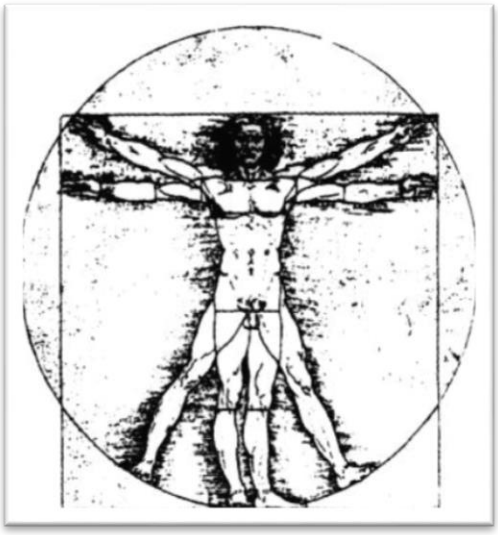
UDC: 61 **ISSN 1409-9837**

ЗАММ



MAMM

ACTA MORPHOLOGICA

INTERNATIONAL JOURNAL OF THE MACEDONIAN ASSOCIATION
OF ANATOMISTS AND MORPHOLOGISTS

Vol. 15 (1) 2018

ACTA MORPHOLOGICA
International Journal of the Macedonian Association of Anatomists and Morphologists (MAAM)
Member of the European Federation of Experimental Morphology (EFEM)
Member of the International Symposium of Morphological Sciences (ISMS)

Published
Twice a year

Corresponding address
Institute of Anatomy, Faculty of Medicine
Ss Cyril and Methodius University of Skopje,
50 Divizija 6, Skopje, R.Macedonia
Tel/fax: +38923125304; +38923125298
e-mail: acta_morphologica@yahoo.com

CONTENT


WEBDOCTOR.com.mk

UDC: 616.36-002-02:578.891]-085.371:616.8-009.836:616.61-008.64-78-052

ORIGINAL ARTICLE

SLEEP DISTURBANCE AND LONGER DIALYSIS VINTAGE WORSEN ANTIBODY RESPONSE TO VACCINATION IN DIALYSIS PATIENTS

Trajceska Lada, Selim GJ, Dzekova P, Rambabova Busletik I, Sikole A
University Clinic of Nephrology, Skopje, Republic of Macedonia

ABSTRACT

Objectives: This study aimed to assess whether sleep disturbance is associated with immune response and could predict magnitude of antibody response to the novel antigen of hepatitis B in vaccinated dialysis patients.

Materials and methods: We prospectively studied the evolution of HBsAg antibody (HBsAb) after primary vaccination (three doses; Engerix B, 40 pg i.m. at 0, one and 6 months) in 91 seronegative dialysis patients in three years period. The mean age of patients was 57.65±12.94; dialysis vintage 47.23±54.63 months; 54% were men. We also assessed dialysis adequacy (Kt/V), pre-dialysis albumin and CRP before vaccination and the anti-HBs titer one month after completed vaccination.

Results: Twenty-one (23%) patients were non-responders (HBsAb<10IU/L); 26 (28%) were weak responders (10-100 IU/L) and 44 (48%) showed good antibody response (>100 IU/L). HBsAb titer showed no association with age, serum albumin Kt/V and CRP ($p > 0.05$). The regression analysis showed that dialysis vintage and presence of sleep disturbance were strongly and inversely associated with immune response ($\beta = - 0.309$, $p=0.01$; $\beta= - 0.252$, $p=0.008$, respectively). In the multivariate model the presence of sleep disturbance and longer vintage predicted lower antibody level, explaining 16% of the population.

Conclusion: Dialysis patients should be vaccinated against hepatitis B as soon as possible at start, or in pre-dialysis period of the disease. Sleep disturbance negatively affects immune response.

Keywords: sleep disturbance, vaccination, HBsAg, antibody response.

INTRODUCTION

The big burden of comorbidities in dialysis patients is sustained by interaction of traditional and non-traditional factors. These patients are prone to infections, which result in frequent hospitalizations and high mortality. Despite infection control polices, hepatitis B is still present among dialysis patients in the regions of developing countries [1]. The effect of immunization against it, presented as quantity of antibodies is different and individual. The mostly connected factor to poor immunization is lower albumin level and age [2, 3]. A recent study in healthy volunteers showed that short sleep duration in the natural environment may negatively affect *in vivo* antibody responses to novel antigens, providing a possible explanation for associations of poor sleep with increased susceptibility to infectious disease [4]. The

literature also supports a relationship between psychological stress and antibody responses to immunizations [5]. Since dialysis patients are permanently exposed to physical, mental stress [6], and frequently suffer from sleep disturbance [7,8], we hypothesized that sleep disturbance could affect the immunological response to vaccination against hepatitis B. This study aimed to assess whether sleep disturbance is associated with immune response and could predict magnitude of antibody response to the novel antigen of hepatitis B in vaccinated dialysis patients.

MATERIALS AND METHODS

We prospectively studied the evolution of HBsAg antibody (HBsAb) after primary vaccination (three doses; Engerix B, 40 pg i.m. at 0, one and 6 months) in 91 seronegative dialysis patients in one dialysis center.

Patients were included if age was above 18 years and dialysis vintage more than three months. The dialysis regime of 4 to 5 hours three times per week was applied for all patients, with blood flow rates of 280 to 300 mL/min using a standard bicarbonate dialysis solution and low flux synthetic membranes with surface area of 1.3 to 1.8 m^2. Data was collected using medical histories and interviews for demographic, social, nutritional and clinical indices. We also assessed dialysis adequacy (Kt/V), pre-dialysis albumin level and CRP before vaccination and the anti-HBs titer one month after completed vaccination.

Sleep disturbance as present or absent was determined by whether the patients reported that they had disturbed sleep with frequent awakenings and felt not rested after the sleep.

RESULTS

The mean age of study participants was 57.65±12.94; dialysis vintage 47.23±54.63 months; 54% of them were men. Sleep disturbance was noted in 24% of patients. One month after completed vaccination, 21 (23%) of patients were non-responders (HbsAb < 10IU/L), 26 (28%) were weak responders (10-100IU/L) and nearly half of the patients (49%) showed good (>100IU/L) antibody response (Figure 1).

The differences between the group of good antibody responders and the rest of the patients are shown in Table 1. Better immunological response was observed in younger patients (p=0.023), less time on dialysis (p=0.001), with higher albumin level (p=0.028) and without presence of sleep disturbance (p=0.001).

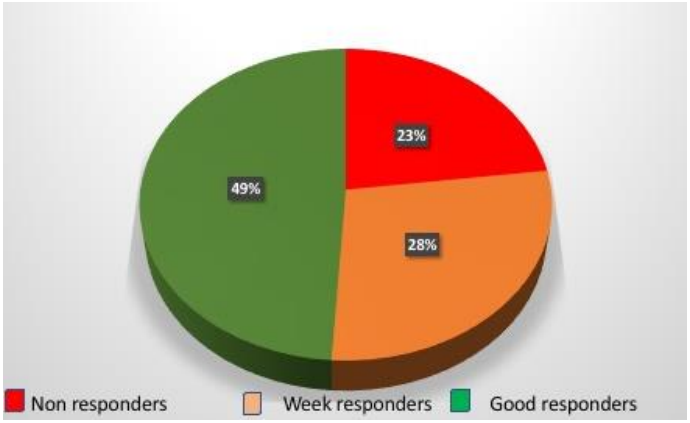


Fig. 1. Distribution of patients by antibody response: non-responders (HBsAb < 10IU/L), weak responders (10-100IU/L) and good antibody response (>100IU/L)

Table 1. Comparative analysis between group of good antibody responders and rest of the patients one month after completed immunization against hepatitis B.

variable	**HBsAb<100IU/L N=47**	**HBsAb>100IU/L N=44**	**p**
Men	26 (55%)	25 (56%)	0.636
Sleep disturbance	16 (34%)	6 (14%)	0.001
Age (years)	58.76 ± 13.87	39.38 ± 3.66	0.023
Dialysis vintage (months)	60.9 ± 62.87	33.5 ± 40.55	0.001
Albumin (g/L)	37.96 ± 3.91	39.38 ± 3.6	0.028
C-Reactive protein (ng/mL)	9.0 ± 14.5	6.6 ± 7.8	0.07
Kt/V	1.44 ± 0.29	1.50 ± 0.25	0.235

In the multivariate analysis the longer dialysis vintage and presence of sleep disturbance were most diminishing factors for adequate immune response in dialysis patients. The model clinically explains 16% of the examined population, as shown in Table 2.

Table 2. Multivariate analysis of immune response predictors in dialysis patients

variable	**β**	**p**
Dialysis vintage	- 0.309	0.001
Sleep disturbance	- 0.252	0.008
R Square 0.16		

In respect of the sustained good antiviral response after six months of vaccination, only 25 patients showed antibodies level above 100IU/L. Continuous drop in antibodies level was observed in both groups of poor and good responders (25.8 ± 29.7 *vs* 20.57 ± 13, p=0.038, 534.0 ± 397.0 *vs* 356.07 ± 395.88, p= 0.027), respectively.

DISCUSSION

Hepatitis B vaccination is recommended for all susceptible chronic hemodialysis patients [9]. Compared with immunocompetent adults, hemodialysis patients are less likely to have protective levels of antibody after vaccination with standard vaccine dosages; protective levels of antibody developed in 67%–86% of patients [10]. In our study anti-HBs seroconversion rate (Anti-HBs > 10 IU/l) was 77% and 49% of patients developed protective antibodies >100IU/L, similar to the Italian study [3].

Serum albumin level was well recognized as an indicator of response to hepatitis B vaccination in dialysis patients in a systematic review and meta-analysis [2]. It seems that albumin, as part of nutritional status, preserves the ability to produce antibodies. Higher albumin level was found in patients with better antiviral response in our study as well, but this association was overwhelmed by other factors in the multivariate model. Also, immunization ability was presented to be better in younger patients in some of the studies [3], but in our case poor sleep and dialysis vintage were most powerful predictors of worse antibody response.

As far as we know, this is the first attempt to connect the quality of the sleep to hepatitis antibody response in dialysis patients. The rate of sleep disturbance was high (24%) and was shown to be an independent predictor of production of antibodies, as we hypothesized. We explain this by prolonging the daily stress patients on dialysis suffer from, also during the night. Patients prone to poor sleep are more depressive, eat less and generally have low immunity.

In our study, patients with shorter duration of dialysis vintage had better results in immunization. Vaccination is recommended for pre-end-stage renal disease patients before they become dialysis dependent [9]. In our dialysis population 25% had been more than 5 years on dialysis, and yet not vaccinated. Strikingly, wast of better responders were less than two years on dialysis treatment. Limited data are available on the duration of immune memory after hepatitis B vaccination in dialysis patients. Our findings showed that with time immune response declines, as associated with age and dialysis vintage. The finding about the association between sleep disturbance and immunogenicity opens new possibilities of handling this problem. Sleep disturbance is a modifiable factor and efforts should be made to prevent and cure it, in order to achieve better response to vaccination in dialysis patients.

CONCLUSION

Dialysis patients should be vaccinated against Hepatitis B in pre-dialysis period of the disease, or as soon as possible at start of dialysis treatment. Sleep disturbance negatively affects immune response. As a modifiable factor, it should be treated in order to provide better quality of life and vaccine response in dialysis patients.

REFERENCES

1. US Renal Data System. Excerpts from the USRDSA.2014 Annual Data Report. Am J Kidney Dis Suppl. 2015;(2):1-274.
2. Ghamar-Chehreh ME, Agah S, Khedmat H, Aghaei A, Alavian SM. Serum albumin level as an indicator of response to Hepatitis B vaccination in dialysis patients: A systematic review and meta-analysis. Caspian J Intern Med. 2017;8(4):250-257.
3. Cordova E, Miglia I, Festuccia F, et al. Hepatitis B vaccination in hemodialysis patients: an underestimated problem. Factors influencing immune responses in ten years of observation in an Italian hemodialysis center and literature review. Ann Ig. 2017;29(1):27-37.
4. Prather AA, Hall M, Fury JM, et al. Sleep and antibody response to hepatitis B vaccination. Sleep. 2012;35(8):1063-9.
5. Cohen S, Miller GE, Rabin BS. Psychological stress and antibody response to immunization: a critical review of the human literature. Psychosom Med. 2001;63(1):7-18.
6. Harris TJ, Nazir R, Khetpal P, et al. Pain, sleep disturbance and survival in hemodialysis patients. Nephrol Dial Transplant. 2011;0:1-8.
7. Mucsi I, Molnar MZ, Retheyli J et al. Sleep disorders and illness intrusiveness in patients on chronic dialysis. Nephrol Dial Transplant. 2004;19:1815-22.
8. Elder SJ, Pisoni RL, Akizawa T et al. Sleep quality predicts quality of life and mortality risk in hemodialysis patients: results from the DOPPS study. Nephrol Dial Transplant. 2008; 23:998-1004.
9. Guidelines for vaccination. Kidney Dialysis Patients and Patients with Chronic Kidney Disease. CDC 2012. http://www.cdc.gov/vaccines/
10. Mast EE, Weinbaum CM, Fiore AE, et al. A Comprehensive Immunization Strategy to Eliminate Transmission of Hepatitis B Virus Infection in the United States: Recommendations of the Advisory Committee on Immunization Practices (ACIP) Part II: Immunization of Adults. MMWR Recommendations and Reports. 2006;55(RR-16):1-33.

UDC: 618.146-006.6-089.85:618.2

ORIGINAL ARTICLE

THE IMPACT OF PREVIOUS SURGICAL TREATMENT CONIZATION ON EARLY PERINATAL OUTCOME

Jovanovska Viktorija, Stojcevski S, Stojovska Lazarova A, Aleksiovska I, Shabani A
University Clinic of Gynecology and Obstetrics, Skopje, Republic of Macedonia

ABSTRACT

Background: At the University clinic for obstetrics and gynecology at the Medical Faculty – Skopje, the most used method in the treatment for cervical intraepithelial neoplasia is the classic conization.

Objective: The goal of the study is to explore the influence of the conization on the early perinatal outcome.

Materials and methods: Retrospective cross - sectional study involving all women that have given birth who had previously made conization and the same number of women that previously had mad conization at the University clinic of gynecology and obstetrics, Faculty of Medicine – Skopje in the period from 01.01.2011 until 31.12.2014 y. Clinical parameters that are being analyzed: Year of birth, year of surgical treatment, gestational stage, Apgar score at 1 minute, Apgar score at 5 minute, birth weight, birth length, premature birth, amniotic fluid and cerclage.

Conclusion: Conization can be a risk factor for poor outcome in subsequent pregnancies respectively increased risk of premature birth, low gestational age, low Apgar score at 1 minute and 5 minute, premature breaking of the forewater shell. Prophylactic cerclage is not always helpful in the prevention of the premature birth.

Keywords: conization, cerclage, perinatal outcome, premature birth.

INTRODUCTION

Cervical cancer is the second most common cause of cancer and the fourth leading cause of death from cancer in women population [1]. The incidence may be reduced by well-organized screening, which is consisted in detecting cytological abnormalities. With this routine screening over to the most of the women in reproductive age is diagnosed with cervical intraepithelial neoplasia CIN, which is a precancerous cervical cancer [2].

To treat CIN, there are several procedures including: conization, cryotherapy, laser vaporization and LEEP. Today, in the treatment of cervical intraepithelial neoplasia are being forced two main therapeutic modalities respectively LEEP (Loop electrosurgical excision) and conization. Both methods are expended respectively exists pathohistological verification of expended lesion [3].

LEEP is the most widely used method in the world. LEEP is cheaper, does not require general anesthesia or operating room and it is a procedure that is done in the outpatient setting and is very convenient for doctor and patient than the classical conization [4]. As a disadvantage occurs higher rate of damaged cones that cannot adequately be evaluated pathologically [5, 6]. On the contrary cones removed by classical conization are with much less damage and are more appropriate for histopathological analysis. Therefore, the University Clinic of Gynecology and obstetrics in the Faculty of Medicine - Skopje preferred method in the treatment of cervical intraepithelial neoplasia is the classical conization.

Lately there is an increased incidence of abnormal cervical cytology, particularly among women of reproductive age. Therefore, there are a higher percentage of pregnancies in previous conization than before [7].

It is still controversial whether the conization of the cervix during the CIN treatment is associated with poor outcome in subsequent pregnancies respectively with the early perinatal outcome including preterm birth, cesarean section, premature breaking of the amnion, low birth weight and length, Apgar, incomplete cervix and cervical stenosis [8].

For those women who want to give birth in the future, it is important to know whether the previous surgery of the cervix would compromise the future pregnancy [9]. Therefore it is important to examine whether the conization as a surgical procedure in the treatment of cervical intraepithelial neoplasia will be of benefit to the patients, but with minimal risks to the outcome of the next pregnancy [9].

Based on these findings, it is created this cross-sectional, retrospective study to be compared the effects and consequences of the conization in the treatment of CIN early on perinatal outcome.

MATERIALS AND METHODS

This study is a retrospective cross-sectional study. In the survey are included all women that has given birth at the University Clinic for gynecology and obstetrics, Medical Faculty - Skopje between 01. 01.2011 to 31.12.2014, where they have previously made conization and the same number of women that has given birth at the University Clinic for gynecology and obstetrics, Medical Faculty - Skopje in the period from 01.01 2011 to 31.12.2014, that haven't made conization previously , and that will be involved in the research at random choice as control group. The sample was divided into two groups:
Group 1: Women that had given birth who had previously made conization. Group 2: Women that had given birth who hadn't previously made conization.
Including criteria were: 1) Patients were < 40 years; 2) all pregnancies are the result of spontaneous conception; 3) all women where previously a conization had been made had a CIN2, CIN3 or CIS biopsy result. Exclusion criteria were: 1) history of repeated cervical excision or ablative treatment; 2) patients with a known risk of premature birth and more forewater pregnancies; 3) patients who become pregnant within the first 6 months after the conization; 4) patients with advanced renal, hepatic or any other major disease (cardiovascular, diabetes or hypertension); 5) alcohol, or tobacco addiction diseases; 6) twin pregnancy.

The study limitation is that it does not include those pregnancies that were terminated before the possibility to bring viable baby to the world and where they had Fetus mortus in utero, and previously conization was performed.

The data in this retrospective study are provided from the obstetric and neonatal histories from the University Clinic for gynecology and obstetrics, Medical Faculty - Skopje. Clinical parameters to be analyzed are: Year of giving birth, year of surgery, gestational age, Apgar score at 1 minute, Apgar score at 5 minutes, way of completion of birth, birth weight, birth length, premature birth, forewater and cerclage.

Statistics is being processed in a statistical program Statistica 7.1 for Windows into processing batches with numerical markings, it is made a descriptive statistics (average, standard deviation, +/- confidence interval, minimum / maximum value). It is tested the distribution of data; in the testing the difference in the analyzed parameters is applied the Mann-Whitney test (Z).

Surgical protocol – Conization

The conization was performed in the operating room where after spinal anesthesia, surgical margin of 2 mm was performed with a scalpel no. 11 and hemostasis was achieved by

the Dexon 1-0 sutures method Sturmdorf. The operating protocol is in accordance with the standards and principles of the work at the University Clinic for gynecology and obstetrics, Medical Faculty – Skopje and was approved by the local ethical committee. In all patients in whom a conization was made, a postoperative cytological examination protocol was made. All patients were recommended to become pregnant after 6-12 months, and no recurrence of CIN 1 was not observed during the process in the last year.

RESULTS

In the group of women who had given birth previously they had made conization, 82 met the inclusion criteria and therefore the received results are for the following parameters that were analyzed: birth weight of newborns varies in the interval 2655.25 ± 887.77 grams; birth interval length varies 46.43 ± 5.50 cm; gestational week interval varies 35.61 ± 4.04 weeks; Apgar score value at 1 minute interval varies from 7.46 ± 1.22 points; the value of Apgar score at 5 minutes, varies in the interval from 8.33 ± 1.46 points.

In the group of women that had given birth and previously hadn't made conization, the obtained results on these parameters analyzed are: birth weight of newborns varies in the interval 3121.25 ± 520.70 grams; birth interval length varies 49.49 ± 2.20 cm; gestational week interval varies 38.78 ± 219 weeks; Apgar score value at 1 minute interval in the range 7.89 ± 0.45 points; value of Apgar score at 5 minutes interval vary 8.86 ± 0.50 points.

The differences of analyzed parameters regarding the group: birth weight of the infants in the control group and Z=-3,03 и p<0,01 (p=0,002), is significantly higher than the group with conization; birth length of the control group and Z=-3,13 и p<0,01 (p=0,002), is significantly higher than the group with conization; gestational week in the control group and Z=-6,09 и p<0,001 (p=0,000), is significantly higher than the group with conization; Apgar score value of 1 minutes in the control group and Z=-2,30 и p<0,05 (p=0,02), is significantly higher than the group with conization; Apgar score value of 5 minutes in the control group and Z=-2,62 и p<0,01 (p=0,009), is significantly higher than the group with conization.

The analyzed parameters birthweight newborns, birth length, gestational week, Apgar score at 1 minute, Apgar score at 5 minutes, are significantly higher in the control group.

DISCUSSION

Cervical cancer is the second most common cause of cancer and the fourth leading cause of death from cancer in women, but the mortality rate is reduced by on time detection and treatment of pre-cancerous lesions. Age with the greatest prevalence of precancerous lesions is 30, so that the need for conservative treatment is increasing [1].

The cervical ccnization, dates from the late 50's, also there are published many studies on cervical conization and perinatal outcomes. Although some authors find no connection, many of them agree that there is a significant correlation between cervical conization and poor perinatal outcome [10].

In this study, the results showed that conization as surgical treatment of cervical pre-cancer is associated with poor perinatal outcome: lower birth weight, lower birth length, lower gestation, lower Apgar score at 1 minute and lower Apgar score 5 minutes. The number of newborns with lower birth weight and length is significantly higher after conization. This is important because these infants are associated with greater morbidity and mortality compared with newborns with adequate birth weight and length corresponding to the mature newborn. Lower Agar score at 1 and 5 minutes, is also associated with higher morbidity and mortality, which still derives from the early gestation and the immaturity of most organ systems [11]. Some meta-analyzes and studies similar to this indicate that conization has more adverse effects on pregnancy, such as increased risk of premature newborns, but they did not say directly that

the conization that leads to PROM may be the main reason for pre term births and low birth weight [12].

The results coincide with previous studies. Michelin and Sor showed that more abortions and premature births occur in patients with previously conization. Also it emphasizes the fact that the interval between birth and conization is inversely proportional to the risk of premature birth [8]. Many studies demonstrate that the healing process is completed 3 months after the conization. Himes and Simhan are emphasizing the fact that the period from short conization to pregnancy, especially if it is shorter than 2-3 months increases the risk of premature birth [13].

It is interesting that the results in this study showed no statistical significance for the early birth time with the elapsed since the conization had been made.

The benefit of prophylactic cerclage in women with a history of conization is not clear. Leiman and Sor concluded that all pregnancies after conization should be treated as high-risk, and it is recommended cerclage in pregnancies followed by extensive biopsy of the cervix [14]. Kullander and Sjoberg concluded that cerclage should be avoided because they could not prove that it reduces the incidence of births after pre term births after the conization [15]. Myllunen, Karjalainen, Zeisler and collaborators have shown that prophylactic cerclage should be used moderately because it does not prevent the preterm birth, while from the other side is being considered that can induce premature uterine contractions.

They found that the cerclage may be a risk factor by itself. Seams can act as a foreign body and can irritate the uterus and make contractions after the procedure. Some authors report significant growth of pathogenic flora in the vagina and cervix after the circlage. They insist that the release of prostaglandins by bacteria in the vagina can cause horioamnionit, premature breaking of membranes, preterm birth and sepsis [16].

In this study the number of patients was insufficient to compare the effectiveness of the cerclage, but the cerclage may not be helpful in preventing premature birth and poor perinatal outcome, and can only add complications followed by operational procedures. In the future, it is good to make a study with a larger number of patients, which will can be seen the effectiveness of the cerclage, according to the cervical length, gestational age at birth and indication for cerclage.

In previous studies of pregnancy outcome after conization, many studies have shown that there are adverse perinatal outcomes, such as preterm birth, low birth weight or the conization itself may be a risk factor for PPI [10]. In this study, emphasis is placed on the associated factors for poor perinatal outcome, and are integrated as a single factor. There are few studies on risk factors for preterm birth after conization [17].

CONCLUSION

At the group of patients with a previous history of cervical conization there is poor perinatal outcome with statistical significance. While the need for prophylactic cerclage remains questionable in this study, this decision is best left to be resolved by the patient during the prenatal counseling.

Obviously it needs to be decided for better treatment that not only will cure CIN, but will give less adverse effects in subsequent pregnancies [18]. This study distinguishes women who may be at risk in their future pregnancies after conization and indicates that the conization may have a higher rate of poor perinatal outcome, and therefore more preterm births and multiple births with low birth weight. Thus, for women who want to become pregnant in the future, it should always be discussed about another method that would sanitize the finding of intraepithelial neoplasia of the cervix.

REFERENCES

1. Ramondetta L. What is the appropriate approach to treating woman with incurable cervival cancer? Natl Compr Netw JNCCN. 2013;11 (3):348-55.
2. Arbyn M, Anttila A, Jordan J, Ronco G, Schenck U, Segnan N, et al. Europian Guidelines for Quality Assurance in Cervical Cancer Screening. Second edition—summary document. Ann Oncol Off J Eur Soc Med Oncol ESMO. 2010;21(3):448-58.
3. Abryn M, Rebolj M, De Kok IMCM, Fender M, Becker N, O'Reilly M, et al. The challenges of organising cervical screening programmes in the 15 old members states of the Europian Union. Eur J Cancer.2009;45(15):2671-8.
4. Jin G, LanLan Z, Li C, Dan Z. Pregnancy outcome following loop electrosurgical excision procedure (LEEP) a systematic review and meta-analysis. Arch Gynecol Obstet. 2014;289(1):85-99.
5. Ortoft G, Henriksen T, Hansen L. After conisation of the cervix, the perinatal mortality as a result of preterm delivery increases in subsequent pregnancy. BJOG Int J Obstet Gynaecol. 2010;117(3):258-67.
6. Guo HJ, Guo RX, Liu YL. Effects of loop electrosurgical excision procedure or cold knife conization on pregnancy outcomes. Eur J Gynaecol Oncol. 2013;34(1):79-82.
7. Klaritsch P, Reich O, Guiliani A, Tamussino K, Haas J, Winter R. Delivery outcome after cold-knife conization of the uterine cervix. Gynecol Oncol. 2006;103(2):604-7.
8. Michelin MA, Merion LM, Franco CA, Matura EFC. Pregnancy outcome after treatment of cervical intraepithelial neoplasia by the loop electrosurgical excision procedure and cold knife conization. Clin Exp Obstet Gynecol. 2009;36(1):17-9.
9. Kristensen J, Langhoff-Ross J, Kristensen FB. Increased risk of preterm birth in women with cervical conization. Obstet Gynecol. 1993;81(6):1005-8.
10. Moinian M, Andersch B. Does cervix conization increase the risk of comlications in subsequent pregnancies? Acta Obstet Gynecol Scand. 1982; 61 (2): 101-3.
11. Mathevet P, Chemali E, Roy M, Dargent D. Long-term outcome of a randomized study comparing three techniques of conization: cold knife, lazer, and LEEP. Eur J Obstet Gynecol Reprod Biol. 2003;106(2):214-8.
12. Kyrgiou M, Koliopoilos G, Martin-Hirsch P, Arbyn M, Prendiville W, Paraskevaidis E. Obstetric outcomes after cons ervative treatment for intraepithelial or early invasive cervical lesions: systematic review and meta-analysis. Lancet. 2006;367(9509):489-98.
13. Himes KP, Simhan HN. Time from cervical conization to pregnancy and preterm birth. Obstet Gynecol. 2007;109(2 Pt 1):314-9.
14. Berghella V, Pereira L, Gariepy A, Simonazzi G. Prior cone biopsy: prediction of preterm birth by cervical ultrasound. Am J Obstet Gynecil. 2004;191(4):1293-7.
15. Kullander S, Sjoberg NO. Treatment of carcinoma in situ of the cervix uteri by conization. A five-year follow-up. Acta Obstet Gynecil Scand.1971;50(2):153-7.
16. Myllynen L, Karjalainen O. Pregnancy outcome after combined amputation and conization of the uterine cervix. Ann Chir Gynaecol.1984;73(6):345-9.
17. Jakobsson M, Gissler M, Paavonen J, Tapper A-M. Long-term mortality in women treated for cervical intraepithelial neoplasia. BJOG Int J Obstet Gunaecil. 2009;116 (6): 838-44.
18. Leiman G, Harrison NA, Rubin A. Pregnancy following conization of the cervix: complications related to cone size. Am J Obstet Gynecol. 1980; 136(1):14-8.

UDC: 618.15-022.7-06:618.39-021.3

ORIGINAL ARTICLE

BACTERIAL VAGINOSIS CORRELATION TO PREGNANCY LOSS PRIOR TO 22ND GESTATION WEEK

Trajkova Kornelija [1], Petrovska M[2], Jankoska G[2], Stojovska Lazarova A [3]
[1]Clinical Hospital Acibadem Sistina, Skopje, Republic of Macedonia
[2]Institute of Microbiology and Parasitology, Medical Faculty, Skopje, Republic of Macedonia
[3]University Clinic of Obstetrics and Gynecology, Skopje, Republic of Macedonia

ABSTRACT

Background: Bacterial vaginosis (BV) is extremely prevalent vaginal condition. It has been related to many pregnancy complications, including pregnancy loss as the most common early pregnancy complication with rate of 10-20%. Beside chromosomal abnormalities as the most common risk factor, maternal risk factors include genital infections and BV, both being risk factors that can be prevented.

Objective: To analyze the correlation of screening tests for BV as well as microbiologically proven *Gardnerella vaginalis* with pregnancy loss prior to 22nd gestation week.

Materials and methods: A study of 72 women with pregnancy before 22nd gestation week, divided in two groups: 36 women with pregnancy loss versus 36 women with normal pregnancy. All women were screened for BV: vaginal secretion analysis using Amsel criteria, measurement of vaginal pH (cut off value of 4.5) and amine test with 10%KOH and vaginal swabs for microbiological analysis.

Results: Of the 36 women in each group, normal findings were registered in 12 versus 22 in the control group; positive swabs and/or screening tests in 24 versus 14 (p=0.018); disturbed vaginal pH in 21 versus 10 (p=0.009) and positive KOH test in 10 of the first group. *Gardnerella vaginalis* was isolated in 10 of 12 tested 2 women (p=0.011).

Conclusion: The signs of BV were more frequently registered among women with pregnancy loss compared to the control group. Disturbance of the vaginal milieu and BV may play causative roles in spontaneous abortion and pregnancy loss during first and second trimesters. In spite of many conducted studies, the role of bacterial vaginosis in pregnancy has not yet been clearly defined. Since these are easily preventable risk factors, additional research in this area may be of great assistance for prevention of unwanted pregnancy outcome of this sort.

Keywords: pregnancy loss, bacterial vaginosis, *Gardnerella vaginalis*.

INTRODUCTION

Pregnancy loss (miscarriage) is a natural death of an embryo or fetus before it is able to survive independently [1]. It is among the most common early pregnancy complications, with the rate of 10-20% after previously confirmed pregnancy and 30-50% immediately after conception [2, 3]. Data indicate that approximately 50 % of the first trimester abortions are result of natural selection [4]. Hypothetically, chromosomal abnormalities result in disturbed embryological development leading to pregnancy loss [5]. Those are risk factors that cannot be prevented.

Thrombophilia, as a cause of thrombosis of the decidua blood vessels that leads to disturbance in the blood supply [6] is among other, quite frequent pregnancy loss risk factors.

Beside numerous research, many pregnancy loss reasons remain unknown [7]. Genital-urinary infections, including vaginal/cervical infections are a group of maternal risk factors that can be predicted, and prevented. Genital infections remain an open field for research and creating strategies.

According to the results of many studies, reduction of the concentration of Lactobacilli leads to disruption of the normal environment to so-called vaginal dysbiosis. If not detected and treated, it leads to bacterial vaginosis - an excellent medium for development of other infections.

Bacterial vaginosis (BV) or anaerobic dysbiosis is a poly-microbial syndrome characterized by a change in the normal vaginal flora, i.e. reduction in the concentration of H_2O_2-producing *Lactobacillus* species with other, mainly anaerobic bacteria such as *Prevotella species, Mobiluncus species, Gardnerella vaginalis, Mycoplasma hominis, Porphyromonas species, Bacteroides species, anaerobic Peptostreptococcus species, Fusobacterium species, Atopobium vaginae, Sneathia, Leptotrichia,* and lately genetically determined organisms from the *Clostridiales* (BVassociated bacterium1 [BVAB1], BVAB2, BVAB3) group, etc.

