with streptozotocin-induced diabetes mellitus subchronically treated with tap water (TW-DM) of water rich in magnesium hydrocarbonate (MW-DM)

Parameter	Groups		*p* value
	TW-DM	MW-DM	
Na^+	134.800 ± 4.902	125.330 ± 17.954	0.042 *
K^+	5.795 ± 0.434	5.423 ± 1.525	0.047 *
Ca^{2+}	2.620 ± 0.252	2.410 ± 0.099	0.316
Mg^{2+}	0.920 ± 0.053	1.000 ± 0.115	0.177
Fe^{2+}	31.000 ± 0.557	40.400 ± 22.345	0.000 **
HCO_3^-	29.333 ± 3.786	22.000 ± 5.657	0.349
Cl^-	92.000 ± 7.810	84.875 ± 9.852	0.774
PO_4^{3-}	2.077 ± 0.047	1.970 ± 0.516	0.042 *

*Results are presented as mean ± SD. * $p < 0.05$, ** $p < 0.001$, t test; Na^+: sodium; K^+: potassium; Ca^{2+}: calcium; Mg^{2+}: magnesium; Fe^{2+}: iron; HCO_3^-: bicarbonate; Cl^-: chloride; PO_4^{3-}: phosphate*

(Table 3). A significant decrease was found for sodium cations (MW-DM: 125.330 ± 17.954; TW-DM: 134.800 ± 4.902); and for potassium cations (MW-DM: 5.423 ± 1.525; TW-DM: 5.795 ± 0.434). The group of rats with streptozotocin-induced diabetes exposed subchronically to magnesium supplementation demonstrated an increase in the concentrations of iron ($p < 0.001$) (MW-DM: 40.400 ± 22.345) in comparison with the diabetic rats who drank tap water (TW-DM: 31.000 ±0.557). Phosphate anions were significantly decreased in MW-DM compared to TW-DM group (MW-DM: 1.970 ± 0.516; TW-DM: 2.077 ± 0.047). (Table 4).

Discussion

Experiments dealing with similar topic found that induction of diabetes with streptozotocin in rats resulted in the impaired glucose metabolism, change in the lipid profile, systemic oxidative stress as well as insulin resistance, because of which use of streptozotocin is considered a highly efficient prototype of diabetes mellitus.[23-25]

Certain results of the present study are in accordance with the literature data, since mean values of serum glycaemia, as well as of the main components of the lipid profile (total and LDL cholesterol, triglycerides) were significantly higher in groups of animals with streptozotocin-induced diabetes (MW-DM, TW-DM), in comparison with both control groups (MW-C, TW-C). Under the conditions of normal metabolism, glucose tolerance is attained by increased secretion of insulin in response to a postprandial hyperglycaemia registered by insulin receptors. Because of that, decreased secretion of insulin and/or loss of sensitivity of insulin receptors results in impaired tolerance to glucose that is found in rats with streprozotocin-induced diabetes.[26] Although the present study has shown the occurrence of hyperglycaemia in diabetic rats, subchronic intake of magnesium hydrocarbonate-rich mineral water has not reduced the glucose concentration. Contrary to that, others have demonstrated that a four-week-long magnesium supplementation leads to the reversal of insulin resistance, improvement of the insulin receptor sensitivity and normalisation of glucose metabolism in general.[27] Similar effect on glucose homeostasis was shown in healthy rats with body hypomagnesaemia and this finding led to the assumption that magnesium might be a natural sensitizer of insulin receptors, which warrants its use in chronic diabetic patients and explains the link between the daily intake of magnesium and the decrease in risk for development of type 2 diabetes mellitus in humans.[28-30]

Although the values of the components of lipid profile (total and LDL cholesterol, triglycerides) were higher in the groups of rats with streptozotocin-induced diabetes, the comparison between these values in rats with and without subchronic intake of magnesium hydrocarbonate-rich mineral water could not detect any significant difference. On the contrary, an increase in the triglyceride levels was found in the MW-C group, which is contradicted by the literature findings that use of magnesium in diabetic patients prevents or reverses the development of diabetes-induced dyslipidaemia.[27] Other studies have shown that the magnesium deficit plays a significant role in the development of lipid imbalance in the process of atherosclerosis, since endothelial cells under *in vitro* conditions and magnesium ion deprivation, express the nuclear factor-*kappa beta*, but also secrete the key factors of atherogenesis, including RANTES (Regulated on Activation, Normal T-cell Expressed and Secreted), IL-8, as well as the platelet-derived growth factor-BB (PDGF-BB), which together cause an impairment of the exchange of serum lipids between blood and the vessel wall, increase in the concentrations of serum triglycerides, accumulation of oxalate in arteries and decreased transport of cholesterol with HDL.[31-32] Other reports also suggest that the magnesium deficit directly affects the lipoprotein metabolism, which contributes to the progression

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of atherosclerosis.[31] Although in some studies a decrease in the serum concentrations of HDL was found as a consequence of a decreased magnesium alimentary intake, the present study did not find a significant increase in the levels of HDL after subchronic intake of magnesium hydrocarbonate -rich mineral water between the control groups and groups with diabetes.[33]

The results of the present study have shown a decrease in the fibrinogen levels due to subchronic intake of magnesium hydrocarbonate -rich mineral water in the control group of rats without diabetes, which suggests that this form of magnesium supplementation may act protectively on the potential thrombosis. Similarly, in various experimental models it was demonstrated the correlation between the magnesium deficit and the general pro-thrombotic state.[31] Parsons and co-workers found that patients with previously diagnosed angina pectoris or previous acute myocardial infarction, had a significant decrease in mortality rate from 30 % to only 1 %, provided they received magnesium sulphate intramuscularly. The same study described the importance of use of magnesium as a therapeutic modality in cardiac patients, with the most prominent advantage being disinhibition of serum plasmin, an enzyme that plays the key role in the process of degradation of fibrin clots.[34] It was shown that use of magnesium in a form of a slow intravenous infusion slows down thrombosis after acute myocardial infarction and reduces the concentration of certain coagulation factors in pregnant women with pre-eclampsia.[35-36] It seems that the key mechanism that explains the anti-thrombogenic effect of magnesium is the inhibition of ADP-induced platelets aggregation.[37] This mechanism could explain the significant decrease in the levels of fibrinogen that results from the subchronic intake of magnesium hydrocarbonate-rich mineral water in the present study. Besides, Paolisso and co-workers also suggested that use of magnesium could reduce the hypercoagulability of platelets in patients with type 2 diabetes mellitus, which also explains the decrease of fibrinogen concentrations found in the present study.[38]

The comparison of the concentration of ions between the group of rats with diabetes showed a decrease in potassium as a consequence of the subchronic intake of magnesium hydrocarbonate -rich mineral water. Various publications showed causality of the connection among the serum concentrations of potassium and magnesium and that hypokalaemia occurs as a regular electrolyte abnormality in the preclinical phase of body hypomagnesaemia.[31] Thiazide diuretics, most frequently prescribed drugs in patients with congestive heart failure, cause an increased renal loss of both potassium and magnesium.[39-42] It was also shown that the normalisation of the intracellular concentrations of potassium in myocytes can be achieved exclusively by regulating the body hypomagnesaemia along with potassium supplementation, in spite of the fact that the supplements on their own cause the normalisation of serum potassium.[39, 43-44] Since use of water rich in magnesium hydrocarbonate brings about increase in the tissue magnesium depots and an increase in the intracellular potassium concentrations, transfer of potassium from extra- to intracellular compartment in order to replenish the tissue deficit could explain the decrease of serum potassium levels found in the present study.

One of the interesting findings in this experimental study was an increase in the serum iron due to subchronic intake of magnesium hydrocarbonate-rich mineral water in rats with diabetes. Similar studies published so far reported a reverse ratio between magnesium and iron levels in erythrocytes. It was hypothesised that magnesium supplementation acts protectively by bringing down the serum ferraemia, which in turn eliminates the additional oxidative tissue injury in diabetic patients.[45] Under physiological conditions, magnesium prevents the exposure of body to high serum iron concentrations by inhibiting the pathological process of haemolysis that would end in iron liberation from the erythrocytes into serum.[46] Some studies found that the decreased magnesium concentrations in erythrocytes were linked to the increased iron concentrations in various tissues and that the decreased alimentary intake of magnesium results in an increased intestinal absorption of iron, but also in decreased number of viable erythrocytes.[47] Under conditions of chronic body magnesium deprivation, erythrocytes with abnormal shape and function, prone to haemolysis, are formed, which explains a decrease in the haemoglobin levels and an increase in serum iron when body contains depleted depots of magnesium.[46-47]

In spite of some significant results, this experimental study has some limitations. An investigation in a larger group of rats is needed to investigate the effect of subchronic magnesium

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supplementation on serum glycaemia and components of lipid profile in diabetes. Besides, a statistical comparison between the complementary control groups and groups of rats with diabetes should be performed to demonstrate the adequacy of the experimental prototype for type 2 diabetes mellitus.

Conclusion

The aim of this study was to ascertain the effects of subchronic intake of magnesium hydrocarbonate-rich mineral water on values of various cardiometabolic parameters, but also on the serum levels of ions in rats with an experimental model of diabetes. Investigation was organised with the assumption that *in vivo* supplementation with magnesium might be a potential therapeutic modality for improvement of levels of cardiometabolic markers in patients with diabetes. Although the results of this study demonstrated the existence of the increased values of serum glycaemia and the impairment of the lipid profile in diabetic rats, in comparison with the healthy controls, subchronic intake of magnesium hydrocarbonate -rich mineral water alone did not result in any significant changes. The results obtained are not completely in accordance with previous studies that reported that the use of magnesium supplementation resulted in the reversal of insulin resistance, improved sensitivity of insulin receptors and general improvement of the metabolism of glucose. The present study however showed that subchronic intake of magnesium hydrocarbonate-rich mineral water in the control group of rats, results in decreased concentrations of fibrinogen, which suggests possible modality in the treatment of various pro-thrombogenic conditions.

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None.

Conflict of interest

None.

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ORIGINAL ARTICLE

Optimised Feature Selection and Cervical Cancer Prediction Using Machine Learning Classification

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Abstract

Background: Screening and early detection play a key role in cervical cancer prevention. The present study predicts the outcome of various diagnostic tests used to diagnose cervical cancer using machine learning algorithms.
Methods: The present study ran various cervical cancer risk factors on a machine learning (ML) classifier to predict outcomes of Hinselmann, Schiller, cytology and biopsy. The dataset is publicly available on the Machine Learning Repository website of the University of California Irvine. The imbalanced dataset was pre-processed using oversampling methods. The significantly varied features between the two levels of a response variable were used to train the machine learning classifiers on MATLAB. The classifiers used were Decision Trees, Support Vector Machine, K-Nearest Neighbours and Ensemble learning classifiers. The performance metrics of the classifiers were expressed as accuracy, the area under the receiver operator characteristic (AU-ROC) curve, sensitivity and specificity.
Results: The Fine Gaussian SVM classifier was the best to classify Hinselmann, cytology and biopsy with the accuracy of 97.5 %, 62.5 % and 98 %, respectively. However, Boosted trees performed best in the classification of Schiller with 81.3 % accuracy.
Conclusion: The present study selected optimised features among multiple risk factors to train various ML classifiers to predict cervical cancer.

Key words: Biopsy; Cervical cancer; Cytology; Hinselmann; Machine learning; Schiller.

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Introduction

Cervical cancer is the fourth most common cancer in women worldwide. An estimated 570,000 cervical cancer cases were diagnosed and 311,000 women died from cervical cancer worldwide in 2018.[1, 2] It is a disease in which healthy cells grow abnormally on the surface of the cervix, forming a mass of cells called a tumour and spreading to other parts of the body such as the bladder, rectum, lungs, vagina and liver. Women under 50 years of age are mostly affected by this disease. Cervical cancer control includes primary prevention (vaccination against human papillomavirus), secondary prevention (screening and treatment of pre-cancerous lesions), tertiary prevention (diagnosis and treatment of invasive cervical cancer) and palliative care.[3] The risk factors for cervical cancer include age, human papillomavirus infection, early sexual activity, long-term use of the hormonal contraceptive pill, sexually transmitted infections and genetics.[4] Screening has a significant role in the early diagnosis of cervical malignancy. Screening procedures for cervical cancer include cytology, Schiller, Hinselmann and the standard biopsy test to recognise cervical cancer.[5]

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Classification of diseases based on artificial intelligence methods helps in prediction of disease and survival rate.[6, 7] Recently, diseases have been classified using computer vision, machine learning (ML) and deep learning (DL) algorithms.[8-10] The various ML classifiers, including Logistic Regression (LR), Decision Tree (DT), Artificial Neural Networks (ANN), Support Vector Machine (SVM) and Naive Bayes (NB), along with feature optimisation methods such as Chicken Swarm optimisation can be used for prediction.[11-14] The present study used ML methods to predict the outcome of various methods used for diagnosing cervical cancer.

Material and Methods

The present study ran various cervical cancer risk factors on a machine learning (ML) classifier to predict the outcome of various diagnostic tests, including Hinselmann, Schiller, cytology and biopsy. The study dataset consisted of a random sample of medical records of 858 patients who attended gynaecology service at Hospital Universitario de Caracas in Caracas, Venezuela, between 2012 and 2013. The dataset was publicly available on the Machine Learning Repository website of the University of California Irvine (UCI ML).[15] Fifteen factors among 32 cervical cancer risk factors were used, including the age of the patient, sexual activity (number of sexual partners and age of first sexual intercourse), number of pregnancies, smoking behaviour, use of contraceptives (hormonal and intrauterine devices) and historical records of sexually transmitted diseases (STDs) to predict indications of response variables. The four response variables include Hinselmann, Schiller, cytology and biopsy (Table 1). Hinselmann's test refers to colposcopy using acetic acid. In Schiller's test, Lugol iodine was used to visualise the uterine cervix.[16]

The dataset contained many missing values as many patients did not answer all the questions for privacy reasons. The dataset was imbalanced and oversampling methods were employed during pre-processing. The significantly different features between the two levels of a response variable were used for ML classification. The ML classifier application on MATLAB 2019a was used for classification.[17]

The classifiers used in this application included Decision Trees, Support Vector Machine (SVM), K-Nearest Neighbours (KNN) and Ensemble learning classifiers. The decision trees included complex, medium and simple tree classifiers. Similarly, the SVMs included linear, quadratic, cubic, fine Gaussian, medium Gaussian and coarse Gaussian classifiers. The ensemble classifiers had boosted trees, bagged trees and RUS boosted tree classifiers.

Table 1: List of predictors and response variables used machine learning classification

SNo	Attribute name	Type	Predictor
1	Age (years)	Integer	Predictor
2	Number of sexual partners	Integer	Predictor
3	Age of first sexual intercourse (year)	Integer	Predictor
4	Number of pregnancies	Integer	Predictor
5	Smokes (yes/no)	Boolean	Predictor
6	Smokes (years)	Integer	Predictor
7	Smokes (packs/year)	Integer	Predictor
8	Hormonal contraceptives (yes/no)	Boolean	Predictor
9	Hormonal contraceptives (years)	Integer	Predictor
10	Intrauterine devices (yes/no)	Boolean	Predictor
11	Intrauterine devices (years)	Integer	Predictor
12	Sexually transmitted disease (STDs) (yes/no)	Boolean	Predictor
13	Number of STDs	Categorical	Predictor
14	STDs (years since the first diagnosis)	Integer	Predictor
15	STDs (years last diagnosis)	Integer	Predictor
16	Hinselmann	Boolean	Response
17	Schiller	Boolean	Response
18	Cytology	Boolean	Response
19	Biopsy	Boolean	Response

Statistical analysis

After assumption checked, the quantitative data were expressed in median (IQR) and compared using the non-parametric Mann-Whitney's U test. The categorical data were expressed in percentage and the relationship between discrete variables was found using a Chi-squared test. The performance metrics of the machine learning classifier were expressed as accuracy, the area under the receiver operator characteristic (AU-ROC) curve, sensitivity and specificity. The JASP version 0.16.2 and MATLAB 2019a were used for statistical analysis.[18] The significance level was considered at 5 %.

Results

Considering Hinselmann as a response variable, the features which were different between the two response levels were age [$W = 2348$; $p < 0.001$],

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Table 2: Comparison of continuous predictors across binary outcomes of Hinselmann, Schiller, cytology and biopsy

	Responses							
	Hinselmann		Schiller		Cytology		Biopsy	
	W	p	W	p	W	p	W	p
Age	2348	< 0.001	1168.5	0.906	1135.5	0.158	1135.5	0.158
Number of sexual partners	2262.5	< 0.001	1341.5	0.152	1229.5	0.414	1229.5	0.414
First sexual intercourse	1559	0.780	962	0.160	1277.5	0.627	1277.5	0.627
Number of pregnancies	1768.5	0.106	1144	0.955	1527.5	0.240	1527.5	0.240
Smokes (years)	1565	0.694	675	< 0.001	1556	0.059	1556	0.059
Smokes (packs/year)	1605	0.487	739.5	< 0.001	1592	0.026	1592	0.026
Hormonal Contraceptives (years)	2237.5	< 0.001	1365	0.101	1188.5	0.277	1188.5	0.277
IUD* (years)	1414.5	0.385	1252	0.204	1142.5	0.057	1142.5	0.057
STDs*: Time since the first diagnosis	1781.5	0.103	1137	0.915	1174	0.244	1174	0.244
STDs*: Time since the last diagnosis	2060.5	< 0.001	1134.5	0.900	1141.5	0.168	1141.5	0.168

**IUD: Intrauterine devices; STDs: Sexually transmitted diseases*

Table 3: Relationship between categorial predictors and binary outcomes of Hinselmann, Schiller, cytology and biopsy

	Hinselmann		Schiller		Cytology		Biopsy	
	χ^2 value	p	χ^2 value	p	χ^2 value	p	χ^2 value	p
Smokes	0.045	0.832	20.308	< 0.001	4.833	0.028	0.437	0.509
Hormonal Contraceptives	18.084	< 0.001	1.510	0.219	2.056	0.152	0.679	0.410
IUD*	0.978	0.323	1.524	0.217	3.690	0.055	1.515	0.218
STDs* (number)	28.195	< 0.001	6.020	0.111	5.975	0.113	2.711	0.438

**IUD: Intrauterine devices; STDs: Sexually transmitted diseases*

Table 4: Performance metrics of the best classifier for Hinselmann, Schiller, cytology and biopsy classification

	Hinselmann	Schiller	Cytology	Biopsy
Features (Serial number based on Table 1)	1, 2, 8, 9, 13, 15	5, 6, 7	5, 7	2, 3, 4, 14, 15
Classifier	Fine Gaussian SVM#	Boosted Trees	Fine Gaussian SVM#	Fine Gaussian SVM#
Accuracy	97.5 %	81.3 %	62.5 %	98 %
AUC*	1	0.83	0.66	0.98
Sensitivity	100 %	69 %	100 %	100 %
Specificity	95 %	94 %	25 %	96 %

**AUC: Area under the receiver operating characteristic curve; #SVM: Support vector machine*

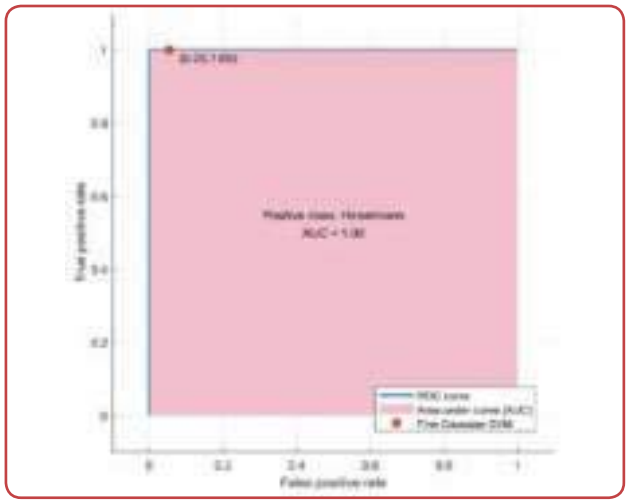

Figure 1: Receiver operating characteristic curve showing performance metrics of Fine Gaussian SVM classifier for Hinselmann classification

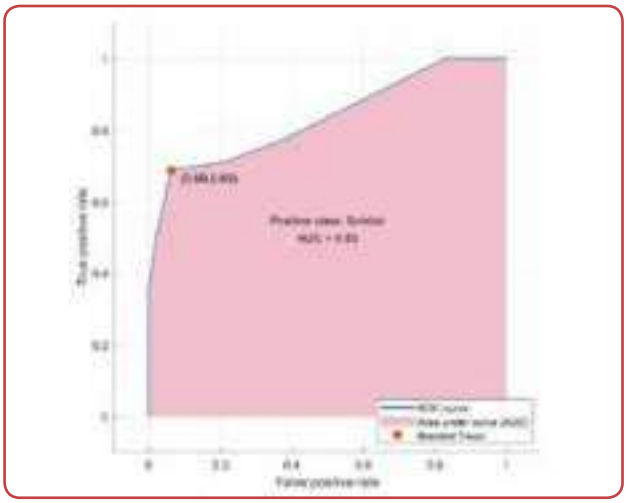

Figure 2: Receiver operating characteristic curve showing performance metrics of Boosted Trees classifier for Schiller classification

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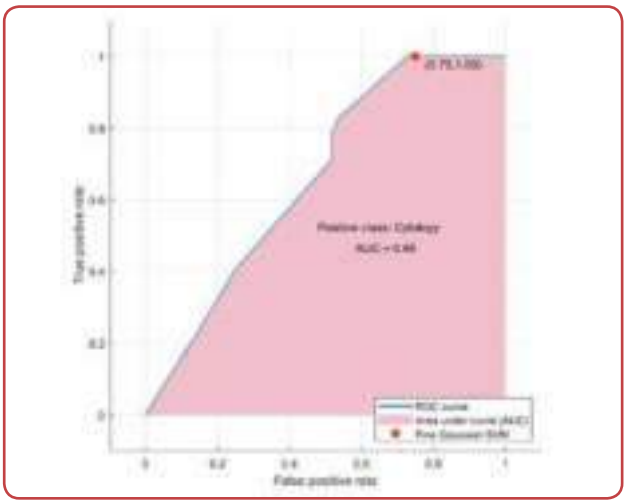

Figure 3: Receiver operating characteristic curve showing performance metrics of Fine Gaussian SVM classifier for Cytology classification

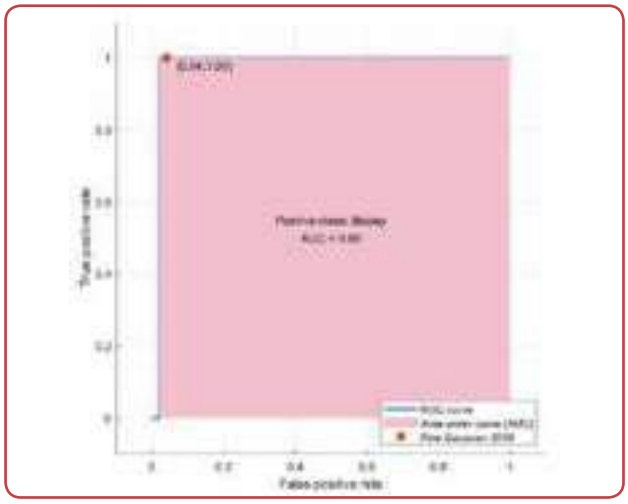

Figure 4: Receiver operating characteristic curve showing performance metrics of Fine Gaussian SVM classifier for Biopsy classification

number of sexual partners [W = 2262.5; p < 0.001], hormonal contraceptives (yes/no) [c^2 = 18.08; p < 0.001], hormonal contraceptives (years) [W = 2237.5; p < 0.001], STDs (number) [c^2 = 28.20; p < 0.001], and STDs: Time since the last diagnosis [W = 2060.5; p < 0.001]. Similarly, for Schiller as the response variable, the features significantly differed were: Smokers (yes/no) [c^2 = 20.31; p < 0.001], Smokers (years) [W = 675; p < 0.001], Smokers (packs/year) [W = 739.5; p < 0.001]. In case of cytology as a response variable, Smokers (yes/no) [c^2 = 4.83; p = 0.028], Smokers (packs/year) [W =1592; p = 0.026] were significantly differed. In case of biopsy, features differed were number of sexual partners [W = 1855.5; p < 0.001], age of the first sexual intercourse [W = 732.5; p < 0.001], number of pregnancies [W = 1647; p = 0.004], STDs (time since first diagnosis) [W = 1603.5; p = 0.013] and STDs (time since last diagnosis) [W = 1567; p = 0.026] (Table 2 and Table 3).

The Fine Gaussian SVM classifier was the best model to classify Hinselmann, cytology and biopsy. However, Boosted trees performed best in the classification of Schiller. Table 4 shows the performance metrics of various classifiers. (Table 4; Figures 1-4).

Discussion

Cervical cancer is a primary cause of premature mortality in women worldwide. Screening and early diagnosis are preventive strategies for better management of cervical cancer. Machine learning methods can be used to process vast amounts of cancer data and is readily accessible to the medical research community to upgrade the survival rate of patients.[19, 20] The present study used various ML algorithms to predict indications for various examinations to diagnose cervical cancer. The Fine Gaussian SVM classifier was the best model to classify Hinselmann, cytology and biopsy. However, Boosted trees performed best in the classification of Schiller (Table 4).

In a similar study, Nagadeepa et al used RF, SVM and Deep Learning (DL) models like Artificial Neural Networks (ANN) and Convolutional Neural Networks (CNN), for cervical cancer prediction. The SVM showed the highest accuracy (97 %), followed by CNN (95/3 %), RF (94 %) and ANN (95.2 %), respectively.[21] Ali et al used ML classifier models to predict cervical cancer from various examinations using clinical data. The random tree classifier showed better results for cytology (98.65 %) and biopsy (98.33 %), whereas the Instance-Based K-nearest neighbour (IBK) with random forest classifier provided higher accuracy for Hinselmann (99.16 %) and Schiller (98.58 %).[22] Nithya et al predict cervical cancer using random forest, rpart, $C_{5.0,}$ KNN and SVM algorithms after optimised feature selection. Contrary to the present study, the random forest and $C_{5.0}$ classifier models showed higher accuracy in predicting cervical cancer.[23] Zahras et al used a deep convolutional neural network to predict the outcome of Hinselmann, cytology, Schiller and biopsy for diagnosing cervical cancer. The results of deep convolutional neural network classification were comparable to the present study, with an accuracy of about 90 % for each target.[24] Asaduzzaman et al developed a system to predict the risk of cervical cancer using machine learning models including AdaBoost, Logistics Regression,

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SVM, Neural Network, kNN, Naïve Bayes, Decision Tree, CN2 rule Inducer, Random Forest and Quadratic Classifier. The most significant factors that contributed to cervical cancer were the number of children, age at first intercourse, age of husband, Pap test and age.[25] Chaudhuri et al developed a 3-Stage Hybrid feature selection approach and a Stacked Classification model to evaluate the cervical cancer dataset obtained from the UCI Machine Learning Repository with 35 features and one outcome variable. In Stage 1, researchers used a Genetic Algorithm (GA) and Logistic Regression Architecture (LRA) for Feature Selection and selected twelve features well correlated with the class but not among themselves. Stage-2 utilises the same GA and LRA for Feature Selection to select five features. In Stage 3, Logistic Regression (LR), Naïve Bayes (NB), Support Vector Machine (SVM), Extra Trees (ET), Random Forest (RF) and Gradient Boosting (GDB) were used with the five features to identify patients with or without cancer. The classifiers showed improved performance metrics with reduced features. In the 66-34 split, all five machine learning methods except NB recorded 97 % accuracy with five features. Also, the Stacked model produced higher than 96 % accuracy with five features in 66-34 and 80-20 splits and 10-fold cross-validation.[26] Sobar et al in a study, predicted cervical cancer using machine learning classifiers based on behaviour and its determinants. The Naïve Bayes and Logistic Regression showed 91.67 % and 87.5 % accuracy, respectively.[27] Ceylan et al predicted cervical cancer early on using a multi-label classification technique. For multi-label classification, problem transformation methods such as Binary Relevance (BR), Classifier Chains (CC), Conditional Dependency Networks (CDN) and Label Combination were used. Sequential Minimal Optimisation, Naïve Bayes, Random Forest and J48 Decision Tree machine learning classifiers were compared for their exact match, accuracy, hamming loss and ranking loss. Except for J48-BR and J48-CDN algorithms, the accuracy percentage and exact match were over 80 %. All algorithms with CC and LC methods had nearly equal accuracy, exact match, hamming loss and ranking loss. RF algorithms based on CC and LC methods showed better performance, followed by J48-CC and J48-LC methods.[28] Gupta et al tried the random forest regression technique for the early detection of cervical cancer. Researchers used recall-based scores to check performance. The aim was to achieve higher recall scores and reduce false-positive values. The recall scores for Hinselmann, Schiller, cytology and biopsy were 0.920, 0.972, 0.912 and 0.996, respectively.[29] High performance can be achieved by reducing variance and bias in ML models. To achieve this, Ahishakiye et al used an ensemble ML classifier including a decision tree, Classification and Regression Trees, Naïve Bayes Classifier, K-Nearest Neighbour and Support Vector Machine. The method showed an accuracy of 87.91 % in cervical cancer classification.[30] Sagala et al applied different data mining algorithms (SVM, Naïve Bayes and KNN) on four different medical tests (biopsy, cytology, Hinselmann and Schiller) as target variables. The Naïve Bayes classifier outperforms other classifiers after evaluation using the 10-fold cross-validation method.[5]

Many datasets have been characterised by low sample size, outliers and multiple risk factors. The dataset issues such as outliers and data imbalance were addressed by Ijaz et al in a random forest classification model. Researchers used density-based spatial clustering of applications with noise (DBSCAN) and isolation forest (iForest) for outlier detection. The synthetic minority over-sampling technique (SMOTE) and SMOTE with Tomek link (SMOTETomek) were used for data imbalance. The four protocols were compared: (1) DBSCAN + SMOTETomek + RF, (2) DBSCAN + SMOTE+ RF, (3) iForest + SMOTETomek + RF and (4) iForest + SMOTE + RF. The iForest with SMOTE and iForest with SMOTETomek had better performance than DBSCAN with SMOTE and DBSCAN with SMOTETomek.[31] Similarly, Ali et al used three feature transformation methods, including log, sine function and Z-score, before performing supervised classification training. Random Tree showed the best accuracy for the biopsy (98.33 %) and cytology (98.65 %) classification, whereas Random Forest and Instance-Based K-nearest neighbour (IBC) was the best for Hinselmann (99.16 %) and Schiller (98.58 %) respectively. The logarithmic method performed best for biopsy datasets, whereas the sine function showed superior performance for cytology. Both logarithmic and sine functions were superior for the Hinselmann dataset, while Z-score performed best for the Schiller dataset.[22] Similarly, Fernandes et al proposed a computationally automated strategy to predict biopsy results from cervical risk factors. The strategy consists of joint and fully supervised optimisation of dimensionality reduction. Further, the approach was instantiated with deep learning architectures, which showed results (AUC = 0.6875) that outperformed previously developed methods, such as denoising autoencoders.[15] Chauhan et al compared various ML classifiers for predicting cervical cancer, including Logistic Regression, Naive Bayes, Support Vector Machine, K-Nearest Neighbour, Linear Discriminant Analysis, Multi-Layer

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Perceptron, Decision Tree and Random Forest. The authors used Synthetic Minority Oversampling Technique for the data imbalance issue. Fivefold cross-validation was used on scaled data and unscaled data obtained by Min-Max scaling, standard scaling and normalisation. RF, SVM and DT showed higher performance in cervical cancer diagnosis. The optimised features were selected using univariate feature selection and Recursive feature elimination (RFE). Overall performance of Random Forest predictor with RFE (RF-RFE) is superior to all others being implemented. The outcome of Random Forest with Recursive feature elimination was greater than other machine learning classifiers.[32] With an appropriate dataset and optimised feature selection, machine learning methods and classification can detect cervical cancer in its early stages.

Conclusion

The present study selected optimised features among multiple risk factors to train various ML classifiers to predict cervical cancer. The results showed the Fine Gaussian SVM classifier is the best model for Hinselmann, cytology and biopsy, whereas Boosted trees performed best in the classification of Schiller.

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None.

Conflict of interest

None.

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ORIGINAL ARTICLE

The Beneficial Effect of Yoghurt Containing *Lactobacillus Rhamnosus* on Caries Prevention in Children With Diabetes Mellitus Type 1

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Abstract

Background / Aim: Children with type 1 diabetes mellitus are thought to have an increased risk of caries. This study aimed to examine the short-term effect (sixty days long) of commercially available yoghurt consumption containing the *Lactobacillus rhamnosus* probiotic culture (LGG yoghurt) on the oral *Streptococcus mutans* count and saliva buffer capacity in children with type 1 diabetes mellitus.
Methods: Children were divided into two groups: the experimental group and the placebo group. Both groups consisted of 50 (N = 50) children with juvenile diabetes, aged 10-15 years, with controlled glucose levels and irregular oral hygiene. At the first examination, every child was evaluated for the caries risk. A sample of unstimulated saliva before yoghurt consumption and after washout of the teeth was inspected for *S mutans* count. The samples were tested for Saliva buffer capacity (Saliva-Check Buffer Testing Mat GC America). The same procedure was repeated after 14 days, 30 days and 60 days after the treatment with probiotic yoghurt.
Results: The results showed decreased number of *S mutans* colonies at the 60-day control examination in the probiotic group. The study also proved a significant increase in saliva buffer capacity in both groups after 60 days.
Conclusion: It could be concluded that daily consumption of LGG yoghurt can improve caries prevention in children with diabetes mellitus type I.

Key words: Probiotics; Diabetes mellitus type 1; *Streptococcus mutans*; Saliva buffer capacity.

Introduction

Diabetes mellitus is one of the most common endocrinological diseases of modern times and as a chronic, non-infectious disease, has its manifestations in the oral cavity. Children with type 1 diabetes mellitus are thought to have an increased risk of caries, among others.[1, 2] According to recent studies, the role of probiotics in these patients has previously proven to be

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effective, especially in the oral cavity tract and they can be administrated in the form of probiotic milk, yoghurt, kefir, chewing gums, toothpaste that affect the reduction of cariogenic microorganisms and accumulation of dental plaque.[3, 4] Prevalence of gingivitis and caries have been demonstrated in patients with poor glycaemic control.[5] Competence with pathogenic microorganisms for the dental surface, aggregation with oral bacteria, interaction with the oral epithelium and consequent modulation of the oral biofilm together with increased IgA levels and thus the immune response, as well as inhibition of anti-inflammatory cytokine production, are characteristics of probiotic strains based on *in vitro* studies.[6] Children with uncontrolled diabetes are considered at high caries risk, lower saliva buffer capacity and lower pH.[7] Milk and yoghurt products have beneficial effects on saliva and inhibition of cariogenic bacteria due to the present casein, sodium, potassium, calcium and phosphorus.[8] The literature proved that yoghurt can increase saliva secretion,[9] and that was the main reason for the hypothesis about the use of LGG yoghurt in this study, where the buffer capacity increased in both groups. Continuous use of probiotic strain seems to be most promising due to its anti-cariogenic effects and a high buffer capacity.[10]

In previous research, it was found that the manifestations of diabetes mellitus in the oral cavity, such as oral candidiasis, halitosis and dental caries have been reduced by using probiotic strains.[5] Daily administration of probiotics is well needed to improve oral health and reduce caries development.[11, 12]

In this study, a randomised clinical trial aimed to determine the efficacy of LGG yoghurt on *Streptococcus mutans* (*S mutans)* levels and modify saliva buffer capacity over two months in children with type 1 diabetes mellitus diagnosed one year before, by comparing two groups, experimental and placebo.

Methods

The study included 50 children with type 1 diabetes mellitus diagnosed one year before, age 10-15, who received insulin as regular therapy and with no other comorbidities. All participants were without oral diseases and antibiotics use over the last two months.

The parents of all patients were informed about the purpose of the study and signed informed consent. The study followed the Declaration of Helsinki and has been approved by the Ethics Committee of the Public Health Institute of Dentistry of Banja Luka (protocol number 01-461-2/18), as well as by the Public Health Institute for Public Health of the Republic of Srpska. At the first examination, each subject was assigned a caries risk according to the Klein-Palmer (Decayed / Missed / Filled – DMF score) epidemiological index. Subjects caries risk was evaluated based on DMF score (low < 1, medium 0-3, high > 3), oral health behaviour and periodical checking. The samples of saliva were taken during the clinical examination, after tooth brushing at home and before starting using yoghurt, by collecting 1 mL in sterile graduated plastic containers. The samples were immediately transferred to a microbiology service for further processing. Each patient consumed 200 mL of yoghurt daily. Experimental group consumed "LGG Dukat, Croatia" and the placebo group (25 children) also consumed 200 mL of "standard Dukat, Croatia youghurt" for 60 days. Samples of saliva were treated with a Vortex mixer before seeding. The 0.1 mL of saliva sample was seeded onto prepared *Mitis Salivarius* agar (manufacturer's recommendation) with a smear (COPAN Spreaders, Italy).

Saliva samples were placed in an incubator (Memmert, 5 % CO_2) at 36 °C for a period of 36 to 48 h. Counting of *S mutans* (CFU/mL) colonies was performed by visual method. The saliva was sampled again after 14 days, one month and given to the microbiology laboratory for re-analysis and results readout after two months. Saliva buffer capacity was determined using Saliva-Check Buffer Testing Mat GC America tests before yoghurt consumption and after the washout period following the manufacturer's instructions.

Frequencies, percentages, the sample mean value with standard deviation were used to describe the parameters of importance depending on their nature. The Chi-square test was used to test the differences between the two categorical variables. ANOVA Repeated Measures was used to test the differences on variables measured in three-time intervals. The influence of the experimental factor on the change of the values of the parameters of importance (first examination,

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30 days and 60 days after the intervention) as well as the influence of gender were tested with the SPANOVA test (Split-Plot ANOVA). The probability level was set at $p < 0.05$. Statistical processing and analysis were performed in the Statistical Package for the Social Sciences (SPSS) v 24 for Windows. The tabular and graphical presentation was done in Excel.

Results

ANOVA Repeated Measurements was applied to determine whether the number of colonies was statistically significantly different when comparing three-time intervals of measurement. Within the placebo group, there was no statistically significant difference in the number of *S mutans* colonies during the three-time intervals ($F = 1.683$, $p = 0.208$). By testing the differences within the experimental group, it was determined that a statistically significant difference exists ($F = 27.336$, $p < 0.001$). By a subsequent comparison of all three-time intervals with one another, it was determined that the average number of *S mutans* colonies at the first examination and after 30 days was not statistically significantly different ($p = 0.627$). However, when comparing the first examination (18.00 ± 10.22) and the examination after 60 days (15.60 ± 9.73), there was a statistically significant difference ($p < 0.001$). By testing the difference between the second (18.08 ± 10.29) and the third examination (15.60 ± 9.73), a statistically significant difference ($p < 0.001$) was obtained. In other words, observing only the experimental group, the average number of *S mutans* colonies was not lower after 30 days, but it was after 60 days, noting that between 30 and 60 days there was a statistically significant decrease in the number of these colonies.

It was also examined whether the buffer capacity of saliva differed in three measured time intervals, separately within the placebo and experimental groups. Within the placebo group, the mean value of the buffer capacity of saliva did not differ if one observes the difference between the first examination (5.00 ± 2.54) and the examination after 30 days (5.16 ± 2.32), ($p = 0.212$). The increase in the average value of the buffer capacity of saliva occurred after 60 days (8.12 ± 1.81), ($p < 0.001$).

Fifty respondents participated in the study, 50 % of whom were in the experimental group, while the other half was a placebo group. Groups were equated according to gender ($p = 0.744$) and to the risk of caries ($p > 0.668$) (Table 1).

Table 1: General data on respondents

	Placebo group (N = 25)	Experimental group (N = 25)	p	All respondents (N = 50)
Gender, N (%)				
male	15 (60 %)	14 (56 %)	0.744[a]	29 (58 %)
female	10 (40 %)	11 (44 %)		21 (42 %)
Caries risk, N (%)				
low	6 (24 %)	7 (28 %)		13 (26 %)
medium	9 (36 %)	11 (44 %)	0.668[a]	20 (40 %)
high	10 (40 %)	7 (28 %)		17 (34 %)

[a] Chi-square test; p = statistical significance.

Table 2: Number of Streptococcus mutans colonies during three-time intervals

Group	Time	M	SD	F	p	Partial Eta²	LSD post hoc test
Placebo group	Time 1: First examination	17.28	9.15	1.683	0.208	0.128	Time 1 : Time 2 (p = 0.298)
	Time 2: 30 days	17.52	9.18				Time 1 : Time 3 (p = 0.103)
	Time 3: 60 days	17.12	9.10				Time 2 : Time 3 (p = 0.134)
Experimental group	Time 1: First examination	18.00	10.22	27.336	**0.000**	0.704	Time 1 : Time 2 (p = 0.627)
	Time 2: 30 days	18.08	10.29				Time 1 : Time 3 (p = 0.000)
	Time 3: 60 days	15.60	9.73				Time 2 : Time 3 (p = 0.000)

ANOVA Repeated Measurement was applied; M = mean, SD = standard deviation, F = Repeated Measure ANOVA, p = statistical significance, bold: statistically significant.

Table 3: Buffer capacity of saliva during three-time intervals

Group	Time	M	SD	F	p	Partial Eta²	LSD post hoc test
Placebo group	Time 1: First examination	5.00	9.15	125.68	**0.000**	0.916	Time 1 : Time 2 (p = 0.212)
	Time 2: 30 days	5.16	9.18				Time 1 : Time 3 (p = 0.000)
	Time 3: 60 days	8.12	9.10				Time 2 : Time 3 (p = 0.000)
Experimental group	Time 1: First examination	5.68	10.22	107.05	**0.000**	0.903	Time 1 : Time 2 (p = 0.029)
	Time 2: 30 days	6.00	10.29				Time 1 : Time 3 (p = 0.000)
	Time 3: 60 days	8.80	9.73				Time 2 : Time 3 (p = 0.000)

ANOVA Repeated Measurement was applied; M = mean, SD = standard deviation, F = Repeated Measure ANOVA, p = statistical significance, bold: statistically significant.

When the differences within the experimental group were tested, the statistically significant difference between any measurements could be confirmed. Thus, at the first examination, the buffer capacity of saliva was the lowest (5.68 ± 2.05), at the measurement after 30 days it was statistically significantly higher (6.00 ± 2.19), while it had the highest value after 60 days (8.80 ± 1.70).

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Table 4: Influence of group and gender on the number of Streptococcus mutans colonies during three measurements

Measurement	Group	Gender	M	SD
First examination	Placebo	Male	17.87	10.12
		Female	16.40	7.92
		Total	17.28	9.15
	Experimental	Male	19.14	8.93
		Female	16.55	11.97
		Total	18.00	10.23
	Total	Male	18.48	10.23
		Female	16.48	9.41
		Total	17.64	9.99
30 days	Placebo	Male	18.20	10.06
		Female	16.50	8.11
		Total	17.52	9.19
	Experimental	Male	19.29	8.84
		Female	16.55	12.17
		Total	18.08	10.30
	Total	Male	18.72	9.34
		Female	16.52	10.18
		Total	17.80	9.66
60 days	Placebo	Male	17.67	10.15
		Female	16.30	7.73
		Total	17.12	9.11
	Experimental	Male	16.64	8.18
		Female	14.27	11.71
		Total	15.60	9.74
	Total	Male	17.17	9.10
		Female	15.24	9.83
		Total	16.36	9.36

Group: F = 18.77, p = 0.000, Partial Eta² = 0.455.
Gender: F = 0.352, p = 0.705, Partial Eta² = 0.015.
Group x Gender: F = 0.024, p = 0.976, Partial Eta² = 0.001.
SPANOVA was applied (Split-Plot ANOVA); M = mean, SD = standard deviation.