Diagnosis is based on two-pronged approach. In clinical practice BV can be diagnosed using the Amsel criteria: thin, white, yellow, homogeneous discharge; clue cells on microscopy, pH of vaginal fluid >4.5; release of a fishy odor on adding 10% potassium hydroxide (KOH) solution. Three criteria at least should be present for a confirmed diagnosis. A modification of the Amsel criteria accepts the presence of two instead of three factors and is considered equally diagnostic [8]. An alternative is to use a Gram-stained vaginal smear, with the Hay/Ison criteria or the Nugent criteria.

The Hay/Ison criteria are defined as follows: Grade 1 (normal): Lactobacillus morphotypes predominate; Grade 2 (intermediate): Mixed flora with some Lactobacilli present, with *Gardnerella* or *Mobiluncus morphotypes* present, also; Grade 3 (BV predominantly *Gardnerella* and/or *Mobiluncus morphotypes* with few or absent Lactobacilli.

Gardnerella vaginalis is the main culprit in BV. *Gardnerella vaginalis* is a short rod (coccobacillus). Hence, the presence of clue cells and gram variable coccobacilli are indicative or diagnostic of BV.

The Nugent Score is now rarely used by physicians due to the time it takes to read the slides and requires the use of a trained microscope reader. A score of 0-10 is generated from combining three other scores: 0–3 is considered negative for BV; 4–6 is considered intermediate; 7+ is considered indicative of BV [9].

The factors responsible for normal microflora disturbance are not fully understood, but the fact is that this condition is a precursor for the development and ascending of genital infections [4].

Reduction of Lactobacilli, in contrast to other microorganisms' growth, is probably the most important predictor for the development of BV [5]. BV is the most common cause of intensified vaginal discharge in women of reproductive age with up to 50 % prevalence in pregnancy , and a relative risk of abortion or premature delivery of 1.4-6.9% [6]. It is associated with many pregnancy complications including spontaneous abortion. BV ascends to the endometrium, but the complete impact of this migration with respect to placental development and early fetal development has not been sufficiently clarified [7].

Results from many studies, including a systematic review of the epidemiology of pregnancy loss, confirm the role of BV as a risk factor [10-13]. The mechanism is probably ascending and spreading an infection with a subsequent inflammatory response [14].

In recent years trend shows that research in the field of genital infections focused vaginal microbiome. Microbiota are aggregates of microorganisms present in different parts of the human body, vaginal microbiome being one of them. There are many ongoing molecular

analysis of the composition among different groups of women or different periods of menstrual cycle or pregnancy in the same woman. Different types of Lactobacilli have been isolated as part of the vaginal microbiome, but it is still not very clear whether all of them play the same role in vaginal environment maintenance.

The goal of the study was analysis of BV screening tests for as well as microbiologically proven *Gardnerella vaginalis* correlation with pregnancy loss prior to 22nd gestation week.

MATERIALS AND METHODS

We conducted a prospective study that included 72 women. Each woman signed informed consent prior to participating in the study. The women included in the study were divided in two groups:

I group: Women with pregnancy loss prior to 22nd gestation week (N=36). Inclusion criteria: Pregnancy with embryo/fetus with heartbeat or Ultrasound finding diagnostic of pregnancy failure, according to the Society of Radiologists in Ultrasound: CRL (crown-rump length) of ≥7 mm, and no heartbeat; mean sac diameter of ≥25мм and no embryo; absence of embryo with heartbeat ≥2 week after scan showing a gestation sac without a yolk sac; absence of embryo with heartbeat ≥11 days after a scan showing a gestation sac with a yolk sac (15).

II group (control): Women with normal pregnancy, prior to 22nd gestation week (N=36).

Inclusion criteria: Normal pregnancy, without expected problems (evaluation based on the medical history).

Women showing any of with the following symptoms were excluded from the study: cervical or uterine bleeding; previously proved genital-urinary infection; uterine anomalies; pregnancy loss over the last 6 months; history of recurrent pregnancy loss; autoimmune diseases; systemic diseases, proven thrombophilia, infectious diseases in the last year (Listeria monocytogenes, mumps, parotitis, TORCH); surgical procedures of the uterine cervix; invasive prenatal procedures.

Protocol

All women were screened for BV including: measurement of the pH of vaginal discharge with indicators strips pH 4.0 - 7.0 Special indicator – Merck KGaA). The strip was attached to the vaginal wall into the area of the vaginal introitus. Compared the indicator with the color chart and read the pH value. pH 4.5 was used as a cut off value; macroscopic vaginal discharge analysis; amine test with 10% KOH. Vaginal swabs were taken for microbiological analysis, especially for presence of *Gardnerella vaginalis*.

Each woman was inquired about presence of any of the symptoms: intensive vaginal discharge and/or vaginal odor; vaginal itching; frequent urination, periodontitis or dental problems.

The results were statistically evaluated by SPSS for Windows using nonparametric statistics (X2 test). The level of significance was set at p<0, 05.

RESULTS

Of the total number of 72 women, normal finding were registered in 34; 12 of them were women from the first group versus 22 from the second (control) group. Abnormal findings, in terms of positive swabs and/or positive screening tests were registered in 38 of the 72 women; 24 (66.67%) in the first group, versus 14 (38.89%) women in the control group (p=0.018) (Fig. 1)

Disturbed vaginal pH was registered in 21(58.33%) women, versus 10 (27.78%) women in the control group (p=0.009).

The amine - KOH test was positive in 10 (27.78%) in the first group versus 4 (11.11%) in the second group (p=0.032). *Gardnerella vaginalis* was isolated in 10 (27.78%) versus 2 (5.56%) women in the control group (p=0.011).

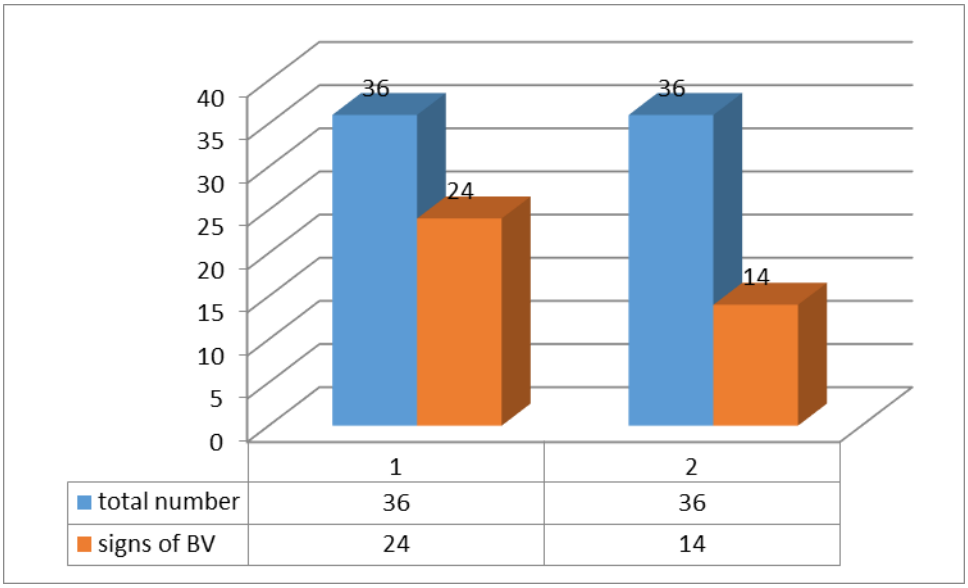


Fig. 1. Distribution of women with positive test in the groups

Ten women in the first group (27.78%) complained about more intense vaginal discharge and vaginal itching, versus 5 (13.89%) in the second group (p=0.147). Only 5 (13.89%) in the first group, versus 2 (5.56%) in the second complained about more frequent urination (p=0.233)

The number of women who complained about some sort of periodontitis or dental problems was 7 (19.44%) versus 5 (13.89%).
The results are shown in Table 1 enclosed below:

Table 1. Abnormal findings among study groups

	I group – pregnancy loss (N=36) **N (%)**	**II group – normal pregnancy (N=36)** **N (%)**	**Total** **(N=72)**	**p-value**
positive swab or screening tests	24 (66.67)	14 (38.89)	38	0,018
increased vaginal pH	21 (58.33)	10 (27.78)	31	0,009
positive KOH test	10 (27.78)	4 (11.11)	14	0,032
Gardnerella vaginalis	10 (27.78)	2 (5.56)	12	0,011
vaginal discharge/ itching	10 (27.78)	5 (13.89)	15	0,147
Frequent urination	5 (13.89)	2 (5.56)	7	0,233
periodontitis/dental propblems	7 (19.44)	5 (13.89)	12	0,527

DISCUSSION

The published results from the previous studies confirm the role of BV as pregnancy loss risk factor [10-13]. BV has a predictive value for pregnancy loss in the second trimester, i.e. in the period from 13 to 15 weeks of gestation [11]. It is also considered as risk factor in the comprehensive epidemiological survey on pregnancy loss published in 2014 [13]. According to the results of the meta-analysis of 2013, BV is associated with a significantly increased risk of preclinical loss of pregnancy, but there are no studies analyzing in more detail first trimester pregnancy loss. The presence of BV in the first antenatal control is associated with early pregnancy loss (RR 5.4, 95% CI, 2.5-11) [14].

Previously published data confirm association between increased pH as one of the signs of BV and pregnancy loss. Pregnant women with elevated vaginal pH in the first trimester have abnormal vaginal flora, abnormal morphology of the lactobacilli and complained about low abdominal pain, which may be understood as warning sign of miscarriage threat [7].

Most of the results in our study are aligned with that data. The study demonstrates a higher incidence of BV signs among women with pregnancy loss compared to the g normal pregnancy group. Incidence of increased vaginal pH as well incidence of positive KOH test and isolated *Gardnerella vaginalis* were significantly higher among women with pregnancy loss, compared to women with normal pregnancies (p=0,009, p=0,032 and p=0,011 respectively). This may confirm the role of BV as well as that of increased vaginal pH as a precursor in the cascade of pregnancy loss through ascending infection.

Concerning subjective symptoms, we did not registered significant difference between the two groups.

Our results have supported the hypothesis that BV is associated with pregnancy loss. Early detection and treatment of disturbed vaginal milieu may reduce the rate of early pregnancy loss.

More research, especially molecular analysis is needed for the purpose of defining the vaginal microbiome among women with normal clinical and microbiological findings, versus women with BV during pregnancy, as well as among population of reproductive age. Also, detailed analysis of the role of BV, and in particular of the vaginal microbiome on the day of embryo transfer is necessary [12]. Use of molecular techniques improved the knowledge of complexity of microbiome ecosystems of different parts of the body, including the vagina [16, 17].

REFERENCES

1. The Johns Hopkins Manual of Gynecology and Obstetrics (4ed) Lippincott Williams & Wilkins. 2012. pp 438-439.
2. Novak E, Berek JS. Novak's gynecology. 13th ed. Philadelphia: Lippincott Williams & Wilkins; 2002, p. 1067-1083.
3. Poorolajal J, Cheraghi P, Cheraghi Z, Ghahramani M, Irani DA. Predictors of miscarriage: a matched case-control study. Epidemiol Health. 2014;36:e2014031.
4. Eckert OL, Moore ED, Patton LD, Agnew JK, Eschenbach AD. Relationship of vaginal bacteria and inflammation with conception and early pregnancy loss following in-vitro fertilization. Infect Dis Obstet Gynecol. 2003;11:11–17.
5. Klebanoff SJ, Hillier SL, Eschenbach DA, et al. Control of the microbial flora of the vagina by H202-generating lactobacilli. J Infect Dis. 1991;164:94–100.
6. McGregor JA, French JI. BV in pregnancy. Obstet Gynecol Surv. 2000;55(5):1–19.
7. Nelson BD, Macones G. BV in Pregnancy: Current Findings and Future Directions. Epidemiol Rev. 2002;24(2):102-108.

8. CDC. Sexually transmitted diseases treatment guidelines. MMWR Recomm Rep. 2015;64(3).
9. Bennett J, Dolin R, Blaser M. Mandell, Douglas and Bennett's principles and practice of infectious diseases. 8th edition. Philadelphia, PA: Elsevier/Saunders; 2015.
10. Ralph SG, Rutherford AJ, Wilson JD. Influence of BV on conception and miscarriage in the first trimester: cohort study. BMJ. 1999 Jul 24;319(7204):220-3.
11. Oakeshott P, Hay P, Hay S, Steinke F, Rink E, Kerry S. Association between BV or chlamydial infection and miscarriage before 16 weeks' gestation: prospective community based cohort study. BMJ. 2002;325(7376):1334.
12. VanOostrum N, DeSutter P, Meys J, Verstraelen H. Risks associated with BV in infertility patients: a systematic review and meta-analysis. Hum Reprod. 2013;28(7):1809-15.
13. Delabaere A, Huchon C, Deffieux X, et al. Epidemiology of loss pregnancy. J Gynecol Obstet Biol Reprod (Paris). 2014 Nov 6;43(10):764-775.
14. Donders GG, Van Bulck B, Caudron J, Londers L, Vereecken A, Spitz B. Relationship of BV and mycoplasmas to the risk of spontaneous abortion. Am J Obstet Gynecol. 2000;183:431-7.
15. Doubilet PM, Benson CB, Bourne T, et al. Society of Radiologists in Ultrasound Multispecialty Panel on Early First Trimester Diagnosis of Miscarriage and Exclusion of a Viable Intrauterine Pregnancy, Barnhart KT, Benacerraf BR, Brown DL, Filly RA, Fox JC, Goldstein SR, Kendall JL, Lyons EA, Porter MB, Pretorius DH, Timor-Tritsch IE. Diagnostic criteria for nonviable pregnancy early in the first trimester. N Engl J Med. 2013;369(15):1443-51.
16. Romero R, et al. The composition and stability of the vaginal microbiota of normal pregnant women is different from that of non-pregnant women. Microbiome. 2014;2:4.
17. Lewis Felicia MT, et al. Vaginal Microbiome and Its Relationship to Behavior, Sexual Health, and Sexually Transmitted Diseases. Obstetrics & Gynecology. 2017;129.4:643-654.

UDC: 615.33.015.8:579.842.17
579.842.17.017.4:615.33

ORIGINAL ARTICLE

VANCOMYCIN RESISTANCE IN INVASIVE AND NON-INVASIVE STRAINS OF *Enterococcus faecium*

Jankoska Gordana, Trajkovska-Dokic E, Petrovska M, Cekovska Z, Kaftandzieva A, Labacevska L, Popovska K, Kotevska V, Panovski N
Institute of Microbiology and Parasitology, Faculty of Medicine, Ss Cyril and Methodius University of Skopje, Skopje, Republic of Macedonia

ABSTRACT

Background: Enterococci are known as cause of urinary tract infections, surgical wound infections, intra-abdominal infections and endocarditis. *Enterococcus faecalis* is isolated from approximately 80% of human enterococcal infections, and *Enterococcus faecium* from most of the remaining infections. Infections with other enterococcal species are rare. Enterococci are resistant to many antibiotics, but vancomycin resistant enterococci (VRE) are a therapeutic problem nowadays. Six different types of resistance are shown in enterococci, but Van-A, Van-B and Van-C have been seen in general clinical practice.

Objectives: The aim of this study was to detect VRE from different human specimens and to determine the genes of resistance in enterococci from urine samples.

Materials and methods: A total of 5340 strains of enterococci in a 6-year period were analyzed: 3616 were isolated from urine samples, 1724 were isolated from blood and wounds. The strains were isolated on Columbia blood agar, UTI chromogenic agar and VRE chromogenic agar. Identification was confirmed by Vitek automated system. Susceptibility was determined by standard disk diffusion method, Vitek system and E-test. The genes for resistance: *vanA, vanB* or *vanC* were detected by PCR only for urine isolated enterococci.

Results: A total of **7**1 (1.97%) strains out of 3616 from urine samples were determined as VRE. All VRE strains were identified as *Enterococcus faecium.* Not a single vancomycin resistant strain of *Enterococcus faecalis* has been isolated at the Institute of Microbiology and Parasitology, Medical Faculty, Skopje, during the 6-year period. The majority of these strains (63) were obtained from hospitalized patients. A total of 90 VRE strains (out of 1724 enterococci) were detected from blood and soft tissue infections. All were identified as *Enterococcus faecium*, except two VRE strains from blood cultures which were identified as *Enterococcus gallinarum*. The most common VRE isolates were obtained from patients hospitalized in the Surgical Intensive Care Units (32.2%). The resistance to vancomycin in all VRE strains was followed by resistance to many other antibiotics such as: penicillin, ampicillin, ciprofloxacin, norfloxacin, moxifloxacin, clindamycin, gentamycin, erythromycin and tetracycline. All strains were susceptible to linezolid and quinopristin/dalfopristin. Examination by PCR of 63 VRE strains (isolated from hospitalized patients) showed that 60 of urinary strains carried *vanA* gene and only 3 strains were *vanB* positive.

Conclusion: Vancomycin resistance was detected in *Enterococcus faecium* and *Enterococcus gallinarum.* All strains were susceptible to linezolid and quinopristin/dalfopristin. VanA resistance was the most frequently detected type of resistance in our isolates.

Keywords: antibiotic resistance, vancomycin, *Enterococcus faecium.*

INTRODUCTION

Enterococci are part of the normal flora that mostly colonize intestinal mucosa, but they could also be found in the urogenital and oral mucosa in humans. They are found in 10^5-10^7 organisms per gram of human feces [1]. Besides that, enterococci are opportunistic bacteria that can cause urinary tract infections, surgical wound infections, intra-abdominal infections and endocarditis [1,2]. Some studies have reported enterococci as a cause of infective endocarditis in 5%–20% of cases [3]. Enterococci are often isolated from patients with chronic and recurrent urinary tract infections, especially associated with morphological and functional abnormalities in the urinary tract [1,4,5]. Enterococci rank third among bacteria isolated in nosocomial urinary tract infection, which is associated with catheterization and instrumentation [1]. *E. faecalis* and *E. faecium* commonly colonize and infect humans in detectable numbers. *E. faecalis* is isolated from approximately 80% of human enterococcal infections, and *E. faecium* from most of the remaining infections. Human infections with other enterococcal species are rare.

Enterococcal infections are often a therapeutic problem due to their antimicrobial resistance. Enterococci are intrinsically resistant to many antibiotics, due to presence of chromosomal genes. Acquired resistance and virulence traits are usually transposon or plasmid encoded. Enterococci show different susceptibility to beta-lactams and quinolones, and high resistance to aminoglycoside, tetracycline and chloramphenicol [4]. *E. faecium* is less susceptible to ß-lactam antibiotics than *E. faecalis*, due to lower affinity of their penicillin-binding proteins for antibiotics [5]. The first VRE was reported in 1986 in France and United Kingdom, and after that VRE strains have been found in different countries [6].

There are six known different types of vancomycin resistance in enterococci: Van-A, Van-B, Van-C, Van-D, Van-E and Van-F. VanA vancomycin resistant strains (resistance to vancomycin and teicoplanin) and VanB strains (resistance to vancomycin alone) are the most common ones [1,6]. Van-C phenotype is susceptible to teicoplanin and only partly resistant to vancomycin. Inducible genes responsible for these phenotypes can be transferred from enterococci to other bacteria and spread resistance [7], for example to *Staphylococcus aureus.* Linezolid and teicoplanin are used to treat VRE if the stain shows sensitivity *in vitro* [2].

The aim of this study was to detect vancomycin resistant enterococci (VRE) from different human specimens and to determine the genes of resistance in enterococci isolated from urine samples.

MATERIALS AND METHODS

A total of 5340 strains of enterococci in a 6-year period (2011-2016) at the Institute of Microbiology and Parasitology, Medical Faculty, Skopje, were analyzed. The majority of the strains (3616) were isolated from urine samples. The other enterococcal strains, 1724, were isolated from blood (1496) and wounds (228) specimens.

The strains were isolated on Columbia blood agar, UTI chromogenic agar (Oxoid, UK) and VRE chromogenic agar (bioMèrieux). Identification and species differentiation was confirmed by Vitek automated system. Isolates were identified by morphology, color of the colonies on chromogenic agar and esculin reaction. Susceptibility was determined by standard disk diffusion method, Vitek system and E-test. The genes for resistance: *vanA, vanB* or *vanC* were detected by PCR only for urine isolated enterococci.

Standard disk diffusion on Müller Hinton agar was used for determination of susceptibility to: penicillin. ampicillin, vancomycin, erythomycin, gentamycin, ciprofloxacin, norfloxacin and nitrofurantoin. Vancomycin resistant strains were placed on VRE chromogenic agar. Identification and confirmation of resistance was finally done by Vitek system, using GP card for identification and GP 586 card for susceptibility of the strains.

E-test (BioMerieux) was also used for suspected VRE strains. The MIC value for VRE strains was more than 16 mg/l.

The genes for resistance, *vanA, vanB* or *vanC*, were detected by the conventional PCR only for the largest number of uro-isolated enterococci.

The following primers were used:	Product size (bp)
vanA	
5'-GGGAAAACGACAATTGC -3'	732
5'-GTACAATGCGGCCGTTA-3'	
vanB	
5'-ATGGGAAGCCGATAGTC-3'	635
5'- GATTTCGTTCCTCGACC-3'	
vanC1	
5'- GGTATCAAGGAAACCTC-3'	822
5'-CTTCCGCCATCATAGCT-3'	
vanC2/C3	
5'- CTCCTACGATTCTCTTG-3'	439
5'-CGAGCAAGACCTTTAAG-3'	

The reaction products were subjected to electrophoresis in 1.5% agarose gel. The size of the product determines the gene and type of resistance.

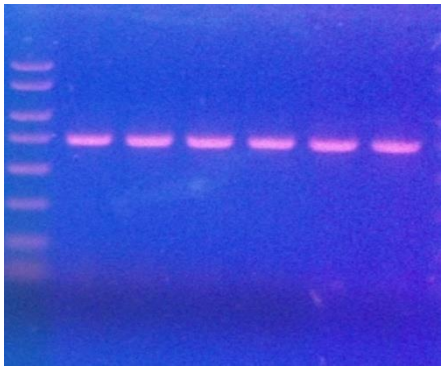


Fig. 1. Agarose gel electrophoresis showing positive amplification of 732 base fragments specific for vanA of VRE

RESULTS

A total of 5340 strains of enterococci in a 6-year period were isolated, 3616 isolates from urine samples and 1724 isolates from blood cultures and specimens of soft tissue infections (Table 1).

Table 1. The incidence of enterococcal isolates in five year period

Enterococci	Urine	Blood cultures, wounds	Total
VRE	**71**	**90**	**161**
VSE	3545	1634	5179
Total no. enterococci	3616	1724	5340

VRE were more often isolated from blood and wounds than urine samples, and this difference was statistically significant (χ^2, $p<0.001$).

VRE strains were isolated from patients hospitalized at the University clinics: Clinic for Infectious Diseases, Surgery Clinics, Nephrology Clinic, Hematology Clinic and Pediatric Clinic (Table 2).

Table 2. Origin of vancomycin resistant enterococci isolated from different specimens

Clinic	Urine	Blood cultures and wounds	Total VRE
Clinic for Infectious Diseases	19	7	26
Surgical Intensive Care Units	8	26	34
Surgery Clinics	18	29	47
Internal Diseases Clinics	9	12	21
Hematology Clinic	8	12	20
Pediatric Clinic	1	3	4
Primary Care Units	8	1	9
Total	**71**	**90**	**161**

The most common VRE strains were isolated from patients hospitalized in Surgery Clinics and Surgical Intensive Care Units. Only a small number of VRE were detected in outpatients, but in most of those cases it was related to their previous stay or intervention in a hospital (Figure 2).

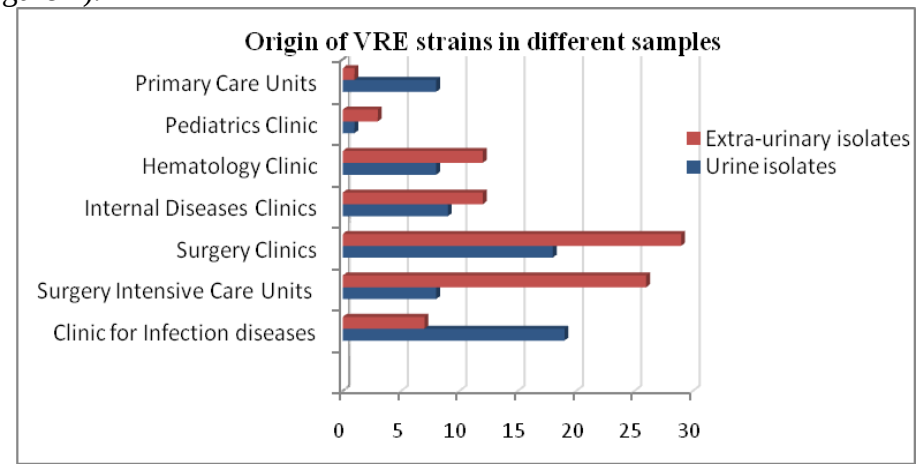


Fig. 2. Origin of VRE isolates in different specimens

All VRE strains were identified as *Enterococcus faecium*. Only two strains from blood cultures were identified as *Enterococcus gallinarum*.

The results from antibiotic susceptibility to antimicrobial agents are shown in Figure 3. The isolates were resistant to almost all examined antimicrobial agents. Susceptibility of 100% was shown only to linezolid and quinopristin/dalfopristin.

Some of the VRE isolates (12 strains from 71) from urine were susceptible to nitrofurantoin, which is in agreement with literature data (8).

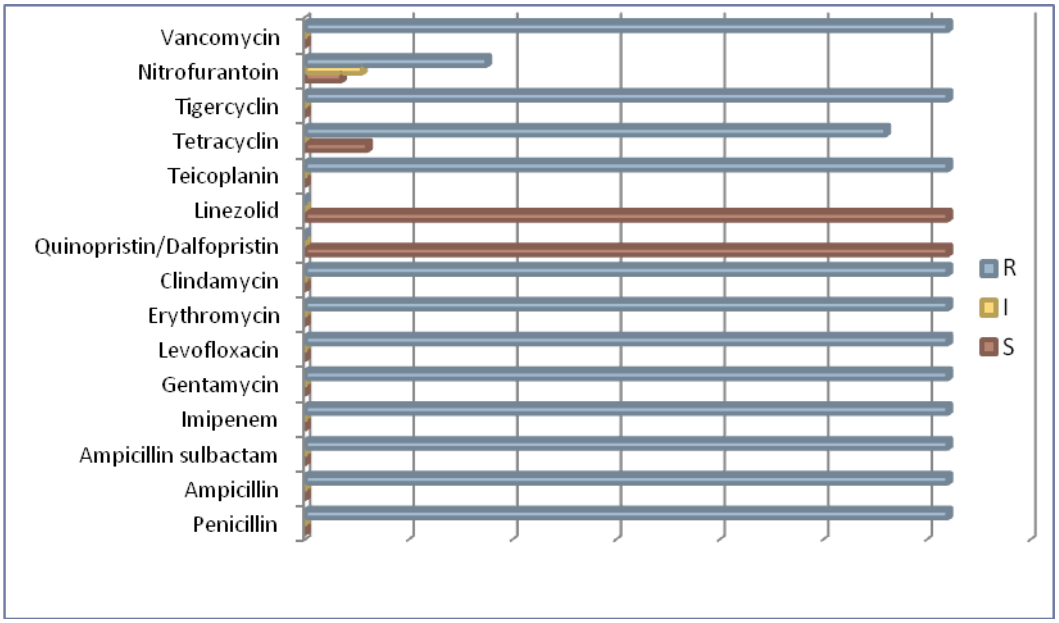


Fig. 3. Susceptibility of VRE isolates to antimicrobial agents

During the years the number of VRE has increased, but without statistical significance (χ^2, $p>0.05$).

The frequency of VRE isolation for six years (20011-2016) is shown in Figures 4 and 5.

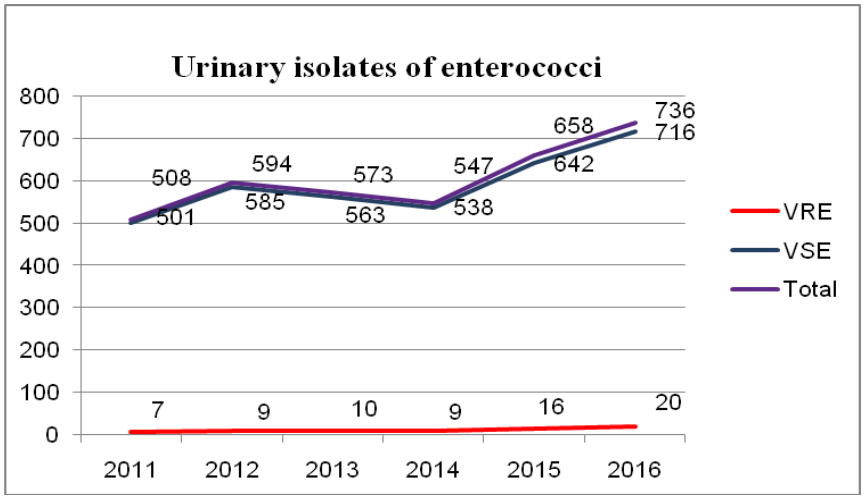


Fig. 4. Distribution of enterococcal urine isolates for six-year period

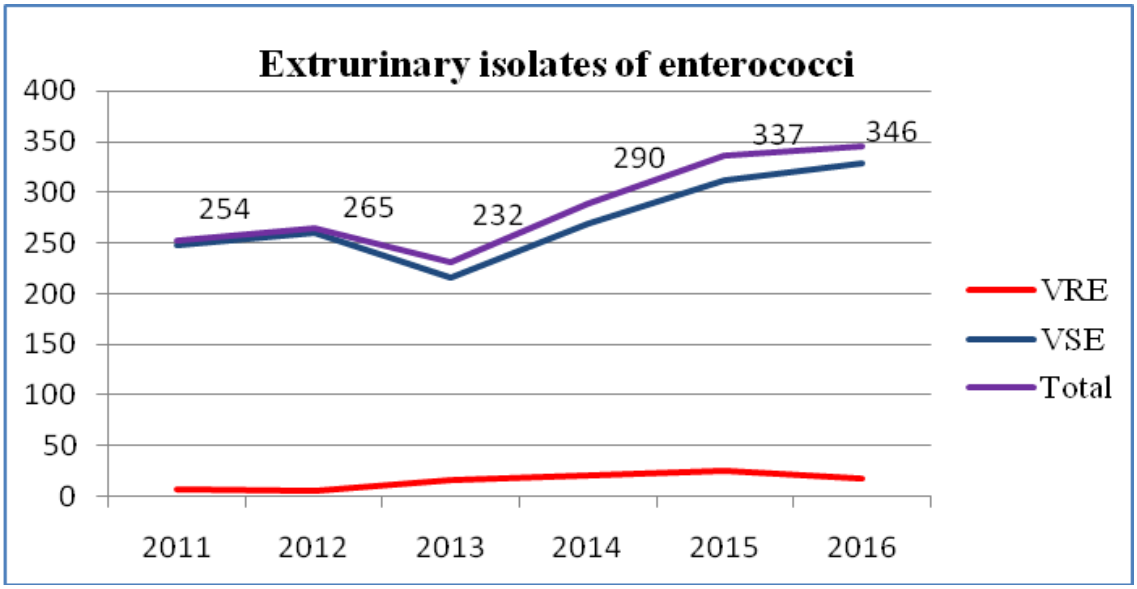


Fig. 5. Distribution of enterococcal isolates from blood and wounds for six year period

Detection of type of resistance was done for enterococci isolated from urine in hospitalized patients. The results are shown in Figure 6.

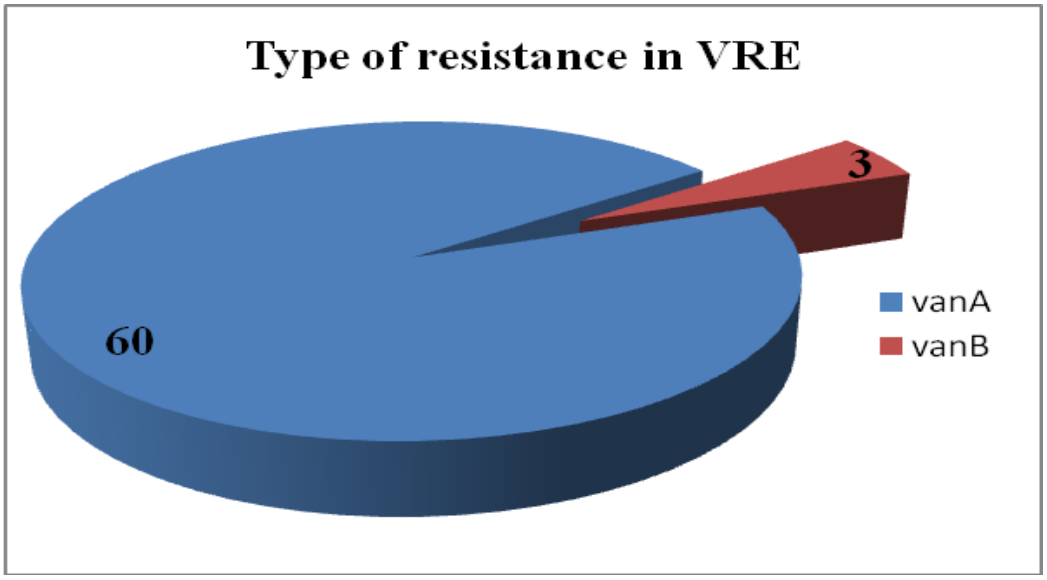


Fig. 6. The type of resistance in VRE strains from urine in hospitalized patients

Most of the strains were vanA positive, only 3 strains were vanB positive.