Table 5: Influence of group and gender on saliva buffer capacity during three measurements

Measurement	Group	Gender	M	SD
First examination	Placebo	Male	5.80	2.88
		Female	3.80	1.32
		Total	5.00	2.55
	Experimental	Male	6.57	2.06
		Female	4.55	1.44
		Total	5.68	2.06
	Total	Male	6.17	2.51
		Female	4.19	1.40
		Total	5.34	2.32
30 days	Placebo	Male	6.00	2.59
		Female	3.90	0.99
		Total	5.16	2.32
	Experimental	Male	7.07	2.02
		Female	4.64	1.63
		Total	6.00	2.20
	Total	Male	6.52	2.35
		Female	4.29	1.38
		Total	5.58	2.28
60 days	Placebo	Male	8.67	2.02
		Female	7.30	1.06
		Total	8.12	1.81
	Experimental	Male	9.36	1.78
		Female	8.09	1.38
		Total	8.80	1.71
	Total	Male	9.00	1.91
		Female	7.71	1.27
		Total	8.46	1.78

Group: F = 0.417, p = 0.662, Partial Eta² = 0.018.
Gender: F = 6.876, p = 0.002, Partial Eta² = 0.234.
Group x Gender: F = 0.576, p = 0.566, Partial Eta² = 0.025.
SPANOVA was applied (Split-Plot ANOVA); M = mean, SD = standard deviation, F = Repeated Measure ANOVA, p = statistical significance

After examining the differences in the average number of *S mutans* colonies in three-time intervals in each group separately (placebo and experimental), the idea was also to examine whether the group affects the change in results over time. In addition to the influence of the group the separate influence of participants gender was examined and their interaction (Group x Gender). The effect was examined by the Combined Analysis of Variance (SSPANOVA). The results imply that the group statistically significantly contributed to the decrease in the number of *S mutans* ($F = 18.77$, $p < 0.001$). Gender did not show a statistically significant influence ($F = 0.352$, $p < 0.705$) and the interaction between gender and the group was not statistically significant either ($F = 0.024$, $p = 0.976$). Thus, the influence of the experimental factor was statistically significant and it did not depend on gender.

After examining the differences in saliva buffer capacity at three-time intervals in each group separately (placebo and experimental), the idea was also to examine whether the group affects the change in results over time. In addition to the influence of the group, the separate influence of gender was examined, as well as their interaction (Group x Gender). The effect was examined by the Combined Analysis of Variance (SSPANOVA). The results showed that the group did not statistically

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significantly contribute to the increase in saliva buffer capacity (F = 0.417, p = 0.662). Gender showed a statistically significant effect (F = 6.876, p = 0.002). The interaction between gender and the group was not statistically significant (F = 0.576, p = 0.566). Thus, the influence of the experimental factor was not a statistically significant factor that was contributing to the change in the buffer capacity of saliva. On the other hand- gender was found to be a statistically significant factor for buffer capacity change. Namely, in men at the first examination, the average buffer capacity of saliva was 6.17 ± 2.50. After 30 days it was 6.51 ± 2.35 and after 60 days 9.00 ± 1.90. The buffer capacity of saliva measured at the first examination in women was: 4.19 ± 1.40. After 30 days it was 4.28 ± 1.38 and after 60 days 7.71 ± 1.27.

Disscusion

Probiotics demonstrated efficiency to reduce *Mutans streptococci* colonies in plaque in the short term studies.[13] This study determined whether the daily intake of probiotic yoghurt drink decrease the CFU/mL of *S mutans* and its influence on the saliva buffer capacity of diabetic children. Results showed a reduction of *S mutans* after two months of probiotic administration in the experimental group and a significant increase of salivary buffer capacity in both groups. Additionally, some previous studies also demonstrated the benefits of milk and yoghurt products for anti-cariogenic effects, increase salivary buffer capacity.[14]

Children with diabetes have shown higher acidogenic of saliva and predisposition for caries development.[15] Dysfunctional salivary flow at the oral cavity of diabetic children is an appropriate environment for the establishment of *S mutans*, especially among the children with uncontrolled diabetes.[16] It is proven that oral administration of probiotics is needed daily and for a long period, because the level of *S mutans* can increase after a short period of use.[17] This study did not follow the pH parameters, because an acidogenic oral environment in diabetic children has been demonstrated previously.[18] The level of *S mutans* decreased during two months of LGG yoghurt intake among the experimental group of children. For this study, two brands of yoghurt were used: *Dukat standard* for the placebo group and probiotic yoghurt, *Dukat LGG* containing 5 x 10^7 CFU/g of *Lactobacillus rhamnosus*. A high level of a probiotic strain is previously reported in the literature and it is between 10^7 and 10^9 in some supplements.[19] Otherwise, results about pH and buffer capacity require more investigations and one of the hypothesis is that yoghurt increase salivary secretion.

Yoghurt consumption was not documented with any harmful side effects and that is the main reason why this food was chosen, enriched by the *Lactobacillus rhamnosus,* to be an experimental factor in this study. Some short-term studies are indicating that *Lactobacillus species* in the form of probiotics can help reduce *S mutans* count.[20] It is also known that *Lactobacillus sp.* can adhere to hydroxyapatite, with variable strength of adhesion to the dental surface.[21] *Lactobacillus* probiotic strain is also related to the activity of arginine deiminase.[22] In this study, after the experimental time of probiotics consumption, the number of *S mutans* slowly increased, indicating the importance of daily administration of probiotics. Additionally, it is noteworthy that the difference between the two groups was not significant in the first and second measurements, but in a longer period of two months, the number of cariogenic bacteria decreased. Long-term probiotic use benefits are reported in the literature[23] and are additionally underlined in this study.

Foods rich in casein phosphopeptides (CPPs) have positive effects on remineralisation of dental enamel and buffering effect on plaque, interfering with *S mutans* on a dental surface.[24] So far, yoghurt and milk products have limited effectiveness in their natural source because they would require large consumption of dairy products. Additionally, it cannot be claimed that in this study CPPs was directly related to an increase of salivary buffer in both groups like in some previous studies.[25] The literature has also shown that salivary buffers can stabilise pH in plaque which can be very useful for the enamel demineralisation.[26]

Dry mouth and reduced salivary flow rates can in diabetic children lead to oral complications. In this study, yoghurt was chosen as an experimental factor due to its influence on saliva secretion induction,[10] which was very useful for this study group. Some studies also suggest that hyposalivation and duration of disease are related to a reduction in the buffer capacity in diabetic children.[1]

This study demonstrated that there was a significant increase of salivary buffer between the first and the last examination. Similar results were found in earlier studies.[27] Additionally, the results demonstrated the influence of participants gender

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on saliva buffer capacity. At first examination, in men, it was 6.17 and after 60 days it was 9, while in women it was 4.19 at first examination and 7.71 after 60 days. The participants' gender influence was something to be further addressed in studies about the diabetic syndrome in the children population.

It seems that *Lactobacillus rhamnosus* does not ferment sucrose.[28] Sucrose has a cariogenic effect by itself and promotes demineralisation.[29] Sucrose is important as a substrate for extracellular (EPS) and intracellular polysaccharides (IPS) formation in the oral cavity and reduces the concentration of Ca, phosphorus (P) and F in dental plaque.[30] The question of caries prevention in the oral cavity of diabetic children with controlled parameters of disease by synergistic administration of probiotic strain and anti-cariogenic food is yet to be investigated. More research in understanding bacterial metabolism is needed to develop therapeutic strategies to define acid accumulation and tooth demineralisation in diabetic children. The improvement of general health in diabetic children using probiotic strain is yet to be demonstrated.

The small sample size determined by a restricted geographical area is one of the studies limitations. All subjects confirmed that they followed study protocol, but this cannot be claimed for all children ages. Also, this study showed effects for children affected by diabetes, but for the adult population, there should be further examination.

Conclusion

Oral administration of probiotic yoghurt may have a large impact on balance in oral microbiota. This study confirmed the effectiveness of short-term use of probiotic strain with metabolic patients and could be a promising preventive factor to caries development.

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Conflict of interest

None.

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ORIGINAL ARTICLE

Relationship Between the Age and Sex of the Patient With the Results of the Indirect Immunofluorescence Test in Patients With Bullous Dermatoses

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Abstract

Background/Aim: Autoimmune bullous diseases are characterised by the production of autoantibodies to epidermal or subepidermal adhesive proteins. The aim of this study was to determine the relationship between age and sex of patients with the results of indirect immunofluorescence test in patients with newly diagnosed bullous dermatoses.
Methods: The investigation presents a retrospective study of newly diagnosed patients with autoimmune bullous diseases at the Clinic for Skin and Venereal Diseases of the University Clinical Centre in Banja Luka in the period 2016-2021. In addition to demographic data, the results of an indirect immunofluorescence test in two titres (≥ 1:10 and ≥ 1:100) were analysed.
Results: In this study, almost the same number of patients with pemphigus (45.2 %) and pemphigoid (54.8 %) was found. There were more women than men in the total sample (p = 0.049). The average age of subjects with pemphigoid was higher than that of patients with pemphigus (p = 0.001). 48.2 % of patients with pemphigus and 51.8 % of patients with pemphigoid had a positive indirect immunofluorescence test. A positive test for epidermal intercellular substance in both sexes at a titre ≥ 1:100 is higher than a titre ≥ 1:10 (p = 0.029). Patients with autoantibody titres ≥ 1:100 to desmoglein-1 were statistically significantly older than patients with titres ≥ 1:10 (p = 0.047).
Conclusion: Number of patients with pemphigus and pemphigoid were similar, with no difference in sex distribution between the two groups of patients, but patients with pemphigoid were older than patients with pemphigus. The difference between high and low autoantibody titres in both sexes was found only in the group of pemphigus on epidermal intercellular substance and desmoglein-1.

Key words: Pemphigus; Pemphigoid; Indirect immunofluorescence test.

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Introduction

Autoimmune bullous diseases are characterised by the loss of tolerance to structural skin proteins, forming as a result autoantibodies to epidermal or subepidermal adhesive proteins.[1] Pemphigus group represents a disease of autoimmune nature which is characterised by the appearance of blisters caused by acantholysis. This group includes *pemphigus vulgaris, foliaceus, fogo salvagem*, drug-induced pemphigus and paraneoplastic pemphigus. The most common types of this group are *pemphigus vulgaris* and *pemphigus foliaceus*. *Pemphigus vulgaris* is more common in Europe and North America, representing 60-90 % of pemphigus cases, whereas *pemphigus foliaceus* is more common in South America and North Africa where endemic

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pemphigus foliaceus is also present. Other clinical forms of pemphigus are less common.

In European countries, pemphigus most often occurs in the age groups between 50 and 60 years, without preference regarding sex.[1, 2] The incidence of *bullous pemphigoid* in Europe is increasing and is associated with aging, in patients with diabetes or malignancy, but it can also be triggered by medication. It most often occurs in people older than 70 years of age. It is more common in women. The mortality rate increases with each decade of life.[3] In pemphigus, IgG autoantibodies are directed against desmoglein-1 and 3, which are part of the intercellular cadherin family, responsible for maintaining intercellular adherence in the epidermis and epithelium of the oral mucosa.

Pemphigus foliaceus is characterised by the presence of antibodies to desmoglein-1, leading to loss of keratinocyte adhesion. Additionally, it is considered that the formation of blisters in pemphigus occurs because of increased presence of proinflammatory mediators or other mechanisms, such as activation of specific muscarinic receptors expressed on keratinocytes, abnormalities in intercellular signalling, or activation of apoptosis. The group of autoimmune subepidermal diseases is characterised by the production of circulating autoantibodies against structural proteins of the basement membrane zone.

Bullous pemphigoid is characterised by the production of autoantibodies directed against BP180 and BP230.[4] Clinically, *pemphigus vulgaris* initially appears on the oral mucosa where it can remain localised for a long time, but also precede skin changes in the form of blisters on unaltered or erythematous skin. After bursting of blisters, painful erosions occur that epithelialise *ad integrum* followed by residual post-inflammatory hyperpigmented macules, which can persist for a long time.[5] The appearance of blisters in *bullous pemphigoid* is preceded by a prodromal phase, with appearance of urticarial, purple, eczema, targetoid, nodular or lichenoid changes. Blisters in *bullous pemphigoid* have a tense covering of various sizes, often causing a subjective feeling of itching. After the rupture of blisters, erosions occur and they epithelialise without scarring. Mucous membranes can be affected in up to 30 % of cases.[6]

The diagnosis of pemphigus and pemphigoid diseases is confirmed based on medical history, clinical manifestations, pathohistological findings, direct immunofluorescence test (DIF) findings and using indirect immunofluorescence (IIF) method, which determines the level of circulating autoantibodies. Monkey oesophagus is most often used as substrate. The enzyme-linked immunosorbent assays (ELISA) are a more sensitive method for measuring antibodies to desmoglein-1 and 3.[7]

The aim of the paper was to determine the relationship between the results of the indirect immunofluorescence test and the age and gender in newly diagnosed patients with bullous dermatoses.

Methods

This research was conducted as a retrospective study that included all newly diagnosed patients suffering from autoimmune bullous diseases treated at the Clinic for Skin and Venereal Diseases of the University Clinical Centre in Banja Luka in the period from 2016 to 2021. The study included a total of 93 patients of both sexes. The patients were divided into two groups. The first group consisted of newly diagnosed patients from the pemphigus group, and the second group consisted of newly diagnosed patients from the pemphigoid group. In all patients, in addition to demographic data, the data on the indirect IIF immunofluorescence test performed in two titres, ≥ 10 and ≥ 100, with the EUROIMMUN immunofluorescence test *Dermatology Mosaic 7* were included. In accordance with the recommendation of the manufacturer EUROIMMUN, in these patients the dilution of samples in titres ≥ 1:10 and ≥ 1:100 from human serum or plasma and electron microscopy were performed. BIOCHIP Dermatology Mosaic 7 contains 6 fields that require fluorescence microscopy. In the first field, in the case of a positive reaction, antibodies to the desmosomes of spinous cells react with surface antigens of keratinocytes. Oesophageal tissue gives granular, reticulated fluorescence in the intercellular substance of the entire spinous layer. In the second field, if the serum contains autoantibodies to the epidermal basement membrane, fine linear dyeing occurs between the

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basement layer and the connective tissue. In the third, fourth and sixth field, if the serum contains autoantibodies desmogelin-1, desmogelin-3 and BP230gC, smooth, fine, granular cytoplasmic fluorescence is visualised, partly with cell membrane fluorescence, on transfected cells of the appropriate array substrate. The nucleus of cells is not dyed or only slightly fluoresces. The fifth field contains BP 2180-NC16A if the serum antibodies are specific for BP180 fluorescent green diamond-shaped fields as opposed to the dark field.

The statistical analysis was performed using the SPSS software package (Statistical Product and Service Solutions) 20. The results were described by average values ($\bar{X}$) and standard deviations (SD) for continuous variables, and incidence and percentages (%) for categorical variables. Differences between average values of variables in groups were analysed using the independent samples t-test, whereas the differences between frequencies of individual groups of patients were tested using Pearson's Chi-squared test. The Pearson correlation coefficient (r) was used to calculate the correlation between the two continuous variables. Binary Logistic Regression was used to examine the effect of gender and age on predicting IIF test results among the study population. P values lower than 0.05 were considered statistically significant.

Results

In the results, the IIF test values in 93 patients of both sexes was monitored. There were 45.2 % of patients with pemphigus and 54.8 % of patients with pemphigoid. Of the total number of patients, 60.2 % were women and 39.8 % were men. The number of affected women was statistically significantly higher than the number of men (p = 0.049) in the total patient population. In the group of patients with pemphigus, 48.2 % were women and 40.5 % were men. In the group of patients with *bullous pemphigoid*, 51.8 % were women and 59.5 % were men. There was no statistically significant difference between the sexes in patients with pemphigus and pemphigoid, (χ (1) = 0.530, p = 0.467).

The average age of the total number of patients in our study was 70.88 ± 14.22 years, in the group of patients with pemphigus 65.64 ± 15.06 years, and in the group of patients with pemphigoid 75.20 ± 11.99 years. The difference in the age of the patients between the two groups of patients was statistically significant, t (91) = -3,405, p = 0.001.

The average age of patients with a negative IIF test was 73.11 ± 14.76 years and are on average 3.70 years older than patients with a positive test. However, this age difference was not statistically significant (t (91) = 1,231, p = 0.222, p > 0.05. The Pearson correlation also indicates that there is a weak, positive correlation between age and IIF test results, but that it is not statistically significant (r = 0.128, n = 93, p = 0.222). The result of the independent t-test indicated that patients with newly diagnosed pemphigus and negative IIF test have an average age of 71.19 ± 16.71 years and are on average 8.96 years older than patients with positive test (62.23 ± 13.06). Pearson correlation indicated that in patients with pemphigus there is a moderate, positive correlation between age and IIF test results, which was not statistically significant (r = 0.292, n = 42, p = 0.060).

The result of the independent t-test indicated that patients with newly diagnosed pemphigoid with a negative IIF test had an average age of 74.57 ± 13.22 years and were on average 1.06 years younger than patients with a positive test (75.63 ± 11.27). However, this difference was not statistically significant, t (49) = -0.308, p = 0.759, p > 0.05. The Pearson correlation indicated that in patients with pemphigoid there was a weak, negative correlation between age and IIF test results, but not statistically significant (r = -0.044, n = 51, p = 0.759).

The number of patients with a positive IIF test was 56 (48.2 % with pemphigus and 51.8 % with pemphigoid). The average age of patients with a positive IIF test was 69.41 ± 13.79. The results of the independent t-test indicated that patients with a positive test belonging to the pemphigus group are on average 13.4 years younger than patients with pemphigoid, which was a statistically significant difference (t (54) = -4,123, p = 0.000). The Pearson correlation indicated that in patients who were positive for the IIF test there was an extremely strong, positive, statistically significant correlation between age and test positivity (r = 0.489, n = 56, p = 0.000) (Table 1).

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Table 1: General patients' characteristics and immunofluorescence test results

Variables	All patients	(Group I) Pemphigus	(Group II) Pemphigoid	*p*-value
Gender, N (%)	93 (100.0)	42 (45.2)	51 (54.8)	0.350*
Male	37 (39.8)	15 (40.5)	22 (59.5)	0.467*
Female	56 (60.2)	27 (48.2)	29 (51.8)	
Age (years) $\bar{X}$ ± SD	70.88 ± 14.22	65.64 ± 15.06	75.20 ± 11.99	0.001**
Negative IIFT, n (%)	37 (100.0)	15 (40.5)	22 (59.5)	0.249*
($\bar{X}$ ± SD)	73.11 ± 14.76	71.19 ± 16.81	74.57 ± 13.22	0.497**
Positive IIFT, n (%)	56 (100.0)	27 (48.2)	29 (51.8)	0.789*
($\bar{X}$ ± SD)	(69.41 ± 13.79)	62.23 ± 13.06	75.63 ± 11.27	0.000**

*N (%) – number (percentage); $\bar{X}$ – average; SD – standard deviation; IIFT – indirect immunofluorescent test; * Chi-square test; ** Independent samples t-test*

Table 2: Relationship between autoantibody titres with the patients' gender and age in the pemphigus group

Autoantibodies	Antibody titre	N	Female N (%)	Male N (%)	*P*	Age $\bar{X}$ ± SD	*P**
EIcS	≥ :10	7	7 (100.0)	-	0.029	58.71 ± 9.21	0.668
	≥ 1:100	15	8 (53.3)	7 (46.7)		61.33 ± 14.50	
Dsg1	≥ 1:10	4	1 (25.0)	3 (75.0)	0.071	56.00 ± 16.55	0.047
	≥ 1:100	9	7 (77.8)	2 (22.2)		69.67 ± 12.61	
Dsg3	≥ 1:10	11	6 (54.5)	5 (45.5)	0.582	58.55 ± 6.19	0.128
	≥ 1:100	9	6 (66.7)	3 (33.3)		60.89 ± 18.26	

N (%) – number (percentage); $\bar{X}$ – average; SD – standard deviation; EIcS - Epidermal intercellular substance; Dsg – desmoglein; P – Chi-squared test; P – Independent t-test*

Table 3: Relationship between autoantibody titres with the patients' gender and age in the pemphigoid group

Autoantibodies	Antibody titre	N	Female N (%)	Male N (%)	*P*	Age $\bar{X}$ ± SD	*P**
EBM	≥ 1:10	13	7 (53.8)	6 (46.2)	0.785	72.54 ± 13.06	0.162
	≥ 1:100	17	10 (58.8)	7 (41.2)		78.29 ± 8.92	
BP180	≥ 1:10	7	3 (42.9)	4 (57.1)	0.629	73.29 ± 15.13	0.576
	≥ 1:100	11	6 (54.5)	5 (45.5)		76.91 ± 11.79	
BP230	≥ 1:10	5	4 (80.0)	1 (20.0)	-	69.80 ± 15.52	-
	≥ 1:100	-	-	-			

N – number (percentage); $\bar{X}$ – average; SD – standard deviation; EBM – epidermal basement membrane; BP – bullous pemphigoid; P – Chi-squared test; P – Independent t-test*

After using the Chi-square test, it was determined that there was a statistically significant difference in the frequency of patients according to gender and the titre of autoantibodies to epidermal intercellular substance (EicS). The number of patients of both sexes with a titre ≥ 1:100 was statistically significantly higher compared to patients of both sexes with a result ≥ 1:10 (p = 0.029). Using the same test, no statistically significant differences in the frequency of patients by sex and titre of autoantibodies to desmoglein-1 were confirmed (Dsg1), (p = 0.071), as well as between patients by sex and titre of antibodies to desmoglein-3 (Dsg3), (p = 0.582). After using an independent t-test, the age difference between positive patients with an autoantibody titre with a titre ≥ 1:10 and a titre ≥ 1:100 was examined. It was determined that the age differences were statistically significant only in patients positive for Dsg1, with patients with autoantibody titres ≥ 1:100 statistically significantly older (69.67 years) than patients with titres ≥ 1:10 (56.00 years), (p = 0.047). In other cases, no statistically significant difference in the patients' age and titre height to EIcs (p = 0.668) and to Dsg3 (p = 0.128) was found. In both cases, patients with a titre ≥ 1:100 were older, but this difference was not statistically significant (Table 2).