DISCUSSION

Vancomycin resistance in enterococci was first reported in Europe in the late 1980s [6]. This resistance is characterized by rapid spreading, limited treatment options and increased mortality rate in patients. The possibility of transferring genes from vancomycin resistant strains of enterococci to other bacteria such as *Staphylococcus aureus* is highlighted [7].

Studies in many countries reported vancomycin resistance in different species of *Enterococcus*. In this study we detected vancomycin resistance only in *Enterococcus faecium* and 2 stains *of Enterococcus gallinarum.*

The first strain of vancomycin resistant *Enterococcus faecium* at our Institute was isolated from urine sample of a hospitalized patient in 2008. In the past decade we have found VRE in different human samples, but more in invasive strains than in urine isolates, with a statistically significant difference. According to the report of the Resistance Surveillance Network (EARS-Net), the rate of VRE varied from 0% in Estonia to 42.7% in Ireland in 2013. The data of the CAESAR network, where our country is included, reported a very high percentage of VRE in our blood cultures. This resistance is probably higher, because the number of examined samples is very small and these isolates are often selected from hospitalized patients that do not respond to therapy.

The most often VRE strains in our specimens were isolatedfrom hospitalized patients. According to literature data enterococci are the most frequently isolated Gram- positive bacteria in nosocomial urinary tract infections. Several risk factors are responsible for developing a hospital VRE infection: close physical proximity to patients; a long period of hospitalization; use of broad spectrum of antibiotics; transplantation; co-morbidities; renal failure or hemodialysis; presence of a urinary catheter or instrumentation of the urinary tract [9]. Eradication of Gram-negative bacteria of the gut microbiota by antibiotic treatment is of great importance for colonization with VRE and an increased possibility of transmission to patients in hospital environment.

VRE strains are not resistant only to vancomycin, but they show resistance to many other antimicrobials. Our VRE isolates were all susceptible only to linezolid and quinopristin/dalfopristin. Some studies reported linezolid resistance in VRE isolated from patients in the intensive care units. Linezolid is commonly used to treat VRE bacteriemia, for example in the USA [2, 10], but in our country this antimicrobial is not yet widely used. The other examined antimicrobials (Figure 3) showed no activity. Some of the uro-isolated VRE were susceptible to nitrofurantoin. The sensitivity of VRE strains to nitrofurantoin was reported by Zhanel G [8].

During the past decade the number of VRE in our specimens was increased, but without statistically significant difference for the analyzed period.

Distribution of phenotypes of vancomycin-resistant enterococci is different, depending on the country, but VanA is predominant [6]. Our results are in agreement with these data; vanA was present in most of uro-isolated enterococci.

CONCLUSIONS

- Vancomycin resistance was detected only in *Enterococcus faecium* and *Enterococcus gallinarum* isolated from human samples
- All examined strains of vancomycin resistant *Enterococcus faecium* were susceptible to linezolid and quinopristin/dalfopristin
- VanA resistance was the most commonly detected type of resistance in our isolates from urine samples (61 of 63 tested strains)
- VanB resistance was detected in only two vancomycin resistant *Enterococcus faecium* isolated from urine samples

REFERENCES

1. Hancock LE, Gilmore MS. Pathogenicity of Enterococci. Gram-Positive Pathogens. Washington, D.C: ASM Publications; 2001.

2. Balli EP, Venetis CA, Miyakis S. Systematic Review and Meta-Analysis of Linezolid versus Daptomycin for Treatment of Vancomycin-Resistant Enterococcal Bacteremia. Antimicrob Agents Chemother. 2014; 58(2): 734–739.

3. Hill EE, Herijgers P, Claus P, Vanderschueren S, Herregods MC, Peetermans WE. Infective endocarditis: changing epidemiology and predictors of 6-month mortality: a prospective cohort study. Eur Heart J. 2007;28(2):196–203.
4. Jankoska G, Trajkovska-Dokic E, Panovski N, Popovska-Jovanovska K, Petrovska M. Virulence factors and antibiotic resistance in Enterococcus faecalis isolated from urine samples. Contributions. 2008. Jul;29(1):57-66.
5. Hill DM, Wood GC, Hickerson WL. Linezolid bladder irrigation as adjunctive treatment for a vancomycin-resistant *Enterococcus faecium* catheter-associated urinary tract infection. Ann Pharmacother. 2015;49(2):250–253.
6. Cetinkaya Y, Falk P, Mayhall CG. Vancomycin-resistant enterococci. Clin Microbiol Rev. 2000;13:686–707.
7. Walters MS, Eggers P, Albrecht V, et al. Vancomycin-Resistant *Staphylococcus aureus.* Delaware. MMWR Morb Mortal Wkly Rep. 2015; 64:1056.
8. Zhanel G**,** H**o**ban JD, Karlowsky JA. Nitrofurantoin Is Active against Vancomycin-Resistant Enterococci. Antimicrob Agents Chemother. 2001;45(1): 1324-326.
9. Sydnor ER, Perl TM. Hospital epidemiology and infection control in acute-care settings. Clin Microbiol Rev. 2011;24:141–173.
10. Bonora MG, Solbiati M, Stepan E, Zorzi A, Luzzani A, Catania MR, Fontana F. Emergence of Linezolid Resistance in the Vancomycin-Resistant *Enterococcus faecium* Multilocus Sequence Typing C1 Epidemic Lineage. J Clin Microbiol. 2006;44:1153–1155.

UDC: 616.133.33-007.64-089.844

ORIGINAL ARTICLE

ENDOVASCULAR TREATMENT OF INTRACRANIAL ANEURYSM ON ANTERIOR CIRCULATION

Lazareska Menka[1], Aliji V[1], Jakimovska Dimitrovska M[1], Businovska J[2], Janevski P[1], Daskalov D[1], Kostov M[3], Papazova M[4]

[1]University Clinic of Radiology, Skopje, Republic of Macedonia; [2] Univeristy Clinic of Anesthesiology, Resuscitation and Intensive Care, Skopje, Republic of Macedonia; [3]University Clinic for Neurosurgery, Skopje, Republic of Macedonia; [4]Institute of Anatomy, Faculty of Medicine, Ss Cyril and Methodius University of Skopje, Skopje, Republic of Macedonia;

ABSTRACT

Intracranial aneurysms are abnormal dilations of the intracranial vessels at the weak spot of arterial wall. Rupture of intracranial aneurysm can cause intracerebral hematoma, intraventricular hemorrhage, rarely subdural hematoma. Evaluation of effectiveness of endovascular treatment of intracranial aneurysm as minimal invasive treatment and assessment of result after endovascular treatment for complete occlusion, residual neck or residual aneurysm.

The study population included 57 patients referred to the University Clinic of Radiology in Skopje, R. Macedonia for endovascular treatment during the period January 2015 to May 2018. This study included 32 females and 25 males, ranging in age from 25 to 74 years.

From total 63 treated IA 33 were ruptured and 30 unruptured, 7 patients was with multiple aneurysm, 5 with 2 and 2 with 3 aneurysms. In this study 34 aneurysms were treated with coiling, 7 aneurysms with stent, 2 aneurysms with flow diverter and complex aneurysms with combined technique, 3 with balloon assisted coiling, 13 with stent assisted coiling and 2 with flow diverter assisted coiling. Two patients with giant aneurysms were treated with occlusion of parent artery after positive balloon occlusive test.

Endovascular therapy is a minimally invasive procedure since it's associated with less risk of bad outcomes, shorter hospital stays and shorter recovery times compared with surgery.

Keywords: intracranial aneurysm, coiling, intracranial stent**,** flow diverter, endovascular treatment

INTRODUCION

Intracranial aneurysms (IA) are abnormal dilations of the intracranial vessels at the weak spot of arterial wall. Usually they arise at the arterial bifurcations, but may arise from the lateral wall or at branch points of larger vessels, rarely can occur at the origin of small perforators. Most of the intracranial aneurysm are true aneurysm and vast majority are saccular or berry aneurysm, although it can be fusiform, mycotic, dissecting aneurysm or side wall-blood blister.

Spontaneous rupture of IA typically results in subarachnoid haemorrhage (SAH) and 10 % of patients die before reaching the hospital. Rupture of IA can cause intracerebral hematoma, intraventricular hemorrhage rarely subdural hematoma. Unruptured can be symptomatic with mass effect of brain stem or causing hydrocephalus, but usually symptoms of cranial nerves dysfunction or

palsy. The best available data suggest that previously unruptured aneurysms carry a risk of hemorrhage of about 1-2 % per year, depending on size, location and other risk factors. The presence of multiple aneurysms and a family history of subarachnoid haemorrhage also raise the risk of rupture. The primary goal of treatment of cerebral aneurysms is to prevent future rupture. Rupture of an intracranial aneurysm is a diagnostic and therapeutic emergency, which requires multidisciplinary management involving neuroradiologists, neurosurgeons and neurology intensive care physicians [1]. Multiple aneurysms and a family history of subarachnoid hemorrhage increse the risk of rupture. An untreated ruptured aneurysm is at high risk of rebleeding [2, 3]. This new rupture may occur very early or several days later. Risk increases over time and the cumulative risk at 4 weeks is 40% [3]. Rebleeding is associated with a poor prognosis and high morbidity and mortality [3]. In the ISAT trial, 59% of patients who suffered an early rebleed died [4]. According to current guidelines, the aneurysm should be excluded promptly, within 72 hours and if possible within 48 hours.

Endosaccular coil embolization was introduced in 1991 by Guglielmi detachable coil (GDC), as minimally invasive technique in the treatment of IA [5, 6]. Coil embolization of very wide-necked or so called complex IA is technically challenging since the coils may protrude from the aneurysm into the parent artery. Intracranial stents, initially launched in 2002, were developed to provide additional support and remodeling of the aneurysm neck, and the concept of parent artery reconstruction was further advanced with flow-diverting stents introduced in 2008 [7- 12]. Goal of endovascular treatment (EVT) is complete exclusion of the aneurysm from the flow of blood.

This technique has gradually overtaken surgical clipping as the first line management of a ruptured aneurysm in light of the results of the International Subarachnoid Aneurysm Trial (ISAT), which compared the two techniques [4, 13, 14]. The indications for surgery are primarily limited to the treatment of ruptured aneurysms causing compressive hematomas, which need to be evacuated. The other main indication, aneurysms which are not accessible to endovascular treatment, is uncommon. The relative risk of death or significant disability at one year for patients treated with coils was 22.6 % lower than in surgically-treated patients. The ISAT trial is the only randomized multicenter international trial that has compared surgery to endovascular coiling for ruptured intracranial aneurysms. Its results supported endovascular treatment and showed a significant reduction in the risk of dependency or death at 1 year [4].

Endovascular techniques can be divided into: parent artery reconstruction with coil deposition (primary coil, balloon-assisted coiling, stent-assisted coiling, and other new techniques such as neck reconstruction devices and intraluminal occlusion devices); reconstruction with flow diversion (FD); and deconstructive techniques with involving parent artery sacrifice with or without bypass. Currently, EVT is considered to be the first line treatment for intracranial aneurysms by most groups.

Aim

Evaluation of effectiveness of EVT of IA as minimal invasive treatment and assessment of results after EVT for complete occlusion, residual neck or residual aneurysm.

Analysis of rate of complication of EVT of aneurysms.

MATERIALS AND METHODS

The study population included 57 patients referred to the University Clinic of Radiology in Skopje, R. Macedonia for EVT during the period January 2015 to May 2018. This study included 32 females and 25 males, ranging in age from 25 to 74 years.

In the study 57 patient were included for EVT of IA on anterior circulation where 7 patients were with multiple aneurysm. In total 63 aneurysms were treated. All interventions were done on Digital subtraction angiography machine Shimatzu at University Clinic of Radiology in Skopje, R. Macedonia.

Materials used in minimal invasive EVT of IA are: Introducer short 11 cm and long 90-110 cm, 5 or 6 F; diagnostic catheters (vertebral, simpson 1, 2, 3); guidewires 25-35 strait or angled tip with different softness; guiding catheters 5-6 F with different tip; intermediate catheter for distal navigation and support; microcatheters 010-028''; microwire 010-014" and platinum coils - spirals with different length, diameter of spiral in 2D and 3D and different softness, coated with hydrogel or other expanding materials. For wide-neck and complex aneurysms intracranial stents; balloons for occlusion and remodeling; flow diverters which are stents with smaller gaps in the mesh, device for flow diversion of blood without occlusion of perforators, but occlusion of aneurysmal sac; Inform consent for this tape of treatment from patient or family.

Anesthesiology and Neurosurgery evaluation; blood test, hemostasis, aPTT and aggregation test if stent or flow diverter is planed with previous preparation 1-5 days double antiplatelet therapy.

Depending of clinic of the patient (ruptured or unruptured) most of the interventions in the last three years were done in IV anesthesia and small number in general anesthesia. First step is in 98% femoral access (puncture of one and rare two femoral artery); rarely radial access in situation (exp. Leriche syndrom or aortic-bifemoral bypass) and placing short introducer-sheath, sometimes long sheath for support.

Diagnostic catheter, guidewire and exchange with guide catheter witch is placed in ICA extra cranial. Continuous flash of guiding (saline plus 5000 IE heparin drop by drop).

Introducing the micro catheter together with micro guidewire with shaping of the tip or pre-shaped, same on contiuous flash. After placing the tip of the microcatheter in the aneurismal sac, microwire is removed and coiling starts, first with 3D or framing coil and after smaller than first one usually helical 2D or 3D from normal to soft and super soft. The goal is to achieve dense packing of the sac without remnant neck or inter-coil gap fillings with contrast.

If there is a need of balloon assistance, balloon catheter is introduced through same guide catheter positioned on the neck of the aneurysm inflated in the moment of placing and packing the coils.

In stent assisted coiling two techniques are available: placing the micro catheter in the sac and another distal in parent artery with stent that is half opened over the neck, then coiling and full deploying of stent after removing the first micro catheter; the other technique is placing and full detachment of stent and throughout gap of stent wall, placing the micro catheter with guidewire in the sac and coiling the sac.

While using the flow diverters for support and specific deployment, application of intermediated catheter is needed for distal access and stability.

If there is a sufficient cross filling from Willis circle or anastomosis after balloon occlusion test of 30 minutes without neurological deficit usually in giant aneurysms, proximal occlusion of parent artery is recommended treatment.

RESULTS

From total of 63 treated IA, 33 aneurysms were ruptured and 30 aneurysms were unruptured, 7 patients were with multiple aneurysm, 5 patients were with 2 and 2 patients with 3 aneurysms. In this study 56.1% were female and 43.9% were male patients. The diameter of the aneurysms was in range of 3 mm to more than 25 mm.

According to the location, the aneurysms in this study on anterior circulation were divided: on internal carotid artery (ICA) segment C4/C5 (cavernous and clinoid segment) 9 aneurysm were found (14.3%); on ophthalmic C6 segment there were 9 aneurysms (14.3%); on communicating segment C7/T there were 14 aneurysms (22.2%); MCA/M1/M2/MCA bifurcation/trifurcation were 16 (25.3%); Acom/pericallosal complex 15 aneurysms were found (23.8%).

Table 1. Type of applied EVT techniques

Coils	**Stent**	**Stent and coils**	**Flow diverter**	**Flow diverter and coils**	**Balloon assisted coiling**	**Occlusion of parent artery**
34 (53.9%)	**7 (11.1%)**	**13 (20.6)**	**2 (3.17%)**	**2 (3.17%)**	**3 (4.76%)**	**2 (3.17%)**

Table 2. Grading of the occlusion of the aneurismal sac

Total occlusion	**Residual neck**	**Residual aneurysm**		**Left to thrombose/stasis**		**Previously threated-recurrence**
		Internal gap	Along the wall	Coil	Stent/FD	
39 (61.9%)	**6 (9.52%)**	**4 (6.34%)**	**1 (1.58%)**	**3 (4.76%)**	**6 (9.52%)**	**4 (6.34%)**

We have obtained good results in treated aneurysms with complication rate equivalent to world published numbers: morbidity 3-10% and mortality 1-2%. Complications during procedure are the following: thrombemboliy in 3 patients resolved, in 2 patients with tirofiban and in 1 patient with thrombectomy - solitere without disability but in one small distal infarction out of motor zone. Angiospasm was found in 5 patients and it was resolved spontaneously or with nimodipine. Rupture was found in 1 patient and instent thrombosis in 2 patients, but with sufficient cross filling. Migration in 3 patients: coil-catch with microsnare, one stent and one FD migration in aneurysmal sac. Complications after procedure: in 2 patients with neurological deficit due to infarction and 1 patient died because of rupture, hydrocephalus and diffuse angiospasm. Two of the patients with complication were with previous ruptured aneurysm.

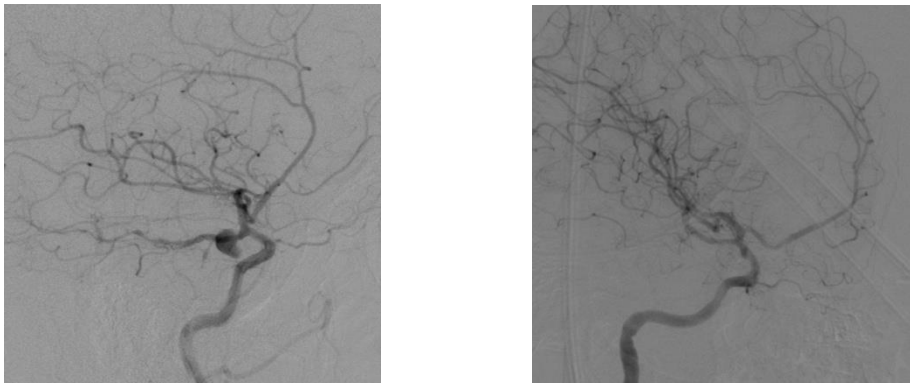


Fig. 1. Ruptured aneurysm of right Pcom(semifetal PCA) treated with coils-complete occlusion

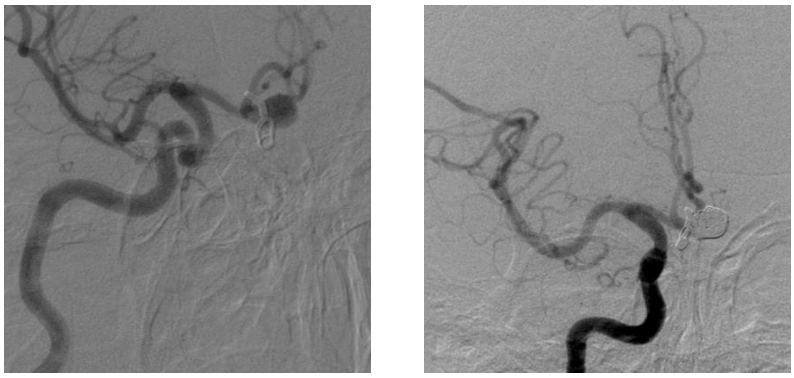


Fig. 2. Clipped right Acom aneurysm aa20 recurrence, treated with coils- total occlusion

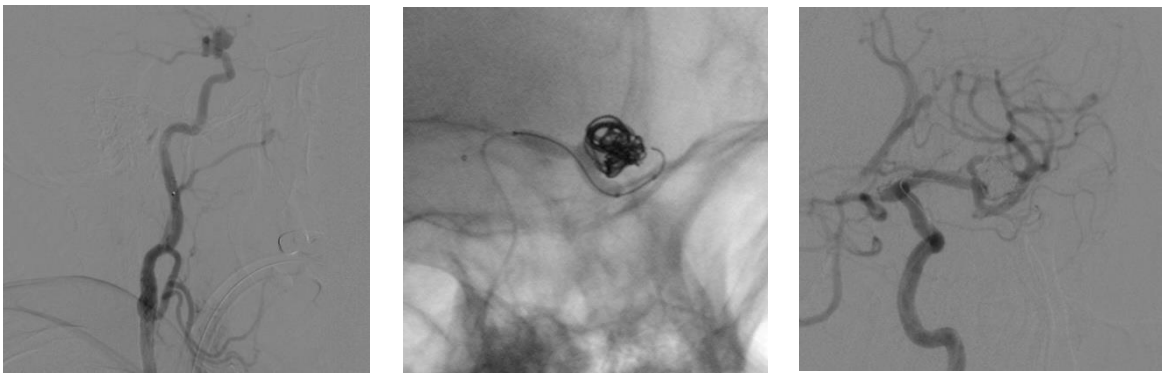


Fig. 3. Carotid stenting and Aneurysm of left MCA bifurcation treated with baloon assisted coiling-residual neck

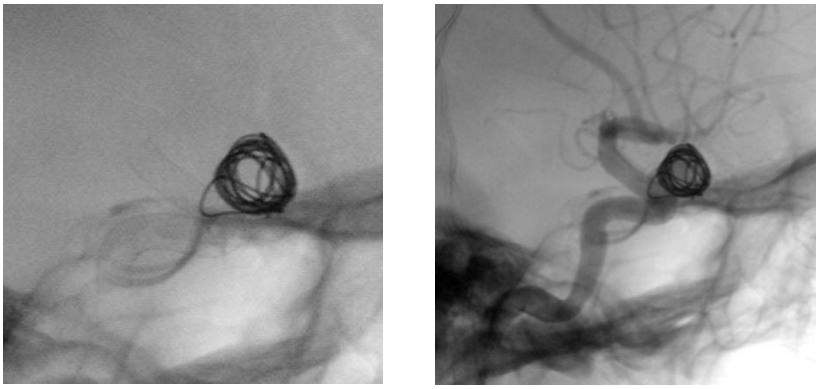


Fig. 4. Unruptured aneurysm of right ophthalmic segment treated with flow diverter and coils-left to thrombose

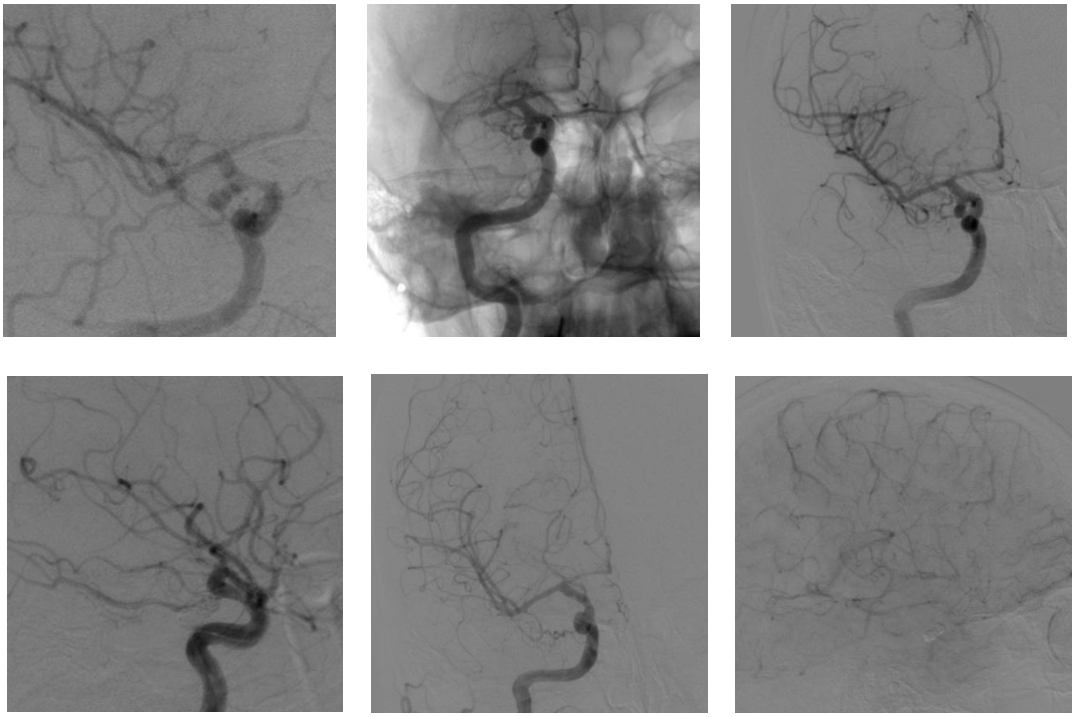


Fig. 5. Ruptured right Pcom aneurysm treated with coiling: thromboembolism of distal M1 with recanalisation; continued coiling-total occlusion; venous faze-distal emboli M3/M4 without neurological deficit

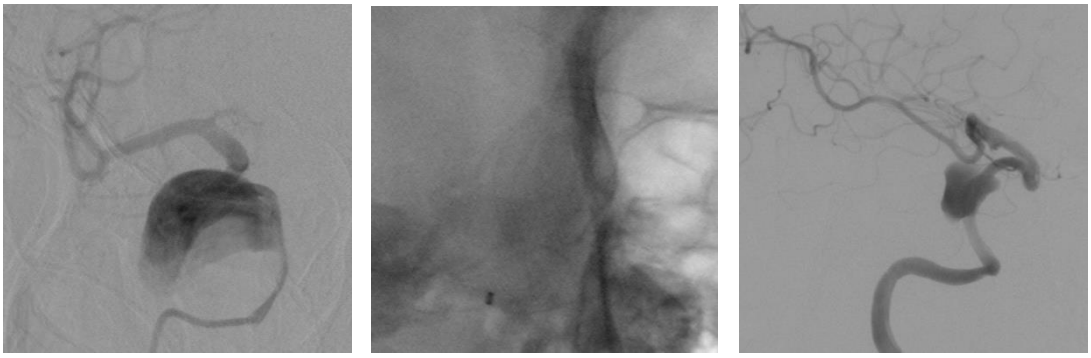


Fig. 6. Unruptured Giant aneurysm on right ICA, cavernous segment treated with flow diverter- sac stasis

Fig. 7. Ruptured aneurysm of Acom from right ICA treated with stent- total occlusion

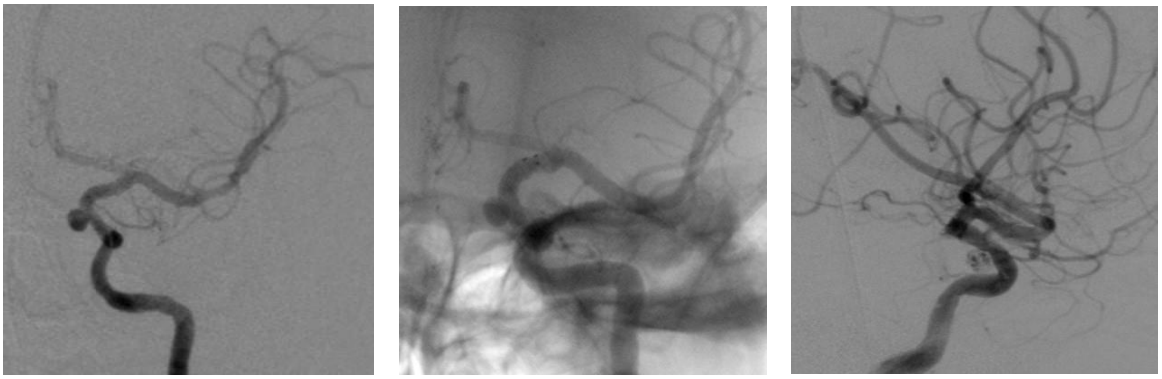


Fig. 7a. Unruptured aneurysm of left ICA treated with stent and coiling- left to thrombose

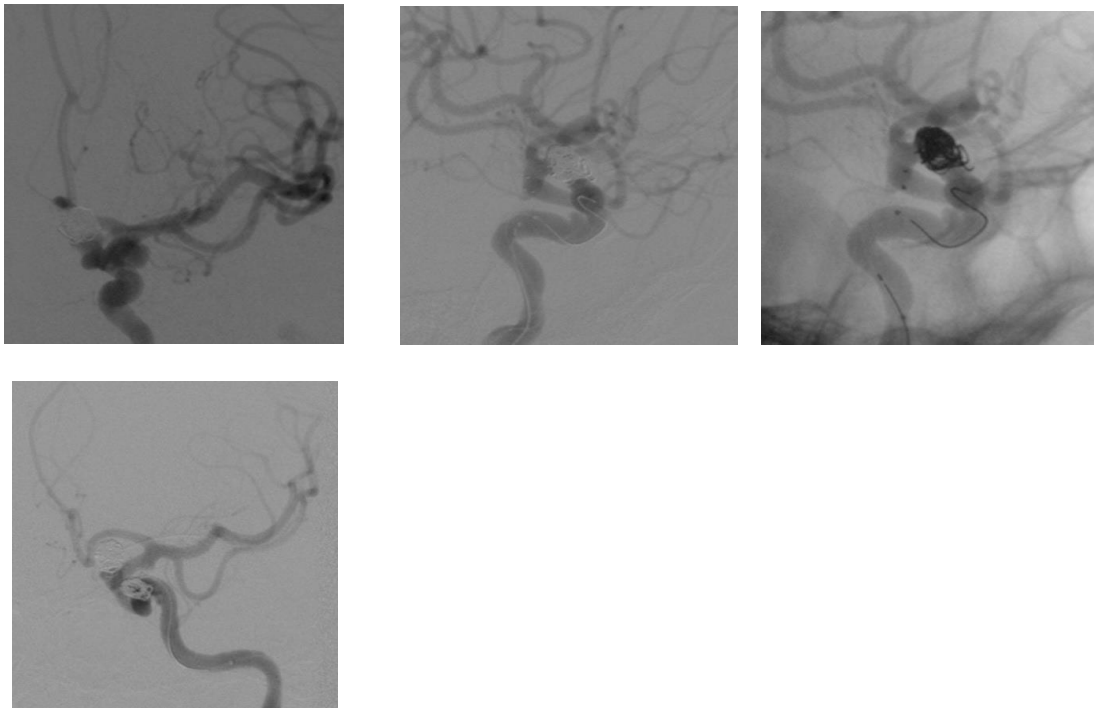


Fig. 8. Residual neck after coiling of ruptured ophthalmic artery and two unruptured aneurysms of left ICA treated with stent and bigger one plus coils- left to thrombose

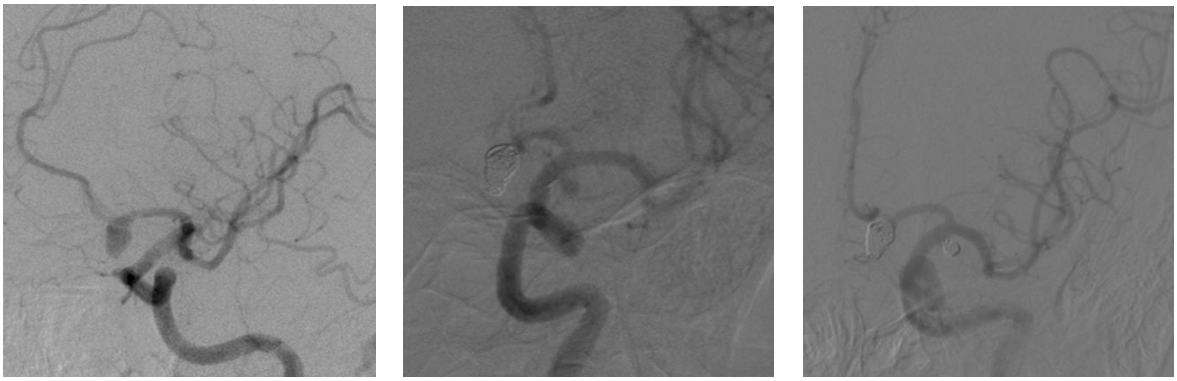


Fig. 9. Ruptured aneurysm of left Acom and C7 unruptured aneurysm, both treated with coiling-total occlusion

DISCUSSION

Approximately 90% of IA arise from the anterior circulation, and 15-30% of these patients have multiple aneurysms. Only 10% of the aneurysms are located on posterior circulation [1].

IA on anterior circulation are 90%, ACA/Acom complex- 30-40%; supraclioid ICA and ICA/Pcom junction-30%; MCA(M1/M2)-20-30%. Five year cumulative risk of rupture of anterior circulation aneurysms: < 7 mm - 0%; 7-12 mm - 2,6%; 13-24 mm - 14,5%; > 25 mm - 40% [13].

Once an aneurysm has ruptured, the chance of re-bleeding dramatically increases. Greatest risk to life is aneurysm re-bleeding although cerebral vasospasm makes a significant contribution to overall morbidity and mortality. Management of small aneurysms is controversial. Less than 7 mm in maximal diameter aneurysms are statistically unlikely to rupture, however there are numerous patients with small aneurysms which have ruptured resulting in subarachnoid haemorrhage. It depends of location too, for example aneurysm at the branching of communication arteries and posterior circulation has greatest risk of rupture. According to the dimensions, IA larger then 25 mm in max diameter are called giant aneurysm. Risk of rupture also depends of the form, lobulation or presence of baby aneurysm, focal outpouching at any weak point of sac usually at dome [13, 14, 15].

The Raymond–Roy occlusion classification (RROC) is an angiographic classification scheme for grading the occlusion of endovascularly treated intracranial aneurysms [16]. It is also known as the Raymond class, Montreal scale or the Raymond Montreal scale. In this scheme class I is defined as complete obliteration, class II as residual neck and class III as residual aneurysm. The scheme was originally created to evaluate aneurysm occlusion class, and not to predict aneurysmal recurrence.

Class III aneurysms exhibit heterogeneous behavior, with one subset likely to occlude over time and another likely to remain patent or to grow. Mascitelli et al. in 2015 proposed a Modified Raymond–Roy Classification (MMRC) or modified Montreal scale, where class III is subdivided to reflect progression to occlusion: class IIIa: contrast opacification within the coil interstices of a residual aneurysm and class IIIb: contrast opacification outside the coil interstices, along the residual aneurysm wall. Mascitelli et al.'s study found that class IIIa aneurysms progress to complete occlusion more than class IIIb aneurysms [16, 17].

A validation study by Stapleton et al. confirmed that the predictive capability of the RROC was improved by the MMRC, showing not only that IIIa occluded more often (53.6% vs 19.2%) but that IIIb lesions would also further recanalize more frequently (65.1% vs 27.4%) [16, 17].

The two main complications of EVT are thromboembolic complications and aneurysm rupture during treatment. Procedural morbidity ranges between 3.7 - 10% and mortality between 0 - 2.1%.

CONCLUSION

Endovascular therapy is a minimally invasive procedure that accesses the treatment area from within the blood vessel. This study provides compelling evidence that, if medically possible, all patients with ruptured brain aneurysms should receive an endovascular consultation as part of the protocol for the treatment of brain aneurysms.

Endovascular coiling of aneurysms is associated with less risk of bad outcomes, shorter hospital stays and shorter recovery times compared with surgery.

REFERENCES

1. Takahashi S. Neurovascular Imaging, MRI & Microangiography. Springer Verlag. (2010) ISBN:1848821336.
2. Bederson JB, Connolly Jr ES, Batjer HH, Dacey RG, Dion JE, Diringer MN, et al. Guidelines for the management of aneurysmal subarachnoid hemorrhage: a statement for healthcare professionals from a special writing group of the Stroke Council, American Heart Association. Stroke. 2009;40(3):994-1025.
3. van Gijn J, Kerr RS, Rinkel GJ. Subarachnoid haemorrhage. Lancet. 2007;369(9558):306-18.

4. Molyneux AJ, Kerr RS, Yu LM, Clarke M, Sneade M, Yarnold JA, Sandercock P; International Subarachnoid Aneurysm Trial (ISAT) Collaborative Group. International subarachnoid aneurysm trial (ISAT) of neurosurgical clipping versus endovascular coiling in 2143 patients with ruptured intracranial aneurysms: a randomized comparison of effects on survival, dependency, seizures, rebleeding, subgroups, and aneurysm occlusion. Lancet. 2005;366(9488):809-17.
5. Guglielmi G, Vinuela F, Sepetka I, Macellari V. Electrothrombosis of saccular aneurysms via endovascular approach. Part 1: electrochemical basis, technique, and experimental results. J Neurosurg. 1991;75(1):1-7.
6. Solander S, Ulhoa A, Vinuela F, Duckwiler GR, Gobin YP, Martin NA, Frazee JG, Guglielmi G. Endovascular treatment of multiple intracranial aneurysms by using Guglielmi detachable coils. J Neurosurg. 1999;90(5):857-64.
7. Guglielmi G, Vinuela F, Dion J, Duckwiler G. Electrothrombosis of saccular aneurysms via endovascular approach. Part 2: preliminary clinical experience. J Neurosurg. 1991;75(1):8-14.
8. Wakhloo AK, Lanzino G, Lieber BB, Hopkins LN. Stents for intracranial aneurysms: the beginning of a new endovascular era? Neurosurgery. 1998;43(2):377–9.
9. Piotin M, Blanc R, Spelle L, Mounayer C, Piantino R, Schmidt PJ, Moret J. Stent-assisted coiling of intracranial aneurysms: clinical and angiographic results in 216 consecutive aneurysms. Stroke.2010;41(1):110–5.
10. Shapiro M, Becske T, Sahlein D, Babb J, Nelson PK. Stent-supported aneurysm coiling: a literature survey of treatment and follow-up. AJNR Am J Neuroradiol. 2012; 33(1):159-63.
11. Lee SJ, Cho YD, Kang HS, Kim JE, Han MH. Coil embolization using the self-expandable closed-cell stent for intracranial saccular aneurysm: a single-center experience of 289 consecutive aneurysms. Clin Radiol. 2013;68(3):256–63.
12. Szikora I, Berentei Z, Kulcsar Z, Marosfoi M, Vajda ZS, Lee W, et al. Treatment of intracranial aneurysms by functional reconstruction of the parent artery: the Budapest experience with the pipeline embolization device. AJNR Am J Neuroradiol. 2010; 31(6):1139-47.
13. Becske T, Kallmes DF, Saatci I, McDougall CG, Szikora I, Lanzino G, et al. Pipeline for uncoilable or failed aneurysms: results from a multicenter clinical trial. Radiology. 2013;267(3):858-68.
14. Wiebers DO, Whisnant JP, Huston J 3rd, Meissner I, Brown RD Jr, Piepgras DG, et al. Unruptured intracranial aneurysms: natural history, clinical outcome, and risks of surgical and endovascular treatment. Lancet. 2003;362(9378):103-10.
15. Molyneux AJ, Birks J, Clarke A, Sneade M, Kerr RS. The durability of endovascular coiling versus neurosurgical clipping of ruptured cerebral aneurysms: 18-year follow-up of the UK cohort of the International subarachnoid aneurysm trial (ISAT). Lancet 2015;385(9969):691-7.
16. Mascitelli JR, Moyle H, Oermann EK, Polykarpou MF, Patel AA, Doshi AH, Gologorski Y, et al. An update to the Raymond-Roy occlusion classification of intracranial aneurysms treated with coil embolization. J Neurointerv Surg. 2015;7(7): 496-502.
17. Stapleton CJ, Torok CM, Rabinov JD, Walcott BP, Mascitelli JR, Leslie-Mazwi TM, et al. Validation of the Modified Raymond-Roy classification for intracranial aneurysms treated with coil embolization. J Neurointerv Surg. 2016;8(9):927-33.

UDC: 616.12-089-06-053.2(497.7)"2013/2015"

ORIGINAL ARTICLE

THE MOST COMMON COMPLICATIONS IN IINTENSIVE CARE UNIT DEPARTMENT AFTER CARDIAC SURGERY IN CHILDREN

Hristina Mandzukovska
University Clinic for Children's Diseases, Skopje, Republic of Macedonia

ABSTRACT

Background: Having regard all the progress in the field of pediatrics, nowadays heart surgery can be performed with excellent outcomes. Serious complications can cause high morbidity and mortality rates. This study has been performed in order to determine the incidence of morbidity and mortality.

Materials and methods: During 2013-2015, the retrospective study was performed with 65 patients undergoing surgery for congenital heart disease which was reviewed for incidence of complications. We used the database of University Clinic for Children's Diseases and University Clinic of Pediatric Surgery in Skopje.

Results: 33 children with complications, have been divided into 2 groups cardiac and extra cardiac complications with mean age of 6,5 ± 0,35 (rang 0,5 - 108 months). The first group included SVT (6,25 %), AV-block (6,25 %), pacing (6,25 %) and pericarditis (9,3 %). In the extra cardiac complications, the most common was bleeding (12,5 %), renal failure (9,3 %), chylothorax (9,3 %), seizures (9,3 %), reintubation after surgery (15.6 %), sepsis (12,5 %) and severe pneumonia (6,25 %). The duration of CPB was 68,32 ± 31,9 minutes, the duration of MV was 2,04 ± 2,42 days, and ICU stay was 3,90 ± 3,45. The incidence of mortality was 9,2 % whereby the most common was sepsis, reoperations for previous lung chronic diseases and renal failure.

Conclusion: Prolonged duration of CPB, duration of MV and ICU stay, are significant risk factors for the increased number of cardiac and extra cardiac complications. It is necessary to apply the measures and careful monitoring of patients to minimize these effects.

Keywords: congenital heart disease, cardiac surgery, complications

INTRODUCTION

The field of pediatric and neonatal cardiac surgery has witnessed major advances in the last 30 years [1,2]. Most of the surgical centers in the world have reported excellent outcomes for neonatal cardiac surgery. This has become possible because of the technologic refinements and development of devices, improved multidisciplinary expertise, and advances in the understanding of the pathophysiology and natural history of CHD [3].

Congenital cardiac diseases are the second most important cause of childhood morbidity and mortality. The incidence is about 5-6 % in the largest cardiac surgery centers. In our country, during cardiac surgery missions the incidence of mortality was 9,2 %. The most common mortality identified was sepsis, than, previous lung chronic diseases, renal failure etc. Postoperative cardiac and extra cardiac complications in pediatric cardiac surgery have been reported and contribute to morbidity and mortality, duration of cardiopulmonary bypass (CPB), duration of mechanical conventional ventilation (MV), duration of intensive care unit stay (ICU stay), cost, and quality of life after pediatric cardiac surgery [4,5].

One of the main procedures during an open-heart surgery is the use of cardiopulmonary bypass (CPB). The safety of cardiopulmonary bypass has improved significantly over the years. Besides it, the major risk itself includes inadequate perfusion of organs or tissues, activation of

a systemic inflammatory response, and embolisation of air or particles. Especially, concerning is potential of embolisation to the brain, but this risk should be quite low - less than one percent in most cases. Activation of inflammatory response causes cardiac and extra cardiac complications with reduce of cardiac output, atrioventricular node block, miocarditis, arrhythmias (requiring temporally or permanent pacing), than lung complications, worsening of renal and liver failure, sepsis, bleeding, vascular damage, neurological complications such as seizures and loss of consciousness, need of reintubation after surgery etc [6,7].

The purpose of our study was to confirm the complications in the postoperative period and to study the correlation of cardiopulmonary bypass (CPB), the duration of mechanical ventilation, postoperative ICU stay, when these complications occure.

MATERIALS AND METHODS

In the retrospective study of 65 children with an age range of 6,5 ± 0,35 (rang 0,5 - 108 months) were reviewed from complications which occurred after heart surgery in congenital diseases. The data base include: age, gender, diagnosis, type of surgery, duration of CPB, duration of MV, ICU stay.

Database containing a list of registered complications after surgery. Postoperative complications were divided into 2 groups; first group cardiac and second extra cardiac complications. The group with cardiac complication includes supraventricular tachycardia - SVT, atrioventricular block - AV-block, pacing, and pericarditis. The second extracardiac group of complications includes chylothorax, bleeding, renal failure, pneumonia, seizures, sepsis, and the need for repeated surgery and the need for endotracheal intubation.

RESULTS

In this study, 65 patients have been studied, 31 male children (48 %) and 34 (52 %) female children. The age range was 6,5 ± 0,35 (rang 0,5 - 108 months). The duration of CPB was with an average of 68,32 ± 31,9 min. (range 10-190 min.) in open surgical procedures. The duration of MV was 2,04 ± 2,42 days (range 1-12 days), and the ICU stay was 3,90 ± 3,45 (range 1-17 days) (Table 1).

Table 1. Demographic characteristics of operated children

	mean ± SD	**number**	**percent %**	**p -value**
age (months)	6,5 ± 0,35	65		p < 0,1
male		31	48	
female		34	52	
duration of CPB (minutes)	68,32 ± 31,9	65	100	p < 0,01
duration of MV (days)	2,04 ± 2,42	65	100	p < 0,01
duration of ICU stay (days)	3,90 ± 3,45	65	100	p < 0,01

Table 2. Types of congenital heart deseases

Congenital heart diseases	number	percentage %
VSD	24	37
ASD	13	20
CAV	5	7.69
TGA	4	6.15
TOF	14	21.5
Stenosis v.mitralis	1	1.53
Stenosis v. aortalis	2	3.07
DORV	1	1.53
Anomalous pulmonary venous return	1	1.53

VSD-Ventricular septal defect, ASD-Atrial septal defect, CAV - Canalis atrioventricularis, TGA – Transposition of the great arteries, TOF-Tetralogy of Fallot, DORV - Double outlet right ventricle.

A cardiac surgery with a thoracotomy and a cardiopulmonary bypassis were made. VSD correction was made with intraventricular patch , the ASD (closure the patch defect), the TOF (closure the VSD with patch, resection of right ventricle outflow tract obstruction, with or without setting a transcanal patch), TGA (Rashkin - atrioseptectomy, and then switch to Janten), CAV (closure the atrial and interventricular septum with patch and mitral valve plastics), DORV (closure of the VSD with patch and replasement of aorta to the left ventricle), and Anomalous venous infusion of the vv. pulmonales (reinplatation of abnormal veins in the left ventricle).

A total of 33 children (50,7 %) of the cases had complications after surgery. Complications were divided into 2 groups: withcardiac and extra cardiac complications. The first group included SVT (6,25 %), AV-block (9,3 %), pacing (6,25 %) and pericarditis (6,25 %). Among those with extra cardiac complications, the most common was bleeding (12,5 %), renal failure 3 (9,3 %), followed by chylothorax 3 (9,3 %). Among those with neurological complications most common were seizures 3 (9,3 %). 15.6 % of the cases required reintubation after surgery, 12,5 % of them suffer from infectious complications with the clinical picture of sepsis and 6,25 % had a severe pneumonia (Table 2).

Table 3. Complications and mortality after cardiac surgery in pediatric population

Complications and mortality	number	percentage %	Number of mortality	percentage %
cardiac	**9**	**28.13**		
STV	2	6.25		
pacing	2	6.25		
AV-block	2	6.25		
pericarditis	3	9.3		
extra cardiac	**23**	**71.87**		
renal failure	3	9.3	2	6.25
chylothorax	3	9.3		
pneumonia	2	6.25		
seizures	3	9.3		
bleeding	3	9.3		
sepsis	4	12.5	2	6.25
reintubation and reoperation	5	15.6	2	6.25

This study covers the duration of treatment of the complications occurring during the ICU stay. SVT was treated with antiarrhytmic agents (3 days), pacing (5 days), AV- block was treated with beta blockers, calcium channel blockers (4 days), and pericarditis with diuretic and corticosteroids agents (7 days). Treatment of extra cardiac complications followed; renal failure was treated with diuretics and correction of electrolytic balance, chylothorax with thoracal drenage and low fat milk nutrition, (16 days), seizures with anticonvulsive therapy (2 days), sepsis with antibiotic therapy (14 days) and bleeding and reoperation were treated immediately in operating room. The mortality percentage in sepsis and reoperation were 6,25 % (2/65), and in renal failure 6,25 % (2/65). In our study, the presence of postoperative complications was significantly associated with a younger age ($p < 0,1$), with prolonged CPB duration ($p < 0,01$), prolonged mechanical ventilation ($p < 0,01$), and prolonged ICU stay ($p < 0,01$).

DISCUSSION

Significant technologic advances have improved outcomes in neonatal cardiac surgery over the last few years. The use of cardiopulmonary bypass, is still necessary for the repair of many congenital cardiac defects and it can still lead to major morbidity, especially in infants, despite a perfect surgical repair [8]. One of the major problems of morbidity is the postoperative complications occurring as a result of this procedure and the prolonged duration of MV and ICU stay.

Our study have demonstrated that postoperative cardiac and extra cardiac complications occurred in 50,7 % of pediatric cardiac surgery patients, and revealed a greater incidence of extra cardiac complications (71,87 %), compared with cardiac complications (23,13 %). The complication rate we observed was greater than the previously reported rates of 29% in neonatal patients confirmed in the study to Kansy A and al. [9].Cardiac complications, arrhythmias such as SVT (6,25 %), atrioventricular node block (6,25 %), and pericarditis (9,3 %) have been confirmed in several cases, and decreased the cardiac output. Therefore, 48 hours postoperative monitoring for heart rytham is very important. Placing the pacing was necessary for establishing the normal sinus rhythm.

High percent of bleeding 9,3 % was one of the reason of reintubation and needed of reoperation in the first few days. Long-term bypass, cardiotomy, suction and hypotermia lead to haemolysis, blood disorders (thrombocytopenia, increased PT and PTT) and vascular complications.

Another complication was the renal failure seen in 9,3 % of patients. Although, during the postoperative ICU stay we used inotropic support, renal failure was manifested as decreasing 24 hour urine output as a result of reduced perfusion. In study of Hornik and al. the incidence of kidney failure in children after heart surgery was less than 10 percent 10].

The postoperative extra cardiac complication in 9,3 % of patients manifested chylothorax as an early postoperative injure of ductus thoracicus. Among the neurological complications, most of the complications observed were seizures in 9,3 % of patients, and of lung complications - pneumonia 6,25 %. According to the study, the incidence of postoperative pneumonia in children is relatively uncommon and the incidence of nosocomial pneumonia (NP) in children after cardiac surgery varied between 9.6 to 21.5% [11]. The incidence of sepsis (12,5%), was in indirect correlation with pneumonia.

The postoperative complications occurred in cardiac surgery confirmed that the established outcome measures, longer CPB duration, the duration of MV and ICU stay, are significantly correlated with the increased number of cardiac and extra cardiac complications.

By increasing the experience in this area, neonatal and pediatric cardiac surgery can be performed with excellent postoperative outcomes. The postoperative complications are one of the main reasons for considerable morbidity and mortality, and the improvements in the postoperative period practices will improve the surgical outcomes further in this challenging age group.

REFERENCES

1. Wernovsky G, Mayer JE Jr, Jonas RA, Hanley FL, Blackstone EH, Kirklin JW, et al. Factors influencing early and late outcome of the arterial switch operation for transposition of the great arteries. *J Thorac Cardiovascular Surg*. 1995; 109:289-301.
2. Wernovsky G, Wypji D, Jonas RA, Mayer Jr JE, Hanley FL, Hickey PR, et al. Postoperative course and hemodynamic profile after the arterial switch operation in neonates and infants. A comparison of low flow cardiopulmonary bypass and circulatory arrest. Circulation. 1995;92:2226-35.

3. Shen I, Giacomuzzi C, Ungerleider RM. Current strategies for optimizing the use of cardiopulmonary bypass in neonates and infants. Ann Thorac Surg. 2003;75(suppl): S729-34.
4. Benavidez OJ, Gauvreau K, Del Nido P, Bacha E, Jenkins KJ. Complications and risk factors for mortality during congenital heart surgery admissions. Ann Thorac Surg. 2007;84:147-55.
5. Pasquali SK, He X, Jacobs JP, Jacobs ML, O'Brien SM, Gaynor JM. Evaluation of failure to rescue as a quality metric in pediatric heart surgery: an analysis of the STS Congenital Heart Surgery Database. Ann Thorac Surg. 2012;94:573-80.
6. Allan CK, Newburger JW, McGrath E, Elder J, Psoinos C, Laussen PC, et al. The relationship between inflammatory activation and clinical outcome after infant cardiopulmonary bypass. Anesth Analg. 2010;111:1244-51.
7. Khabar KS, Barbary MA, Khouqeer F, Devol E, al-Gain S, al-Halees Z. Circulating endotoxin and cytokines after cardiopulmonary bypass: differential correlation with duration of bypass and systemic inflammatory response/multiple organ dysfunction syndromes. Clin Immunol Immunopath. 1997;85:97-103.
8. Irving Shen, Carmen Giacomuzzi, and Ross M. Ungerleider; Current Strategies for Optimizing the Use of Cardiopulmonary Bypass in Neonates and Infants. Ann Thorac Surg. 2003;75:S729–34.
9. Kansy A, Tobota Z, Maruszewski P, Maruszewski B. Analysis of 14,843 neonatal congenital heart surgical procedures in the European Association for Cardiothoracic Surgery Congenital Database.Ann Thorac Surg. 2010; 89:1255-9.
10. Hornik CP, HeX, Jacobs JP, Li JS Jaquiss RD Jacobs ML, O Brien SM, Peterson ED, Pasquali SK Complications after the Norwood operation: an analysis of The Society of Thoracic Surgeons Congenital Heart Surgery Database. Ann Thorac Surg. 2011. 92(5):1734-40.
11. Healy F, Hanna B.D, Zinman R. Pulmonary Complications of Congenital Heart Disease. Paediatr Respir Rev. 2011;13(1):10–15.

UDC: 616.94-053.31(497.7)"2017"

ORIGINAL ARTICLE

MOST COMMON FACTORS AFFECTING OCCURRENCE OF NEONATAL SEPSIS - CHALLENGE AND EXPERIENCE FOR TREATMENT AT NEONATAL INTENSIVE CARE UNIT

Najdanovska-Aluloska Natasa, Mandzukovska H, Angelkova N, Kirovski I
University Clinic for Children's Diseases, Skopje, Republic of Macedonia

ABSTRACT

Background: Neonatal sepsis remains a serious complication, especially among preterm infants. Neonatal sepsis is divided into early- and late-onset sepsis, based on timing of infection and presumed mode of transmission. Early - onset sepsis is defined by onset with in the first week of life, to infections occurring in the first 72 hours due to maternal intrapartum transmission of invasive organisms. Late - onset sepsis is defined as infection occurring after one week and is attributed to pathogens postnatally acquired.

Materials and Methods: We have investigated neonatal sepsis in our NICU from 1 January till 31 December 2017, for one-year period, in order to determine mortality associated with sepsis and to identify the dependent predictors for morbidity and mortality. A total 216 infants were admitted in the NICU. Data were collected regarding the primary reason for NICU, maternal condition, gender, gestational age, length of NICU stay, duration of MV and non-invasive ventilation, using of umbilical catheter, and peripherally vein line.

Results: Early - onset sepsis was detected in 15 neonates (12 %) within the first 72 hours. Late - onset sepsis was detected in 18 neonates (3,2 %) after 72 hours. Premature infants 23 (70 % range 26 - 37 gestational age) were more exposed to sepsis than term infants 10 (30 %; range 38-40 gestational age). 15 neonates (45,5%) were exposed to the early - onset sepsis, and 18 neonates (54,5%) were exposed to late-onset sepsis. The most frequent isolates were *Staphylococcus aureus* 7 (21,2 %), followed by *Acinetobacter* 6 (18,1 %), *Meticillin- resistant Staphylococcus aureus* 5 (72 % from total 7 *Staphylococcus aureus*) and *Staphylococcus epidermidis* 5 (15,1%), followed by *Klebsiella pneumoniae* 2 (6,06 %), *Serratia* 2 (6,06 %) and *Pseudomonas aureginosa* 2 (6,06 %). Late - onset sepsis was significantly more common in premature infants. We confirmed that neonatal sepsis resulted with increase duration of NICU stay and duration of MV. Early diagnosis, followed by appropriate antibiotic treatment, short hospital stay and restricted use of invasive devices should be the aims to reduce the risk of late - onset sepsis during the stay in the NICU.

Conclusion: Neonatal sepsis is a major cause of death in infants despite sophisticated neonatal intensive care. Early and adequate antibiotic therapy decreases the risk of morbidity of hospitalized patients.

Keywords: sepsis, ICU, morbidity, antibiotic treatment, risk factor

INTRODUCTION

In hospitals the incidence of neonatal sepsis is highest in the neonatal intensive care units (NICU), and these are suitable areas for targeting efforts to reduce it. Infection rates are standard indicators of quality and safety in all the healthcare settings all over the world and their monitoring is increasingly regarded as an important contributor to safe and high-quality healthcare [1].

Blood culture remains the gold standard in diagnosing causative pathogens; however, the average time to detection of positive cultures is 16 hours and may be as long as 24 to 48 hours is increasing the risk of complications. For this reason, those with suspected sepsis are commenced on empiric antibiotic therapy until sepsis can be ruled out. [2].

Various fetal, maternal and NICUs environmental factors contribute toward causing infections in newborns. Apart from poor skin integrity and the immature immune system, some of the fetal factors that predispose neonates to infections are: low birth weight, gestational age and Apgar score, prolonged hospital stay, invasive procedures such as endotracheal intubation, placement of umbilical catheter, peripherally inserted central catheters (PICC) and peripherally vein line, preparation of parenteral nutrition, lack of adequate hand washing by the hospital personnel and indiscriminate use of antibiotics [3,4,5].

Diagnosis of sepsis in neonates is challenging due to nonspecific signs and symptoms. The clinical features include systemic signs of infection such as fever, hypothermia, tachycardia, jaundice, failure to thrive, lethargy, irritability, and restlessness. In addition, laboratory diagnosis until recently was time-consuming. This therefore necessitates the initiation of empirical antibiotic therapy pending a definitive diagnosis.

Some of the maternal factors that predispose neonates to infections are: the premature rupture of membrane, maternal fever within two weeks prior to delivery, urinary infections, meconium-stained amniotic fluid, foul smelling liquor and instrumental delivery, etc. [6].

According the outcomes and database of many hospitals in the world, the vast majority of infections were caused by Gram-positive organisms (*Staphylococcus* and *Spreptococcus pneumoniae*) over 70%, and Gram-negative organisms (*Klebsiella, Enterococcus, Proteus mirabilis* and *Pseudomonas*) over 20% [7,8].

There is difference between neonatal sepsis rates in low-middle income countries and high-income countries. Rates in low-middle income countries are two to three times higher, than those of high-income countries. In high-income countries, sepsis surveillance plays a major role in infection control as it is essential for decreased of morbidity and mortality at NICU [9].

The aim of this study is to determinate the incidence, onset, risk factors, and causative agents associated with neonatal sepsis. Also to promote a strategy to reduce the rate of neonatal and hospital infections and need for aggressive antibiotic therapy.

MATERIALS AND METHODS

A retrospective study to the tertiary care neonatal unit (NICU) at the University Pediatric Clinic in Skopje performed from 1 January until 31 December 2017. During the study period of one year, a total of 216 neonates were admitted in NICU.

Neonatal sepsis was classified as early onset sepsis with positive culture within the first 72 hours of life, and late - onset sepsis with positive blood culture obtained after 72 hours of life with clinical signs of sepsis. Neonates with clinical or laboratory signs suggestive of sepsis but with negative blood cultures or contaminated blood cultures without clinical or laboratory signs of sepsis were excluded.

Blood cultures were confirmed at the Institute of Public Health of the Republic of Macedonia. We used automated method with BacT-Alert system. The positive results are

processed with standard microbiological techniques and methods such as automated Vitek-2 system for identification of microorganisms and determining their resistance to antimicrobials.

Blood cultures were performed on neonates with a positive history of obstetric risk factors (chorioamnionitis, prolonged rupture of membranes) or those who presented clinical signs of suspected sepsis, such as apnoea, lethargy, respiratory distress, jaundice, hypotension, poor feeding, glucose imbalance, and seizures. Blood cultures were obtained by peripheral puncture. A sterile 0.5 ml blood sample was sent for culture prior to commencing antibiotic therapy. The growth of bacteria within 48 h after blood culture was considered clinically significant. Pathogenic bacteria were identified using standard conventional microbiological methods.

For obtaining information we used the clinical database. It included obstetric risk factors for infection, antenatal care, duration of NICU stay, gender, birth weight, gestational age, duration of non-invasive ventilation and mechanical ventilation, duration of NICU stay, umbilical catheterization, peripherally vein line and duration of parenteral nutrition.

RESULTS

Two hundred and sixteen neonates were admitted in NICU and in 33 neonates, neonatal sepsis was confirmed. Out of them, 54,5 % (18/33) were male, and 45,5 % (15/33) were female.

A total of 33 neonates were suspected for neonatal sepsis; 15 (45,5%) of them were suspected to have early-onset sepsis, while the remaining 18 (54,5%) were suspected to have late-onset sepsis.

The overall incidence of neonatal sepsis was 15,2 % (33/216). The incidence of sepsis among preterm neonates was 70% (23/33) compared to term neonates, for whom the incidence was 30 % (10/33).

The following risk factors were significantly associated with microbiologically confirmed sepsis in the multivariate analysis: antenatal maternal condition (infections and premature rupture of membranes), gestational age, preterm neonates with lower birth weight were more exposed to sepsis than term infants (67% versus 33 %). The details are shown in Table 1.

Table 1. Multivariate analysis of neonatal and maternal characteristics associated with neonatal sepsis in study of 33 neonates

	number or median range	**percentige (%)**
male gender	18	54.5
female gender	15	45.5
antenatal maternal condition		
infection	15	45.5
premature rupture of membranes	7	21.2
gestational age in weeks	38.3 (27 - 40)	
27 - 30	6	18.1
28 - 32	7	21.2
33-38	10	33
> 38	10	30.3
birth weight (g)		
1000-1500	5	15.1
1501 - 2000	**7**	21.2
2001-2500	8	24.2
> 2500	16	48.5
early-onset sepsis	**15**	**45.5**
preterm	9	27.2
term	6	18.2
late-onset sepsis	**18**	**54.5**
preterm	14	42.4
term	4	12.1

The following risk factors of NICU procedures were associated with neonatal sepsis such as duration of non-invasive ventilation and mechanical ventilation, duration of NICU stay, umbilical catheterization, peripherally vein line (table 2).

Table 2. Analysis of NICU procedures associated with neonatal sepsis in study of 33 neonates

	number of neonates	**median range of duration and procedures in days**	**Range (days)**
duration of mechanical ventilation	25	7	5-15
duration of NICU stay	33	10	8-11
duration of non-invasive ventilation	8	5	4-7
umbilical catheterization	7	13	12-14
peripherally vein line	26	9	7-10

During the study period, the first-line empiric choice of antibiotic therapy was a third generation cephalosporin (Cefotaxime) with an aminoglycoside (Amikacin). Empirical antibiotic therapy was initiated according to antibiotic guidelines for NICU and after that it was changed according of antibiogram outcomes.

The most common isolate overall was *Staphylococcus aureus* in 7/33 (21,2 %), followed by *Acinetobacter* 6/33 (18,1%), than *Meticillin- resistant Staphylococcus aureus* 5/33 (15,1 %) and *Staphylococcus epidermidis* 5/33 (15,1 %). With a lower percentile of 6,06 % were *Streptococcus pneumoniae, Enterococcus, Pseudomonas aeruginosa, Serratia marcescens* and *Klebsiella pneumoniae.* Gram-negative infections were significantly more frequent in preterm neonates compared to term neonates and caused the late-onset sepsis ($p < 0,01$). Table 3

Table 3. Bacterial isolates in study of 33 neonates

Organism	number of patients	Percentige %
Gram - positive	**21**	**63.6**
Staphylococcus aureus	7	21.2
Meticillin- resistant Staphylococcus aureus	5	15.1
Staphylococcus epidermidis	5	15.1
Streptococcus pneumoniae	2	6.06
Enteroccoccus	2	6.06
Gram - negative	**12**	**36.3**
Klebsiella pneumoniae	2	6.06
Serratia marcencens	2	6.06
Acinetobacter	6	18.1
Pseudomonas aeruginosa	2	6.06

DISCUSSION

Sepsis in the neonatal intensive care unit still remains one of the most common causes of morbidity and mortality, especially in preterm newborns. Neonatal sepsis is divided into early-onset (defined as the onset of sepsis within the first 72 hours of life) and late-onset sepsis (after 72 hours of life).

Early-onset sepsis is commonly caused by organisms acquired from the mother's genital tract around the time of delivery (Gram-positive organisms like group B streptococcus and enteric Gram-negative organisms, predominantly *Escherichia coli* and *Klebsiella pneumoniae)* [10, 11].

Late-onset sepsis is usually caused by pathogens acquired during the course of hospitalization or during delivery, including *Coagulase-negative staphylococcus* (CoNS), *Staphylococcus aureus, Enterococcus species,* and *Enterobacteriaceae* [12].

Blood culture is still the gold standard for definitive diagnosis of neonatal sepsis, in spite of some drawbacks of blood cultures, low sensitivity and possible contamination.

Analyzing several studies during the years, several risk factors that interfere in the onset of neonatal sepsis have been identified. Maternal genitourinary infections and premature rupture of fetal membranes were the main gestational risk factors involved in neonatal sepsis [6, 13].

They are very closely related to early-onset sepsis and associated with higher neonatal mortality and morbidity [14].

Usually, these conditions contribute to preterm labor with all risks of uncertain outcomes. In a study of Levine, a 3,5 times increased risk of a sepsis in a premature rupture of membranes in premature neonates was reported [15].

Previous studies have shown that a low gestational age and very low birth weight were the most prevalent predictive factor for neonatal sepsis [16].

The factors related to the NICU setting include the average length of the NICU stay, duration of MV, and non-invasive ventilation, use of invasive devices such as umbilical catheter, peripherally vein line, which are aggravated by the immunological immaturity of the newborns. The extensive use of invasive devices for caring for the immunologically immature neonates especially preterm is the main cause of sepsis [17].

In our study, a number of preterm neonates is twice as high as than the number of term neonates and the incidence of neonatal sepsis in preterm is nearly two times higher than in term neonates (69, 7 % versus 30,3 %). 15 of them were male (54,5 %), and 13 (45,5 %) female.