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In patients positive for epidermal basement membrane (EBM), there were no statistically significant differences in the frequency of patients according to gender and autoantibody titre values (p = 0.785). The same was found in BP180-positive patients (p = 0.629). In the case of BP230, it was not possible to use Chi-squared test because there were no patients with a titre ≥ 1:100. Using an independent t-test, differences in average age between positive patients with different titre values were analysed. In cases with positive autoantibodies to EBM and BP180, patients with a titre ≥ 1:100 were older, but this difference was not statistically significantly greater than in patients with a titre ≥ 1:10 (Table 3).

Discussion

This research confirmed that an indirect immunofluorescence test was performed on 93 patients with bullous dermatoses over a five-year period. This test quantitatively determines the level of circulating autoantibodies in patients and it is important in the diagnosis, differential diagnosis of autoimmune bullous diseases and monitoring the results of administered therapy. There were 45.2 % of patients suffering from pemphigus and 54.8 % of patients diagnosed with pemphigoid. Out of the total number of patients, 60.3 % were women and 39.8 % were men. The number of women was statistically significantly higher compared to the number of men (p = 0.049) in the total patient population. In the group of patients diagnosed with pemphigus there were 48.2 % women and 40.5 % men, whereas in the group of patients diagnosed with pemphigoids there were 51.8 % women, and 59.5 % men. The results showed no statistically significant difference between the sexes in newly diagnosed patients in both groups (p = 0.467).

The study of Arbache et al included 421 patients, 277 patients in the pemphigus group and 144 patients in the subepidermal autoimmune disease group. In serology, elevated titres have been reported for *vulgar* and *pemphigus foliaceus* that correlate with earlier stages of the disease or active disease. The results confirmed the importance of direct and indirect immunofluorescence in the diagnosis of autoimmune bullous diseases. As for IIFT, lower values were found for *bullous pemphigoid*. This deviation may be a consequence of technical problems, especially in terms of the nature of the substrate used.[8]

Kutlubay and his associates analysed 346 new cases of patients with autoimmune bullous dermatoses in the period from 2003 to 2019, and the research included 151 men and 195 women. The average age of patients was 54.4 years and the average duration of the disease was 85.6 months. The most common diagnoses were *pemphigus vulgaris* 70 %, *bullous pemphigoid* 13 %, and *pemphigus foliaceus* 6 %, and *pemphigus vulgaris* mostly appeared in women in a ratio of 1:4. Patients with *bullous pemphigoid*, with an average age of 75.6 years, were present in a lower percentage compared to *vulgar pemphigus*, and the highest incidence rates of pemphigoid in both sexes were registered in the age groups 70 to 79, especially in people over 80 years of age, which is in accordance with the data from literature, as well as the results of the present research.[9]

The average age of patients in this study in both groups was 70.88 ± 14.22. The average age of patients suffering from pemphigus was higher than the average age of patients diagnosed with pemphigoid (p = 0.001). This study included a higher percentage of women compared to men in both groups of patients. According to the data of similar studies, pemphigus occurs more often in women, and when it comes to age, it is most often diagnosed between the ages of 50 and 60 in European countries, whereas in other countries worldwide, it occurs between the ages of 30 and 50. *Bullous pemphigoid* occurs in the age of 70 and older, as it did in the patients presented in this study. Although the overall incidence is slightly higher in women, after 80 years it is more common in men, according to Alpsoy et al.[10] In the study of Milinković and his associates in Serbia, *pemphigus vulgaris* appears in a higher percentage in patients who are 80 years old or older and is more common in women.[11]

In Schmidt et al, *pemphigus vulgaris* occurs in 70-80 % cases, *pemphigus foliaceus* in 20 %, paraneoplastic pemphigus in 5 % and IgA pemphigus in 1-3 % of all described bullous autoimmune diseases. In Germany, their prevalence is around 40,000 cases across the country, and their incidence is around 20 new cases per million inhabitants per year.[12]

In the study of De and his associates, a total of 268 cases of autoimmune bullous diseases were

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registered at the Immunobullous Disease Clinic, where 50 patients (18.7 %) were subepidermal, and 20 of them (40 %) were patients with *bullous pemphigoid* with an average age of 59 (33- 80) years, and the ratio of men to women was 1.2:1. *Bullous pemphigoid* is the most common subepidermal autoimmune bullous disease in India as well as in Western Europe, although the proportion appears to be lower, at least in their group of patients, with the disease starting earlier in life. In the present study, patients with pemphigoid are older than 59 years, opposite to the results indicated in De's paper.[13]

In this study, the number of patients with positive IIFT was 56, with and the average age of patients was 69.41 ± 13.79 years. Patients from the pemphigus group were on average 13.4 years younger than patients belonging to the pemphigoid group, which is a statistically significant difference ($p < 0.05$). Following the analysis of IIFT and patients' gender in these results, a statistically significant connection in both groups of patients was not found ($p < 0.05$).

Creedin and Bergman carried out a retrospective cohort study that included 770 patients in whom IIFT was performed for the presence of intercellular autoantibodies in the monkey oesophagus in the period 2000-2017. 176 patients were diagnosed with *pemphigus vulgaris*, and 29 patients with *pemphigus foliaceus*. The sensitivity of this immunoassay was significantly higher for the *pemphigus vulgaris* diagnosis ($p = 0.018$). Among female patients, 65.9 % had *pemphigus vulgaris* and 34.5 % had *pemphigus foliaceus*, which is in line with the results of this study in which the predominance of female patients in the group with pemphigus vulgaris was determined.[14] The Pearson correlation indicated that in both groups of patients with positive IIFT in this study, there was an extremely strong, positive correlation with the patients' age ($p = 0.000$).

The retrospective study of Askin and associates included 320 patients with autoimmune bullous diseases and 70 % of them had a positive IIFT result for *pemphigus vulgaris*. Another result of the study showed that the disease is more common in women and has a peak incidence in the fifth decade, but that there was no statistically significant difference between age and sex of patients with IIFT results.[15]

The results indicate a statistically significant difference in patient frequency by sex and autoantibody titre to EicS. The number of patients of both sexes with titre ≥ 1:100 was statistically significantly higher compared to patients of both sexes with ≥ 1:10 ($p = 0.029$). The average age of the patients was 58.71 ± 9.21 in patients with a titre ≥ 1:10 and 61.33 ± 14.50 with a titre ≥ 1:100. These results are in accordance with the results of a study conducted by Hashimoto and associates, which included about 5,000 cases. The study included 30 patients with IgG and IgA autoantibodies to the intercellular substance, among which clinically, there was no prevalence between men and women, and average patients' age was 55.6 years.[16]

These results did not show any statistically significant difference in the age and sex of patients according to the titre of autoantibodies to desmoglein-1 ($p = 0.071$) and desmoglein-3 ($p = 0.582$). Delavarian and associates conducted a study that included 19 patients diagnosed with *pemphigus vulgaris*, 9 men and 10 women, with an average age of 47.26 ± 15.11, opposite to the patients presented in this study, whose average age was significantly higher (65.64 ± 15.06). Among 19 patients with pemphigus, desmoglein-3 positive autoantibodies were present in 95 % of patients and desmoglein-1 positive in 63 % of patients.[17]

There were not any statistically significant differences according to the sex and age of the patients and the values of autoantibody titres in patients in this study who tested positive for EBM and BP180. In the case of autoantibodies to BP230, there were not any patients with a titre of ≥ 1:100.

The study of Muhammed N and associates included 30 patients with a *bullous pemphigoid* diagnosis, the patients' average age was 56.33 ± 13.69. Women were present in a higher percentage than men. Autoantibody titres to BP 180 and 230 were performed in patients. Patients with higher autoantibody titres to BP180 had more severe disease, whereas autoantibody titres to BP230 had no correlation with the disease severity, and this study had no patients with autoantibodies to BP230 at titres ≥ 1:100. The patients were older, and there was no predominance according to gender.[18]

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Conclusion

Number of patients with pemphigus and pemphigoid were similar, with no difference in sex distribution between the two groups of patients, but patients with pemphigoid were older than patients with pemphigus. The difference between high and low autoantibody titres in both sexes was found only in the group of pemphigus on epidermal intercellular substance and desmoglein-1.

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Conflict of interest

None.

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Olaleye et al. Scr Med 2022 Sep;53(3):229-34. DOI:10.5937/scriptamed53-39374
ORIGINAL ARTICLE

Challenges of Antiretroviral Therapy Among Children in Free State Province, South Africa

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Abstract

Background/Aim: Antiretroviral therapy (ART) is an important intervention for survival among children in Sub Saharan Africa where HIV infection rates are comparatively high. Only few studies have explored issues relating to paediatric ART initiation and maintenance. This study was conducted to explore the perceptions and experiences of trained professional nurses regarding paediatric ART.
Methods: Six focus group discussions (FGDs) were conducted among trained professional nurses in selected health facilities in Free State Province, South Africa. Verbatim transcripts were analysed with a thematic approach.
Results: The participants of this study reported counselling as an important component of paediatric ART in health facilities. The problem of non-disclosure, migration, incomplete records from referral health facilities, inadequate health workforce and difficulty in record keeping were cited as barriers against paediatric ART.
Conclusion: This study showed that initiation and adherence to antiretroviral therapy among eligible children faces a significant challenge.

Key words: Children; Antiretroviral; Therapy; Challenges; Problems.

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Introduction

Globally, an estimated 2.1 million children are living with HIV and almost 9 in 10 are in Sub-Saharan Africa.[1] While about 160,000 new HIV infections occurred in 2016, an estimated 120,000 deaths due to AIDS were recorded among children.[2] The introduction of antiretroviral therapy (ART) has been associated with significant changes in the natural course of HIV infection including improved clinical status and increased longevity.[3] Furthermore, ART has also been associated with improved clinical states of children living with HIV. A prospective study reported a decline in the incidence of AIDS- and non-AIDS-defining illnesses among children.[4] However, less than half of children living with HIV are currently accessing ART worldwide.[5, 6]

In Sub-Saharan Africa where the impact of HIV-infection is very significant, studies have shown that transportation and supplementary food costs, poor attitudes of health workers, fear of stigma and discrimination are barriers to uptake of ART and adherence to therapy.[7, 8] However, most of the studies on ART were conducted among adults. While knowledge of the barriers and facilitators of treatment access may help in addressing concerns and optimising health care delivery services, there is paucity of studies on the uptake of paediatric ART in health care settings. The objective of this was to explore and describe issues relating to initiation of HIV-infected children on Antiretroviral Therapy (ART) and identify enablers as well as barriers to ART initiation among eligible children in Free State Province, South Africa.

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CONTENT

Methods

The present study was part of a study whose findings have been described elsewhere.[9] The present study was conducted out in the three sub-districts of Manguang metropolitan district (Thaba Nchu, Botshabelo and Bloemfontein) of Free State Province. The prevalence of HIV infection among children aged 0-4 and 5-14 years in this province were 1.7 % and 2.7 %, respectively.[10] The study was conducted among purposively-selected participants who were nurse-initiated management of antiretroviral therapy - NIMART-trained professional nurses.

A qualitative approach using focus group discussion (FGD) was adopted as a data collection method suitable for exploring and describing perceptions, attitudes and practices about health and social issues. Two sessions of FGD were held in each of the three sub districts of Mangaung and data collection took place over a period of three days. The conduct of FGD sessions were led by the principal author and demographic sheets were used for profiling of the characteristics of the participants. The interview guide included open-ended questions regarding the practice of initiation and maintenance on ART among eligible children in their respective health facilities, their experiences about paediatric ART and, recommendations for improvement in ART services in the province. Interview questions were followed with prompts to gain an in-depth understanding of participants' experiences, thoughts, and perceptions. Each FGD session lasted about one hour.

Audio recordings were transcribed by professional transcribers. Audio recordings were replayed and corrections made to transcriptions where appropriate. Field notes were also triangulated with transcriptions from audio recordings.

Based on emerging topics, data coding was done and verbatim transcripts were analysed with a thematic approach. Data were analysed using Atlas.ti version 7 software.[11]

Validity and reliability

The lead facilitator is experienced in qualitative research methods and familiar with policy discourses in health systems and all efforts were made to ensure objectivity in this research. All original data were re-assessed after analysis to ensure any concept or information had not been missed. Findings were also shared with participants for validation.

Ethical Considerations

Ethical clearance (ECUFS NR 52/2013) for the conduct of the study was obtained on 21 May 2013 from the Research Ethics Committee of the University of Free State (UFS). The conduct of the study conforms to international ethical guidelines. Informed consent was obtained from all participants. Data was anonymised ensure that no sociodemographic variable was able to potentially identify any particular participant and access to the data was restricted only to the members of the research team. No compensation or direct benefits were given to the participants.

Results

Sociodemographic variables

The age of the 47 participants in the study ranged from 38 to 60 years with a median of 50 years. Majority (95.7 %) of them were females. All participants were trained professional nurses with experience in paediatric ART.

Theme and categories

Based on the analysis of the study data, the findings of this study are reported under the following themes:

1. Process of paediatric ART initiation and adherence in health facilities

a. Counselling as an important component of paediatric ART in health facilities

HIV counselling and testing (HCT) is usually followed by the conduct of other tests prior to initiation of patients on ART. A participant stated thus:

"...you can initiate after doing the test, the viral load and CD4 and explain to the mother..."

In addition to HCT and other laboratory tests, participants also explain to their clients the importance of adherence with medications.

"...explain compliance with taking the medicine every day.... you can tell the mother and you can say that your child will be healthy, you can be healthy, just comply and come to the clinic every month for your medicine and everything will be fine..."

Health workers often make comparisons between HIV infection and other chronic diseases to encourage or assist caregivers cope with challenges of adherence with ART among children.

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"...So, I try to explain to people that we have to look at HIV the same way that people have to look at diabetes, they have to take the medicine every day. If you have heart disease, you have to take the medicine every day, so you can have a normal life. So, most of the people still have this mind set of if I have HIV, I have to go and apply for a policy for a funeral or whatever because that's things of the past and you have to change that perception of I am going to die. No, you are not going to die, you stay with me, you come to my clinic and you will have a normal life and you can be healthy. So, that is good news and people start to take it. It's easier for them to comply..."

b. *Tracing of defaulters - an enabler of adherence*

Health facilities support adherence with ART by promoting defaulter tracing. A participant explained: ."

"...Within our facility, the patients are coming every month because we have to fill these every month. So on the book list, we have, we are seeing the attendees, and also the data capturer is capturing that this patient was here today, so the following month, on this day, the patient will be/is supposed to be there. So, if the patient is not here, they are doing some follow ups; we are asking people to help us trace the patient and sometimes we are going, or we are phoning from our own cell phones and following up, following up on the patient..."

2. Challenges of ART initiation and adherence among eligible children

a. *Caregiver-related problems*

i. Problem of non-disclosure

Oftentimes, caregivers do not disclose the HIV status of children to them even when they are old enough to know. This prevents adequate follow up of children on the therapy (Figure 1).

"The main challenge there is that mothers who are on ART, even their children, they don't know how to tell these kids why they are taking these medicines. But it's very difficult and they want to always come to the clinic to collect for the kids, they don't want to bring the children to the clinic."

Participants explained as follows: *"...Children don't really know why they are taking this medication and if you ask them... you will find out that they*

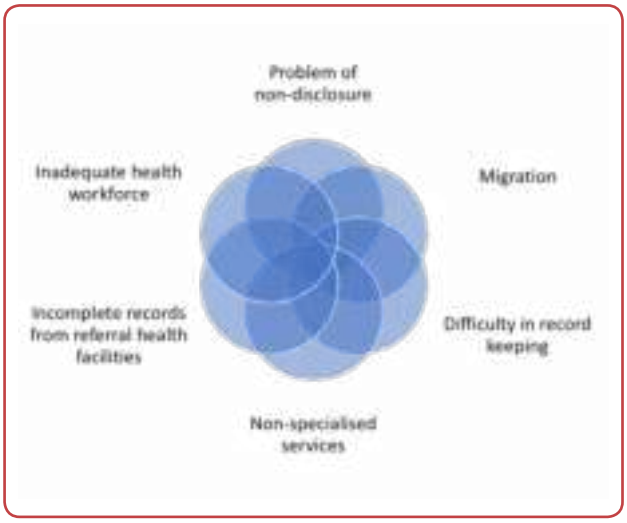

Figure 1: Challenges of antiretroviral therapy (ART) initiation and adherence among eligible children

really are not saying anything to the kids. The kids just know that they are taking these pills, and they don't know what these are.....and sometimes they don't want even to take them. So, I think that's a challenge, because parents are afraid to talk about things to their children; starting from sex and everything, whatever. So HIV is something is like a taboo for parents to talk about with their children."

"... that is why the mother end up saying that: You know, I am not ready for my child to know about her status... we also do consider that as a hindrance.... It is even difficult with the mothers..."

"...I remember the one that we have now... She was not taking treatment because she was afraid other children will mock her. She was not taking treatment gradually and surely, she was deteriorating. Now as I'm talking to you, she is bed-ridden with a shunt. She had TB meningitis. Her parents were not even aware that she was not taking treatment. She's thirteen years old – bed-ridden. She was saying children were discriminating, so she decided not to take the tablets any longer..."

ii. Migration

Majority of the participants view inability to locate caregivers and their children as a major hindrance to paediatric ART:

"...The other challenge is that people are not staying at one place, you get the people starting at this clinic, and then they go to another clinic, say for instance the mother goes to the antenatal clinic at [...] but now the mother is not staying at [...], she is staying at one of the places. Then for immunisation and everything, she will go to another clinic..."

"...Sometimes, the parents are changing addresses. So, sometimes, we fail to initiate in time..."

CONTENT

b. Health system-related problems

i. Incomplete records from referral health facilities

Participants explained that when patients are referred to their facilities without adequate referral documents, initiation or follow up on ART is often hindered:

"If a client is referred from the hospital to the clinic, some of the records are not completed, it is only the profile of the client, the rest has to be done at the clinic."

ii. Inadequate health workforce

Participants cited the issue of few health workers attending to a large number of patients attending health services for several health conditions including paediatric ART as a major barrier to adherence:

"...Because, when you are using one pharmacist assistant, when he's not there, like us in our case now, that sister who's seeing that long queue, is even dispensing ARVs. So it's so difficult and when they lose patience, the patients just go away; they can't stay for long."

"..... that other person who is positive is still going back to the clinic for continuation of care. So shortage is clearly an issue, it's the main issue..."

iii. Non-specialised services

Participants reported that having to offer care to all categories of clients limit their ability to provide quality services to children eligible for ART initiation or maintenance:

"... it's very difficult to attend properly to clients if you are not a person who is dealing with children on a daily basis, so you will find that you will be doing some stuff, like seeing hypertensives or antenatal patients. All of a sudden there is a child, you know, you tend to forget these problems... It is very difficult to follow the protocol correctly and to keep all the statistics correctly...There has to be someone who's responsible for this programme..."

"...The children and adults want to see one sister. Really it is not going to be fair for those clients because sister will be seeing a hypertensive patient and then enter in the register. After that patient comes the child. The child is for immunisation, she is doing that. After that is the IMCI who is sick, after that is the one who is to be initiated. That sister is not really going to manage to do all these things at the same time. It is true she must know all but now to master every program is not going to be possible...."

iv. Difficulty in record keeping

Difficulty in the use of monitoring and evaluation tools was reported as a major barrier to paediatric ART initiation and maintenance:

"We visited one of the clinics, the professional nurse was working alone, initiating, seeing all other patients, the clinic was full. So we assessed the form, this form we fill for initiating, the forms were terrible. We struggled, I think we couldn't get eight files for the patients, the ones we got hold of, all the forms were terrible, so I think that this initiation form is not flexible."

"There is a lot of information which is needed, but you will find that the professional nurse has just left the spaces blank...the forms were all incomplete, some of the information, I think it was impossible for the poor registered nurse to fill in the form, because she was just hurrying to fill in the form and initiate."

3. Strategies for improving paediatric ART initiation and maintenance

Participants also identified possible strategies that may improve the coverage and quality of paediatric ART services as follows:

a. Community education to reduce stigma

Reducing stigma was highlighted as an important step towards improved care for children living with HIV:

"...This issue of stigma, should also be reduced because it is the only one, the possibility of the child to be helped out when necessary, because now, even this morning, somebody read us an item from the newspaper that we are, you know sort of discriminating these people who are HIV positive. Here in [...] you are having caravans where we isolated the people, so if you can just raise the health education of stigma, confidentiality and all that, may be that...will improve..."

" .....More education maybe on radios or stuff because the problem is like, here, people come from far then they are scared, I don't know, they don't want to go to their clinic where they live because people will see them..."

b. Increase trained workforce

Recruiting and training health workers on paediatric ART is important for improved care among eligible HIV infected children:

"On ARVs training matter, but at the moment we are

CONTENT

unable to go and train because we are short-staffed. So if you get more staff and then they should be trained on ARVs initiation."

"...I think training of nurses will be a priority issue, big priority... Nobody else will say that, the nurse does everything..."

c. *Inclusion of HIV/AIDS in Primary School Curriculum*

Including items on HIV into primary school education was cited by some participants as a strategy to improve care:

"...School health I think is where it comes in. Like now including it in the primary school curriculum and the school health professional nurses go into schools giving kids information about HIV and AIDS..."

Discussion

This study focused on exploring the perceptions and experiences of trained professional nurses on the practice of initiation and maintenance of eligible children on ART and strategies for improving care for HIV-infected children. The findings of this study are important in that only few qualitative studies exist on issues related to ART among children in Sub Saharan Africa. In the interactions, the study found that caregivers often hide children's own HIV status from them even when they are emotionally mature enough to know; migration of parents/caregivers hinder initiation and maintenance of children on paediatric ART; there is inadequate workforce to adequately cater to the needs of eligible HIV infected children; and community education including introduction of HIV-related topics into primary school curriculum will go a long way towards improving ART among children.