Maternal infections (45,5 %), and premature rupture of fetal membranes (21,2 %) were most common main risk factors for neonatal sepsis.

We confirmed that premature neonates with birth weight 1500-2500 g. are most sensitive and most vulnerable to neonatal sepsis. As we said in our study, the incidence of late-onset sepsis was higher in preterm neonates than in term neonates with early-onset sepsis. This finding is similar to other previous studies [18].

The environment and medical devises in the NICU increased the risk of neonatal sepsis and it is, therefore, no surprise that the NICU stay duration, duration of MV, non-invasive ventilation, duration of using umbilical catheters and peripherally vein line are associated with an increased risk of sepsis. The NICU stay duration was associated with an increased risk of neonatal sepsis. The NICU is considered to be important focus areas for follow the conditions and treatment of neonates. In our study the median duration of the NICU stay was 10 days (range 8-11days) ($p > 0,01$).

In relation to the risk factors for neonatal sepsis in several studies, was found high rates of MV and CPAP. Confirming this data, other researchers show that the use of MV is associated with a higher risk of late-onset sepsis [5,19].

One Brazilian study showed that newborns that developed sepsis were more exposed to invasive procedures, including the use of MV, PICC, and 80% of the newborns with diagnosed sepsis underwent MV [20].

In our study, the median duration of MV was 7 (5 - 15) days and median range of duration of non-invasive ventilation was 5 (4-7) days ($p < 0,01$). The risk of late-onset sepsis increases as the number of MV days increases. One multicentre research trial in America showed that 50% of the newborns that underwent MV for 28 days or more developed late-onset sepsis, while only 9% of the newborns that underwent MV for 7 days or more developed late-onset sepsis [7].

One study conducted in Norway showed that neonatal sepsis was more frequent among the newborns who used CPAP in the first 24 hours than among the newborns who underwent MV for the same period. The reason, according to the authors is nosocomial, since after the third day of age, the upper airways of newborns are colonized by *Staphyloccocus aureus*, while the lower airways of intubated newborns are considered sterile [21].

Most of the neonates used umbilical catheters and peripherally vein line for many days, which is the key risk factor for neonatal sepsis. In a few studies of the risk factors for neonatal sepsis, it was found that there were more cases of infection among the neonates who were submitted to umbilical catheters for more than 11 days (76.2%) than among the newborns who were not submitted to umbilical catheters (23.8%) ($p < 0.0001$). Consequently, umbilical catheters in that study were considered a significant predictor for neonatal sepsis [22].

In our study, the median duration of using umbilical catheters was 13 (range 5 - 15) days and the median duration of peripherally vein line was 9 (range 7-10) days ($p < 0,01$) and we confirmed correlation with the increased rate of neonatal sepsis.

Most nosocomial infections in the NICU are the result of procedures to preserve the life of these neonates, and it is not acceptable to consider these infections as a consequence of neonatal intensive care.

Following several studies during the last few years, Gram-negative microorganisms are responsible for 60–70% of blood-culture-positive infections in the neonatal period [23,24,25].

In our study, in all neonates admitted to the NICU over a year period, *Staphylococcus aureus* was the leading pathogen of sepsis (21.2 %). *Meticillin- resistant Staphylococcus aureus* and *Staphylococcus epidermidis* with incidence (15,1 %) and *Streptococcus pneumoniae* and *Enteroccoccus spp*. with incidence (6,06 %) were the most common microorganisms which caused neonatal sepsis.

Although, in few studies the sepsis caused by *Acinetobacter* is considered rare, in our study it is very common and it has a high morbidity rate. With incidence of 18,1% it is considered one of the most resistant microorganisms to the antibiotics that are routinely used in the NICU. Late-onset sepsis is predominantly caused by *Acinetobacter spp*, and the antibiotic treatment with Collistin is adequate. *Klebsiella pneumoniae* (6,06 %), *Serratia marcenscens*(6,06 %), *Pseudomonas aeruginosa* (6,06 %), were the most common bacteria isolated of Gram - negative and responsible for neonatal sepsis.

The environment and intravascular devises in the NICU increased the risk of neonatal sepsis and it is, therefore, no surprise that the NICU stay duration, duration of MV, non-invasive ventilation, duration of using umbilical catheters and peripherally vein line are associated with an increased risk of sepsis.

Therefore, it is concluded that identification of the risk factors associated to the diagnosis of neonatal sepsis may contribute to interventions and research that helps to reduce neonatal mortality resulting from these risks.

REFERENCES

1. Pittet D. Infection control and quality health care in the new millenium. American J Infect Control. 2005;33(5):258-267.
2. Manzoni P, De Luca D, Stronati M, Jacqz-Aigrain E, Ruffinazzi G, Luparia M, Tavella E, et al. Prevention of Nosocomial Infections in Neonatal Intensive Care Units. Amer J Perinatol. 2013;30(02):81-88.
3. Brady MT. Health care-associated infections in the neonatal intensive care unit. Am J Infect Control. 2005;33(5):268–275.
4. Simonsen, KA, Anderson-Berry AL, Delair SF, Davies HD. Early-onset neonatal sepsis. Clin Microbiol Rev. 2014;27:21–47.
5. Vergnano S, Sharland M, Kazembe P, Mwansambo C, Heath PT. Neonatal sepsis: An international perspective. Arch Dis Child Fetal Neonatal Ed. 2005;90(3): F220–F224.
6. Allegranzi B, Bagheri Nejad S, Combescure C, Graafmans W, Attar H, Donaldson L, Pittet D. Burden of endemic health-care-associated infection in developing countries: Systematic review and meta-analysis. Lancet. 2011;377(9761):228–241.
7. Stoll JB, Hansen N, Fanaroff AA, Wright LL, Carlo WA, Ehrenkranz RA, et al. Late onset sepsis in very low birth weight neonates: the experience of the NICHD neonatal research Network. Pediatrics. 2002;110(2Pt1):285-91.
8. Patel SJ, Saiman L. Antibiotic resistance in neonatal intensive care unit pathogens: mechanisms, clinical impact, and prevention including antibiotic stewardship. Clinics in Perinatology. 2010;37(3):547–563.

9. Folgori L, Bielicki J, Sharland M. A systematic review of strategies for reporting of neonatal hospital-acquired bloodstream infections. Arch Dis Child Fetal Neonatal Ed. 2013;98(6):F518–F523.
10. Simonsen KA, Anderson-Berry AL, Delair SF, Davies HD. Earlyonset neonatal sepsis. Clin Microbiol Rev. 2014;27(1):21-47.
11. Hornik CP, Fort P, Clark RH, Watt K, Benjamin Jr DK, Smith PB, et al. Early and late onset sepsis in very-low-birth-weight infants from a large group of neonatal intensive care units. Early Hum Dev. 2012;88(Suppl2):S69-74.
12. Lim WH, Lien R, Huang YC, Chiang MC, Fu RH, Chu SM, et al. Prevalence and pathogen distribution of neonatal sepsis among very-low-birth-weightinfants. Pediatr Neonatol. 2012;53(4):228-34.
13. Verstraete EH, De Coen K, Vogelaers D, Blot S. Risk Factors for Health Care-Associated Sepsis in Critically Ill Neonates Stratified by Birth-Weight. Pediatr Infect Dis J. 2015;34(11):1180-6.
14. Pugni L, Pietrasanta C, Acaia B, Merlo D, Ronchi A, Ossola MW, Bosari S, Mosca F. Chorioamnionitis and neonatal outcome in preterm infants: A clinical overview. J Matern Fetal Neonatal Med. 2016;29(9):1525–1529.
15. Levine CD. Premature rupture of the membranes and sepsis in preterm neonates. Nurs Res. 1991;40(1):36–41.
16. Edwards MS, B.C. Sepsis in the newborn. In Krugman's Infectious Diseases of Children, 11th ed.; Gershon, A.A., Hotez, P.J., Katz, S.L., Eds.; Mosby: Philadelphia, PA, USA, 2004; pp. 545–561.
17. Nambiar S, Singh N. Change in epidemiology of health care-associated infections in a neonatal intensive care unit. Pediatr Infect Dis J. 2002;21(9):839–842.
18. Gandhi S, Ranjan KP, Ranjan N, Sapre N, Masani M. Incidence of neonatal sepsis in tertiary care hospital: an overview. Int J Med Sci Public Health. 2013;2:548-552.
19. Polin RA, Denson S, Brady M T, et al. Strategies for prevention of health care-associated Infections in the NICU. Pediatrics. 2012;129(4):e1085-93, 2012
20. Silva SMR. Características dos recém-nascidos pré-termo com peso inferior a 1.500g e sepse neonatal tardia [dissertação]. Porto Alegre (RS): Universidade Federal do Rio Grande do Sul; 2014
21. Rønnestad A, Abrahamsen TG, Medbø S, Reigstad H, Lossius K, Kaaresen PI, et al. Septicemia in the first week of life in a Norwegian national cohort of extremely premature infants. Pediatrics. 2005;115(3):e262-8.
22. Freitas BAC, Peloso M, Manella LD, Franceschini SCC, Longo GZ, Gomes AP, et al. Sepse tardia em pré-termos de uma unidade de terapia intensiva neonatal: análise de três anos. Rev Bras Ter Intensiva. 2012; 24(1):78-85.
23. Lavoie PM. Earlier initiation of enteral nutrition is associated with lower risk of late-onset bacteremia only in most mature very low birth weight infants. J Perinatol. 2009;29(6):448-54.
24. Obiero CW, Seale AC, Berkley JA. Empiric treatment of neonatal sepsis in developing countries. Pediatr Infect Dis J. 2015;34(6):659–661.
25. Khan AM, Morris SK, Bhutta ZA. Neonatal and perinatal infections. Pediatr Clin N Am. 2017;64(4):785–798.

UDC: 618.146-006.6-022.6:578.827]-084-057.875(497.7)

ORIGINAL ARTICLE

CERVICAL CANCER AND HUMAN PAPILLOMAVIRUS KNOWLEDGE AMONG STUDENTS IN THE REPUBLIC OF MACEDONIA

Simeonovski Viktor, Kostovski M
Faculty of Medicine, University of Ss. Cyril and Methodius of Skopje, Skopje, Republic of Macedonia

ABSTRACT

Background: Cervical cancer (CC) is the second most common cancer in females worldwide and first most common cancer of the genital tract. The high incidence of CC cases in women from less developed countries remains a global public health issue.

Objective: To evaluate the knowledge and awareness of CC and HPV infection among students in Macedonia.

Materials and methods: A cross sectional study was conducted on 1007 students through a self-developed pre-validated anonymous questionnaire. The questionnaire comprised of 52 questions categorized in 4 sections: demographic information, knowledge, prevention and risk factors about HPV and CC. The Knowledge section consisted of 10 questions. The difference in knowledge about CC and HPV was compared between gender, age, medical background (medical students-MS vs. non medical students-NMS), religion and residency.

Results: The majority of students proved to have general knowledge about CC. Only 50.2 % of the MS and 25.7% of NMS who have self-reported to know what HPV, answered correctly by giving correct definition of HPV. The MS were 2.919 times more likely to provide the correct definition about HPV in comparison to NMS. (OR=2.919[95%CI (1.79-4.749)). Although most of the students were familiar with the fact of who can be affected by HPV (68.6%), the knowledge considering HPV infections in an important number of students remains to be generally low.

Conclusion: Our results showed that there are some gaps and misconceptions regarding students' knowledge about HPV infections. Therefore, different public health strategies, in order to provide more effective educational programs for improving knowledge of HPV among students are needed.

Keywords: cervical cancer, HPV, knowledge, students;

INTRODUCTION

Cervical cancer is the second most common cancer in females worldwide and first most common cancer of the genital tract. What presents a global public health issue are the high numbers of incidence of new cancer cases in women living in less developed countries – 445,000 new cases in 2012 (84% of the new cases worldwide) [1]. Cervical cancer is caused by sexually transmitted infection with certain types of human papillomavirus (HPV), more precisely oncogenic subtypes 16 and 18, which are believed to cause 70% of the precancerous and cancer lesions of the reproductive systeM [1,2].

The, human papillomavirus is the most common sexually transmitted disease. HPV is transmitted only by skin-to-skin contact, both in men and women [3]. It is believed that the range of newly infected women worldwide is 2 to 44%. The prevalence rate of over 80% and >50% of all the sexually active women show positive results to any HPV testing at one point in time [2, 3]. Moreover, 60% of the men in Europe are affected by HPV [4], with substantial heterogeneity by country [2]. What is most peculiar about this infection is that most of the infections are asymptomatic [5]. Although relatively low, up to 10% of all the infections can cause serious health implications, ranging form genital warts to respiratory papillomatosis or genital cancer. Hence, several preventive measures are advised against HPV infection, of which most notable are education programs, use of condoms during sexual intercourse and vaccine prophylaxis [6]. Interestingly, since the introduction of the HPV vaccine in 2006 [7], a territorial discrepancy in incidence and awareness is still to be noted, even among the targeted groups where the vaccination is fully implemented [8, 9]. In Macedonia, the quadrivalent HPV vaccine has been introduced into the national immunization program, since 2009 [9, 10].
The aim of this study is to evaluate the knowledge and awareness of cervical cancer and HPV infection of students in Macedonia.

MATERIALS AND METHODS

Study design and participants

A cross sectional study was conducted in the period of February to March 2015 among students of different academic backgrounds from several Universities in the Republic of Macedonia. Data was collected from 1007 respondents (342 males, 665 females, with mean age of 20.66±1.811) through a self-developed pre-validated anonymous questionnaire. No specific criteria for inclusion or exclusion were made for this study and the questionnaire was given to all the students. The participants were approached in between courses, after gaining permission from the faculty of record and were administrated the survey. Prior to filling out the survey, the students were given a brief introduction on its purpose and anonymity and were given instructions how to proceed. For maximum effect, this introductory information was also included on the first page of the questionnaire. As the survey was completely anonymous, only verbal consent was taken as an ethical requirement and no written consent was necessary. The willingness to participate of the students was taken into account and those, who were of personal reasons unwilling to participate in the survey, were excused from partaking.

Survey Instrument

A questionnaire for the study was designed consisting of 52 questions, which were believed to be important on the basis of the topic. The survey was divided in four main sections: Demographic information, Knowledge, Prevention and Risk factors about HPV and cervical cancer. We pre-validated the survey for clarity and comprehension and revised it upon students' feedback. The survey took approximately 15 minutes to fulfill. For the purpose of this study, only the sections Demographics and Knowledge about HPV were analyzed. The survey included a brief introduction before every abovementioned section. The questions about personal information included questions about Gender, Age, Residency, Field of Study and Religion. The Knowledge section consisted of 10 questions. The first 4, were general multiple choice questions about cervical cancer and were mandatory for all the participants. The fifth question was of a dichotomous type where respondents were asked to self-report their

knowledge of what HPV is. There was also an additional mandatory open-end question only for the students answering positively to the previous one, evaluating the self-reported knowledge of HPV. Respondents were asked to briefly provide their definition of HPV. Their answer was then evaluated according to the definition for HPV, proposed by the WHO: Human papillomavirus (HPV) is the most common sexually transmitted viral infection of the reproductive tract. Most sexually active women and men will be infected at some point in their lives. Skin-to-skin genital contact is a well-recognized mode of transmission. Cervical cancer is by far the most common HPV-related disease [1].The next four questions were more detailed about HPV and only mandatory to people who answered positively on the fifth question.

Statistical analysis

For data analysis, International Business Machines Statistical Package for Social Sciences (IBM SPSS) version 24 was used. Descriptive statistics was used for processing the demographic entries, as well as the knowledge about HPV. The Pearson's chi-square test was used for the analysis of categorical data. The difference in knowledge about cervical cancer and HPV was compared between 5 groups: gender (Male and Female), age groups (Adolescents (<19years old) and Young adults (>19years old), medical background (Medical and Non-medical students), religion (Christian, Muslim and Atheist) and residency (City (100.000+ inhabitants), Town (1000-100.000 inhabitants) and Rural (<1000 inhabitants)) [11]. The p-value of less than 0.05 was considered statistically significant.

RESULTS

Presented on Table 1 is the frequency of incorrect answers among all of the participants. The lowest percent of incorrect answers was observed for the 4th question (26.0%), while the highest number of incorrect answers was obtained for the 8th question. Concerning the issues about cervical cancer, participants had higher than 50% correct answers on all questions.

Table 1. Frequency distribution of incorrect answers among study participants

Question	n (%) of students answering incorrectly
1. Risk factors of cervical cancer are:	437 (43.3)
2. In your opinion, what place does cervical cancer take among neoplastic diseases in your country, concerning morbidity?	400 (39.6)
3. In your opinion, what place does cervical cancer take among neoplastic diseases in your country, concerning mortality?	458 (45.4)
4. Women at what age are at the highest risk of cervical cancer?	262 (26.0)
5. Do you know what the HPV (Human Papilloma Virus) is?	420 (41.6)
6. The HPV may infect… ?	187 (31.4)
7. If the HPV infection occurs in male, the symptoms are .. ?	271 (61.3)
8. If the HPV infection occurs in female, the symptoms are: .. ?	509 (89.0)
9. How many HPV infections lead to serious health complications?	484 (82.0)

Table 2 represents the differences of the frequency distribution among students according to different study groups. It is of note that higher percentage of incorrect answers was seen among medical and non-medical students considering questions 2-4. Hence, medical students showed significantly higher knowledge of these questions. Interestingly, non-medical students (65.7%) were able to provide more risk factors for cervical cancer when compared to medical students (46.9%).

Table 2. Frequency distribution (%) of correct responses to question 1 through 4.

Question	Sample size	1.Risk factors of cervical cancer are:		2.In your opinion, what place does cervical cancer take among neoplastic diseases in your country, concerning morbidity?*		3. In your opinion, what place does cervical cancer take among neoplastic diseases in your country, concerning mortality? *		4. Women at what age are at the highest risk of cervical cancer?*	
		n (%) of correct responses	p-value	n (%) of correct responses	p-value	n (%) of correct responses	p-value	n (%) of correct responses	p-value
Gender	1007								
Male		143 (41.8)	0.46[1]	86 (25.1)	0.47[2]	68 (19.9)	0.09[3]	179 (52.3)	**0.002[4]**
Female		294 (44.2)		209 (31.4)		145 (21.8)		400 (60.2)	
Age	1007								
Adolescents		199 (58.5)	0.404[5]	91 (26.8)	0.477[6]	65 (19.1)	0.559[7]	188 (55.3)	0.370[8]
Young Adults		372 (55.8)		203 (30.4)		147 (22.0)		391 (58.6)	
Field of study	1003								
Medical		230 (46.9)	**0.000[9]**	188 (38.4)	**0.000[10]**	120 (24.5)	**0.000[11]**	299 (61.0)	**0.000[12]**
Non-medical		337 (65.7)		105 (20.5)		91 (17.7)		275 (53.6)	
Religion	994								
Christian		415 (55.9)	0.792[13]	235 (31.7)	**0.001[14]**	150 (20.2)	0.105[15]	441 (59.4)	**0.001[16]**
Muslim		83 (58.9)		28 (19.9)		35 (24.8)		80 (56.7)	
Atheist		66 (59.5)		23 (20.7)		22 (19.8)		47 (42.3)	
Residency	1004								
City		348 (55.2)	0.414[17]	193 (30.6)	0.482[18]	142 (22.5)	0.304[19]	354 (56.2)	0.737[20]
Town		219 (59.3)		98 (26.2)		69 (19.0)		214 (58.8)	
Rural		5 (50.0)		2 (20.0)		2 (20.0)		7 (70.0)	

*Obtained from χ^2 test for comparison of categorical variables; [1]Pearson $\chi^2 = 0.46$, df=1; [2]Pearson $\chi^2 = 0.47$, df=2; [3]Pearson $\chi^2 = 4.82$, df=2; [4]Pearson $\chi^2 = 12.370$, df=2; [5]Pearson $\chi^2 = 0.697$, df=1; [6]Pearson $\chi^2 = 1.420$, df=2; [7]Pearson $\chi^2 = 1.164$, df=2; [8]Pearson $\chi^2 = 1.986$, df=2; [9]Pearson $\chi^2 = 35.867$, df=1; [10]Pearson $\chi^2 = 46.841$, df=2; [11]Pearson $\chi^2 = 24.897$, df=2; [12]Pearson $\chi^2 = 18.844$, df=2; [13]Pearson $\chi^2 = 0.792$, df=2; [14]Pearson $\chi^2 = 19.792$, df=4; [15]Pearson $\chi^2 = 7.668$, df=4; [16]Pearson $\chi^2 = 18.365$, df=4; [17]Pearson $\chi^2 = 1.765$, df=4; [18]Pearson $\chi^2 = 3.470$, df=4; [19]Pearson $\chi^2 = 4.841$, df=4; [20]Pearson $\chi^2 = 1.995$, df=4;

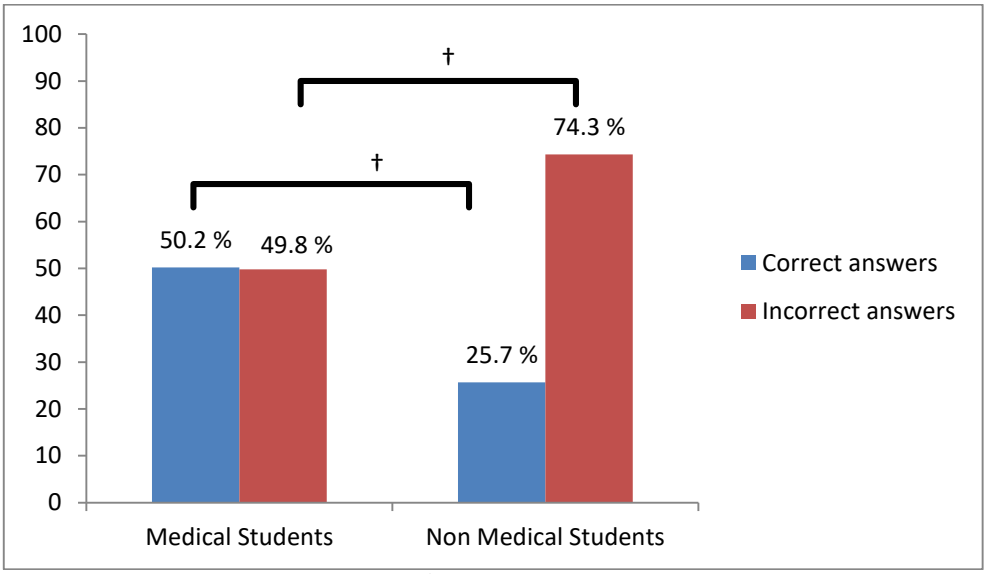
Table 3 shows the distribution of self-reported knowledge of HPV among different study groups. Namely, statistically significant higher number of positive answers was observed in young adults in comparison to adolescents, females against males and medical students opposed to non-medical students. (p=0.000). There was no significant difference in knowledge between different religions or residencies.

Table 3. Frequency distributions of self-reported knowledge about HPV among different study groups

Characteristic	**Sample size**	**Knowledge**		**p-value**
		Know n (%)	Don't know n (%)	
Age	1006			
Adolescents		163 (48.1)	176 (51.9)	**0.000[1]**
Young adults		423 (63.4)	244 (36.6)	
Gender	1005			
Male		156 (45.6)	184 (54.5)	**0.000[2]**
Female		430 (64.9)	233 (35.1)	
Residency	1002			
City 100.000+		375 (59.6)	254 (40.4)	0.423[3]
Town <100.000		201 (55.4)	162 (44.6)	
Rural <1000		6 (60.0)	4 (40.)	
Religion	993			
Christian		443 (59.8)	289 (40.2)	0.149[4]
Muslim		74 (52.5)	67 (47.5)	
Atheist		59 (53.2)	52 (46.8)	
Field of study	1001			
Medical		374 (76.5)	115 (23.5)	**0.000[5]**
Non-medical		210 (41.0)	302 (59.0)	

*Obtained from χ^2 test for comparison of categorical variables; [1]Pearson χ^2 = 21.736, df=1; [2]Pearson χ^2 = 34.366, df=1; [3]Pearson χ^2 = 1.720, df=2; [4]Pearson χ^2 = 3.801, df=2; [5]Pearson χ^2 = 129.453, df=1;

Figure 1 illustrates the difference in the percentage of correct and incorrect answers about HPV, by giving a definition of HPV, between medical and non-medical students. The medical students are 2.919 times more likely to provide the correct definition about HPV in comparison to non-medical students. (OR=2.919[95%CI (1.79-4.749))



†Statistically significant (p<0.05), obtained from χ^2 test for comparison of categorical variables;
Pearson χ^2 = 19.381, df=1, **p=0.000**;

Fig. 1. Differences in knowledge about HPV between medical and non- medical students.

The frequency distribution of correct answers among students of different study groups, who have self reported to know what HPV is is shown on Table 4. Significantly higher percentage of correct answers was seen in young adults (73.8%) compared to adolescents (54.9%), (p=0.000) considering the question evaluating whether both genders may get infected by HPV. Interestingly higher percentage of students answered incorrectly considering the percentage of serious health complications following HPV infection. However, young adults (p=0.005) and males (p=0.000) showed significantly higher knowledge considering this question.

Table 4. Frequency distribution (%) of correct responses to questions 6 through 9, after self assessed knowledge of HPV.

Characteristic	Sample size	Gender			Sample size	Age			Sample size	Field			Sample size	Religion				Sample size	Residency			
Question		Male n (%) of correct responses	Female n (%)of correct responses	p- value		Adolescents n (%) of correct responses	Young adult n (%)of correct responses	p-value		Medical N (%) of correct responses	Non-medical n (%)of correct responses	p-value		Christian n (%)of correct responses	Muslim n (%) of correct responses	Atheist n (%)of correct responses	p-value		City n (%)of correct responses	Town n (%)of correct responses	Rural n (%)of correct responses	p-value
6. The HPV may infect… ?	595	110 (68.8)	298 (68.5)	0.955[1]	595	90 (54.9)	318 (73.8)	**0.000**[2]	593	263 (69.9)	144 (67.0)	0.512[3]	584	316 (70.2)	42 (56.0)	41 (60.5)	*0.049*[4]	541	268 (70.3)	132 (64.7)	5 (83.3)	0.276[5]
7. If the HPV infection occurs in male, the symptoms are?	442	41 (33.1)	130 (40.9)	0.130[6]	442	36 (34.3)	135 (40.1)	0.289[7]	440	114 (40.1)	55 (35.3)	0.314[8]	432	136 (40)	10 (20.8)	17 (38.6)	**0.037**[9]	439	108 (37.1)	59 (41.3)	2 (40.0)	0.704[10]
8. If the HPV infection occurs in female, the symptoms are: .. ?	572	18 (11.8)	45 (10.7)	0.145[11]	572	21 (13.1)	42 (10.2)	0.315[12]	570	36 (9.8)	27 (13.2)	0.215[13]	562	43 (9.9)	8 (11.3)	9 (16.4)	0.334[14]	568	40 (11.0)	22 (11.2)	0 (0.0)	0.688[15]
9. How many HPV infections lead to serious health complications?	590	45 (28.7)	61 (14.1)	**0.000**[16]	590	21 (13.0)	85 (19.9)	**0.005**[17]	588	7.6 (20.2)	30 (14.2)	0.066[18]	579	78 (17.4)	16 (21.9)	9 (15.5)	0.577[19]	586	66 (17.5)	36 (17.7)	1 (16.7)	0.996[20]

*Obtained from χ^2 test for comparison of categorical variables; [1]Pearson $\chi^2 = 0.003$, df=1; [2]Pearson $\chi^2 = 19.699$, df=1; [3]Pearson $\chi^2 = 0.430$, df=1; [4]Pearson $\chi^2 = 6.049$, df=2; [5]Pearson $\chi^2 = 2.572$, df=2; [6]Pearson $\chi^2 = 2.298$, df=1; [7]Pearson $\chi^2 = 1.125$, df=1; [8]Pearson $\chi^2 = 1.015$, df=1; [9]Pearson $\chi^2 = 6.594$, df=2; [10]Pearson $\chi^2 = 0.710$, df=2; [11]Pearson $\chi^2 = 0.145$, df=1; [12]Pearson $\chi^2 = 1.010$, df=1; [13]Pearson $\chi^2 = 1.540$, df=1; [14]Pearson $\chi^2 = 2.194$, df=2; [15]Pearson $\chi^2 = 0.749$, df=2; [16]Pearson $\chi^2 = 16.607$, df=1; [17]Pearson $\chi^2 = 3.793$, df=1; [18]Pearson $\chi^2 = 3.371$, df=1; [19]Pearson $\chi^2 = 1.099$, df=2; [20]Pearson $\chi^2 = 0.008$, df=2;

DISCUSSION

Assessing the knowledge and awareness of cervical cancer, HPV and their mutual relation has been a main focus of numerous studies [12-33]. Most of these focus particularly on females' perspectives and do not include comparisons of knowledge between men and women [12-17]. Similarly, there are a number of studies that evaluate only the knowledge of students with medical background [14, 18-23]. Since HPV is the most common sexually transmitted disease worldwide that affects both genders and is the causative agent for cervical cancer, [3] the present study includes both male and female participants and also students from various academic backgrounds.

In the present study, the majority of students have answered correctly to most study questions. This shows that, great majority of students among all study groups appear to be generally informed about the aspects of CC. However, the participants neither showed knowledge about the severity of HPV infection symptoms in both men and women, nor the number of HPV related serious complications, notwithstanding their socio-demographic differences. 48.6% of students expected that symptoms which appear in women due to HPV were severe. The majority of students also expected the complications of HPV to be quite serious. Similarly, a study from Pakistan presented that 68% of their respondents rejected the statement that if infected with HPV, any symptoms may not occur at all [24]. Accordingly, one study conducted in the UK reports limited knowledge about the virus among their participants (30%) [12], while another study from China illustrates lack of detailed knowledge about HPV infection and CC where only 47.2% of the respondents have correctly answered over 10 of total 22 questions [18].

The current study highlights that medical students had significantly higher number of correct answers on most questions considering cervical cancer, which was also in accordance to some other studies [21, 24-27]. Interestingly, significantly higher percentage of medical students (76.5%) than their non-medical counterparts (41%) has self-reported positive knowledge of HPV.

Wardle et al. (2001) have established that respondents most commonly associate five risk factors with cervical cancer [28]. Although it was expected medical students to associate more risk factors with CC, due to their medical background, in our study significantly higher percentage of non-medical students (65.7%) have associated higher number of risk factors for CC. As Pitts et at. (2002) showed in their study, high number of sexual partners (67.8%) and early age of sexual initiation (60.3%), were among the most frequent answers about risk factors for CC [12]. The same risk factors presented as most frequent in the present study as well.

Contrary to several other studies that Kamzol et al. (2012) reviewed, where poor awareness of risk factors was described [29], our study verifies much higher percentage of 67.7% knowing that CC is associated with multiple risk factors. Several other studies highlight low levels of risk factor association among participants [13], low ability for detection of risk factors (14) or lack of understanding about risk factors connected to CC [30].

In a study carried out in Portugal, it was noticed that merely 20.7% of all the students knew the correct morbidity rate of CC, and only 17% of mortality, regardless of the academic background [31]. In our study, medical students had significantly higher knowledge of both morbidity and mortality rates ($p=0.000$). One study carried out in India, that evaluated

knowledge about the incidence of CC among 630 female students has come to a conclusion where only 20% of the students have reported its incidence correctly [15]. Another study, has reported inconsistent knowledge considering CC incidence [19].

Studies in the literature indicate that significant differences in the knowledge about CC might be expected between males and females [18, 31]. This might be due to the fact that women pay more attention to this issue [18]. Remarkably, in the current study the only significance in results where females were more avert is for the age of the highest risk of CC ($p=0.002$).

In the present study, a significantly higher number in positive answers of self reported knowledge about HPV in medical students (76.5%), females (64.9%) and young adults (63.4) was noted. ($p=0.000$). There are several studies in agreement with the results in our study. Namely, Medeiros et al. proved significantly higher knowledge scores in female students (64.1%), as well as significant difference in knowledge between health and non-health students ($p<0.001$) [31]. Drewry et al. came to conclusion that older women are more likely to be more knowledgeable about HPV with odds ratio [OR] 2.54; 95% confidence interval [CI] 1.34, 4.78) [17]. Moreover, a study about medical students in South Africa found out that adolescents had lower knowledge about HPV, than their older colleagues [20].

It is of note that 76.5% of medical students have self reported to know what HPV is, but after careful evaluation of the definition given by these students almost 50% of them have incorrect answers. Although 41% of the non-medical students reported to know what HPV is, 74.3 % of them have failed to provide a correct definition, about what HPV exemplifies for them, which lead us to conclude that they have indeed insufficient comprehension of the HPV problem.

Concerning the question of who can be affected by HPV, in the present study, significantly higher percent was seen among the majority of the participants (68.6%), which is supported throughout the literature [21, 24, 25, 32, 33]. Significantly higher number of correct answers among young adults, compared to adolescents was also noted ($p=0.000$). According to Blödt et al. less than 15% of the males knew that HPV can affect both men and women [32]. Another study in Belgium discovered statistically significant difference in the comparison between medical and non-medical student groups [33].

Although the majority of the participants showed less knowledge considering the HPV infection related complications, adolescents seemed to be less informed compared to the young adults. Namely 87% of them answered incorrectly to the study question. Furthermore, male participants of the study presented more knowledgeable about the complications related to HPV infection than women ($p=0.000$). Females reported that HPV related complications were much more frequent than the usual nature of the disease and expected higher percentage of HPV related complications than men. Particularly, 52.9% of the female respondents answered that more than 50% of the HPV infections are followed by serious health complications. Unlike the present study, a study in Portugal showed a significant association between being a female student and selecting milder symptoms of HPV infection ($p<0.001$ in health sciences students) [31].

It is worth mentioning that our study showed several limitations. This study is of a cross sectional design and thus the associations drawn from our results should be interpreted with

caution. It used convenience sampling method, and therefore the study sample might not be representative of the studied population. However, conclusions drawn from the current study could be applied as a solid base for a future more comprehensive research as well as to challenge Public Health authorities for improving public health strategies for better and more efficient educational and preventive programs concerning attitudes towards cervical cancer and HPV.

In conclusion, our study showed that the majority of students have general knowledge about cervical cancer. Although most of the students are familiar with the fact of who can be affected by HPV their knowledge considering HPV infections remains to be generally low. Medical students have significantly higher knowledge of cervical cancer. Although medical students were more able to give a correct definition of HPV, medical background concerning the rest of the questions about the general knowledge about HPV infections did not show to have important role. Results from our study indicate that there are some gaps and misconceptions regarding students' knowledge about HPV infections.

REFERENCES

1. WHO. Human Papillomavirus (HPV) and cervical cancer, 2018 (accessed May 25 2018); Available from (http://www.who.int/news-room/fact-sheets/detail/human-papillomavirus-(hpv)-and-cervical-cancer)
2. Baseman JG, Koutsky LA. The epidemiology of human papillomavirus infections. J Clin Virol. 2005;32(Suppl 1):S16-24.
3. Shew ML, Fortenberry JD. HPV infection in adolescents: natural history, complications, and indicators for viral typing. Semin Pediatr Infect Dis. 2005;16(3):168-74.
4. Hebnes JB, Olesen TB, Duun-Henriksen AK, et al. Prevalence of genital human papillomavirus among men in Europe: systematic review and meta-analysis. J Sex Med. 2014;11(11):2630-44.
5. Stanley M. Pathology and epidemiology of HPV infection in females. Gynecol Oncol. 2010;117(2 Suppl):S5-10.
6. C.D.C. Genital HPV Infection, 2017 (accessed May 23, 2018); Available from (https://www.cdc.gov/std/hpv/stdfact-hpv.htm)
7. The FUTURE II Study Group. Quadrivalent Vaccine against Human Papillomavirus to Prevent High-Grade Cervical Lesions. N Engl J Med. 2007;356:1915-1927
8. Schmotzer G, Reding K. Knowledge and Beliefs Regarding Human Papillomavirus Among College Students at a Minority-Serving Institution. J Community Health. 2013;38(6):1106-1114.
9. Poljak M, Seme K, Maver PJ, et al. Human papillomavirus prevalence and type-distribution, cervical cancer screening practices and current status of vaccination implementation in Central and Eastern Europe. Vaccine. 2013;31;31 Suppl 7:H59-70.
10. Seme K, Maver PJ, Korać T. Current status of human papillomavirus vaccination implementation in central and eastern Europe. Acta Dermatovenerol Alp Pannonica Adriat. 2013;22(1):21-5.
11. Doxiadis CA. Ekistics, the science of human settlements. Science. 1970;170(3956):393-404.
12. Pitts M, Clarke T. Human papillomavirus infections and risks of cervical cancer: what do women know?. Health Educ Res. 2002 Dec;17(6):706-14.

13. Koç Z. University students' knowledge and attitudes regarding cervical cancer, human papillomavirus, and human papillomavirus vaccines in Turkey. J Am Coll Health. 2015;63(1):13-22.
14. Akpo D, Deji P, Idiake V, et al. Cervical Cancer: Knowledge, Screening Practices and Vaccines among Female Medical Students in the Common Wealth of Dominica. Arch Med. 2016;8:5
15. Saha A, Chaudhury AN, Bhowmik P, et al. Awareness of cervical cancer among female students of premier colleges in Kolkata, India. Asian Pac J Cancer Prev. 2010;11(4):1085-90.
16. Drewry J, Garcés-Palacio IC, Scarinci I. Awareness and Knowledge about Human Papillomavirus among Latina Immigrants. Ethnicity & disease. 2010;20(4):327-333.
17. Sherman SM, Nailer E, Minshall C, et al. Awareness and knowledge of HPV and cervical cancer in female students: A survey (with a cautionary note). J Obstet Gynaecol. 2016;36(1):76-80.
18. Wen Y, Pan XF, Zhao ZM, et al. Knowledge of human papillomavirus (HPV) infection, cervical cancer, and HPV vaccine and its correlates among medical students in Southwest China: a multi-center cross-sectional survey. Asian Pac J Cancer Prev. 2014;15(14):5773-9.
19. Mehta S, Rajaram S, Goel G, et al. Awareness about Human Papilloma Virus and its Vaccine Among Medical Students. Indian Journal of Community Medicine: Official Publication of Indian Association of Preventive & Social Medicine. 2013;38(2):92-94.
20. Hoque M, Van Hal G. The knowledge about, beliefs and attitudes of medical students regarding vaccination against the human papillomavirus, in South Africa: A Cross-Sectional Study. Biomedical Research 2015; 26 (1): 65-70
21. Fu CJ, Pan XF, Zhao ZM, et al. Knowledge, perceptions and acceptability of HPV vaccination among medical students in Chongqing, China. Asian Pac J Cancer Prev. 2014;15(15):6187-93.
22. McCusker S M, Macqueen I, Lough G, et al. Gaps in detailed knowledge of human papillomavirus (HPV) and the HPV vaccine among medical students in Scotland. BMC Public Health, 13, [ARTN 264].
23. Pandey D, Vanya V, Bhagat S, et al. Awareness and Attitude towards Human Papillomavirus (HPV) Vaccine among Medical Students in a Premier Medical School in India. Wu JT, ed. PLoS ONE. 2012;7(7):e40619.
24. Khan MT; Buksh MA; Rehman IU, et al. Knowledge, attitudes, and perception towards human papillomavirus among university students in Pakistan. Papillomavirus research. 2016;2405-8521:2:122-127
25. Rathfisch G, Güngör İ, Uzun E, et al. Human papillomavirus vaccines and cervical cancer: awareness, knowledge, and risk perception among Turkish undergraduate students. J Cancer Educ. 2015 Mar;30(1):116-23.
26. Yam PWA, Lam PL, Chan TK, et al. A Cross Sectional Study on Knowledge, Attitude and Practice related to Human Papillomavirus Vaccination for Cervical Cancer Prevention between Medical and Non-Medical Students in Hong Kong. Asian Pac J Cancer Prev. 2017 Jun 25;18(6):1689-1695.
27. Borlu A, Gunay O, Balci E, Sagiroglu M, et al. Knowledge and Attitudes of Medical and Non-Medical Turkish University Students about Cervical Cancer and HPV Vaccination. Asian Pac J Cancer Prev. 2016;17(1):299-303.
28. Wardle J, Waller J, Brunswick N et al. Awareness of risk factors for cancer among British adults. Public Health. 2001;115(3):173-4.

29. Kamzol W, Jaglarz K, Tomaszewski KA, et al. Assessment of knowledge about cervical cancer and its prevention among female students aged 17-26 years. Eur J Obstet Gynecol Reprod Biol. 2013;166(2):196-203.
30. Giles M, Garland S. A study of women's knowledge regarding human papillomavirus infection, cervical cancer and human papillomavirus vaccines. Aust N Z J Obstet Gynaecol. 2006;46(4):311-5.
31. Medeiros R, Ramada D. Knowledge differences between male and female university students about human papillomavirus (HPV) and cervical cancer: Implications for health strategies and vaccination. Vaccine. 2010;29(2):153-60.
32. Blödt S, Holmberg C, Müller-Nordhorn J, et al. Human Papillomavirus awareness, knowledge and vaccine acceptance: a survey among 18-25 year old male and female vocational school students in Berlin, Germany. Eur J Public Health. 2012;22(6):808-13.
33. Deriemaeker H, Michielsen D, Reichman G, et al. Knowledge about human papillomavirus and the human papillomavirus vaccine in Belgian students. Central European Journal of Urology. 2014;67(4):410-41.

UDC: 616.314-002-084:546.161

ORIGINAL ARTICLE

THE INFLUENCE OF FLUORIDE TREATMENT ON Ca, P CONTENT AND Ca/P RATIO OF THE STARTING PHASE OF EARLY CHILDHOOD CARIES

Kokoceva Ivanovska Olga
Department of Pediatric and Preventive Dentistry, Faculty of Dentistry, Ss. Cyril and Methodius University of Skopje, Skopje,
Republic of Macedonia

ABSTRACT

Circular caries occurs in the early childhood (1-1.5 year), immediately after the eruption of primary (deciduous) teeth. According to the latest world glossary, this term is also known as early childhood caries (tooth decay in early childhood).

The aim of this study was to examine the mineral content changes at the starting stage-initial lesion, before and after topical application of fluoride preparation.

Forty extracted primary incisors–teeth samples were assigned into three groups: I group - twenty healthy mandibular incisors, control group: II group - ten untreated maxillary incisors with initial lesion - white spot (macula alba), and group III - ten maxillary incisors with initial lesion - white spot (macula alba) treated with topical fluoride preparation, with energy dispersed spectrometer (EDS). .

This study included qualitative and quantitative microanalysis of changes in the mineral content of calcium (Ca), phosphorus (P), Ca/P ratio in teeth samples in the three groups.

The results have shown that the mineral content in individual groups of samples showed a significant effect of the topical fluoride treatment by increasing the values of calcium and phosphorus in the tooth enamel. This indicates significant remineralization process in the initial lesion. In some cases the mineral composition of the enamel reached values similar to healthy teeth.

Timely detection of the decay in its initial stage known as initial lesion-white spot (macula alba) and its early treatment with a topical fluoride preparations may completely compensate the mineral loss with a full reparation of the initial lesion (restitutio ad integrum).

Keywords: early childhood caries, enamel, initial lesion, remineralization, fluoride treatment, energy dispersed spectroscopy

INTRODUCTION

In the early age of the child (1-1.5 year), immediately after the eruption of primary teeth, a special form of tooth decay that affects only primary teeth may occur. Much higher prevalence of the circular caries has been reported since the last decade [1-3]. This has caused major problems for children, parents and dentists, which require special attention. In the latest world caries terminology, this form of tooth decay is known as "early childhood caries" [4 -8].

During the initial phase of the early caries lesion (macula alba), there is a mineral loss in enamel underlayers. Calcium and phosphate ions are the first to diffuse from the enamel into the oral medium, under the influence of cariogenic hazards [9].

Timely detection and treatment of the early childhood caries [10] is the key to its complete elimination.

The objective of this study was to examine the changes in the mineral content of the enamel in the initial stage (macula alba), before and after topical application of fluoride preparation.

The qualitative and quantitative analyses included:

- Qualitative and quantitative microanalysis of the content of Ca, P, Ca/P ratio in the enamel of primary teeth with circular caries in its initial lesion and comparative analysis of healthy primary teeth**.**
- Qualitative and quantitative microanalysis of minerals: Ca, P, Ca/P ratio in the enamel of primary teeth with initial lesion treated with a topical fluoride treatment and comparative analysis with untreated one (teeth with initial lesion)**.**

MATERIALS AND METHODS

The study comprised 40 samples of extracted teeth during their stage of physiological change, divided into three groups:

I group - twenty healthy mandibular incisors, or control group,

II group - ten untreated maxillary incisors with initial lesion - white spot (macula alba),

III group - ten maxillary incisors with initial lesion- white spot (macula alba) treated with aminofluoride solution, once a week in a period of six months **[**1 ml solution aminofluoride contained 10 mg fluoride (1%): olaflur (0.925%) and decafluoride (0.075)].

The qualitative and quantitative analyses were made at the Institute of Medical Diagnostics and Research in the Biomedical and Natural Sciences, at the Faculty of Medicine in Nis. The tests were performed with energy dispersed spectrometer (EDS**),** EDS X-Ray Analyser (Qx2000), Link Analytical, 1992, U.K. Applying the EDS System analysis, all samples from all groups underwent determination of the mineral content of Ca, P, and Ca/P ratio in the enamel [10].

Microanalyses with EDS X-Ray analyser are based on the measurement and determination of the characteristic X-ray which is generated during the interaction of electrons between the primary pencil and the given sample.

Actually, with the help of the EDS software, based on high peaks it is possible to calculate every element in percentage (%). If we compare the results obtained for the two different minerals, we get Ca/P ratio.

All laboratory testing were done in:

1. Healthy enamel (mandibular incisors-control group).
2. The initial lesion (maxillary incisors of both examined groups).
3. Healthy part the surrounding enamel with an initial lesion (maxillary incisors of both examined groups)

A comparative analysis between the control group and both examined groups was also performed.

Statistical analyses were made with the statistical program using basic statistical methods: Student's t-test, Analysis of variance (ANOVA) and Tukey HSD post-hoc test. The value of $p<0.05$ was considered to be statistically significant.

RESULTS

The results of the qualitative microanalysis system with EDS are presented with diagrams of the obtained peaks of mineral presence. The quantitative microanalyses are presented in percentages (%).

1. EDS-qualitative microanalysis of test samples

The results obtained for tested minerals with the qualitative microanalysis system with EDS-diagrams are shown in Figures 1-3.

Explanation of EDS-diagrams:

X- axis is the energy, ranging from 0 to 20 kilo electron volts,

Y-axis shows how high is every peak and it is used to see which mineral is present in the sample.

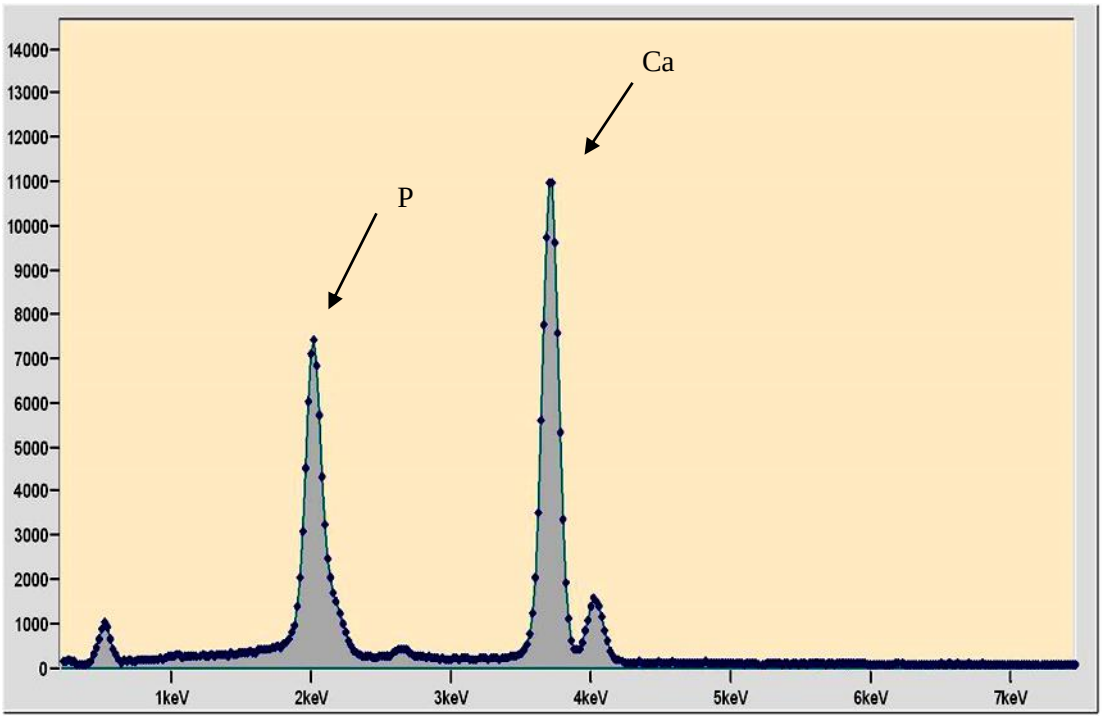


Fig. 1. EDS - qualitative analysis of Ca and P in the control group (healthy teeth) - untreated sample

Untreated sample

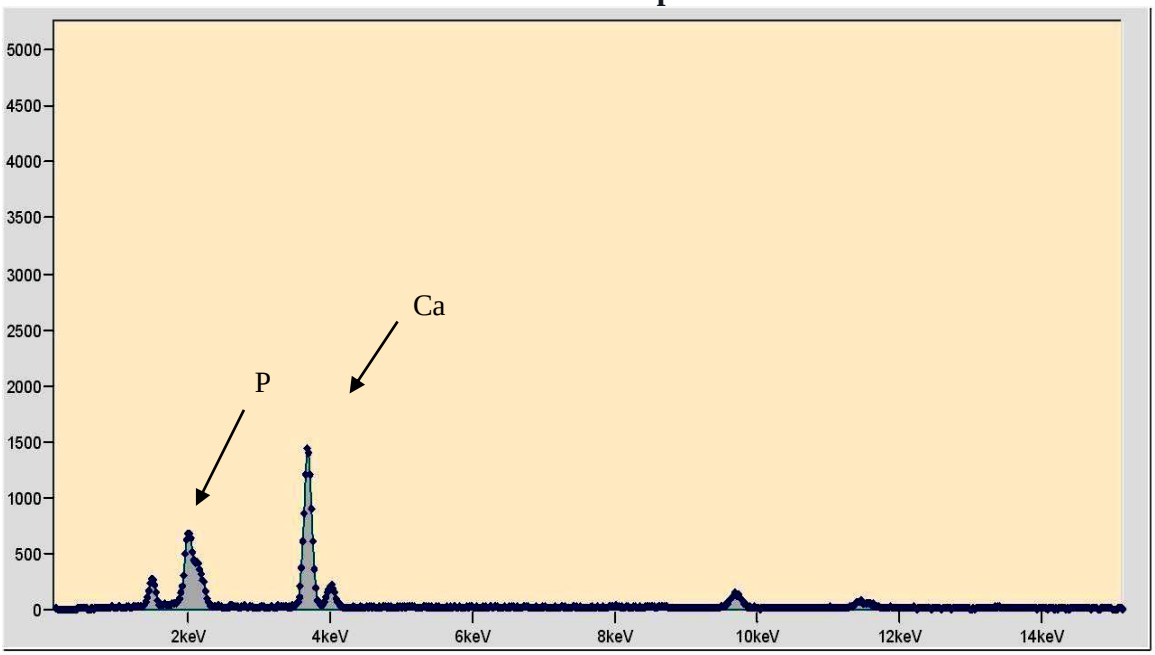


Fig. 2. A) EDS - qualitative analysis of Ca and P in the area of enamel with initial lesion in untreated sample

Treated sample

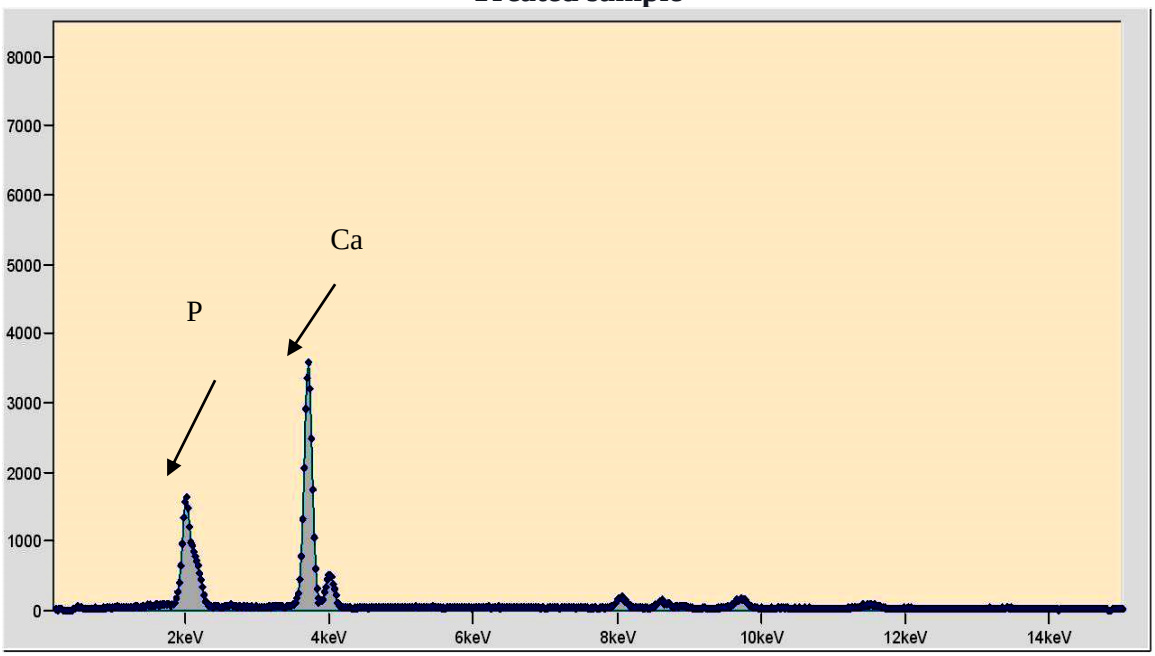


Fig. 2. B) EDS - qualitative analysis of Ca and P in the area of enamel with initial lesion in treated sample

Untreated sample

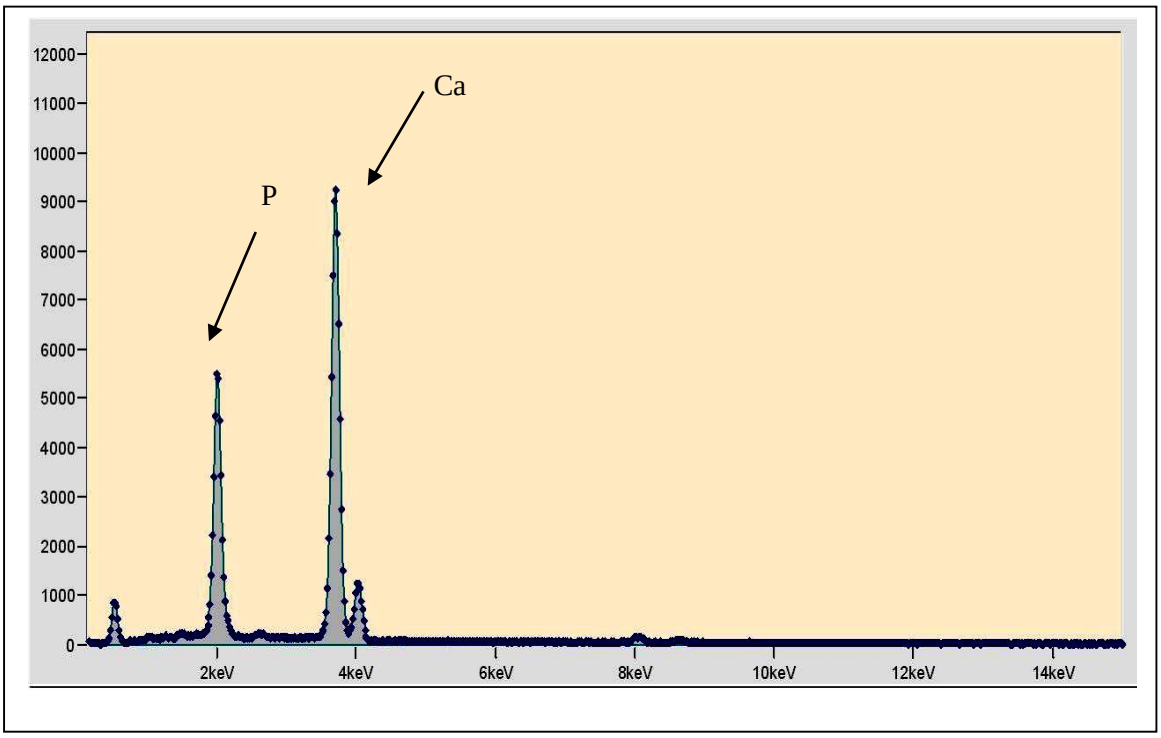


Fig. 3. B) EDS - qualitative analysis of mineral content of Ca and P in the common section - surrounding enamel with initial lesion in treated sample

Treated sample

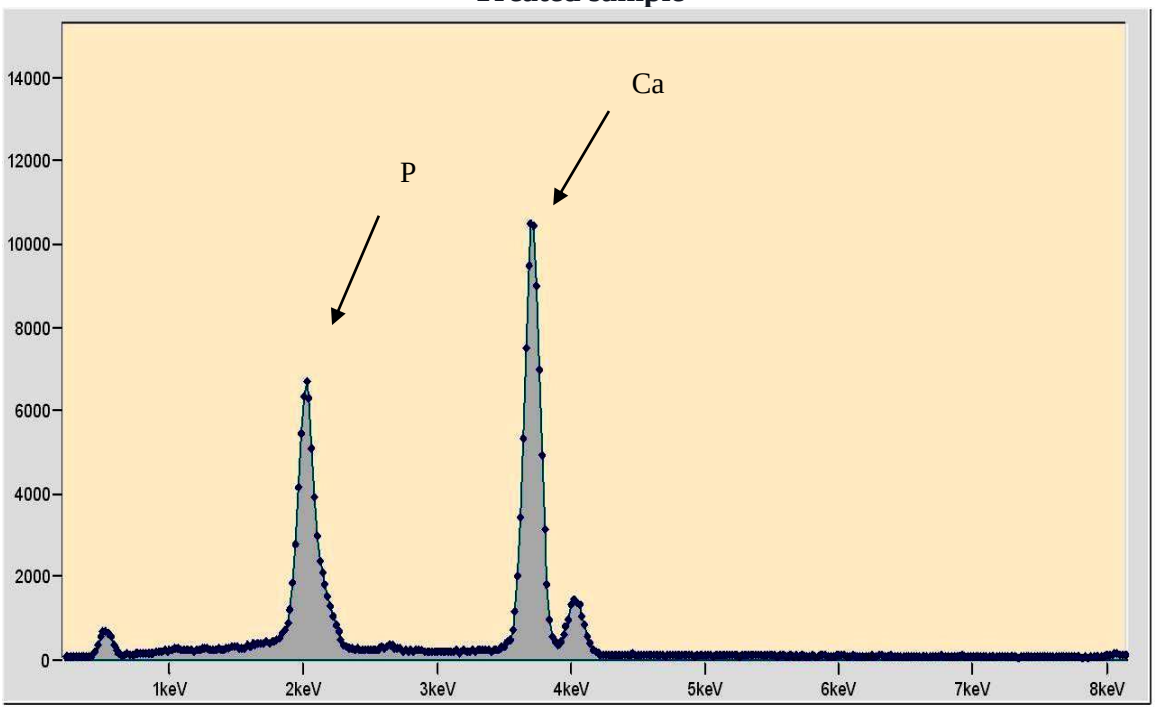


Fig. 3. B) EDS - qualitative analysis of mineral content of Ca and P in the common section surrounding enamel with initial lesion in treated sample

2. EDS - quantitative microanalysis

The results from the overall EDS quantitative microanalysis and the statistically analyzed data are presented in Tables 1-3.

Table 1. Content of Ca (%) in the enamel of untreated and treated teeth

Localization of the probe	N	Content of Ca%				Student t-test
		Untreated teeth		**Treated teeth**		
		Mean	**±SD**	**Mean**	**±SD**	P
Healthy enamel of mandibular incisors (control group)	**20**	**31.722**	**1.410**	/	/	
Part of enamel with initial lesion (maxillary incisors)	**10**	**24.020**	**1.073**	**26.885**	**1.522**	0.001
Healthy part – the surrounding enamel with an initial lesion (maxillary incisors)	10	29.778	0.914	31.109	1.568	0.0015

The analysis of variance (ANOVA) showed statistically significant differences of the average Ca values between the untreated teeth and the healthy mandibular incisors (F=84.999; p=0.0001) and between the treated teeth and the healthy mandibular incisors (F=31.054; p=0.00015) **(Table 1)**. The Tukey HSD post-hoc test confirmed that there was no significant difference between the treated teeth with an initial lesion in the healthy surrounding enamel and the control group of teeth.

Table 2. Content of P (%) in the enamel of untreated and treated teeth

Localization of the probe	N	Content of P%				Student t-test
		Untreated teeth		**Treated teeth**		
		Mean	**±SD**	**Mean**	**±SD**	P
Healthy enamel of mandibular incisors (control group)	**20**	**16.110**	**0.936**	**/**	**/**	
Part of enamel with initial les ion maxillary incisors	**10**	**14.188**	**0.523**	**15.573**	**0.853**	0.00004
Healthy part – the surrounding enamel with an initial lesion (maxillary incisors)	10	15.400	0.452	15.916	1.052	n.s.

The analysis of variance (ANOVA) showed statistically significant differences of the average P values between the untreated teeth and the healthy mandibular incisors (F=14.836; p=0.000049) **(Table 2).** The difference between the treated teeth and the healthy mandibular incisors was F=1.393; p=0.2567. According to the Tukey HSD test there were statistically significant differences only in the untreated samples with an initial lesion, between the part with the lesion and the healthy surrounding enamel. A significant difference was observed between the part with the initial lesion and the control group, as well.

Table 3. Ca/P ratio values in the enamel of treated and untreated teeth

Localization of the probe	**N**	**Ca/P ratio**				**Student t-test**
		Untreated teeth		Treated teeth		
		Mean	±SD	Mean	±SD	**P**
Healthy enamel of mandibular incisors (Control group)	20	1.970	0.045	/	/	
Part of enamel with initial les maxillary incisors ion	10	1.692	0.025	1.726	0.027	0,00001
Healthy part – the surrounding enamel with an initial lesion maxillary incisors	**10**	**1.933**	**0.40**	**1.958**	**0.092**	0,00019

The analysis of variance (ANOVA) confirmed the statistically significant differences of the average Ca/P values (%) between the untreated teeth and the healthy mandibular incisors (F= 129.366; p=0.00001), and between the treated teeth and the healthy mandibular incisors (F=49.610; p=0.00019) **(Table 3).** The Tukey HSD test confirmed that there were no statistically significant differences between the healthy surrounding enamel of the initial lesion and the control group.

DISCUSSION

The examinations carried out by the EDS system, when testing the percentage of presence of certain minerals in the mineral composition of the control group (healthy mandibular incisors) showed the following average values: Ca (31.722 %), P (16.110 %), Ca/P=1.97 (9).

The study of Sabel *et al.* on the mineral content of the enamel in healthy primary teeth showed average values as follows: Ca (33.82%) and P (17%) [11]. The authors implemented the method XRMA (x-ray micro analyses) for determination of the mineral content, similar to the method used in the present study. Our method is also based on microanalysis with X – rays and this fact makes the comparison of the respective minerals in the same kind of examined teeth most appropriate. The average value of Ca/P ratio in the present study was 1.970 in the healthy mandibular incisors, which is also consistent with the results presented in the study of Sabel *et al.*, who reported an average value of 1.99.

Lakomma and Rytomma [12] in their study compared the mineral content of the enamel in healthy primary and permanent teeth. They reported higher average values of the mineral

content in permanent teeth. The values for primary teeth mineral amount were: Ca (34%), P (18.5%). Regarding the mineral content of the enamel in healthy permanent teeth, Lakomma and Rytomma found the following values: Ca (35%) and P (18.6%). The study of Derise and Retief [13] found Ca values of 36.2-38%) and P of 18.2-19.2%.

The amount of present calcium and phosphorus in the enamel of permanent teeth ranged within the following limits: Ca (37.03-39%); P (18.1-19%). Hoyer *et al.* (14) obtained similar results: Ca (35.19-38.99%); P (16.45-18.55%), while George *et al.* reported the following values: Ca (36.16 - 39.97%); P (16.37-17.57%).

The results from the mentioned studies on the mineral content of the enamel in permanent teeth have confirmed that primary teeth are less mineralized than permanent teeth. The present study has also pointed out the same finding.

Tests with samples of teeth with an initial form of circular caries revealed a change in the mineral composition, compared to the control group of teeth.

The amount of Ca, P and Ca/P ratio in subjects with initial lesions that were not treated with a topical fluoride treatment was significantly lower than the proportion of these minerals in healthy teeth and was within the following limits:

- Calcium amount of 24.020% in the part of the enamel of the initial lesion, to 29.778% in the common section - surrounding enamel with initial lesion.
- Phosphorus amount of 14.188% in the enamel of the teeth with initial lesion, to 15.400% in common share - surrounding enamel with initial lesion.
- Ca/P ratio was lowest in the area of enamel in the teeth with initial lesion, with an average value of 1.692.

The analysis of the mineral composition of the surrounding healthy enamel next to the initial lesion showed higher values of the present minerals. This prompts the conclusion that the effect of topical fluoride treatment is significant in subjects with initial lesion. In some of the samples there was a complete repair of the mineral composition, approximately the same as the control group. The results imply that the presence of Ca, P and Ca/P ratio in subjects with initial lesions that were not treated with a topical fluoride treatment, was significantly lower than the proportion of these minerals in healthy teeth [9].