Caregivers' disclosure of infected children's own HIV status to them is an important component of the long term management of paediatric HIV/AIDS. While WHO recommends that children of school age should be informed of their status,[12] findings of this study show that non-disclosure of children's own HIV status limits the capacity of the health workforce to provide quality health care and support to eligible children. This is in keeping with the results of a systematic review which showed the rates of own HIV status disclosure to children as ranging between 0 % and 69 % in resource-limited settings.[12] The review also reported improved adherence to medication after disclosure.[13]

Migration was a factor cited as a major barrier to paediatric ART in this study. When a caregiver migrates to a new area with their HIV-infected children without updating their contact details in the attending health facility, follow up on treatment and support becomes difficult. Studies have also reported migration as one of the causes of loss to follow up on ART among people living with HIV.[14-16]

Inadequate capacity of the health workforce in terms of number of professional nurses compared to patients reported as a major barrier to quality healthcare for HIV infected children in the present study was also observed in similar studies.[17, 18] Participants in a qualitative study among health workers in Lesotho reported insufficient manpower and increased workload as one of the major difficulties facing health workers responsible for HIV care and support.[19] While ratio of health workers to patients are often low in developing countries, lack of adequate training and poor infrastructure often complicate their tasks.

Community education aimed at reducing stigma was recommended by majority of the study participants as a useful tool for improving paediatric ART. This recommendation is important in that it has been shown that community mobilisation and education was associated with improved acre for HIV infected pregnant women and their exposed infants in a study in Uganda.[20] Community education is capable of enlightening the public and creating synergies between healthcare facilities and the society towards mobilising for improved care of HIV infected children. Having a primary school curriculum with inclusion of appropriate HIV-related information was also cited as a strategy for improving paediatric ART.

This study provides data about perceptions and experiences of professional nurses regarding initiation and maintenance of HIV-infected children on ART. Participants highlighted enablers as well as barriers to ART among eligible children in the study area. The findings of this study are important as it is one of the few qualitative studies focusing on paediatric ART. However, this study was conducted only among professional nurses. Hence, perspectives of other health care workers were not explored.

CONTENT

Conclusion

This study showed that initiation and adherence to antiretroviral therapy among eligible children faces a significant challenge.

Acknowledgements

None.

Conflict of interest

None.

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ORIGINAL ARTICLE

Oncological Safety of Breast Conserving Surgery in Breast Cancer

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Abstract

Background/Aim: Breast-conserving surgery is a type of surgery used as a treatment option for breast cancer. It was introduced at the end of the 20th century following and in accordance with relevant clinical studies. With heightened public awareness of breast cancer and the introduction of new diagnostic procedures, despite the proven oncological safety of this type of surgery, a growing number of women choose to undergo total mastectomy. The aim of this study was to confirm the oncological safety of breast-conserving surgery performed on breast cancer patients at the University Clinical Centre of the Republic of Srpska.

Methods: This study analysed 305 female patients with I and II stage of breast cancer, operated on between March 2009 and December 2013. One group of patients underwent breast-conserving surgery (BCS), followed by adjuvant radiation therapy and the other total mastectomy (MX). The patients were followed up for 5 years after the surgery. Analysed herein were the local-regional recurrence, distant metastases, disease-free survival and overall survival rates.

Results: After a five-year follow-up, the local-regional recurrence rate for patients in the BCS group was 4.3 %, while for the MX group it was 4.2 %. The overall survival rate of patients in the BCS group was 90.9 %, as opposed to 89.1 % for MX patients.

Conclusion: After a five-year follow-up, no statistically significant difference was observed between the two groups of patients regarding the local-regional recurrence ($p = 0.967$) and overall survival rates ($p = 0.610$). Breast-conserving surgery is an oncologically safe surgical treatment for breast cancer.

Key words: Breast cancer; Mastectomy; Breast-conserving surgery; Breast centre; Radiation therapy.

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Introduction

The 20th century saw a gradual decrease in the number of radical mastectomy procedures as the treatment of choice for breast cancer. This decrease reflected in the trend that began with the Halsted radical mastectomy as the gold standard at the turn of 20th century, to mid-century modified radical mastectomy, to breast conserving surgery at the end of last millennium. Breast conserving surgery was introduced in the 1970s, as recommended by the World Health Organisation (WHO). The first clinical trials (Milan I, NSABP B-06) ensued, which sought to compare the overall survival rates (OSR) of breast conserving surgery (BCS) and radical surgery (MX) patients. Breast conserving procedures, which conserved the breast tissue, included post-

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operative radiation therapy (RT).[1, 2] In the late 20th century, axillary lymphadenectomy also became less radical, following the introduction of sentinel lymph node biopsy in breast cancer patients.[3] Today, the results of trials (AMAROS, Z0011) showed that axillary dissection can be safely omitted in case of metastatic sentinel lymph node followed by RT.[4, 5]

The introduction of screening mammography has shown that diagnosing breast cancer at an early stage, thus also performing surgery early on, has an impact on the disease prognosis. Since screening mammography was introduced in the EU, the mortality rate in some member states has dropped by up to 30 %, a piece of data that also indicates the importance of timely breast cancer surgery.[6] By contrast, when it comes to breast cancer that has already led to distant metastases, local surgical treatment produces little effect, as studies conducted thus far have not proven the benefits of surgical treatment for patients with metastatic cancer.[7]

Although BCS has been shown to be oncologically safe when compared to MX, there is an upward trend in the number of radical mastectomies in developed countries.[8, 9] Several reasons are reinforcing the trend, among which one is certainly better-quality diagnostic radiology. It has been easier to detect multifocal tumours since the introduction of breast magnetic resonance imaging (MRI). Also, the introduction of BRCA1 and BRCA2 gene mutation tests has resulted in a growing number of radical surgical procedures, including prophylactic surgery, which mainly comes down to mastectomy, with or without breast reconstruction. With raised public awareness about breast cancer and constant fear of recurrence of the disease on the one hand and women being inadequately informed on the other, more women opt for MX rather than BCS.[10]

Following recommendations of the European Society of Breast Cancer Specialists (EUSOMA) and other professional organisations, as well as resolutions adopted by the European Parliament to establish units and centres to focus exclusively on breast pathology, the University Clinical Centre of the Republic of Srpska (UCC RS) established a Breast Centre.[11] Prior to the establishment of the UCC RS Breast Centre in 2009, more precisely, between 2004 and 2008, a mere 3.4 %, or 14 out of 410 breast cancer patients treated at the UCC RS underwent BCS. In the first five years after the establishment of the Breast Centre, the number of patients who underwent BCS rose to about 40 % and in 2013 it surpassed the number of mastectomised women. Also, during that period, a conserving surgery in the axilla was introduced using the sentinel lymph node biopsy procedure.[12]

The aim of this study was to confirm the oncological safety of BCS in comparison with MX, as performed at the UCC RS, irrespective of the molecular subtype of breast cancer.

Methods

The trial was retrospective study on patients who underwent surgery between March 2009 and December 2013 and conducted at the UCC RS (Banja Luka, Bosnia and Herzegovina) after it was approved by the UCC RS Ethical Committee as the authorising body. The patients were examined prior to the surgery and their cases presented to the Tumour Board. All surgeries were performed under general anaesthesia. According to the type of surgery, the patients were divided into two groups. The first group consisted of patients who had BCS and the second of patients who had MX (skin-sparing mastectomy, nipple-sparing mastectomy, simple mastectomy). BCS group was followed by RT no later than two months after surgery or adjuvant chemotherapy. The Nottingham score was used to determine histological grade of the tumour. The resection margins were examined under the microscope and those that showed 'no ink on tumour' were considered negative.

The study included the patients who met the following requirements: female patients, diagnosed with invasive breast cancer, stages I or II according to the AJCC (The American Joint Committee on Cancer, 8th ed) classification, free resection margin (R0), radiation therapy conducted following BCS and available medical reports on post-operative follow-up. The study did not include patients who had been administered neoadjuvant chemotherapy.

SPSS Statistics 24 software was used to interpret the obtained data statistically and to present the results in tabular form. The χ^2-test was used with a significance level of $p = 0.05$. The survival proportion was estimated using the Kaplan-Meier

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method and compared using the log-rank test. The rates of local-regional and distant recurrence were calculated for a five-year follow-up, from the time of the surgery to the disease recurrence. The disease-free survival rates (DFSR) were obtained for a five-year follow up, from the surgery to the moment of local and/or distant recurrence. The overall survival rates (OSR) were also calculated for a five-year follow-up, from the time of the surgery to the patient's death.

Results

The study included a total of 305 female patients with primary invasive breast cancer. The first group (BCS) comprised 186 patients (61 %) and the MX group 119 patients (39 %). Most of the followed patients in both groups were between 50 and 69 years old, with only very few younger than 50 (BCS = 11.3 %; MX = 6.7 %). Most patients (72.1 %) who underwent BCS had a tumour of up to 2 cm, while for the MX group it was half the patients (50 %). No statistically significant difference was observed between the two groups regarding the lymph node status. Most patients (BCS = 75.8 %; MX = 68.1 %) had no metastases in axillary lymph nodes. Also, according to the histological grade of the tumour, there was no significant difference between the groups, with most patients (60 %) belonging to histologic grade 2. Hormone oestrogen and progesterone receptor positivity (ER/PR) was near equal for both groups (BCS = 74.8 %; MX = 75.6 %). Expression of the HER2 (human epidermal growth factor receptor) oncogene was greater in the MX group (23.5 %), as opposed to the BCS group (16 %) (Table 1).

Table 1: Clinical and pathological characteristics of patients

Characteristics	BCS n = 186	MX n=119	*P* value
Age			
≤ 50	21 (11.3 %)	8 (6.7 %)	.362†
51 - 69	105 (56.4 %)	74 (62.2 %)	
≥ 70	60 (32.3 %)	37 (31.1 %)	
pT stage			
T1a	3 (1.6 %)	0 (0 %)	
T1b	27 (14.6 %)	9 (7.6 %)	
T1c	104 (55.9 %)	51 (42.8 %)	
T2	52 (27.9 %)	57 (47.9 %)	
T3	0 (0 %)	2 (1.7 %)	
pN stage			
N0	141 (75.8 %)	81 (68.1 %)	.139†
N1	45 (24.2 %)	38 (31.9 %)	
Stage (AJCC)			
I	130 (69.9 %)	53 (44.5 %)	.000†
II	56 (30.1 %)	66 (55.5 %)	
Grade			
G1	27 (14.5 %)	12 (10.1 %)	.515†
G2	112 (60.2 %)	74 (62.2 %)	
G3	47 (25.3 %)	33 (27.7 %)	
ER/PR status			
Positive	152 (81.7 %)	97 (81.5 %)	.964†
Negative	34 (18.3 %)	22 (18.5 %)	
HER2 status			
Positive	29 (16.0 %)	28 (23.5 %)	.083†
Negative	157 (84.4 %)	91 (76.5 %)	
Histological type			
IDC NST	139 (74.8 %)	90 (75.6 %)	.323†
ILC	9 (4.8 %)	10 (8.4 %)	
Other types	38 (20.4 %)	19 (15.9 %)	

† χ^2 test; BCS: breast conserving surgery; MX: mastectomy; AJCC: The American Joint Committee on Cancer classification, 8th ed.; ER: oestrogen receptor; PR: progesterone receptor; IDC NST: invasive ductal carcinoma no special type; ILC: invasive lobular carcinoma

Table 2: Analysis of LRR, DM, DFSR and OSR according to operative techniques

	BCS n = 186	MX n=119	*P* value
LRR	8 (4.3 %)	5 (4.2 %)	.967†
DM	16 (8.6 %)	20 (16.8 %)	.030†
DFSR	165 (88.7 %)	97 (81.5 %)	.078†
OSR	169 (90.9 %)	106 (89.1 %)	.610†

† χ^2 test; LRR: loco-regional recurrence; DM: distant metastases; DFSR: disease-free survival rate; OSR: overall survival rate; BCS: breast conserving surgery; MX: mastectomy

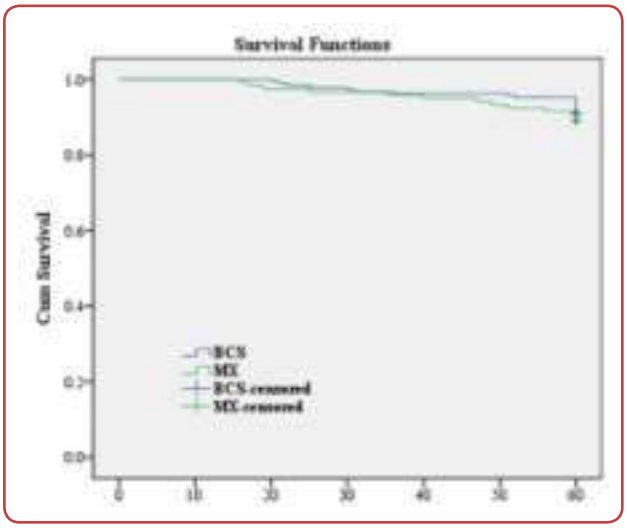

Figure 1: Overall survival proportion by operative techniques (months)
BCS: breast conserving surgery; MX: mastectomy

After a five-year follow-up, local-regional recurrence (LRR) was confirmed in 4.3 % patients who had undergone BCS and in 4.2 % patients who had MX. Over a five-year follow-

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up, 17 (9.1 %) patients from the BCS group died, compared to 13 (10.9 %) patients in the MX group (Table 2). Using the Kaplan-Meier method, no statistically difference (Log Rank test = 0.307; p= 0.580) was obtained in the survival proportion between the BCS and MX groups.

Discussion

The results of first clinical trials in the last decades of 20th century indicated that even after a twenty-year follow-up, there were no significant differences between the OSR of patients who underwent BCS followed by RT over those who had MX. The Milan I trial showed that OSR was 75.7 % in MX patients relative to 73.9 % in patients with BCS.[1, 2] Also, a study by the National Cancer Institute (NCI USA) that followed up patients for ten years returned no significant statistical results regarding OSR following BCS (77 %) and MX (75 %).[13] In a 22-year follow-up study, Ariagade and colleagues found the OSR was significantly higher among BCS patients (60.3 % vs 49.5 %).[14] The results of long-term (10-20 years) randomised studies that found no significant statistical difference in OSR between BCS and MX groups are consistent with the findings of this study, confirming no significant difference in survival between BCS and MX patients. After a ten-year follow-up, the Gustave Roussy Oncology Institute (France) published data that claims an 80 % survival rate following MX and 79 % survival rate following BCS.[14] An 18-year follow-up study by the NCI claimed a 54 % OSR following conserving surgery and a 58 % OSR following radical surgery.[15]

The study found the rate of distant metastases significantly higher among the MX group (16.8 %), as opposed to patients who underwent BCS (8.6 %), a finding consistent with the fact the MX patients had tumours that were biologically more aggressive. Studies in which patients were followed during long-term intervals (20 years) returned a similar rate of distant metastases in the two groups.[1, 2, 14, 16] Limitation of this study was a relatively short-term follow-up study (5 years), compared to those previously cited, which followed patients for much longer.

In the Milan I trial after a twenty-year of follow-up the rate of LRR in BCS patients was 8.8 % and 2.2 % in MX group.[2] In NSABP B-06 trial after ten-year follow up in patients with BCS rate of LRR was 6.5 %.[17] Plichta and colleagues in study with younger breast cancer patients found the LRR was higher among MX group (8.7 % vs 4 %).[18] The results of two large European randomised trials showed similar rates of LRR between BCS and MX groups (10 % vs 9 %).[19] When it comes to the rate of LRR of the disease, the study presented herein did not find a significant difference between the compared groups, ie between BCS and MX. The results show the same incidence of LRR of the disease after a five-year follow-up between BCS and MX groups.

The resection margin status appears to be a factor with a significant impact on the local recurrence rate. Today, the margin status after resection is considered negative when ink applied to the specimen reveals no cancerous cells touching its edge ('no ink on tumour').[20] A meta-analysis of studies aimed at assessing the influence of the resection margin width, namely, of 1, 2 and 5 mm, on the local recurrence rate did not find a significant difference between the different widths considered. The 'no ink on tumour' standard has also helped reduce the number of re-excisions done for the purpose of extending the surgical safety margin.[17]

The results of new trials suggest that post-operative RT in BCS reduces not only the risk of local and regional recurrences but also reduces the risk of breast cancer death.[21] The results of a meta-analysis by EBCTCG (Early breast cancer trialists' collaborative group), which included 17 randomised trials, showed that adjuvant RT administered after BCS significantly reduced the potential of breast cancer cells to local recurrence and distant metastases.[22] The study of Danish breast cancer cooperative group (DBCG) on a large population-based material suggest that patients with BCS have a better survival than patients who had mastectomy.[23] Also, Swedish national trial with six-year follow-up concluded that BCS followed by RT yielded better survival than MX patients irrespective of RT.[24]

Conclusion

The results of the study confirmed the oncological safety of BCS in relation to MX in terms of LRR rates. Also, the results showed that MX group had a significantly higher rate

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(p = 0.030) of distant metastases in comparison with the BCS group. No statistically significant difference was found between the observed groups in terms of the LRR rate, DFSR and OSR (p > 0.05).

With adequate preoperative radiological diagnostics, histopathology reports, disease staging and an educated team of physicians, BCS followed by RT is an oncologically safe procedure. It is equally important for the patient to participate actively in her treatment in accordance with clearly presented information and data, which allow adequate decision-making.

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Conflict of interest

None.

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ORIGINAL ARTICLE

Correlation of Hysterosalpingography and Laparoscopy in the Detection of Tubal Infertility Factor

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Abstract

Background/Aim: Around 15-20 % of couples worldwide struggle with infertility, a difficult and aggravating gynaecological issue. Conception occurs in both partners, male and female, as they are both responsible for conception. This study aimed to evaluate the diagnostic accuracy of hysterosalpingography (HSG) in the detection of tubal infertility factors, by comparing the findings of HSG with the findings of laparoscopy (LPSC).
Methods: A retrospective study from 1st January 2018, to 31st December 2019, is presented. Infertile patients who underwent LPSC, HSG and ultrasound to evaluate sterility during this timeframe were included in the research.
Results: The study involved 63 infertile patients with a mean lifespan of 33.3 ± 4.7 years. The conclusions of LPSC and HSG, in general, were in good correlation and the percentage of agreement among the diagnostic procedures was 77.8 %. In the case of dichotomous categories, there is a good alignment between LPSC and HSG results with a percentage of 85.7 %. Hydrosalpinx: The findings of LPSC and HSG are quite similar. The percentage of agreement among the diagnostic techniques used was 79.2 %. Canal obturation: LPSC and HSG results are in good correlation with a percentage of 78.6 %. Terminal obturation: LPSC and HSG results are also in good agreement with the calculated percentage of 82.1 %.
Conclusion: In determining tubal sterility factors, there is considerable consistency between LPSC and HSG results ($\kappa = 0.68$; 95 % CI 0.54-0.83). There is a good correlation between LPSC and HSG findings of dichotomous categories ($\chi^2 = 0.63$; 95 % CI: 0.41-0.86). 86.2 % of subjects with peritubular appendages and 8.8 % of subjects without peritubular appendages had hydrosalpinx, which is a statistically significant difference ($\chi^2 = 37.957$; $p < 0.001$). Between LPSC and HSG data, there is a good agreement in the diagnosis of hydrosalpinx ($\kappa = 0.64$; 95 % CI: 0.38-0.91).

Key words: Infertility; Hysterosalpingography; Laparoscopy; Hydrosalpinx.

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Introduction

Infertility is a serious and rapidly increasing gynaecological issue that affects 15-20 % of couples worldwide and is defined as the inability to conceive within 12 months of regular unprotected intercourse.[1] Infertility always unites the problem of two people[2] because conception is the responsibility of both, the male and female partners. 48.5-72.4 million couples globally suffer from primary or secondary infertility. Meta-analysis calculated the percentage of infertility for different countries and cultures which ranges from 5-30 %, but most often is 15-20 %.[3]

Many factors affect fertility. The most common causes of infertility are changes in lifestyle, environmental pollution, delayed birth of the first

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child at an advanced age of a female partner, separation of partners due to professional reasons, anovulation, reduced ovarian reserve, sexually transmitted diseases, male partner problems, anxiety and stress.[4] The causes are almost equal in male and female partners. The male partner is a cause of infertility in 30-40 % of cases, female in 40-55 % of cases and both are responsible for 10 % of cases. The remaining 10 % of the causes stay unexplained.[5] According to FIGO classification, causes of infertility in women are divided into several groups: the problem with the fallopian tube and pelvic peritoneum (25-35 %), anovulation or infrequent ovulation (30-40 %), endometriosis (1-12 %), uterine abnormalities (6 %) and unexplained infertility (14 %). Examining tubal factors of infertility, or to be precise, fallopian tube patency is the initial step in discovering female infertility factors. They are detected using some or all of the following methods: hysterosalpingography (HSG), laparoscopy (LPSC), and ultrasound.[6] Each method of diagnosis has its advantages and disadvantages, while the combination of these methods gives the most accurate and most reliable diagnosis. This is why the degree of their correlation is extremely important and is leading us to a decision on which patients should expect pregnancy by natural conception or when is necessary to apply one of the methods of assisted reproductive technology. Of all infertility causes in women, the tubal factor accounts for about 30 %.[5, 7] The aetiological factors of tubal pathology are usually infections with bacteria and viruses, the existence of intrauterine devices, endometriosis, tuberculosis of the genital organs and previous surgery.[8]

As said, one of the first stages in determining a woman's infertility is to examine her fallopian tube function by HSG, LPSC, and ultrasound.[6] HSG is indicated in the diagnosis of marital infertility, or female infertility, especially after previous surgeries or anamnesis indicating previous inflammatory diseases of the genital organs, a clinical finding that indicates the presence of endometriosis or suspicion of uterine anomalies. Indications for HSG are also a history of tubal pregnancy, corrective surgery on the fallopian tubes and a ruptured appendix. There are diagnostic and therapeutic indications for performing HSG, and among them, infertility is the most important. Diagnostic indications for HSG are an examination of infertility suspicion of congenital anomaly of the internal genital organs of a woman, examination of the condition of the uterine cavity, examination of defects in the uterine wall, examination of tubal structure, intrauterine localisation insert, repeated abortions and abnormal uterine bleeding. Therapeutic indications for the use of HSG are intrauterine adhesions and tubular occlusions. Contraindications for HSG are bleeding and allergy to contrast agents, while the absolute contraindications are intrauterine pregnancy and acute pelvic inflammatory disease.

Reasons to perform LPSC include infertility, ectopic pregnancy, endometriosis, chronic pain in the lower abdomen, acute and subacute problems in the pelvis and malformations of the internal genital organs. Indications for extended LPSC are infertility, endometriosis, neoplastic and non-neoplastic ovarian tumours, genital and intra-abdominal tuberculosis and sterilisation of tubes. Since LPSC is conducted under general anaesthesia, most cases in which LPSC is contraindicated are due to anaesthesia, mainly diseases of the cardiovascular and respiratory systems. The absolute contraindication for LPSC is haemorrhagic diathesis. The greatest danger is posed by puncture of the abdomen "blindly". The tip of the needle can penetrate layers of the anterior abdominal wall where various forms of emphysema and injuries to blood vessels can occur during insufflation. Complications of LPSC can be divided into intraoperative complications (emphysema, injuries of blood vessels, intestines, etc.) and postoperative complications (pain, bleeding, infections and the occurrence of postoperative adhesion).

This study aimed to evaluate the diagnostic efficacy of HSG in identifying tubal infertility causes.

Methods

The results of a retrospective study conducted at the Department of Sterility at Obstetrics and Gynaecology Clinic "Narodni Front", Belgrade, the Republic of Serbia, between 1st January 2018 and 31st December 2019 were recorded. An ultrasound, HSG, and LPSC were performed on 63 patients as part of the study's sterility assessment. The patient's age, sterility type, length, laparoscopic findings, past pregnancies and abortions, patency of the fallopian tubes, location of obstruction of the fallopian tubes and kind of corrective surgical procedures performed on the fallopian tubes during LPSC were examined.

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Depending on the kind of variables and the normality of the distribution, the data is shown as n (%), arithmetic means ± standard deviation, or median (range, min-max). The techniques applied for statistical hypothesis testing are the exact probability Fishers test and the Chi-square test. Weighted Kappa coefficient and Kappa coefficient were taken for measurement agreement and the SPSS program was used for the statistical analysis. Statistical hypotheses were tested at the level of statistical significance (alpha level) of 0.05.

Results

The average age of all researched participants was 33.3 ± 4.7 years. The youngest participant was 23 years old, while the oldest was 41. The majority of those who participated in the research had no prior pregnancies (87.3 %) or abortions (87.3 %). Ectopic pregnancies were reported by 3 (4.8 %) respondents. The study's participants were more likely to suffer from primary sterility (81.0 %). The average length of sterility was two years. In the subjects with fallopian tube obstruction, the most common place was found to be a terminal obstruction (63.6 %). Of all respondents included in the study, 29 (48.3 %) had peritubular adhesions. The most common corrective surgery was bilateral salpingoneostomy (42.9 %). 22.2 % of participants had a good HSG result and 30.2 % of participants had a good result at the LPSC (Figure 1 and 2).

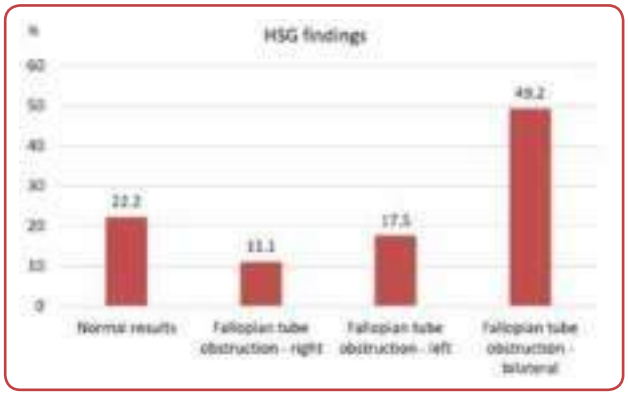

Figure 1: Distribution of respondents according to hysterosalpingography (HSG) findings

There is good agreement between LPSC and HSG findings (κ = 0.68; 95 % CI: 0.54-0.83). The proportion of agreement with the applied diagnostic procedures was 77.8 % (Table 1).

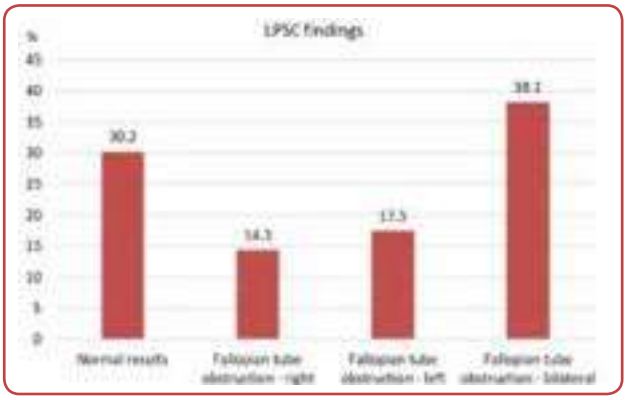

Figure 2: Distribution of respondents according to laparoscopy (LPSC) findings

Table 1: Absolute frequencies of laparoscopy (LPSC) and hysterosalpingography (HSG) category findings

HSG Result	LPSC Result				
	Normal result	Fallopian tube obstruction right	Fallopian tube obstruction left	Fallopian tube obstruction bilateral	In total
Normal result	12	1	1	0	14
Fallopian tube obstruction right	1	6	0	0	7
Fallopian tube obstruction left	4	0	7	0	11
Fallopian tube obstruction bilateral	2	2	3	24	31
In total	**19**	**9**	**11**	**24**	**63**

There is good agreement between LPSC and HSG findings of dichotomous categories (κ = 0.63; 95 % CI: 0.41-0.86). The proportion of agreement between applied diagnostic procedures of dichotomous categories was 85.7 % (Table 2).

Table 2: Absolute frequencies of laparoscopy (LPSC) and hysterosalpingography (HSG) findings of dichotomous categories

HSG Result	LPSC Result		
	Regular	Obstruction	In total
Regular	12	2	14
Obstruction	7	42	49
In total	**19**	**44**	**63**

86.2 % of subjects with peritubular appendages and 8.8 % of subjects had hydrosalpinx without peritubular appendages, which is a statistically significant difference (χ^2 = 37,957; p < 0.001) (Table 3).

There is good agreement between LPSC and HSG findings (κ = 0.64; 95 % CI: 0.38-0.91). The proportion of agreement of the applied diagnostic procedures was 79.2 % (Table 4).

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Table 3: Distribution of subjects by hydrosalpinx - the presence of peritubular growths

	The presence of peritubular appendages			
	Yes		No	
Hydrosalpinx	n	%	n	%
Yes	25	86.2	3	8.8
No	4	13.8	31	91.2
In total	**29**	**100.0**	**34**	**100.0**

Table 4: Absolute frequencies of LPSC and HSG finding categories (hydrosalpinx)

HSG Result	LPSC Result			
	Hydrosalpinx right	Hydrosalpinx left	Hydrosalpinx bilateral	In total
Hydrosalpinx right	1	0	1	2
Hydrosalpinx left	0	9	1	10
Hydrosalpinx bilateral	2	1	9	12
In total	**3**	**10**	**11**	**24**

Table 5: Distribution of subjects according to fallopian tube obstruction – the presence of peritubular adhesions

	Fallopian tube obstruction			
	Yes		No	
Peritubular appendages	n	%	n	%
Yes	28	63.6	0	0.0
No	16	36.4	19	100.0
In total	**44**	**100.0**	**34**	**100.0**

Peritubular adhesions were found in 63.6 % of subjects with tubal obstruction and none (0.0 %) subjects without fallopian tube obstruction, which is a statistically significant difference (χ^2 = 21,764; p < 0.001) (Table 5).

Discussion

Some authors have found in their research a slightly higher incidence of primary than secondary infertility. The percentage of primary infertility ranged from 60–72 % and secondary from 28–40 %.[9] When resizing groups according to inclusion criteria, a 2001 research included 328 infertile women who were subjected to diagnostic LPSC to investigate infertility, including 191 unexplained infertile patients. There were 106 (55.5 %) primarily infertile and 85 (44.5 %) secondary infertile patients. The mean age of infertile patients was 28 ± 5 years, respectively.[10] In this research which included 63 participants 81 % were primary infertile, while 19 % were secondary infertile.

The tubal factor is responsible for infertility in 25-35 % of cases. HSG and LPSC are the two most important and most widely used diagnostic methods for examining fallopian tube patency. Tubal obstruction diagnosed by HSG in this study was 77.8 %, of which unilateral obstruction was found in 28.6 % and bilateral obstruction in 49.2 % of cases. Tubal obstruction diagnosed laparoscopically was 69.8 %, of which unilateral obstruction was present in 17.5 % and bilateral obstruction in 38.1 % of cases. Left fallopian tube obstruction was diagnosed more often both by HSG and LPSC than right fallopian tube obstruction and was 17.5 %. The agreement of HSG and LPSC findings in diagnostics fallopian tube obturation in this study was 77.8 %, based on which it can be concluded that there is a good correlation between LPSC and HSG findings.

In a study that was done and which consists of a reviewed series of 200 consecutive hysterosalpingograms to assess infertility in patients attending the clinic at Hillbrow Hospital in Johannesburg, fallopian tube anomaly was found in 81.8 % cases and terminal hydrosalpinx present in 64.4 %. After completing the research, it was found that there is a good correlation between HSG and laparoscopic findings, the degree of correlation in this study was 75 %.[8]

Routine HSG and LPSC have been performed at a certain time for any patient who has had primary infertility for more than two years. The clinical aspects of 433 such patients' findings were reviewed retrospectively. An agreement between the two diagnostic methods was found in 70.9 %. Almost half of the population experienced HSG or LPSC abnormalities.[11] In a two-year prospective study comparing the diagnostic accuracy of HSG and LPSC, 143 infertile women were evaluated. The agreement between these two methodologies' results was 66.4 %.[12] In a prospective cohort study including 11 Canadian centres, unilateral fallopian tube obstruction was detected in 14 % of infertile patients and bilateral obstruction in 24 %, whereas laparoscopic unilateral and bilateral fallopian tube obstruction was diagnosed in 12 % of each.[13] Comparing the presented results with the previously mentioned, it was concluded that there is a good alignment between them.

HSG and LPSC are complementary methods of examination of infertile patients. As mentioned, there is a high correlation between HSG and LPSC

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findings in the diagnosis of hydrosalpinx in this study. In 2019 research involving 102 operated women, obstruction of the fallopian tubes was found in 94 (92.1 %) patients. One-sided hydrosalpinx was found in HSG in 16 (15.7 %) subjects and bilateral hydrosalpinx in 30 (29.4 %) women. Unilateral hydrosalpinx was found by LPSC in 17 (16.1 %) patients and bilateral hydrosalpinx in 32 (31.4 %) subjects. Matching between findings obtained by HSG and LPSC in the diagnosis of unilateral hydrosalpinx was 76.5 % and bilateral 70.4 %. This difference was not statistically significant.[14]

The most significant distinctions between ultrasonography, HSG, and LPSC are evident in the diagnosis of peritubular appendages, which LPSC is the most accurate at diagnosing. Peritubular appendages are considerably more common in patients with tubal obstruction (63.6 %), while peritubular 76.5 % of respondents with past surgical operations and 34.8 % of respondents without previous surgery had increments pelvis. It may be assumed that pelvic surgery causes the creation of peritubular adhesions, which are most reliably identified by LPSC and that the same adhesions are the cause of fallopian tube occlusion in the majority of cases. The level of fallopian tube destruction can be assessed more precisely laparoscopically than with ultrasound and HSG, which is important in deciding whether to perform surgery on the fallopian tubes as well as which infertile patients can expect natural conception and which require treatment with IVF procedures.

Conclusion

In determining tubal sterility factors, there is considerable consistency between LPSC and HSG results. There is a good correlation between LPSC and HSG findings of dichotomous categories. 86.2 % of subjects with peritubular appendages and 8.8 % of subjects without peritubular appendages had hydrosalpinx, which is a statistically significant difference. Between LPSC and HSG data, there is a good agreement in the diagnosis of hydrosalpinx. The most significant distinctions between ultrasonography, HSG, and LPSC are evident in the diagnosis of peritubular appendages, which LPSC is the most accurate at diagnosing.

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Conflict of interest

None.

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REVIEW ARTICLE

The Evidence for a Role of Bacteria and Viruses in Cardiovascular Disease

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Abstract

Inflammation plays a critical role in atherosclerosis and cardiovascular disease. Bacteria and viruses are major causative agents of inflammation in the body which normally develops as a response to infection. It is a logical extention, therefore, to believe bacterial and viral infections may be involved in a variety of presentations of cardiovascular diseases. The purpose of this review is to describe the data and conclusions to date on the involvement of these infectious agents in the induction of cardiovascular disease. The review also discusses the various specific bacteria and viruses that have been implicated in cardiovascular disease and the mechanisms, if known, that these agents induce cardiovascular disease.

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Infections Associated with Cardiovascular Disease

A number of risk factors have been identified as important in determining the expression of cardiovascular disease (CVD). Some are modifiable and others like genetic predisposition, time and race are not. Among the more recent environmental factors that are modifiable is infection. Over time, it has become dogma in the cardiovascular research field that infection and its associated inflammatory reactions are principle factors in the development of many CVDs. The primary type of CVD in which this presents itself is atherosclerotic CVD. The hypothesis that infection and inflammation plays a critical role in atherosclerosis created an initial surge of research to identify the specific infectious agents that may cause atherosclerotic disease. The results from these studies were somewhat surprising and disbelieved for many years. One concern for the studies was that these causative infectious agents identified were from very unlikely sources. Bacteria and viruses commonly associated with diseases of non-cardiac origin were implicated in CVD. However, the evidence to strongly support their involvement has accumulated over many years and now even includes the virus involved in the

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most recent COVID-19 pandemic. The purpose of this review is to discuss the various bacteria and viruses that have been implicated in CVD and the mechanisms, if known, that these agents induce CVD.

Bacterial Infections Associated with Cardiovascular Disease

A variety of bacteria, commonly associated with diseases of non-cardiac origin, have nonetheless been implicated in CVD. All are Gram-negative bacteria. For example, although *Helicobacter pylori* is most commonly associated with persistent gastritis,[1] these bacteria have also been identified as causative agents in CVD. *Porphyromonas gingivalis* and *Actinobacillus actinomycetemcomitans* are causative bacteria for periodontal disease but have also been implicated in CVD. *Chlamydia pneumoniae* and *Mycobacterium tuberculosis* are well known for their respiratory complications,[2] however, these bacteria have also been identified as causative agents in CVDs.

Chlamydia pneumoniae is the most intensely studied pathogen with regard to its role in CVD. *C pneumoniae* antibodies have been detected within the serum of patients with coronary artery disease.[3, 4] DNA and elementary bodies of *C pneumoniae* have also been found in the atherosclerotic plaque.[2] Most importantly, a causal relationship and the mechanisms of action for *C pneumoniae* infection and atherogenesis has been tested and established in some cases,[5-9] although some aspects remain in dispute.[10, 11] Heat inactivated *C pneumoniae* is not atherogenic.[12]

H pylori has been strongly correlated with atherosclerotic heart disease and coronary artery disease and stroke clinically,[13-15] particularly in patients with hyperhomocysteinaemia.[16] Causal evidence for a role for *H pylori* in atherogenesis and coronary artery disease is lacking.

P gingivalis is one of several bacterial pathogens commonly associated with dental plaque and gum disease. This includes *Actinobaccillus actinomycetemcomitans, Treponema denticola* and *Bacteroides forsythus*. Again, there is strong evidence of a close correlation of *P gingivitis* with coronary artery disease with DNA evidence of its presence in up to 30 % of carotid atheromas.[17, 18] Furthermore, CVD risk has been correlated with the severity of the periodontal infection.[19]

The Mechanisms Whereby Bacterial Infections Induce Cardiovascular Disease

Further studies have clearly demonstrated that the relationship between infection and atherosclerosis is not a simple indirect association but an important cause and effect relationship.[5-7, 20-25] The mechanism whereby infections induce an atherogenic lesion is unclear. Many of these infectious agents either have stimulatory effects on cellular proliferation[8, 9, 23, 26, 27] or inhibitory effects on apoptosis.[28] These actions may be involved in their atherogenic effects.[23, 26, 28] The susceptibility of atherogenesis to such a broad spectrum of infectious agents, however, suggests that a pathway common to infection in general may be a key player in this phenomenon. Heat shock proteins have been identified as one potential mediator that would link the atherosclerosis process with the infection/inflammatory condition.[28-32] It is also clear that lipids have an important role to play in modifying the atherogenic effects of infections.[5, 9, 33] It has been identified that the atherogenic potential of *C pneumoniae* is dependent upon a high cholesterol environment.[5, 7] Feeding the LDL receptor knockout mouse a high cholesterol diet induced atherosclerotic plaque formation. Conversely, infection of mice on a normal diet with *C pneumoniae* did not induce an atherosclerotic effect. However, if the animals were placed on the cholesterol-enriched diet to induce atherosclerosis and then infected with *C pneumoniae*, a significantly greater stimulation of atherosclerotic plaque formation[5, 7] than cholesterol could induce on its own was observed. These data strongly suggested a synergistic interaction between the infection and cholesterol. This model is attractive because it links an important conventional atherogenic risk factor (cholesterol) with the infection process and may explain why some people develop clinically significant atherosclerosis after an infection and others do not. Relevant to the present topic, it also strongly points to the importance of diet in modulating the atherogenic action of an infectious agent. Significantly, a

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clinical investigation has documented the potent stimulatory effect of additional risk factors like hypercholesterolaemia on the cardiovascular effects of infection with *C pneumoniae* in a clinically relevant patient population.[34]

Antibiotic Use to Prevent CVD

If these bacteria do play a role in atherosclerotic CVD, the next logical step would be to determine if antibiotic usage can prevent or deter the clinical symptoms of CVD. *C pneumoniae* strains are susceptible to antibiotics like tetracycline, fluoroquinolones and macrolides in *in vitro* studies.[35, 36] However, the results from clinical trials testing the capacity of antibiotics to prevent major CVD events have been disappointing. Retrospective studies in patients treated with antibiotics prior to a myocardial infarction have yielded no clear conclusions.[37, 38] A detailed description of the clinical trials using antibiotics to prevent CVD is found elsewhere.[39] In summary, although the first few small antibiotic trials with azithromycin on patients with cardiovascular disease resulted in a positive protective effect versus the incidence of myocardial infarctions and indices of CVD,[40-43] several more extensive trials like the ACADEMIC study (The Azithromycin in Coronary Artery Disease: Elimination of Myocardial Infection with Chlamydia),[44] the WIZARD trial (Weekly Intervention With Azithromax Against Atherosclerotic-Related Disorders),[45] the CLARICOR trial (CLARIthromycin for patients with stable CORonary heart disease),[46] the ACES trial (Azithromycin and Coronary Events Study),[47] the ANTIBIO trial (Antibiotic Therapy in Acute Myocardial Infarction),[48] the AZACS trial (Azythromycin in Acute Coronary Syndrome)[49] and the PROVE-IT trial (Pravastatin Or Atorvastatin Evaluation and Infection Therapy)[50] all concluded that antibiotic treatments did not result in any reduction in cardiovascular events. As a result, the use of antibiotics to prevent CVD have been largely abandoned as a viable approach. The reason for the lack of prevention may be multifactorial. It is clear that macrolides do not impact persistent *Chlamydial* infection during its persistent stage[39] and this persistence of bacterial infection would negate any antibiotic effect of the macrolides tested. Murine models of atherosclerosis have identified a high cholesterol environment as being critical to the atherogenic effects of *C pneumoniae* infection.[5] It is possible, therefore, that antibiotic treatment may be best utilised in a clinical population with high circulating cholesterol levels prior to the appearance of clinically significant cardiovascular disease. Some clinical studies would lend support to this hypothesis[34] but further work is required to substantiate this conclusively. Additionally, it is possible that a critical window of time exists for the preventative effect of the antibiotics. Antibiotics delivered to rabbits shortly after bacterial inoculation inhibited *C pneumoniae*-induced atherosclerosis but was ineffective if delivered 6 weeks post-inoculation.[51] Clinical trials using antibiotics were delivered in patients with well established cardiovascular disease. The alternative approach of testing the delivery of antibiotics chronically in order to prevent the appearance of atherosclerotic plaque formation before it is established has been proposed but never tested. Today, with the ever growing emergence of multidrug resistance in pathogenic bacteria as a serious health problem, an approach of using antibiotics over an extended period of time when there is an absence of clinical evidence of an infection would not be feasible. This strategy for the prevention of cardiovascular disease would undoubtedly promote the acceleration of antibiotic resistance in pathogenic bacteria and this would be an unacceptable consequence.

Other approaches besides antibiotics for the treatment of infection-induced atherosclerotic disease could be advanced. The afore-mentioned relationship of cholesterol with *C pneumoniae*-induced infection may offer intriguing options. An oxidation mechanism has been proposed to be involved in the association of cholesterol with *C pneumoniae*-induced atherogenesis. It is well known that *C pneumoniae* can induce a stimulation of intracellular oxidative processes.[52, 53] For example, *C pneumoniae* and other infectious processes can induce oxidation of LDL in *in vitro* and *in vivo* settings.[52-56] Oxidized LDL is thought to play an important role in atherosclerosis.[57-59] Oxidized LDL can potentiate the mitogenic actions of *C pneumoniae*.[9] It is possible, therefore, that the oxidation induced by infectious agents like *Chlamydia pneumoniae*[9] is critical to the atherogenic event and preventing this process may retard or block the atherogenic action of *Chlamydia* and other infectious organisms. Blocking the oxidation by treating the *C pneumoniae*-induced cardiovascular disease with

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antioxidants has not been tested but may provide positive results.