The overall analysis of mineral content in individual groups of samples with EDS revealed a significant effect of topical fluoride treatment on improving the presence of minerals. The EDS-qualitative microanalysis showed an increase in the values in the tooth enamel, which was confirmed by EDS-quantitative microanalysis [15]. This indicates that a significant process of remineralization in the initial lesion happens, as one of the major benefits from the application of fluoride in dentistry [16]. In some cases, the reached mineral composition of the enamel in teeth with initial lesion was similar to that in healthy teeth [10].

In cases of early childhood caries, the timely detection in its initial stage known as initial lesion and its treatment with a topical fluoride treatment at that time, may lead to complete reparation [17]. The complete repairment and remineralization is only possible at this stage of the development of the tooth decay [14,18]. The ultimate effect is complete extinction of the white spot in the initial lesion and recovery of mineral loss i.e. restutio ad integrum [19].

It is crucial for the pedodontists to diagnose the initial stage of the disease as early as possible. The prevention with fluoride treatment [20-22] accompanied with the application of other preventive measures will provide maximum benefits [23-27].

CONCLUSIONS

The results obtained in this study have yielded better understanding of mineral changes on the enamel surface, in the initial phase of early childhood caries. It can be concluded that:

- Values of calcium and phosphorus were significantly lower in samples with initial lesion teeth of early childhood caries, compared to healthy teeth samples (control group).
- In the samples with initial lesion, treated with a topical fluoride treatment, the values of those two minerals were statistically significantly higher in comparison to the untreated samples of teeth.
- Early detection of early childhood caries in the initial phase and early prevention with topical fluoride treatment may result in remineralization of the initial lesions and extinction of the white spot, with possible complete reparation of the mineral loss, i.e. restutio ad integrum.
- Consumption of fluoride supplements is advised; varnishes and fluoride tooth pastes must also be provided;
- Caries risk assessment and parental education for prevention of oral diseases must be implemented.

All of the above presented conclusions can help in creation of the strategy for successful prevention of early childhood caries.

REFERENCES

1. Srkoč O., Brajan M., Stilinović D. Prilog etiologiji cirkularnog karijesa. Acta Stomatol Croat 1989; 23(2):159-164.
2. Carević M., Vulović M, Šindolić M. Integrated Approach in Combating Early Childhood Caries. Balk J Stom. 2009;13:15-20.
3. Kokoceva-Ivanovska O. Ethio-pathogenetics and preventive aspects of the circular caries on the deciduous teeth. Master Thesis, 2002:57-73. Faculty of Dental Medicine, University"Ss Cyril& Methodius", Skopje, R.Macedonia.
4. Louloudiadis K. Prevention of Early Childhood Caries. Balk. J. Stom. 2001;5(2):77-82.
5. Atanasov N, Markova N. Early Childhood Caries (ECC)-Our point of view. Balk J Stom 2001;5(2):83-7.
6. Ismail A. Prevention of ECC. Comin Dent OralEpidemiology. 1998; 26(Suppl):49-61.
7. Moss JS. The relationship between diet, saliva and baby bottle tooth decay. Int Dent J. 1996; 46(Suppl 1):399-402.
8. Tinanoff N., Reisine S. Update on early childhood caries since the Surgeon General's Report. Academic Paediatr. 2009; 9(6):396-403.
9. Kokoceva-Ivanovska O., Mijoska A., Gligorova D. EDS-Quantitative Microanalysis of Calcium and Phosphate in The Initial Lesion of a Circular Caries before and after the topical fluoride treatment. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2016;7(3): 2403-2406.
10. Kokoceva-Ivanovska O. Early childhood caries: Following of the early developing stages and possibilities for its prevention. Ph.D. Thesis, 2011:137-159. Faculty of Dental Medicine, University Cyril & Methodius, Skopje, Macedonia.
11. Sabel N et al. Elemental composition of normal primary tooth enamel analyzed with XRMA and SIMS. Swed Dent J. 2009; 33:75-83.

12. Lacoma EL, Rytomaa I. Mineral composition of enamel and dentin of primary and permanent teeth in Finland. Scand J Dent Res. 1997;85(2):89-95.
13. Derise NL., Ritchey SJ. Mineral composition of normal human enamel and dentine and the relation of composition to dental caries: Microminerals. J Dent Res. 1974; 53:853-858.
14. Hoyer I, Gaengler P, Bimberg R. In vivo remineralization of human enamel and dental calculus formation. J Dent Res. 1984;63(9):1136-1139.
15. Caltabiano C, et al. Carious and noncarious lesions of the hard dental tissues. Ultrastructural (SEM) and microanalytical (EDS) analyses of teeth from the 3rd century B.C. Minerva Stomatol. 1996;45(5):197-204.
16. CarčevM. Preventive stomatology, Скопје, 2006.
17. Kokoceva Ivanovska O, Jankulovska M, Mijoska A, Zabokova-Bilbilova E, Pavlevska M, TodorovskaG. Ultrastructural Changes of the Initial Lesion at Early Childhood Caries. Journal of International Dental and Medical Research. 2017;10(1):36-41.
18. Linton LJ. Quantitative measurements of remineralization of incipient caries. Am J of Orthod Dentofac Orthop 1996; 110:590-7.
19. Vulovic M, Beloica D, Gajic M, Stefanovic R. Preventive Stomatology, Beograd: 2002
20. Verkamp JSJ, Weerheijm KL. Nursing bottle caries: The importance of a development perspective. J Dent Child. 1995; 62(6):381-385.
21. Hicks J, Wild T, Flaitz CM, Seybold S. Fluoride varnishes and caries development in primary tooth enamel: an in vitro study. J Dent Child. 2001; 68(5-6):304-310.
22. Weintraub JA, Ramos-Gomez F, et al. Fluoride varnish application prevents caries in preschool children. J Evid Base Dent Pract. 2007; 7:23-24.
23. Fidya, Rachmawati R, Effendi MC., Ferianti Dewi NKA. The effect of NAF 5% and NANONaF to the permanent tooth endurance toward dental caries. Journal of International Dental and Medical Research. 2015;8(2): 34-39.
24. Blumenshine SL., Vann WF, Gizlice Z, Lee JY. Children's school performance: Impact of general and oral health. J Public Health Dent. 2008;68(2):82-7.
25. American Academy of Paediatric Dentistry. Symposium on the prevention of oral disease in children and adolescents. Chicago, Ill; November 11-12, 2005: Conference papers. Paediatr Dent 2006; 28(2):96-198.
26. Weinstein P, Harrison R., Benton T. Motivating parents to prevent caries in their young children. One-year findings. JADA. 2004; 135(6):731-737.
27. Tinanoff NT., Kanellis MJ, Vargas CM. Current under-standing of the epidemiology mechanism, and prevention of dental caries in preschool children. Paediatr Dent. 2002; 24(6):543-51.

UDC: 618.15:544.362]-084:618.3

ORIGINAL ARTICLE

VAGINAL pH VERSUS CERVICAL LENGTH IN THE SECOND TRIMESTER OF PREGNANCY AS A PREDICTOR OF PRETERM BIRTH

Stojovska Lazarova Aleksandra[1], Jankoska G[2], Jovanovska V[1], Jovanovska E[3], Kostovski M[4]
[1]University Clinic of Gynecology and Obstetrics, Skopje, Republic of Macedonia; [2]Institute of Microbiology and Parasitology, Medical Faculty, Ss. Cyril and Methodius of Skopje, Skopje, Republic of Macedonia; [3]University Clinic of Radiology, Skopje, Republic of Macedonia; [4]Medical Faculty, Ss. Cyril and Methodius of Skopje, Skopje, Republic of Macedonia;

ABSTRACT

Objective: The aim of the study was to evaluate the diagnostic value of vaginal pH and cervical length measurement in the second trimester of pregnancy as a preterm labor (PTL) predictor.

Materials and methods: During a prospective cohort study 225 uncomplicated singleton pregnant women between 20 and 24 weeks of gestation were assesses regarding vaginal pH and cervical length. Vaginal pH was measured using Ph - indicator strips and cervical length was determined using transvaginal ultrasound. The cut - off values for vaginal pH and cervical length were defined as 5 and < 30 mm respectively.

Results: Vaginal pH of 5 and above was found in 57/225 women (25.33%) while cervical length <30mm was found in 19/225 (8.7%). The incidence of PTL < 37 weeks was 12.2% while the incidence of early PTL < 34 weeks was 17.5%. Predictive value of higher vaginal pH was significantly more (29.7%) than vaginal PH<5 (13%) in predicting PTL. As a result, alkaline vaginal PH significantly increases the odds of preterm labor (OR=3.06). Shortened cervical length is better predictor of PTL than higher vaginal pH with positive predictive value of 71% and negative predictive value of 85%. Cervical length less than 30 mm nearly 14-fold increases odds of preterm birth (OR=13.9)

Conclusion: Compared to alkaline vaginal pH, shortened cervical length has better value to predict PTL overall. However, regarding early or late PTL, vaginal PH is more accurate to predict late PTL, while cervical length measurement is more appropriate to predict early PTL (<34 weeks).

Keywords: bacterial vaginosis; cervical length; preterm birth; second trimester.

INTRODUCTION

Preterm labour and birth are a major cause of perinatal morbidity and mortality. Despite modern advances in obstetric and neonatal management, the rate of preterm birth in the developed world is increasing. It occurs in 5-18 % of all deliveries worldwide with most developed countries reporting an increased incidence over the last three decades [1]. Advances in perinatology and neonatology in the past decade have resulted in increased survival rates, but unfortunately the associated morbidity for the survivors remains significant where one fifth to one quarter will suffer at least one major disability including chronic lung disease, impaired mental development, cerebral palsy, deafness or blindness [2,3]. Even late preterm infants (born between 32 and 36 weeks of gestation) have a greater risk of respiratory distress syndrome, feeding difficulties, temperature instability, jaundice and delayed brain development [2,3].

Preterm birth may be iatrogenic or spontaneous. Iatrogenic premature birth is result of medical intervention due to a fetal and/or maternal condition (e.g., praeeclampsia, intrauterine fetal growth restriction, placental abruption) necessitating early delivery. By contrast, spontaneous premature birth often occurs despite best effort to prolong the pregnancy.It is estimated that up to 80 percent of premature births fall into this category. The major goal of the obstetrician in this regard is therefore to prevent preterm birth. Failing in this,it is crucial to delay preterm birth long enough to optimize the outcome for the fetus. That means to have time to administer corticosteroids to enhance fetal lung maturation and / or to give magnesium for fetal neuroprotection. A prerequisite for the success of this strategy is the reliable prediction / identification of women at risk of preterm birth.

Evidence suggests that spontaneous preterm labour and delivery are a heterogeneous condition with many triggers or precipitating factors including infection, cervical insufficiency, malnutrition, multifetal pregnancy and idiopathic uterine contractions [4].

Early detection of pregnant women at risk of premature labour will help to reduce the occurrence of prematurity-related mortality and morbidity. Bacterial vaginosis and cervical insufficiency are two items that recently known play an essential role in preterm delivery. They can be diagnosed using safe, simple and reliable methods.These problems are also potentially treatable [4]. Bacterial vaginosis (BV) is a lower genital tract infection characterized by change in normal vaginal flora and replacement by vaginosis-associated anaerobic microorganisms. BV is a leading cause of many undesirable fetal outcomes including premature birth and premature rupture of fetal membranes [5,6]. This problem leads to an increase in vaginal pH that may be diagnostic [7]. Cervical insufficiency describes a functional weakness of the cervix associated with short cervical length, less than 3 cm. [8].

Transvaginal ultrasound assessment of the cervix has been used as a diagnostic measurement for prediction of preterm labour. The question is wether all pregnant women should be measured. A previous preterm birth is amongst strongest risk factors for subsequent preterm birth. However, a nulliparous women have no past obstetric history to call upon, any such previous history risk factor-based assessment is inapplicable in their situation. Overall, risk factor role alone is unreliable, as over 50 % of pregnancies that deliver preterm will fail to be identified. So, among these and recent evidences suggests that more effective screening of premature birth can be provided by sonographic measurement of cervical length at second trimester of pregnancy [9,10]. On the other hand some studies propose that prevention of PTL is possible by vaginal pH screening [11,12]. Maybe it is best to do both methods together, but this study was designed to compare the accuracy of a sign of bacterial vaginosis by increased vaginal pH and a sign of cervical insufficiency by measuring CL less than 3 cm in predicting preterm labour.

Aim of the study

The aim of the study was to evaluate the diagnostic value of vaginal pH and cervical length measurement in the second trimester of pregnancy as a preterm labour (PTL) predictor.

MATERIALS AND METHODS

This prospective cohort study was conducted on 225 pregnant women with uncomplicated singleton pregnancy between 20-24 weeks of gestation. All cases were recruited in the study as they came to Univesity Clinic of Gynecology and obstetrics – Skopje for routine pregnancy follow up during 1 calendar year. Informed written consent was received from all participants.

The exclusion criteria were : suspected chorioamnionitis (fever > 38.5), vaginal bleeding, history of cervical procedure on the cervix, autoimmune disease, cervical cerclage,

early intrauterine growth restriction, hypertension or diabetes mellitus, major congenital fetal anomalies that were diagnosed and sexual intercourse or use of products that can affect vaginal pH over the past 24 h.

Gestational age was determined using last menstrual period and a correction made based on ultrasound measurements performed at first trimester of pregnancy. Vaginal pH was measured to assess bacterial vaginosis using pH-indicator strips (Merck KGaA, 64271 Darmstadt, Germany) after speculum insertion in lithotomy position. No lubricants were used in order to possible interaction with the results. pH measurement was performed by doctors .The vaginal pH of 5 was considered as cut-off point and defined as elevated in our study population. This cut-off point has been used in many other studies [14, 15, 16]. After the measurement of vaginal pH, transvaginal ultrasound examination was performed using Voluson E-8 expert ultrasound imaging system (GE) to measure the length of the cervix, from internal to external os. To avoid differences in the level of medical care in both groups of the study, it was tried that the patient, physician and the investigator remain blind to the vaginal pH and the cervical length until delivery time.

However, to prevent health problems in patients, related codes were given to the physician if needed. All patients were followed until delivery and gestational age was determined at delivery time. SPSS software version 17 was used for statistical analysis. After primary clearing and correction of missing data, variables were described using descriptive statistics. Risk of preterm delivery among the study population was calculated in general and in terms of pH and cervical length and predictive value of each item was reported. Chi-square test and t-test were used for comparing results. Regression modeling was used to adjust for the effect of confounders. P-value less than 0.05 was considered statistically significant.

RESULTS

Vaginal pH and cervical length were measured at 20-24 weeks of gestation in 225 pregnant women. Mean (SD) maternal age was 26.80 (4.55) years. Mean gestational age at enrollment was 21.4 weeks. Mean (SD) gestational age at delivery was 38 (2.1) weeks. Minimum gestational age at delivery was 22 weeks and maximum was 41.5. According the both parameters the incidence of preterm labour <37 weeks in our cohort was 20% while the incidence of early PTL<34 weeks was 11%.

Our study showed alkaline vaginal pH and short cervical length (<30mm) significantly increases the odds of preterm labour (OR=3.06 and OR=14 respectively). Compared to vaginal pH, cervical length had a greater positive predictive value (PPV) and positive likelihood ratio to predict PTL (Table-I). In addition, compared to cervical length, vaginal pH was more accurate in predicting early (34-37 weeks) than late (<34 weeks) PTL (Table II and III). Multivariate logistic regression showed that even after adjusting for effect of age and parity, there is a significant relationship between vaginal pH and cervical length and preterm delivery (P<0.0001).

Table 1. Indices for Vaginal PH≥5 and Cervical Length <30mm as Preterm labour predictors.

	Sen % (CI)	**SP % (CI)**	**PPV % (CI)**	**NPV % (CI)**	**+LR(CI)**
PH≥5	58.5(47.6-69.1)	68.4(63.2-73.2)	31.5(24.4-39.2)	**Variable**	1.85(1.47-2.34)
Cervical length<30mm	31.0(21.5-41.9)	96.8(94.4-98.4)	71.1(54-84.6)	85.0(81.1-88.3)	9.90(5.12-19)
P Value	<0.0001	<0.0001	<0.0001	0.427	<0.0001

Abbreviations: Sen,; Sensitivity, CI; Confidence Interval, SP; Specificity, PPV; Positive Predictive Value, NPV; Negative Predictive Value; +LR; Positive likelihood Ratio.

Table 2. Frequency of early versus late PTL regarding vaginal PH.

		Early preterm	**Late preterm**	**Normal delivery**	**Total**
PH<5	Number	11	11	146	168
	%	6.5%	6.5%	87.0%	100.0%
PH ≥5	Number	10	7	40	57
	%	17.5.%	12.2%	70..3%	100.0%
Total	Number	21	18	186	225
	%	**9.33%**	**8%**	**82.67%**	**100.0%**

Table 3. Frequency of early versus late PTL regarding cervical length.

Variable	B	S.E	Odds Ratio	95%CI	P
Vaginal PH	1.002	0.169	2.73	1.96 - 3.79	<0.001
Cervical Length	-0.116	0.32	0.89	0.84 - 0.95	<0.001
Parity	0.961	0.237	2.61	1.64 - 4.16	<0.001
Age	-0.28	0.039	0.92	0.90 – 1.05	0.475

DISCUSSION

This study showed a significant correlation between alkaline vaginal pH and shortened ultrasound cervical length measurement regarding the prediction of PTL. Preterm labour in vaginal pH higher than 5 was seen to be three times more than vaginal pH<5(OR=3.06) and it is also concluded that cervical length less than 30 mm almost 14-fold increases odds of pretrm birth(OR=13.9). Series of studies were performed regarding the correlation of vaginal pH, cervical length and preterm labor [13-17]. The findings of the current study is almost consistent with those of Lim KH et al.who found that women who were referred for preterm labour had approximately doubled chance of being diagnosed with bacterial vaginosis compared to control [15].

Kumar S et al. conducted a prospective trial and recruited 120 women in their study, 60 women in spontaneous preterm labour were compared to 60 women in spontaneous labour in term pregnancy with or without rupture of membranes. pH testing of vaginal discharge and vaginal smear were used for diagnose of bacterial vaginosis (BV). They concluded that attributive risk of preterm labour in women with BV was 5.0476 (CI 95% 1.9677 to 12.952) [16].

Sendag F et al.via a prospective study not only found that there is a significant correlation between vaginal pH>5 and increased risk of preterm delivary, but also simultaneously found that there is a significant correlation between an elevated pH (>5) and shortened cervical length (r = -0.59, p <0.001) [17].

Our results further support the idea of almost all of the mentioned studies. However, poor informations from the literature were found in the question of effect of vaginal pH on premature labour regarding early and late separately. Since based on our findings odds ratio of early and late PTL were 2.63 and 3.82 respectively, it is observed that BV increases the risk of late PTL more than early one. Our findings is comparable with the result of Pires CR et al.and Thomas S et al.regarding correlation of cervical length and preterm labour and negligible differences in results may be explained by the different cut-off point for short cervical length (less than 25mm) and number of cases [18,19]. Matijevic R et al.during a prospective cohort study for evaluation of diagnostic value of vaginal pH and cervical length on prediction of PTL showed that elevated vaginal pH has better accuracy [20].

These results differ from our findings regarding accuracy of vaginal pH and cervical length, since we concluded that short cervix is more valuable than vaginal pH as a PTL predictor. A possible explanation might be that they used cervical length <25mm as their cut-off point. Even shorter cervical length(<15 mm) were considered as a risk factor for PTL in some studies and also showed it has a relatively limited value as a single measure for predicting PTL [21,22]. This inconsistency may be due to a number of reasons like different methodology and analysis.

CONCLUSION

Based on our study either cervical length measurement or vaginal pH assessment can be considered as a preterm labour predictor in low risk population, cervical length measurement has better diagnostic value overall. However, vaginal pH appeared to be more accurate in the prediction of PTL, while cervical length measurement was more appropriate to predict early PTL<34 weeks.

REFERENCES

1. Goldenberg RL, Culhane JF, Iams JD, Romero R. Epidemiology & causes of preterm birth. Lancet. 2008;5(371) 9606:75–84.
2. Slattery MM, Morrison JJ. Preterm delivery. Lancet.. 2009;360:1489-1497.
3. Krueger PM, Scholl TO. Adequacy of prenatal care and pregnancy outcome. J Am Osteopathol Assoc. 2007; 100(8):485-92.
4. Grimes-Dennis J, Berghella V. Prediction of preterm delivery. Curr Opin Obstet Gynecol. 2007;19(2):191–195.
5. Kurki T, Sivonen A, Renkonen OV, Savia E, Ylikorkala O. Bacterial Vaginosis in Early Pregnancy and Pregnancy Outcoms. Obstet Gynecol. 2012;80(2):173–177.
6. Hillier SL, Martius J, Krohn M, Kiviat N, Holmes KK, Eschenbach DA. A Case-Control Study of Chorioamnionic Infection And Histologic Chorioamnionitis In Pregnancy. N Engl J Med. 2008;319(15):972–978.
7. Hay PE, Lamont LR, Taylor-Robinson D, Morgan DJ, Ison C, Pearson J. Abnormal Bacterial Colonization Of The Genital Tract And Subsequent Preterm Delivery And Late Miscarriage. British Med Journal. 2014;308(6924):295–298.
8. Celik E, To M, Gajewska K, Smith GC, Nicolaides KH. Cervical length & obstetric history predict spontaneous preterm birth: development and validation of a model to provide individualized risk assessment. Ultrasound Obstet Gynecol. 2008;31(5):549–554.
9. Souka AP, Papastefanou I, Michalitsi V, Salambasis K, Chrelias C, Salamalekis G, et al. Cervical length changes from the first to second trimester of pregnancy and prediction of preterm birth by first-trimester sonographic cervical measurement. J Ultrasound Med. 2011;30(7):997–1002.
10. Lakhani PD, Bansode AS, Nanavati MS, Desai SV. Role of Cervical Length as Predictor of Preterm Labor & a Comparison of Transabdominal versus Transvaginal Ultrasound in Determining the Accuracy of Cervical Length. J South Asian Federation Obstetr Gynecol. 2010;2(2):129–131.
11. Leitich H, Bodner-Adler B, Brunbauer M, Kaider A, Egarter C, Husslein P. Bacterial vaginosis as a risk factor for preterm delivery: a meta-analysis. Am J Obstet Gynecol. 2003;189(1):139–147.
12. Platz-Christensen JJ. Bacterial vaginosis & pregnancy. Acta Obstetricia et Gynecologica Scandinavica. 1994;73(9):741–742.

13. Simhan HN, Caritis SN, Krohn MA, Hillier SL. Elevated vaginal pH & neutrophils are associated strongly with early spontaneous preterm birth. Am J Obstet Gynecol. 2003;189(4):1150–1154.
14. Hauth JC, Macpherson C, Carey JC, Klebanoff MA, Hillier SL, Ernest JM. Early pregnancy threshold vaginal pH & Gram stain scores predictive of subsequent preterm birth in asymptomatic women. Am J Obstet Gynecol. 2013;188(3):831–835.
15. Lim KH, Brooks H, McDougal R, Burton J, Devenish C, De Silva T. Is there a correlation between bacterial vaginosis & preterm labour in women in the Otago region of New Zealand Aust N Z J Obstet Gynaecol. 2010;50(3):226–229.
16. Kumar S, Suri V, Sharma M. Bacterial vaginosis in preterm labor. Int J Gynaecol Obstet. 2016;95(1):40–41.
17. Sendag F, Kazandi M, Akercan F, Kazandi AC, Karadadas N, Sagol S. Vaginal fluid pH, cervicovaginitis & cervical length in pregnancy. Clin Exp Obstet Gynecol. 2016;37(2):127–130.
18. To MS, Skentou CA, Royston P, Yu CK, Nicolaides KH. Prediction of patient-specific risk of early preterm delivery using maternal history & sonographic measurement of cervical length: a population-based prospective study. Ultrasound Obstet Gynecol. 2006;27(4):362–367.
19. Pires CR, Moron AF, Mattar R, Diniz AL, Andrade SG, Bussamra LC. Cervical gland area as an ultrasonographic marker for preterm delivery. Int J Gynaecol Obstet. 2006;93(3):214–219.
20. Matijevic R, Grgic O, Knezevic M. Vaginal pH versus cervical length in the mid-trimester as screening predictors of preterm laborin a low-risk population. Int J Gynaecol Obstet. 2010;111(1):41–44.
21. Berthelot-Ricou A, Tur S, Guidicelli B, Capelle M, Gamerre M, Courbiere B. Prognostic risk assessment of delivery, in patients admitted for threatened preterm birth, in a tertiary care maternity center. J Gynecol Obstet Biol Reprod (Paris). 2014; 43(8):600-9.
22. Melamed N, Hiersch L, Domniz N, Maresky A, Bardin R, Yogev Y. Predictive value of cervical length in women with threatened preterm labor. Obstet Gynecol. 2017;122(6):1279–1287.

UDC: 611.716.087.1

REVIEW ARTICLE

EVALUATION OF SKELETAL AND SOFT TISSUE FACIAL VARIATIONS WITH THE USE OF CEPHALOMETRIC AND ANTHROPOMETRIC ANALYSES - REVIEW ARTICLE

Dimitroska Snezana, Kanurkova L, Pop Stefanova-Trposka M.
[1]Faculty of Dentistry, European University, Skopje, Republic of Macedonia
[2]Faculty of Dentistry, Department of Orthodontics, Ss. Cyril and Methodius University of Skopje, Skopje, Republic of Macedonia

ABSTRACT

The growth and development in the orofacial region presents a dynamic and complex process that appears in different periods of time and at different intensities during a person's development. Any deviation from this process leads to formation of different morphological deviations of this system.

Therefore, early diagnosis is a key factor when treating patients with orthodontic anomalies and provides a proper approach to the plan and therapy of orthodontic malocclusions.

The purpose of this study is to establish the best diagnostic method which can be used in order to determine morphological changes in skeletal and dental structures in patients with sagittal irregularities, to determine the degree of their expression, facial aesthetics, and analysis regarding possible correlation of different cephalometric and anthropometric measurements.

INTRODUCTION

The human body and its proportions as well as the anatomic-morphological parts of the head and face were subject to many analyses made by painters and sculptors from ancient Egypt, while in Europe this artistic approach reached its peak in the Renaissance period. Big part of the geometrical researches by Da Vinchi and Durer, regarding facial features, later found a direct application in the X-ray cephalometry [1]. In fact they had set the proportions for drawing proper facial features [2].

Many other Renaissance artists (eg. Francesca and Pacioli, Cennini, etc.) contributed to the overall scheme of proportions that later became norms, which were used by other artists, and later by surgeons and orthodontists [3].

Researches made by Farcas, among other things, have confirmed the fact that the face is divided in three thirds, and that the lower third of the face has bigger proportions than the rest of the face. Many methods have been used to investigate facial structures in order to evaluate facial changes, including cephalometry, anthropometry, and photometry. The essential diagnostic method that is applied in orthodontic practice and enables accurate detection of morphological deviations of skeletal and soft tissue structures of the face is the analysis of the craniometric and cephalometric images. In clinical practice, the quantitative evaluation of morphology of facial structures is determined by anthropometric methods using soft tissue points as well as skeletal cephalometric points.

There are many cephalometric studies by authors who apply their own methods of analysis. They suggested average values of the tested parameters. Furthermore, they established norms and values for these parameters which represent the starting point for

comparison of cephalometric analysis of patients that we examine. As a result, we can obtain an accurate diagnosis of the concrete malocclusion.

The imperative for every orthodontist is to monitor changes in the growth and development of cranial facial structures that will further benefit in developing a treatment plan for orthodontic malocclusion [4].

MATERIALS AND METHOD

Searching for references, we used literature from Medline's Pub Med's contemporary research bases, using the following keywords: *("anthropometry", "face", "cephalometry", "soft tissue analysis", "soft tissue profile", facial growth.)*

Moreover, we came across studies in which anthropometric parameters and indexes were used as well as cephalometric standard skeletal and soft tissue analyses in subjects with sagittal irregularities.

RADIOGRAPHIC CEPHALOMETRY

The success of any orthodontic therapy depends on a well-established treatment plan, including a selective use of any information provided by cephalometric radiographic analyses. These frequently used methods are mainly applied for analyzing craniofacial structures in orthodontic clinical practice.

William Profit, in the latest edition of Contemporary Orthodontics, defines the purpose of cephalometric analyses, which in fact is an evaluation of vertical and horizontal relation between five following major functional components: cranial base, maxilla, mandible, teeth, and processus alveolaris in both jaws [2].

Many analyses made by Steiner, Rickets, Tweed, Bjork, Harvold and Wits., have greatly contributed in determining morphological changes of the maxillary and mandibular skeletal and dental structures in patients with sagittal irregularities.

Steiner's analysis can be found in most of the surveys which include measurement of craniofacial structures, for example in Loster's cephalometric study [5], which uses the angle and linear distance to the NA and NB lines to determine the position of the incisors in Poland's population.

Steiner analysis is considered as first of the modern cephalometric analyses (1950), that can establish a relation between the jaws by determining the angles of SNA, SNB, ANB and the position of the incisors regarding the N / A and the N / B line, setting their relative protrusion. This analysis contains a selection of clinically significant parameters, and presents a basis and an example for many other future analyses.

Another significant cephalometric analysis, often used in researches regarding craniophacial structures, is the Rickett's analysis.

The Ricketts analysis is more comprehensive than other investigations and has undergone many modifications, but the most commonly used parameters in orthodontic practice are: the facial angle that determines the facial height, the convexity of the face (concave, convex and proper facial features), the ratio of the upper and lower incisors to A-Pog, and the relation of the lips according to the aesthetic line [6].

Nowadays, in order to determine the facial type many studies include the Rickett's Vert index that contains the following cephalometric measurements: facial angle (the angle between the Ba-Na line and the Pt-Gn line), facial depth (the angle formed from the N-Pog , Po-Or), the lower facial height (the angle between ANS-Xi and Xi-MP lines), mandible angle (the angle formed by Po-Or and Go-Me), and mandibular arc (the angle formed by Dc-Xi Xi -MP).

The Vert index is also used in studies made by Martins et al. [7] and Paranhos et al. [5] in which according to the index, a person's face can be classified as: mesophical, doligofacial, and brachyphalic.

In his study regarding facial soft tissue changes of people aged 5 to 45; Bishara uses the facial angle and angle of the convexes to evaluate changes in the facial profile.

Yogosawa, as well uses Rickets's parameters in order to evaluate the connection between the skeletal structures and soft tissues [8].

In addition to Steiner's parameters SNA, SNB, ANB, Harvold and Wits's analysis [5] was used in determining the sagittal ratio of the jaws and the position of the incisors.

SOFT TISSUE ANALYSES

The evaluation of **soft-tissue facial structures** is equally essential in establishing a good orthodontic diagnosis, since the position of the bones and teeth directly affects the soft tissues appearance.

One of the objectives to be achieved at the end of the orthodontic treatment is the facial aesthetics. Therefore, there is a need of good qualitative and quantitative facial analysis [9], when developing a treatment plan. By using the soft-tissue cephalometric variables, we can establish the facial aesthetics and harmony.

The analysis of the soft tissue profile can help in determining the necessary orthodontic treatment in order to maintain or improve facial aesthetics. We know that the position of the lips is intimately related to the orthodontic norms of aesthetics, stability, and function.

It is known that soft tissues cover the skeletal structures of the head and face and define the final physiognomy. For this reason orthodontists use the soft tissue analysis primarily for establishing a diagnosis.

According to Martins and his assoc. In recent years the emphasis in diagnosis and therapy has been shifted from hard to soft tissue, therefore, nowadays, the treatment decisions are mostly based on the attainment of facial aesthetics rather than on skeletal and dental relations [7].

Many authors in their cephalometric studies have also inserted parameters for description and analysis of soft tissues. For a successful evaluation of facial balance and harmony, a facial profile analysis is required, therefore, profile analysis here have a dominant role. For this purpose, establishing a relation between the nose, lips and chin is of great importance [10]. These analyses are found in a number of surveys, such as: Nanda et al, his study includes analysis of growth changes in the facial profile, using the following parameters: nose height, nose depth, upper lip height, lower lip height, Ls-E, lip thickness, nasolabial angle, mentolabial angle [5].

Bishara et al , analyzing soft tissue structures in patients aged 5 to 45, applied the following parameters; the angle of convexity including the nose (Gl-Pr-Pog), the convex angle excluding nose (Gl-Sls-Pog), Holdaway's angle (Ls-Pog: NB), the upper lip according to the Ricketts aesthetic line (Ls: Pr -Pog), lower lip according to Ricketts's aesthetic line (Li: Pr-Pog) [1].