Other alternative pathways have been identified as associated with the atherogenic actions of infectious stimuli like *C pneumoniae*. It has been suggested[39] for example, that it may be more effective to target downstream pathways induced by the *Chlamydial* infection rather than the bacteria itself. Targeting the inflammatory and mitogenic pathways that are activated by the infection to generate the atherogenesis may be more beneficial, particularly those involving heat shock protein 60,[27, 29] specific pattern recognition receptors, transporters or other target proteins.[39]

Viral Infections Associated with Cardiovascular Disease

Bacteria are not the only infectious agents that have been associated with cardiovascular diseases. Herpes simplex virus (HSV),[23, 24] Epstein-Barr virus DNA and cytomegalovirus (CMV),[28, 60] have been tied to both atherosclerotic cardiovascular and cerebrovascular lesions. More recently, another viral infection, the Coronavirus 2019 (COVID-19), has also been strongly linked to a variety of cardiovascular diseases by a host of new studies.

Siscovick and colleagues found that herpes simplex virus type 1 (HSV) IgG antibodies were associated with a 2 fold increase in the risk of incident myocardial infarction and coronary heart disease in older adults.[61] Another study discovered elevated levels of HSV, CMV and Epstein-Barr virus DNA in coronary artery atherosclerotic plaques obtained by end-arterectomy.[62] A large investigation, the ARIC study (Atherosclerosis Risk in Communities), reported that high levels of CMV antibodies were significantly associated with incident coronary heart disease.[63] In another large study of over 14,000 patients, CMV was associated with a significant increase in the risk for all-cause mortality and, when combined with elevated CRP levels, the risk for all-cause mortality and CVD-associated mortality also increased.[64] Serum antibody levels to HSV and two periodontal infections were measured in over 1100 Finnish and Russian participants.[65] They found the risk for CVD was increased when the antibody levels for the three infectious agents were elevated. These studies have led to the hypothesis that the entire burden of simultaneous chronic infectious disease may be the most important factor in the induction of CVD.[66]

Conflicting evidence arguing against a role for HSV and CMV in CVD has been reported. The prevalence of CMV and HSV in coronary artery disease patients was not elevated.[67] Siscovick et al[61] did not find that an elevation in IgG antibodies to CMV was associated with CVD risk among the elderly. Ridker et al[68] also found no correlation between HSV or CMV IgG antibody titers and subsequent risk for CVD in postmenopausal women. More negative results have been reported for CMV in another recent study.[69] The conflicting evidence, particularly for CMV, would lead one to conclude that there is a need for further large controlled studies to resolve this situation.

The emergence of the COVID-19 pandemic has resulted in the evolution of strong evidence for another association between an infectious agent and CVD. Indeed, it may be argued that the controversy that has arisen in the field as to the involvment of viruses in CVD has been answered unquestionably by the clear association of the COVID-19 virus with both acute and chronic manifestations of CVD. The COVID-19 zoonotic viral protein acts initially by binding to the angiotensin converting enzyme 2 (ACE2) receptor to ultimately gain entry to the cell.[70] The strong evidence for a role for the ACE2 receptors in the CVD induced by COVID-19 infection should not come as too big a surprise. ACE2 receptors have long been associated with CVD indiependently of COVID-19 infection.[71-73] This includes the overexpression of ACE2 activity in failing hearts.[73] The resultant systemic inflammation caused by COVID-19 infection induces an intense immune and cytokine response followed by plaque destabilisation, acute coronary syndrome, hypoxia, myocarditis, arrhythmias, cardiomyopathy, cardiogenic shock, myocardial damage, cardiac arrest and ultimately heart failure.[74] Venous thromboembolism and platelet aggregation have also been observed in COVID-19-infected patients.[70]

Some 20-30 % of patients admitted to hospitals with COVID-19 infections have cardiovascular complications.[75] Pre-existing CVD appears to exacerbate the effects of COVID-19 infection.[75] Even in patients who had recovered from COVID-19 infection, magnetic resonance imaging detected myocardial damage and myocardial inflammation in 78 % and 60 %, respectively, of these patients.[76, 77] The cardiovascular

pathologies may be linked to an increased risk of venous and arterial thromboembolic events.[77] Arrhythmias persist post COVID-19 recovery and the emergence of cardiomyopthies subsequent to infection have also been reported.[75] This further supports the contention that COVID-19 infection is associated with a persistent and chronic CVD.[75] Futhermore, the strikingly higher male mortality incidence in COVID-19 infected patients may be explained by the sexual dimorphism in pre-existing cardiovascular comorbidities.[78]

Conclusion

The majority of evidence over decades of investigation supports the involvement of both bacterial and viral infection as having an important role to play in inducing CVD. It is also possible now to come to a tentative conclusion that different bacteria and different viruses have very different capacities to induce CVD and, furthermore, they will induce varying severities of CVD. Perhaps the most dramatic example of a viral infection being associated with CVD is the recent pandemic with COVID-19. The infection has been associated with both an immediate as well as a chronic manifestation of a panapoly of CVDs. This is likely due to its uniquely facile access to the cardiovascular tissue through the ACE-2 receptor. Unfortunately, the serious vascular and cardiac effects of the current COVID-19 pandemic may only be a harbinger of the future challenges facing the cardiovascular system when multidrug resistance to antibiotics escalates even further and unleashes a horde of pathogenic bacteria on a defenceless cardiovascular system.[79]

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Conflict of interest

None.

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HISTORY OF MEDICINE

Public Pharmacies on the Territory of Bosnia and Herzegovina Over the Austro-Hungarian Rule

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Abstract

Background/Aim: With the adoption of legislation over the Austro-Hungarian rule (1878–1918) apothecary in Bosnia and Herzegovina (B&H) became a regulated profession, which enabled the arrival of graduated pharmacists. The aim of the paper was to present in which towns on the B&H territory public pharmacies were opened over this period and their owners.
Methods: A retrospective and descriptive research was conducted at the Archives of the Republic of Srpska, the Museum of the Republic of Srpska and the Archives of Bosnia and Herzegovina. The method of the qualitative secondary data analysis was applied.
Results: With the arrival of Masters of Pharmacy from all parts of the Austro-Hungary, an increasing number of public pharmacies began to open. Concessions for the opening pharmacies were initially granted to foreigners and among the settlers, pharmacists there were mostly Czechs, Croats, Poles, Hungarians, Slovaks, who completed pharmacy studies at universities in Vienna, Zagreb, Prague, Lviv, Graz, Innsbruck, Krakow. In the beginning, there were no locally educated pharmacists and the first appeared at the end of the 19th and the beginning of the 20th century. During this period at least one pharmacy was opened in many towns, two worked in Banja Luka, Mostar, Tuzla and Bijeljina and seven in Sarajevo. In the first years, each pharmacy was staffed by only one pharmacist and over time there were more pharmacy staff in the pharmacies. Twenty years after the occupation, public pharmacies owned by Masters of Pharmacy were opened in thirty three towns around B&H and in 1918 there were forty eight public pharmacies in thirty eight towns.
Conclusion: The number of public pharmacies and qualified pharmacy staff in B&H increased over the Austro-Hungarian rule from 1878 to 1918, which contributed to the improvement of the profession, health and social conditions in the country during this period.

Key words: History of pharmacy; Public pharmacies; Austro-Hungarian rule; Bosnia and Herzegovina.

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Introduction

Bosnia and Herzegovina (B&H) was under the rule Austro-Hungary Monarchy (Monarchy) from 1878 to 1918, when health conditions in the country changed significantly and the Europeanisation of medicine and pharmacy began. The Austro-Hungarian government found an extremely bad social and economic situation, a lack of health education of the population and unsettled health conditions in the country.[1] The Austro-Hungarian administration immediately started the organisation of the health service according the canons that ruled in the Monarchy,

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including passing the necessary legislation for the regulation of this area.[2, 3] The Order of the Provincial Government from July 1879 on performing medical practice of doctors, ranches, veterinarians, dentists and midwifes stipulated that the preparation and dispensing of medicines was the exclusive right of pharmacists, which the doctors or ranches could independently perform only with a special authorisation.[4] Apothecary activity was regulated as early as in February 1879 by the Order of the Provincial Government[5] and then more comprehensively by adopting the Law on Pharmacies in 1907.[6] According to these regulations, the owner or provisor of the pharmacy could only be a person with a doctor's degree in chemistry or a master's degree in pharmacy obtained at an Austro-Hungarian university. Concession for the opening of the pharmacy was given by the Provincial Government. Regulated apothecary activity, the opportunity for easy employment and earning enabled the arrival of graduated pharmacists in the country from Austro-Hungarian university centres and an increasing number of public pharmacies had started to open. The concession was mostly received by pharmacists from other parts of the Monarchy, as in the beginning there were no locally trained pharmacists. The first appeared at the end of the 19th and the beginning of the 20th century. The aim of this paper is to present in which towns on the B&H territory public pharmacies were opened over this period and their owners.

Methods

A retrospective and descriptive research was conducted at the Archives of the Republic of Srpska and the Museum of the Republic of Srpska from November 2019 to August 2020 and at the Archives of Bosnia and Herzegovina in September 2021 in order to explore archival materials about apothecary activity and pharmacies that worked on the territory of B&H over the Austro-Hungarian rule. The research covered the occupation (1878–1908) and annexation (1908–1918) period of Austro-Hungarian rule, which were presented as a whole.

Manual documentary analysis of original sources and desk research analysis from the secondary sources were conducted. The material available in its original from the Archives of the Republic of Srpska, the Museum of the Republic of Srpska and the Archives of Bosnia and Herzegovina was used. Company registers, books (Administration Reports, Collections of Laws), address books and newspapers were used from these sources, as well as other sources, as books, published papers in the journals and the Internet.

Pharmacies were shown according to the then territorial division of the country into six districts, namely Banja Luka, Bihać, Mostar, Sarajevo, Travnik and Tuzla. The name of the city or town from the Austro-Hungarian period was given in parentheses if it is different from today's. All owners or lessees of the pharmacies mentioned in the paper were Masters of Pharmacy who completed their studies at an Austro-Hungarian university, which is why "Master of Pharmacy" or "pharmacist" it is not stated next to their name.

Results

In the Banja Luka district, public pharmacies were opened in Banja Luka, Prijedor, Gradiška (Bosanska Gradiška), Derventa, Doboj, Novi Grad (Bosanski Novi), Kozarska Dubica (Bosanska Dubica), Brod (Bosanski Brod), Tešanj and Prnjavor. The first modern public pharmacy in Banja Luka was opened in April 1879 by an immigrant from Hungary, Moritz Brammer, an Ashkenazi Jew, on the basis of a concession from December of the previous year. From 1892, "To the Golden Snake" (*K zlatnoj zmiji*) pharmacy was continued to be run by his son Robert Brammer, after whose death in 1916 his wife and sons became the owners. One of them, Ernest, later took over his father's pharmacy.[7] The second pharmacy "To the Golden Lion" (*K zlatnom lavu*) was opened in 1890 by an immigrant from Croatia, Otto Lőschner.[8, 9] His house and pharmacy were bought in 1907 by Tomo Mirković, a native of Bijeljina.[10] A pharmacy in Prijedor was opened in 1879,[2] probably by Jakob Greif, because according to a newspaper advertisement from 1884, the advertised tooth preparations could also be bought in Prijedor, at the pharmacist Greif.[11] In 1900, Isidor Chorzemski from Galicia bought the pharmacy from the owner, the widow Maria Greif,[12] and sold it 1910 to Ludwig Hirchmann from Croatia (Figure 1).[13]

Stefan Variačić from Croatia opened a pharmacy in Gradiška in 1881.[9, 10] In 1885, Josef Oltvany from Hungary received a concession for a pharmacy

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Figure 1: Ludwig Hirschmann

Figure 2: Alexander Sussmann

in Derventa,[14] which was in 1894 leased by Alexander Sussmann from Galicia (Figure 2).[15] He bought the pharmacy "To the Savior" (*K spasitelju*) five years later.[16] The pharmacy in Doboj was opened in 1884,[2] and since Josef Čech was the owner of the pharmacy registered in 1891,[17] it is possible that he opened the pharmacy. It is not known when Josef Čech left, but "The City Pharmacy" (*Gradska apoteka*) was opened in 1896 by Josip Matković from Croatia,[9] which he sold in 1906 to his compatriot Đuro Vojković.[18] The pharmacy in Novi Grad was opened in 1892 by Gyula Keller from Hungary,[9] and Emil Starwer opened "To the Black Eagle" (*K crnom orlu*) pharmacy in Kozarska Dubica in 1894 or a year earlier.[2, 19] Adolf Barzal from Bohemia opened a pharmacy in Brod in 1894,[9] which was taken over by Nikola Radovanić in 1916.[20] Pero Misita, a native of Mostar, opened a pharmacy in Tešanj in 1896,[21] which was after his death in 1907 run by Bedřich Marek,[10] Filip Bohaček[22] and from 1915 Guido Fichtner.[23] In Prnjavor, a pharmacy was opened by Pole Michael Finkelstein on the basis of a concession from 1901.[24]

In the Bihać district, public pharmacies were opened in Bihać, Bosanski Petrovac, Bosanska Krupa and Sanski Most. Eduard Rhein opened a pharmacy in Bihać in 1879 and after his father's death, his son Eduard took over the management of the pharmacy "To the Black Eagle" (*K crnom orlu*) in 1899.[24] In Bosanski Petrovac, Ivan Gjuričić opened a pharmacy in 1895.[9] It was later bought by Nikola Turić from Croatia when it was called "To the Golden Lion" (*K zlatnom lavu*).[10, 24] Based on the concession from 1899, Leopold Cisař from Bohemia opened a pharmacy in Bosanska Krupa.[25] In 1909, the Provincial Government announced a tender for the opening of a pharmacy in Sanski Most,[26] and in 1910 Moriz Kirtner opened the pharmacy "At the Star" (*Kod zvijezde*).[27]

In the Mostar district, public pharmacies were opened in Mostar, Konjic, Ljubuško, Stolac and Trebinje. Two pharmacies were operating in Mostar during this period. In 1879, Julius Thonhauser from Hungary opened the pharmacy "At the Imperial Eagle" (*Kod carskog orla*) (Figure 3),[28, 29] which was bought in 1894 by Wenzel Mikan from Bohemia.[30] The second pharmacy was registered by Nikola Zovetti in 1890 and two years later it was bought by Czech Martin Houška.[10] Since 1911, the lessee of his pharmacy was Lovre Blažević from Croatia. Joachim Boglić opened a pharmacy in Trebinje in 1883,[30] Šimun Madirazza from Dalmatia in Ljubuški in 1886,[10] and the Pole Waclaw Babinski in Konjic in 1893.[9] According to one source, the pharmacy in Stolac was opened in 1882,[2] which was taken over from Luigi Cornioni in 1898 by Vinzenz Giancovich from Dalmatia.[9]

In the Sarajevo district, public pharmacies were opened in Sarajevo, Goražde, Foča, Visoko, Višegrad and Rogatica. During this period, several pharmacies were operating in Sarajevo.[31] The Austro-Hungarian government found a modern pharmacy in Sarajevo where Eduard Pleyel worked, the Czech who came to Sarajevo

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Figure 3: Julius Thonhauser's house (left) and Military Post (right)

in 1877.[3] He worked as an assistant in the pharmacy owned by Josef Sumbul, an unqualified apothecary, which he later took over from him[3, 19] and registered the pharmacy "To the Emperor of Austria" (*K caru austrijanskom*) in 1884.[32] From 1912, his son Eduard took over the pharmacy. Soon, Stefan Dobóczky also opened a pharmacy in the city called "To Help" (*K pomoći*), which was registered in 1884.[33] This pharmacy was later taken over by his son Stefan Dobóczky Junior, while Stefan Dobóczky Senior was the owner of a seasonal pharmacy in Ilidža.[25, 34] In 1905, Heinrich Sclesinger bought the seasonal pharmacy from the heiress, the widow Magdalene.[35] The city pharmacy was bought by Stevan Romčević from the heiress, the widow Sofieand registered at the end of 1911.[36] "At Sarajevo City" (*Kod grada Sarajeva*) pharmacy was opened in 1881 by Jakov Sumbul (Figure 4),[37] who completed his studies in Constantinople at the Faculty of Medicine. The Provincial Government did not recognise his diploma and demanded the nostrification to run a pharmacy, thus forcing him to take a provisor in his pharmacy. He employed Heinrich Schlesinger from Slavonska Požega (Figure 5)[19] as a provisor in 1887, who became the owner of this pharmacy in 1891.[27]

In addition to his medical practice, doctor Josef Koetschet opened a pharmacy in 1864 or 1866.[38]

Figure 4: Jakov Sumbul

Figure 5: Heinrich Schlesinger

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Figure 6: Filip Bohaček

Figure 7: Moriz Kirtner

He sold it to Ljudevit Matić, who became the owner of the pharmacy "To the eagle" (*K orlu*) on the basis of a concession from 1886.[39] In 1897, Matić sold the pharmacy to Czech Franz Ružička,[39] whose business partner Czech Josef Patera became the owner of this pharmacy in 1900.[40] After Josef Patera's death in 1913, the owners of this pharmacy became the heirs and the lessee was Rudolf Hübner.[24] The owner of the pharmacy at Čemaluša street number 165 was Friedrich Herzig but it is not known when he received the concession. The lessees were Eustach Weidenhoffer and Karl Chornitzer,[9,41] and from 1900 the owner of the pharmacy "To Mary the Helper" (*K pomoćnoj Mariji*) became Viktor Benischko.[42] Born in Sarajevo, Đorđe Besarović opened the pharmacy "At the Lion" (*Kod lava*) in 1907,[27] and Nikola Ivošević opened the pharmacy "To the Star" (*K zvijezdi*) in 1913, the seventh public pharmacy in the city of Sarajevo.[43]

Eduard Jecewicz opened a pharmacy in Goražde based on a concession from 1893,[10] which he managed until 1916, when the owners became his heirs.[44] Nikola Kus opened a pharmacy in Foča in 1898,[9] which was owned by the Czech Karel Fuhrich in 1901.[25] His property was deleted from the Commercial Register in 1911,[45] in the meantime the heirs of Bedřich Marek became the owners of the pharmacy and Anton Balek the lessee.[27] According to one source, the pharmacy in Visoko was opened on the basis of a concession from 1887, which was transferred to the then owner Josef Zeleny in 1898.[25] According to Zeleny's letter from 1902, the first pharmacy was opened in 1883 on the basis of a concession given to Heinrich Schlesinger, after whose departure several owners changed until Zeleny's takeover.[22] The pharmacy with inventory and all supplies was bought from Zeleny in 1906 by Jan Halla,[46] and from him in 1914 by Czech Filip Bohaček (Figure 6).[22] Wenzel Koza received the concession for a pharmacy in Višegrad based on a tender in 1907,[47] and Heinrich Moravec opened a pharmacy in Rogatica in 1914.[19]

In the Travnik district, public pharmacies were opened in Bugojno, Jajce, Livno, Travnik, Zenica and Tomislavgrad (Županjac). In Travnik Michael Bardasz opened the pharmacy "To God's Providence" (*K božjoj providnosti*) in 1884,[9] in Livno Anton Kluczenko opened the pharmacy "Faithful Shepherd" (*Vjernom pastiru*) in 1892,[9,10] and in Bugojno Theodor Heydušek from Moravia opened the pharmacy "To the Golden Eagle" (*K zlatnom orlu*) in 1893.[9,24] Martin Houška ran the pharmacy in Zenica from 1886 to 1890,[10] when it was taken over by the new owner Josip Šilhabl,[40] and then from 1913 by Otto Weiss.[48] According to a newspaper advertisement from 1884, the advertised preparation against stomach problems could also be bought in the "To God's Eye" (*K božjem oku*) pharmacy in Jajce,[49] while another source states that the pharmacy was opened in 1892.[2] It was not known who opened this pharmacy, whose owner was Karl Chornitzer from Moravia in 1899,[41] and where Dinko Mistura from Dalmatia worked two years

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Figure 8: Gavro Peciković

Figure 9: Simon Zaloscer

before.[47] Moriz Kirtner (Figure 7)[50] from Hungary opened the pharmacy in Tomislavgrad based on a concession from 1895.[9] After he opened a pharmacy in Sanski Most in 1910, this one was closed and did not work until 1918.

In the Tuzla district, public pharmacies were opened in Tuzla, Bijeljina, Brčko, Gračanica, Gradačac, Šamac (Bosanski Šamac), Maglaj and Zvornik. According to some sources, the first pharmacy in Tuzla was opened during the Turkish rule, which was run by the military doctor Pantelaki for a time, then Siminiati, from whom Gavro Peciković (Figure 8) from Vojvodina bought the pharmacy.[3, 19, 51] In the inspection report from 1906, it was stated that the pharmacy was opened in 1863 based on the concession of the Ottoman Government,[52] and Gavro Peciković opened the "District Pharmacy" (*Okružnu apoteku*) in 1882.[25] The second pharmacy in Tuzla, "To the Star" (K zvijezdi), was opened in 1894 by Simon Zaloscer (Figure 9) from Galicia.[9, 53]

The pharmacy in Brčko was opened in 1879 by Anton Dobrzański.[9] From 1900, Gustav Proche from Bohemia became the owner of the city

Figure 10: Gustav Proche

Figure 11: Waleryan Rittermann's house with pharmacy

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pharmacy "To the Austrian Eagle" (*K austrijskom orlu*),[48] whose spacious office is shown in Figure 10.[54] Hungarian Antal Čapo opened a pharmacy in Bijeljina in 1880,[2, 55] and Anton Hudovski from Slavonia became its owner from 1896.[9] The pharmacy in Maglaj was probably opened by Moriz Kirtner, because he worked there for five years before he opened the pharmacy in Tomislavgrad in 1895.[56] In 1896, Rudolf Loebl from Bohemia became the owner of the pharmacy "To the Rose" (*K ruži*).[9]

A pharmacy in Gračanica was opened in 1894 on the basis of the concession given to Johann von Bersa, which was by the Decree of 1896 withdrawn and assigned to Johann Stanislaw Niemczyk from Austrian Silesia.[40] In Gradačac Slovak Michal Hodža opened a pharmacy in 1893,[9] and in Šamac Pole Waleryan Cyril Rittermann in 1897 opened a pharmacy "To the Guardian Angel" (*K anđelu čuvaru*), which was located in a large family house (Figure 11).[9, 57] Based on a concession from 1885, Franz Ivanuš from Croatia was the owner of a pharmacy in Zvornik which he bought from Michael Hedvig.[9] From 1910, the widow Marie became the owner and the pharmacy was managed by lessees (Figure 11).

Discussion

With the arrival of Masters of Pharmacy from all parts of the Monarchy, an increasing number of public pharmacies began to open. Concessions for opening pharmacies were initially granted to foreigners and according to data from this research, among the settlers there were mostly Czechs, Croats, Poles, Hungarians, Slovaks, who completed pharmacy studies at universities in Vienna, Zagreb, Prague, Lviv, Graz, Innsbruck, Krakow. Over time, more favourable conditions were created for the education of the local population. Societies with cultural and educational goals played a role in this process, helping and educating poor pupils and students through scholarships and aid, thus creating a domestic intelligentsia.

There were attempts to open pharmacies in some other towns or a larger number of pharmacies in towns where one or more of them had already been opened. For almost 40 years, only two pharmacies worked in Banja Luka, in the city centre. In 1912, Nikola Ivošević, then the lessee of the pharmacy in Zenica, tried to open a third one, which was not supported by the owners of the existing pharmacies. His request was rejected because in order to open a new pharmacy, in addition to considering the local need for opening a pharmacy, the survival of already existing pharmacies had to be taken into account.[58] In May 1918, Czech Josef Kos, employed in the pharmacy military service in Sarajevo, also submitted an application for the granting of a concession for a new pharmacy in Banja Luka.[59] In Kotor Varoš, in the Banja Luka district, in 1916, Guido Fichtner, the lessee of the pharmacy in Tešanj and Adolf Barzal, the owner of the pharmacy in Brod, tried to get a concession for a pharmacy. The requests were rejected with the explanation that they would most likely apply for the advertised competition because almost all the young pharmacists had served in the military and that the District hospital handles the task of dispensing medicines better.[60] In the City of Sarajevo, five pharmacies worked for almost thirty years and two new ones were opened in 1907 and 1913. The concession of the eighth pharmacy was foreseen in 1914,[61] but the concession was not filled because in 1918 seven pharmacies were operating in Sarajevo.[62] In the Mostar district, there were attempts to open pharmacies in Čapljina and Bileća. In Čapljina, Dinko Mistura from Ljubuški and Vinzenz Giancovich from Stolac submitted applications for a concession for the pharmacy, which were rejected due to the negative opinion of the Stolac District Office on their necessity and profitability.[63] In 1912, Spasoje Radmilli from Dubrovnik asked for a concession for a pharmacy in Bileća and the request was rejected with the explanation that the Municipal clinic fully meets the needs of the population and that the pharmacy would do poorly without some other business.[64] A competition for the opening of a pharmacy in Mrkonjić Grad, in the Travnik district, was announced in 1899,[65] but the pharmacy was not opened in this period.

Accustoming the people to being treated with medicines that they could get in pharmacies went slowly, so many pharmacists were also engaged in other jobs. Eduard Rhein from Bihać, Stefan Variačić from Gradiška, Theodor Heydušek from Bugojno, Gyula Keller from Novi Grad, Alexander Sussmann from Derventa, Stanislaw Niemczyk from Gračanica, Michal Hodža from Gradačac, Dinko Mistura from Jajce, Waclaw Babinski from Konjic, Anton Kluczenko from Livno, Rudolf Loebel from Maglaj, Wenzel Mikan from Mostar, Gavro Peciković and Simon Zaloscer from Tuzla and

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Moriz Kirtner from Tomislavgrad were engaged in the production of soda water in addition to the apothecary business. A liquor factory was owned by Gustav Proche from Brčko, a grocery store by Michael Finkelstein from Prnjavor, Đuro Vojković from Doboj, Karel Fuhrich from Foča, who also had a registered large production facility for the distillation of fruit and brandy and Anton Kluczenko from Livno, who also had a photographic craft.

In the first years, only one qualified pharmacist worked in each pharmacy and over time there were more pharmacists in the pharmacies. Twenty years after the occupation, in 33 towns throughout B&H, 40 Masters of Pharmacy had registered pharmacies.[34] In 1905, the pharmacy staff consisted of 65 people, of which 38 were pharmacy owners, five provisors, nine assistants and 13 interns, who worked in 44 public pharmacies.[66] In 1914, 48 public pharmacies worked in B&H,[67] as well as at the end of 1918, when pharmacies were distributed in 38 towns.[62]

Conclusion

With the opening of an increasing number of pharmacies in B&H during the period of Austro-Hungarian rule from 1878 to 1918, the number of qualified pharmacists grew, which contributed to the improvement of the profession, health and social conditions in the country over this period. As an educated class of people, they played a significant role in public, economic and social life, bringing modern trends to our region.

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Conflict of interest

None.

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CASE REPORT

Simple Trachelectomy Following Neoadjuvant Chemotherapy in Cervical Cancer Over 2 cm in Size - a Case Report and Review of Literature

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Abstract

In the past few decades fertility preservation has emerged as a treatment modality for cervical cancer patients. Different surgical methods have been described, such as open or minimally invasive trachelectomy and gross cervical conisation combined with laparoscopic lymphadenectomy. A thirty-year-old nulliparous woman with uterine cervical cancer FIGO stage IB2 (classification from 2009) underwent neoadjuvant chemotherapy. After three cycles of chemotherapy with cisplatin and iphosphamide there was no colposcopic findings of cervical invasion, therefore a conservative surgery was performed. The patient underwent laparoscopic pelvic lymphadenectomy, cervical amputation and the endocervical curettage. The histopathology confirmed a complete response to chemotherapy.

Key words: "Bulky" cervical cancer; Fertility sparing surgery; Neoadjuvant chemotherapy.

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Introduction

Of all surgically treated stage IB cervical cancers around 45 % occur in women younger than 40.[1] Key factors that make a patient a candidate for radical trachelectomy are: patients younger than 40 years of age, strong motivation to preserve fertility, no history of infertility, tumours smaller than 2 cm in diameter, FIGO stages IA2-IB, that the upper part of the endocervical canal is not involved and no evidence of metastasis to the regional lymph nodes.[2] A radical trachelectomy is an operation to remove most of the cervix with parametria and the upper part of the vagina such as in standard radical hysterectomy but the uterus is left in place with utero-vaginal anastomosis. Approach for this operation could be abdominal or vaginal. Some authors have proposed more conservative treatments with the same oncologic outcomes as radical trachelectomy. The proposed treatments are cervical conisation or simple trachelectomy, followed by pelvic lymphadenectomy in cases of low-risk patients (tumours less than 2 cm in size, no lymphovascular invasion and a negative lymph nodes.[3] Results comparing these two techniques show some differences in pregnancy outcomes however both success rates are acceptable.[2, 4-7]

Case History

A thirty-year-old nulliparous woman with uterine cervix cancer FIGO stage IB2 underwent neoadjuvant chemotherapy. After her routine

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gynaecological exam at the September of 2017 gynaecologist took a PAP smear. PAP smear was high-grade squamous intraepithelial lesion (H SIL) and as examination showed suspicious clinical lesion multifocal gynaecologist did the punch biopsy and cervical squamous carcinoma, G2 was diagnosed (Figure 1).

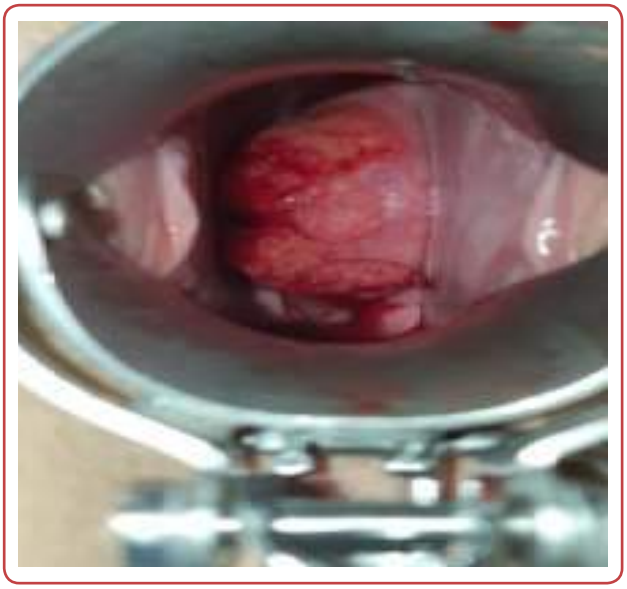

Figure 1: Gynaecological exam before neoadjuvant chemotherapy treatment

The lesion was measured at 30 mm in maximal diameter and the proliferation index was not assessed. Magnetic resonance (MR) of the pelvis and abdomen that was performed, as well (Figure 2). Based on the results patient was sent to the Gynaecological Oncology Tumour Board at the Oncology Institute of the Vojvodina. The standard operative radical surgery was proposed with preservation of the ovaries. A strong desire to preserve fertility was expressed by the patient and she refused the treatment that could jeopardise fertility, such as radiotherapy.

The patient was informed about neoadjuvant chemotherapy treatment as not standard of care, that only a few such cases have been reported in recent literature and that no randomised trials have been conducted among these high-risk patients that could confirm the benefits of fertility preserving treatment in this setting. After counselling the patient about the procedure and algorithm of the treatment she signed the informed consent about fertility preserving. Due to lack of the experience in laparoscopy and sentinel node detection was not initially performed. However, the patient insisted on fertility preservation.

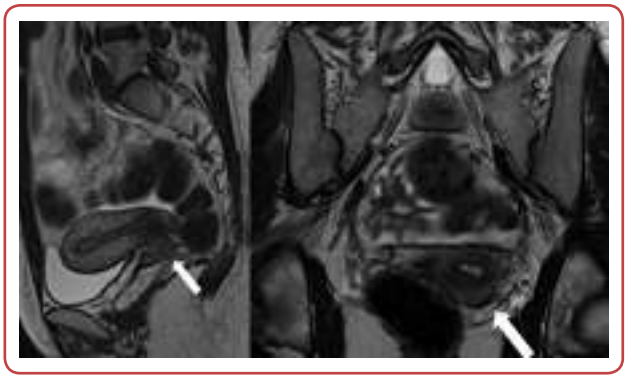

Figure 2: T2W sagittal and paraxial magnetic resonance tomograms on cervix (arrow) hyperintensity zone of infiltration on anterior part of the cervix, diameter 25x29 mm, stomal invasion about 8 mm in depth. Infiltration of parametria was not found and cervical ring was without disruption.

After informing patient about the chemotherapy protocol and receiving informed consent, neoadjuvant chemotherapy (NACT) was administered. The performance status was 0. Medical history showed no other malignancy.

Chemotherapy protocol

Patient received three cycles in 10-12 day interval chemotherapy protocol of cisplatin (75 mg/m^2) and iphosphamide (1800 mg/m^2), in accordance with the Prague protocol for squamous carcinoma,[8] during the October 2017. This protocol was chosen because paclitaxel-based regimens for cervical cancer are not covered by national health insurance in Serbia. A clinical exam along with an MR evaluation was performed three weeks after the last chemotherapy cycle (Figure 3 and 4).

Due to complete response, the patient had surgery four weeks after the last NACT cycle, 16 November 2017. A simple trachelectomy followed by laparoscopic pelvic lymphadenectomy was performed. The surgery lasted four hours with no intra- or postoperative complications. Total blood loss was up to 100 mL. No samples were sent for a frozen section examination.

Final pathological findings resulted in only H-SIL, no cancer was found. There were no metastases in any of the 21 harvested lymph nodes (Figure 5).

The patient was discharged 7 days after operation. The first follow up was one month after operation and showed an adequate length of the uterine cervix over 2 cm. After six months the first a PAP test showed no intraepithelial lesions of malignancy (NILM) and patient had follow up

Figure 3: Two weeks after III cycle of neoadjuvant chemotherapy treatment

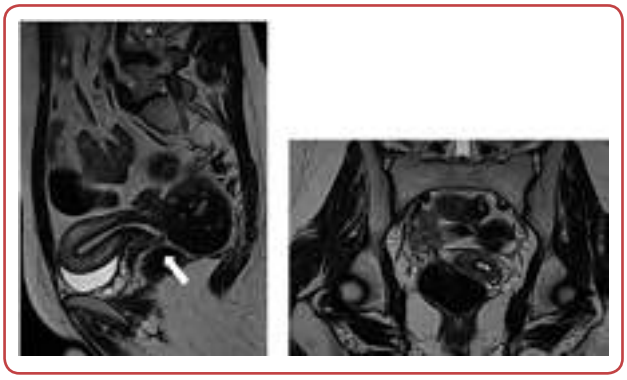

Figure 4: T2w sagittal and paraxial MR tomograms of small pelvis (no pathological findings). Complete therapeutic response (arrow) after III cycles of chemotherapy.

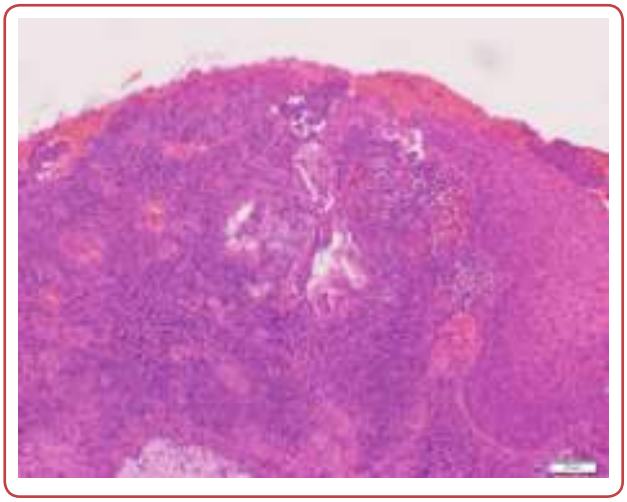

Figure 5: Highly dysplastic cervical squamous epithelium after neoadjuvant chemotherapy. There is a brisk stromal inflammatory response. No tumour stromal infiltration. Haematoxylin and eosin, 100 x.

exams every two months. A pregnancy was diagnosed one year after surgery and a healthy male child was delivered via Caesarean section at 38 gestation week. The pregnancy was followed by obstetrician and no needed for cerclage. Patient was followed in standard 3-4 months interval after delivery with gynaecological examination and PAP smear. The latest follow up in August 2022, 56 months, has shown a NILM PAP smear and imaging with no evidence of disease.

Discussion

Good oncologic and reproductive outcomes have been published in the last three decades with fertility sparring treatment in the early cervical cancer setting.[2, 7] Various surgical approaches have been described, ranging from vaginal and open to minimally invasive surgery. Adequate oncologic and obstetric outcomes have been reported with all approaches. For low risk, early lesions even more conservative treatments plans can show promising results.[9] There is still a subset of patients with a tumours larger than 2 cm in size that have a strong desire to preserve fertility. A key goal for gynaecologic oncologists is to trial fertility sparring approaches for these patients to drive a better balance of oncologic and obstetric outcomes.

Many papers have reported a good chemotherapy response rate for cervical cancer. NACT not only reduces tumour volume, which might lead to less challenging surgery, but also lowers lymph node positivity. Also, a good response to chemotherapy leads to disease downstaging which offers certain subsets of patients an option for fertility sparring treatment.[10] Seven patients with large FIGO IB – IIA-1 tumours (diameter 30 to 45 mm) were presented by Marchiole et al and all patients were treated with neoadjuvant chemotherapy followed by laparoscopic pelvic lymphadenectomy and vaginal radical trachelectomy (VRT). After NACT, all patients were evaluated by clinical examination, colposcopy and MRI. A 50 % reduction of tumour volume was shown in 5 of 7 patients (71 %) and a partial response (40 % volume reduction) was noted in the remaining two cases. All 7 patients underwent VRT. No signs of relapse shown in the follow up period with a median of 22 months (range 5-49 months).[11] A large

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Table 1: Neoadjuvant chemotherapy and different techniques of fertility sparing surgery in literature

	Cervical cancer ≥ 2 cm	NACT protocol	Conservative surgery	Optimal pathological response (CR+PR1) No (%)	Recurrences No	Pregnancy No
Marchiole et al[11]	7	TIP (or TEP for Ad Ca)	VRT+PL	4/7 (57 %)	0/7	1/7
Robova et al[12]	15	IP+(Dox for Ad Ca)	ST+PL	9/12* (75 %)	3/12	7/12
Maneo et al[13]	8	TIP (or TEP for Ad Ca)	Conisation + PL	6/6& (100 %)	0/6	NR
Plante et al[14]	3	TIP	VRT+PL	3/3 (100 %)	0/3	3/3
Palaia et al[15]	1	TIP	ST+PL	1/1 (100 %)	0/1	0/1
Kobayashi et al[16]	1	BOMP	Conisation	1/1 (100 %)	0/1	1/1

NACT: neoadjuvant chemotherapy; TIP: cisplatin, iphosphamide, paclitaxel; Ad Ca: adenocarcinoma; BOMP: bleomycin, vincristine, methotrexate, cisplatin; VRT: vaginal radical trachelectomy;

series of 15 patients was published by Robova et el which included tumours larger than 2 cm treated with NACH followed by vaginal trachelectomy. The Prague protocol was used, 3 cycles of cisplatin (75 mg/m^2) and iphosphamide (2 g/m^2) in case of squamous cancer and cisplatin (75 mg/m^2 and doxorubicin (35 mg/m^2) in case of adenocarcinoma every 10 days. That was followed by simple vaginal trachelectomy with laparoscopic lymphadenectomy. Complete response was obtained in 5 patients on the final histopathological report, while 6 patients had microscopic residual disease and 4 had macroscopic residual disease. A relapse occurred in the 3 cases with suboptimal response, all 3 patients in question were diagnosed with adenocarcinoma. A patient affected by ovarian relapse died, while two patients presented with endocervical recurrence but are alive without evidence of disease. In terms of obstetric outcomes, there were 7 reported pregnancies resulting in 7 children, 2 of which were preterm (26 and 35 weeks of gestation).[12] Table 1 presents thirty cases of patients reported in the literature diagnosed with cervical cancer larger than 2 cm in size that were treated with NACT followed by fertility sparing surgery.

Different approaches in young cervical cancer patients were also published. Lanowska et al initiated treatment with laparoscopic lymphadenectomy and after confirming no lymph node metastasis neoadjuvant chemotherapy was administered. Sentinel lymph node detection was performed in all patients. Neoadjuvant chemotherapy was comprised of 2-3 cycles of paclitaxel/iphosphamide/cisplatin regimens. That was followed by radical vaginal trachelectomy. Twenty patients were enrolled with a mean tumour size of 3 cm. Radical vaginal trachelectomy was performed in 18 patients. Complete response was noted in 9 cases, while chemoradiation was recommended in 2 cases due to an insufficient response. One relapse was reported in the mean follow up of 23 months. In terms of obstetric outcomes there were 5 patients reported 7 pregnancies. The authors concluded that the approach of laparoscopic lymphadenectomy followed NACT and RTV in the node negative subset could be adequate in terms of oncologic and obstetric outcomes.[17]

While NACT followed by surgical treatment is performed in some centres, it must be noted that there is no standard in terms of chemotherapy protocols and surgical approaches are even more variable. An acceptable approach with NACT and fertility sparring surgery in the setting of bulky cervical cancer was identified by these three small studies. An oncological safe approach was presented by Lanowska et al for patients at high risk of lymph node metastasis.[17] They used SLN detection or complete pelvic lymphadenectomy to identify candidates for NACT and fertility sparring surgery. Developing a sentinel node detection procedure, this approach will become more acceptable. As mentioned, a further aspect that is not standardised is chemotherapy. Most protocols are double or triple combinations that are based on platinum agents. The paclitaxel/cisplatin is reported to be the most promising regiment and should be the basis for future studies. According to some authors a dose dense NACT interval (12-14 days) has shown a better therapeutic response.[12]

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One further important aspect that requires consideration is ovarian function after chemotherapy. Is an examination of ovarian reserve before treatment necessary or is a normal pregnancy outcome expected? An expected normal outcome seems more likely, but further research is warranted. At the recent review article by Buda et al, authors reviewed a total of 20 articles and 114 women with IB2 disease, possible candidates for NACT prior to FS. In this review they found that uterine conservation was achieved only in 76.7 % of them. An optimal pathological response to NACT was achieved in 60.9 % of cases. A TIP protocol (cisplatin, iphosphamide and paclitaxel) regime was related to the best response. Authors notice that suboptimal response to NACT appeared to be an independent negative prognostic factor. Besides, up to 9.2 % of patients recurred with a median 7.4 months and 4.6 % of patients died of disease. In conclusions authors notice that NACT prior to FS surgery could be an option, but the literature about this issue is still weak and FS should be carefully discussed with patients.[18]

Squamous carcinoma is the most frequent cervical malignancy. However, cervical adenocarcinoma has increased incidence in the younger woman and is responsible for 20 to 25 % of all cervical cancers.[19, 20] The histological type of tumour should not influence a decision for surgery based on recent clinical recommendations, with exceptions of rare histological types such as small and neuroendocrine cervical cancer.[2] Both squamous cell and cervical adenocarcinoma receive the same standard treatment, some research has shown that adenocarcinoma has a negative impact on survival in the setting of both early and advanced-stage disease.[21-23]

The diameter of the tumour has been shown as a very important prognostic factor, too. Gil-Ibañez at al presented a total of 111 patients were included, 82 (73.9 %) with tumours < 2 cm and 29 (26.1 %) with tumours 2–4 cm. Authors concluded that the tumour size over 2 cm is the most important negative prognostic factor in this multicentre cohort of patients with early cervical cancer and who underwent fertility spearing surgery in Spain.[24]

Further studies are warranted, both with a larger patient census and a longer follow up to validate this more conservative approach and to best define subsets of patients with bulky cervical cancer that can have the most benefit from this type of treatment. The clinical trial named CONTESA hope will answered on some questions. Trial design is pre-menopausal women diagnosed with stage International Federation of Gynaecology and Obstetrics (FIGO) IB2, 2–4 cm cervical cancer who wish to preserve fertility will receive three cycles of platinum/paclitaxel chemotherapy. Patients with complete/partial response will undergo fertility-sparing surgery. Patients will be followed for 3 years to monitor outcome. Patients with suboptimal response (residual lesion ≥ 2 cm) will receive definitive radical hysterectomy and/or chemoradiation. Authors expected complete accrual in 2022 with presentation of results by 2025.[25]

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None.

Conflict of interest

None.

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