During the process of research of numerous studies related to craniofacial structures, where the subject is soft tissue facial profile and its growth changes , predicting facial profile changes with an orthodontic treatment, facial aesthetics and harmony, the most frequently applied parameters include the Ricketts's aesthetic line, the Holdaway's angle (H angle builds the NB line and the line that connects Pg and Ls) [4], the Z angle of Merrifield (a method in which the reference line is the Frakfurth horizontal and the profile line is used to estimate the facial aesthetics) and Burstone's analysis. It determines the ratio of dento-skeletal structures

to soft tissue structures by using variables that can establish the horizontal dimensions of soft tissues compared to skeletal.

The results of the studies support the tendency for obtaining morphological analysis using facial photographs in orthodontics and facial surgery as an essential tool to establish an accurate diagnosis and a satisfying result [3, 9, 11].

ANTHROPOMETRIC METHODS

In addition to cephalometric radiography orthodontists often use anthropometric measurements to determine facial proportions. Although in the past years they have been replaced only with cephalometric measurements, present requirements for soft tissue proportions have restored soft tissue measurements into use [2].

The use of anthropometric methods along with the cephalometric has been mentioned by several [3, 6, 9, 10, 12-15]. In orthodontics, the focus is mostly on facial profile and therefore many studies in literature are dedicated to determining the soft tissue facial profile, especially the lower third, the measurement of the inclination and the angles on the cephalograms and lateral photographs.

Regarding the fact that certain craniofacial morphological features are associated with certain malocclusions, anthropometric measurements can provide valuable information related to the orthodontic therapy plan.

Establishing a facial type presents an important factor for one orthodontic treatment, since it can affect the anchorage system, can predict the growth of the maxillo-mandibular structures, the muscular strength, and treatment stability [16].

Anthropometric surveys (measurements performed on human body) allow orthodontists to determine the current values and changes in facial proportions, as well as growth predictions without using X-rays [9].

Dissatisfied with the process of establishing morphological changes on head and face by applying visual procedures on patients with cleft lip and palate, Farkas G, begins to explore the use of classical anthropometric methods for quantitative facial analysis [15]. Anthropometric proportional indexes were used before and after surgery on patients with cleft lip and palate in order to determine the effects of the intervention itself [15].

His comprehensive research in the field of anthropometry contributed to formation of a craniofacial base which is based on accurate anthropometric measurements, including a larger number of different ethnic groups. In his study, 1470 subjects were analyzed, from five different regions of the world, Europe, the Middle East, Asia, Africa and North America [5].

In many studies where anthropometric measurements were used, the parameters were taken from the highly relevant database by Farkas (1994) and Farkas and Munro (1987).
In one of his numerous studies “Anthropometric proportions of the upper lip, lower lip and chin in young adults", he investigated the linear vertical measurements of upper and lower lip, chin, length of upper and lower vermillion, in order to show the interaction of the vertical measurement of the lips and chin with linear measurements of the lower third of the face [14].

He believes that the possibility of a quantitative analysis of facial appearance and any improvement achieved after the completion of treatment would benefit in setting objective goals for quality treatment [13], which from an orthodontic aspect is very significant.

The Farkas research is focused on vertical projected linear anthropometric and cephalometric measurements of the facial profile. Six anthropometric and cephalometric parameters are used to determine the facial height (n-gn), the upper facial height (n-sto), the lower facial height (sn-gn), the height of the mandible (sto-gn), the height of the nose (n-sn) and the height of the upper lip (sn-sto).

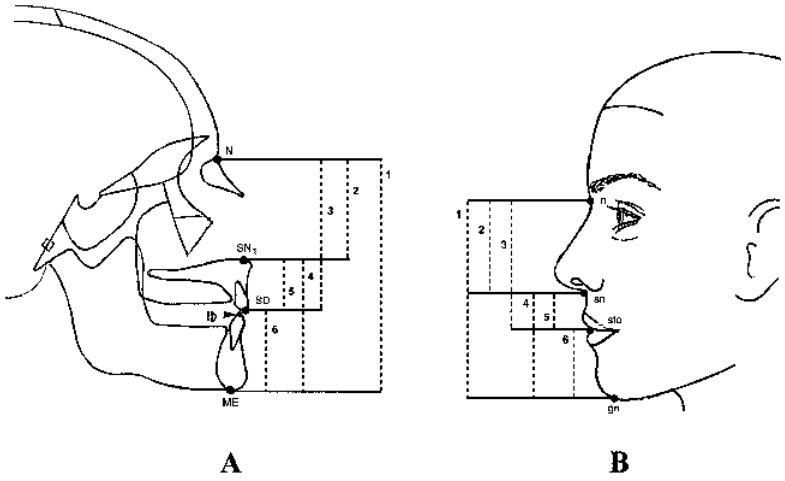


Image. 1 Cephalometric (A) Anthropometric (B) facial parameters.
(A) 1. Facial height (N-ME); 2.Nose height (N-SN1); 3.Upper facial height (N-SD); 4.Lower facial height (SN1-ME); 5. Height of upper allveolar ridge (SN1-SD); Height of the lower alveolar ridge (ID-ME).
(B) 1. Face height (n-gn); 2. Nose height (n-sn); 3.Upper face height (n-sto); 4. Lower face height (sn-gn); 5. Height of the upper lip (sn-sto); 6. Height of the mandible (sto-gn).

The correlation between anthropometric and cephalometric measurements was made by Maria Budai, Farkas and assoc. This study showed that there aren't any differences between vertical anthropometric and cephalometric measurements in a healthy population [10]. In this study, most of the measurements were made in the lower third of the face, using the same points but with different measurement methods, and then they were all compared.

CONCLUSION

The anthropometric measurements same as cephalometric measurements provide reliable information for analysis of craniofacial skeletal and soft tissue structures.

It is believed that anthropometry will gradually become a method of choice when diagnosing orthodontic anomalies and as a tool for scientific research studies, especially in younger population, avoiding harmful radiographic radiation.

In order to optimize the working hours, to avoid diagnosis errors, it is also important to investigate the possible correlation between these cephalometric and anthropometric measurements, which would later serve as reference parameters in the diagnosis of orthodontic anomalies and their use in further clinical practice.

By presenting anthropometric parameters and standard cephalometric and craniometrics analyses, it is possible to determine the morphological changes in the maxillary and mandibular skeletal and dental structures in patients with sagittal irregularities, to determine facial aesthetics by analyzing soft tissue cephalometric variables and to determine the correlation between these diagnostic parameters.

REFERENCES

1. Bishara SE, Jakobsen JR, Hession TJ, Treder JE. Soft tissue profile changes from 5 to 45 years of age. Am J OrthodDentofacialOrthop. 1998; 114(6):698-706.
2. Bergman RT, Waschak J, Borzabadi-Farahani A, Murphy NC. Longitudinal study of cephalometric soft tissue profile traits between the ages of 6 and 18 years. Angle Orthod. 2014;84(1):48-55.
3. Edler R, Agarwal P, Wertheim D, Greenhill D. The use of anthropometric proportion indices in the measurement of facial attractiveness; Eur J Orthod. 2006;28(3):274-81. Epub.
4. Halazonetis DJ. Morphometric correlation between facial soft-tissue profile shape and skeletal pattern in children and adolescents. American Journal of Orthodontics and Dentofacial Orthopedics. 2007;132(4) 450-132.
5. Loster JE, Williams S, Wieczorek A, Loster BW. The Polish face in profile: a cephalometric baseline study. Head Face Med. 2015;11:5.
6. Ozerović B. Rendgenkraniometrija i rendgenkefalometrija, I izdanje, dr Borka Ozerović, Beograd, 1982.
7. Martins L, Vigorito J. Photometric analysis applied in determining facial type. Dental Press J Orthod. 2012;17(5):71-5.
8. Tsunori M, Mashita M, Kasai K. Relationship between facial types and tooth and bone characteristics of the mandible obtained by CT scanning. Angle Orthod. 1998;68(6):557-62.
9. Farkas LG, Katic MJ, Hreczko TA, Deutsch C, Munro IR. Anthropometric proportions in the upper lip-lower lip-chin area of the lower face in young white adults. Am J Orthod. 1984;86(1):52-60.
10. Budai M, Farkas LG, Tompson B, Katic M, Forrest CR. Relation between anthropometric and cephalometric measurements and proportions of the face of healthy young white adult men and women. J Craniofac Surg. 2003;14(2):154-61.
11. Edler RJ; Background considerations to facial aesthetics, J Orthod. 2001; 28(2):159-68.
12. Torres-Restrepo AM, Quintero-Monsalve AM, Giraldo-Mira JF, Rueda ZV, Vélez-Trujillo N, Botero-Mariaca P. Agreement between cranial and facial classification through clinical observation and anthropometric measurement among envigado school children. BMC Oral Health. 2014;14:50.
13. Farkas LG, Katic MJ, Forrest CR, Alt KW, Bagic I, Baltadjiev G, Cunha E, et al. International anthropometric study of facial morphology in various ethnic groups/races. J Craniofac Surg. 2005;16(4):615-46.
14. Farkas LG1. Accuracy of anthropometric measurements: past, present, and future. Cleft Palate Craniofac J. 1996;33(1):10-8; discussion 19-22.
15. Farkas LG1, Hajnis K, Posnick JC. Anthropometric and anthroposcopic findings of the nasal and facial region in cleft patients before and after primary lip and palate repair. Cleft Palate Craniofac J. 1993;30(1):1-12.
16. Hönn M1, Göz G; Reference values for craniofacial structures in children 4 to 6 years old: review of the literature. J Orofac Orthop. 2007;8(3):170-82.

UDC: 616.314-77-06

REVIEW ARTICLE

OVERVIEW OF PROSTHODONTIC COMPLICATIONS RELATED WITH DENTAL IMPLANTS

Stavreva Natasha, Kapusevska B.
Department of Prosthodontics, Faculty of Dental Medicine, Ss. Cyril and Methodius University of Skopje, Skopje, Republic of Macedonia

ABSTRACT

Implant dentistry has evolved as a standard of care in the past few decades. It has been beneficial to patients with predictable outcomes. Although higher success rates have been reported in the implant dentistry, there are several complications that do occur. Most of the complications can be minimized if the proper diagnosis and treatment plan is executed. The dentist should have a thorough knowledge regarding all the complications associated with dental implants, prevention strategies, and their management. Follow up after every 6 months has been advocated for the dental implant restorations. The patient should be informed about the anticipated complications and the probable outcome of the treatment. The purpose of this article is to describe the prosthodontic complications associated with dental implants and to discuss their prevention and management. Success of the dental implant has its own limitations.

Key words: dental implants, biological, mechanical, aesthetic complications.

INTRODUCTION

Dental implants have been used to rehabilitate edentulous and partially edentulous patients with fixed prostheses for over 30 years [1].

According to the International Congress of Oral Implantologists (Italy 2007), the dental implant with bone loss of ≤ 2mm after the initial surgery with no signs of pain and mobility could be regarded as a successful implant.

Complications rarely result in the complete failure of treatment, their management can be frustrating, expensive and time consuming for the patient, clinician and technician. This review discusses the incidence, aetiology, prevention and management of common prosthodontic complications, which may occur following the delivery of implant retained crowns and bridgework.

Prosthodontic complications can be broadly categorized as biological, mechanical and aesthetic [2].

BIOLOGICAL COMPLICATIONS

Peri-implant mucositis

Peri-implant mucositis is the inflammatory condition of the soft tissues around the dental implants, analogous to gingivitis, affecting the marginal soft tissues surrounding osseo-integrated dental implants [3,4]. Peri-implant mucositis may affect around 50% of implants and is characterized by bleeding on probing [5] (Figure 1).

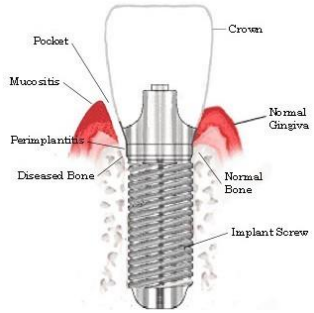


Fig. 1. Mucositis

Peri-implant mucositis usually resolves with improved homecare, routine dental checkup, antiseptic mouthwash and recently waterpeak cleaning [5,6]. Very useful in its treatment could also be a local application of dental ozone.

Peri-implantitis

Peri-implantitis is an inflammatory lesion, analogous to periodontal disease, affecting the supporting bone in addition to the soft tissues around an implant [7,8] (Figure 2).

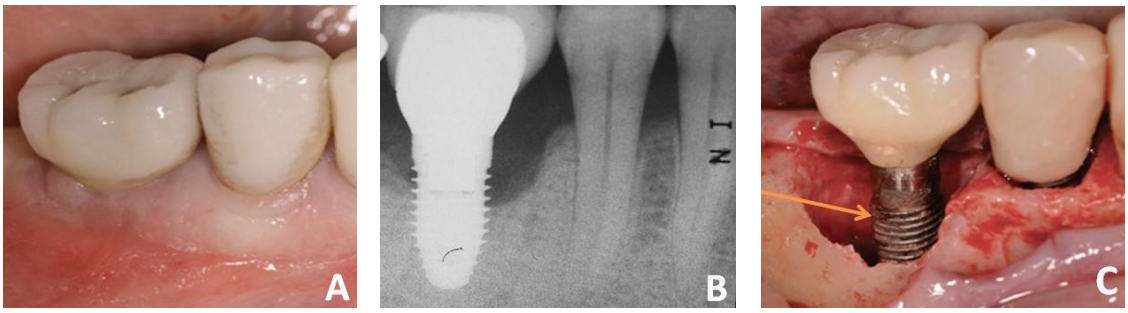


Fig. 2. Peri-implantitis

Pain, mobility, bleeding on probing, increasing probing depths over a period of time, exudation, recession and exposed threads of the implant are the clinical findings in the case of peri-implantitis [9]. Exudation around the dental implant for >2 weeks requires immediate intervention. Putative risk factors for peri-implantitis include smoking, diabetes mellitus, poor oral hygiene, a history of periodontal disease, interleukin-1 genotype, alcohol, rough surfaced implants, retained cement [10,8,11], bruxism, osteoporosis, hyperparathyroidism, history of radiation therapy and genetics [12].

Treatment can be instituted by local and systemic methods. Surgery is required in few cases. Local treatment methods include mechanical debridement, sub-gingival irrigation using the antiseptic solution such as chlorhexidine and local drug delivery. In untreated severe cases, local treatment should be accompanied with systemic antibiotics. Surgical treatment includes opening the flap for the debridement and decontamination of dental implants using various pharmaceutical agents. Lasers are also used for this purpose [13].

Fistulae

Only 1-2% cases of the fistulae have been reported at the interface between the dental implant and abutment [14].

They are usually associated with loose abutments screws or ill-fitting frameworks [10]. These fistulae usually resolve following removal of the prosthesis, irrigation with 0.2% chlorhexidine gluconate and replacement of the prosthesis at the correct torque. In case of ill-fitting prosthesis, restoration with a new prosthesis should be considered [15] (Figure 3).

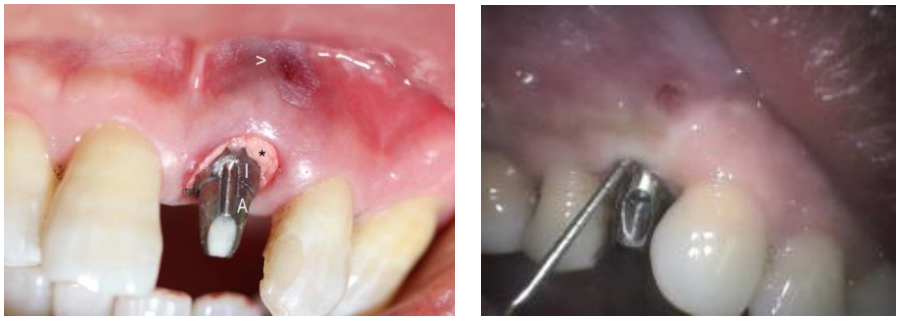


Fig. 3. Fistulae

Soft tissue hyperplasia

Incidence rate of 15-20% has been reported in a 9-year follow-up study. Hyperplastic growth of the soft tissues may occur due to poor oral hygiene and plaque accumulation around the dental implants [16], framework misfit, dead space beneath frameworks and a lack of attached gingivae [10] (Figure 4).

Fig. 4. Hyperplasia of soft tissue

Hyperplasia usually resolves following correction of predisposing factors, improved oral hygiene and mechanical debridement. However, it may occasionally be necessary to surgically resect the hyperplastic tissue.

Implant loss

Implant losses are categorized as early or late [10]. Early implant loss has been associated with overheating during implant placement, infection, poor bone quantity and poor bone quality [17]. Late implant loss occurs following functional loading because of a breakdown of an established osseo-integration [17] (Figure 5).

Fig. 5. Loss of implant

The reported survival rates for implants supporting crowns and fixed partial dentures exceed 95% over 5 years [18,19,20] and 93% over 10 years [19,20]. When an implant fails an

alternative treatment may be considered, the site may be allowed to heal before placing a new implant or a new, wider implant may be placed immediately [21].

Bone loss

The level of the crestal bone is a significant marker for the implant health and stability. Bone loss may occur secondary to the excessive occlusal loading or bacteria. Due to continuing overloading of the implant and bone loss, crevicular sulcus becomes deeper and is populated by anaerobic bacteria, leading to excessive periodontal destruction (Figure 6).

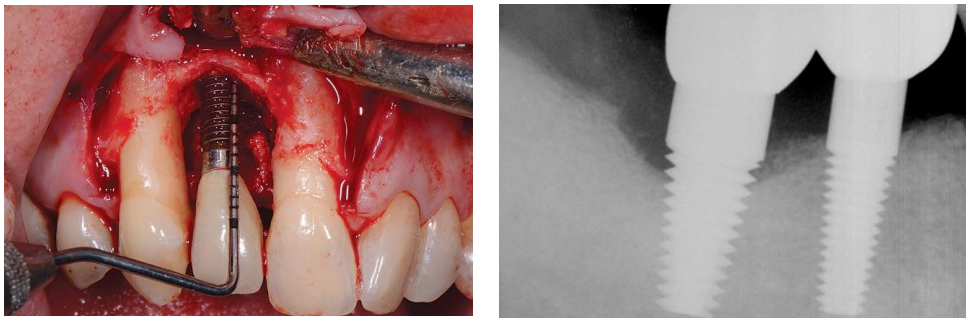


Fig. 6. Bone loss

To avoid bone loss, occlusal loading should be within the physiological limits of the bone and adverse occlusal forces should be minimized [22].

MECHANICAL COMPLICATIONS

Natural dentition is cushioned with the periodontal ligament to adapt against occlusal forces. When occlusal forces exceed the adaptive capacity of the oral tissues, it may result in trauma from occlusion [23]. Mechanical complications occur when the capacity of the prosthesis to withstand applied forces is exceeded. The precise complication will depend upon the magnitude and the direction of the applied force. However, the weakest parts of the system, that is, the screws and veneering material, are usually affected [24]. Mechanical complications have also been associated with the inappropriate selection or use of materials, manufacturing imperfections, ill-fitting frameworks and trauma [10,25,26].

Screw loosening and screw fracture

According to several systematic reviews, screw loosening is the most common mechanical complication which is associated with the single implant-supported restoration [27]. Screw loosening usually occurs as a result of micro-movement at the joint interface. This micro-movement may be associated with an inadequate initial torque, ill-fitting frameworks and occlusal overload [28] (Figure 7).

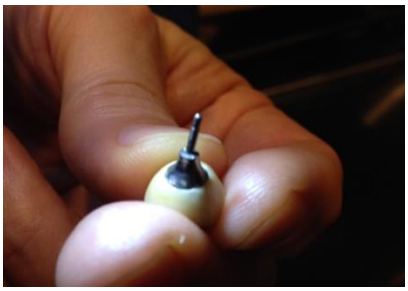


Fig. 7. Screw loosening

Fracture of the implant abutment and prosthesis screw may occur as a consequence of repeated screw loosening due to the metal fatigue. Improper implant components, reusing the

loosened screw, excessive load, ill-fitting prosthesis are the prime causes for the screw fracture. If the screw gets fractured it should be retrieved [29] (Figure 8).

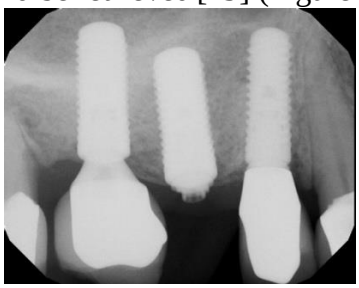


Fig. 8. Screw fracture

Loss of retention and decementation

Loss of retention is a complication specific to cement retained prosthesis. Loss of retention is commonly associated with abutments that provide inadequate retention or resistance form. Selection of the implant abutment should be done with caution to prevent this complication. In case of inadequate space available for the restoration, implant should be placed at increased depth within the anatomical and physiological limits of the bone. Decementation of the implant supported restoration can be prevented by using glass-ionomer and resin cements [30].

Chipping/fracture of the restoration (veneering material or framework)

Fracture of the veneering material is one of the most common mechanical complications reported in the literature, whereas it is the second most common mechanical complication reported in metal and metal- ceramic single implant restorations [31] (Figure 9,10).

Fig. 9. Chipping of veneering material

Fig. 10. Fracture of veneering material

If a small fracture is present in the posterior non-esthetic zone, it should be smoothened whereas larger defects are repaired. Several systems for the intra-oral repair of the metal ceramic restoration have been introduced [32]. Chipping/ fracture of the restoration can be minimized by:

• Reducing size of the occlusal table
• Providing uniform thickness and support for veneering ceramic
• Shallow cusp height
• Avoiding excessive occlusal contacts [33].

Implant fracture

Fracture of the implant is a rare complication. Studies show that only 3-6% cases in maxilla and 3% cases in mandible have been associated with fracture of the implant [34] (Figure 11).

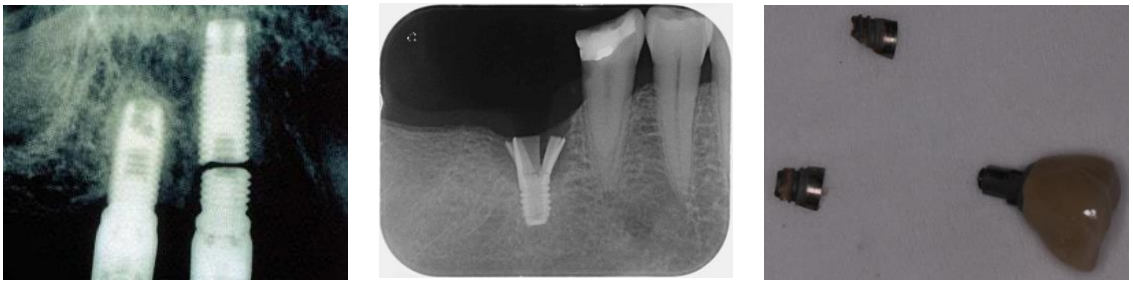


Fig. 11. Implant fracture

The implant fracture may occur due to following reasons:
• Defective material and design of the implant
• Absence of passive fit of the restoration
• Occlusal overload
• Narrow diameter
• Para-functional habits
• Long cantilever design.

It is proposed that large diameter implant should be used in molar and premolar region to reduce the chances of fixture fracture.

Treatment of fractured implant includes its removal and placement of larger diameter implant. After successful osseo-integration of the replaced implant prosthesis should be given [35].

It is suggested that careful, periodic occlusal evaluation and analysis before and after implant restorations should be carried out. A sound adoption of biomechanical principles must also be considered in the planning of combined tooth and implant supported partial dentures [36].

Abutment fracture

Abutment fracture is a rare complication associated with the dental implants with only in 0.5% of the cases reported. To prevent implant abutment fracture following factors should be considered:

• Abutment should have adequate mechanical strength to resist occlusal forces and metal fatigue
• It should have a well-fitting surface with the other components
• In case of cement-retained implant restoration, adequate retention and resistance form should be present

Till now, titanium has proven to be the most successful abutment material except few mechanical complications [37].

AESTHETIC COMPLICATIONS

Aesthetic results of the dental implants cannot be predicted. Some of the common esthetic complications reported include:

- Poor restoration contour and shade mismatch
- Missing interdental papillae, mucosal recession
- Malposed implant.

Aesthetic outcomes are highly subjective and care should be taken to avoid imposing our own aesthetic parameters on patients. Studies have revealed that there is often poor agreement between patients and professionals regarding aesthetic outcome [37] and that patients are usually highly satisfied with the aesthetic outcome [38,39]. Several reviews have reported an overall aesthetic complication rate approaching 10% [2,18].

The final aesthetic outcome is largely dependent upon the pre-existing state of the hard and soft tissues [38,39,40] the care with which surgical procedures are performed and lip position [41,42].

The outcome of the treatment is more appreciated by the patient compared to the dentist hence the patients should be informed about the limitations of the aesthetic results [43].

CONCLUSION

The success of the dental implants has its own limitations. Although implant retained crowns and bridgework are highly successful they may be associated with a variety of biological, mechanical and aesthetic complications. The dentist should have a thorough knowledge regarding all the complications associated with the dental implants, prevention strategies, and their management. Follow up after every 6 months has been advocated for the dental implant restorations. Clinicians providing implant treatment should be aware of the potential complications and the strategies by which they can be prevented and managed. Potential complications should be communicated to the patient before commencing with the treatment.

REFERENCES

1. Jemt T, Lekholm U, Adell R. Osseointegrated implants in the treatment of partially edentulous patients: a preliminary study on 876 consecutively placed fixtures. Int J Oral Maxillofac Implants. 1989;4:211–217.
2. Goodacre C J, Bernal G, Rungcharassaeng K, Kan J Y. Clinical complications with implants and implant prostheses. J Prosthet Dent. 2003;90:121–132.
3. Alani A, Bishop K. Peri-implantitis. Part 2: Prevention and maintenance of peri-implant health. Br Dent J [Internet]. Nature Publishing Group; 2014;26;217(6):289–97.
4. Renvert S, Persson GR. Periodontitis as a potential risk factor for peri-implantitis. Journal of Clinical Periodontology. 2009;36:9–14.
5. Lindhe J, Meyle J. Peri-implant diseases: consensus report of the sixth European workshop on periodontology. J Clin Periodontol. 2008;35:282–285.
6. Renvert S, Roos-Jansåker A M, Claffey N. Non-surgical treatment of peri-implantitis and peri-implantitis: a literature review. J Clin Periodontol. 2008;35:305–315.
7. Heasman P, Esmail Z, Barclay C. Peri-implant diseases. Dental Update. 2010;37:511–512,514-516.
8. Heitz-Mayfield L J. Peri-implant diseases: diagnosis and risk indicators. J Clin Periodontol. 2008;35:292–304.
9. Alani A, Kelleher M, Bishop K. Peri-implantitis. Part 1: Scope of the problem. Br Dent J. 2014;26;217(6):281–7.

10. Goodacre C J, Kan J Y, Rungcharassaeng K. Clinical complications of osseointegrated implants. J Prosthet Dent. 1999;81:537–552.
11. Chee W, Jivraj S. Screw versus cemented implant supported restorations. Br Dent J. 2006; 201:501–507.
12. Dawson DR, Jasper S. Key Systemic and Environmental Risk Factors for Implant Failure. Dental Clinics of North America. 2015;59(1):25–39.
13. Lekholm U, Adell R, Lindhe J, Brånemark P-I, Eriksson B, Rockler B, et al. Marginal tissue reactions at osseointegrated titanium fixtures. International Journal of Oral and Maxillofacial Surgery. 1986;15(1):53–61.
14. Vere J, Bhakta S, Patel R. Prosthodontic complications associated with implant retained crowns and bridgework: a review of the literature. Br Dent J. 2012;212(6):267–72.
15. Lang N P, Berglundh T. Peri-implant disease: where are we now? Consensus of the seventh European workshop on periodontology. J Clin Periodontol 2011;38:178–181.
16. Goodacre CJ, Kan JYK, Rungcharassaeng K. Clinical complications of osseointegrated implants. The Journal of Prosthetic Dentistry. 1999;81(5):537–52.
17. Esposito M, Hirsch J M, Lekholm U, Thomsen P. Biological factors contributing to failures of osseointegrated implants (I). Success criteria and epidemiology. Eur J Oral Sci. 1998;106:527–551.
18. Jung R E, Pjetursson B E, Glauser R, Zembic A, Zwahlen M, Lang N P. A systematic review of the 5-year survival and complication rates of implant supported single crowns. Clin Oral Implants Res. 2008;19:119–130.
19. Aglietta M, Siciliano V I, Zwahlen M et al. A systematic review of the survival and complication rates of implant supported fixed dental prostheses with cantilever extensions after an observation period of at least 5 years. Clin Oral Implants Res. 2009; 20:441–451.
20. Pjetursson B E, Tan K, Lang N P, Brägger U, Egger M, Zwahlen M. A systematic review of the survival and complication rates of fixed partial dentures (FPDs) after an observation period of at least 5 years. Clin Oral Implants Res. 2004;15:625–642.
21. Grossmann Y, Levin L. Success and survival of single dental implants placed in sites of previously failed implants. J Periodontol 2007;78:1670–1674.
22. Oh T-J, Yoon J, Misch CE, Wang H-L. The Causes of Early Implant Bone Loss: Myth or Science? Journal of Periodontology. 2002;73(3):322–33.
23. Schwarz MS. Mechanical complications of dental implants. Clinical Oral Implants Research. 2000;11:156–8.
24. Davies S J. Occlusal considerations in implantology: good occlusal practice in implantology. Dental Update. 2010; 37:610–612, 615-616, 619-620.
25. Kreissl M E, Gerds T, Muche R, Heydecke G, Strub J R. Technical complications of implant-supported fixed partial dentures in partially edentulous cases after an average observation period of 5 years. Clin Oral Implants Res 2007;18:720–726.
26. Virdee P, Bishop K. A review of the aetiology and management of fractured dental implants and a case report. Br Dent J. 2007;203:461–466.
27. Cavazos E, Bell FA. Preventing loosening of implant abutment screws. The Journal of Prosthetic Dentistry. 1996;75(5):566–9.
28. Al-Sabbagh M, Bhavsar I. Key Local and Surgical Factors Related to Implant Failure. Dental Clinics of North America. 2015;59(1):1–23.
29. Yilmaz B, McGlumphy E. A technique to retrieve fractured implant screws. The Journal of Prosthetic Dentistry. 2011;105(2):137–8.

30. Bragger U, Karoussis I, Persson R, Pjetursson B, Salvi G, Lang NP. Technical and biological complications/failures with single crowns and fixed partial dentures on implants: a 10-year prospective cohort study. Clin Oral Implants Res. 2005;16(3):326–34.
31. Örtorp A, Jemt T. Clinical Experiences of Implant-Supported Prostheses with Laser-Welded Titanium Frameworks in the Partially Edentulous Jaw: A 5-Year Follow-up Study. Clinical Implant Dentistry and Related Research. 1999;1(2):84–91.
32. Chee W, Jivraj S. Screw versus cemented implant supported restorations. Br Dent J. 2006;201(8):501–7.
33. Sadid-Zadeh R, Kutkut A, Kim H. Prosthetic Failure in Implant Dentistry. Dental Clinics of North America. 2015;59(1):195–214.
34. Tagger Green N, Machtei EE, Horwitz J, Peled M. Fracture of Dental Implants: Literature Review and Report of a Case. Implant Dentistry. 2002;11(2):137–43.
35. AL Quran FAM, Rashan BA, AL-Dwairi ZN. Management of Dental Implant Fractures. A Case History. Journal of Oral Implantology. 2009;35(4):210–4.
36. Muroff FI. Removal and Replacement of a Fractured Dental Implant: Case Report. Implant Dentistry. 2003;12(3):206–10.
37. Pjetursson BE, Brägger U, Lang NP, Zwahlen M. Comparison of survival and complication rates of tooth-supported fixed dental prostheses (FDPs) and implant-supported FDPs and single crowns (SCs). Clinical Oral Implants Research. 2007;18:97–113.
38. Buser D, Martin W, Belser U. Optimising esthetics for implant restorations in the anterior maxilla: anatomic and surgical considerations. Int J Oral Maxillofac Implants 2004; 19 Suppl:43–61.
39. Choquet V, Hermans M, Adriaenssens P, Daelemans P, Tarnow D P, Malevez C. Clinical and radiographic evaluation of the papilla level adjacent to single-tooth dental implants. A retrospective study in the maxillary anterior region. J Periodontol. 2001;72:1364–1371.
40. Tarnow D P, Cho S C, Wallace S S. The effect of inter-implant distance on the height of inter-implant bone crest. J Periodontol. 2000; 71:546–549.
41. Palacci P, Ericsson I. Esthetic implant dentistry soft and hard tissue management. Chicago: Quintessence Publishing Co Inc., 2001.
42. Sclar A G. Soft tissue and esthetic considerations in implant therapy. US: Quintessence Publishing Co Inc., 2003.
43. Almog DM, Torrado E, Meitner SW. Fabrication of imaging and surgical guides for dental implants. The Journal of Prosthetic Dentistry. 2001;85(5):504–8.

These guidelines are in accordance with the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals". (Complete document available at www.icmje.org)

Manuscripts are accepted for processing if neither the article nor any essential part, tables or figures, has been or will be published or submitted elsewhere before presenting in Acta Morphologica. This restriction does not apply to abstracts or press reports related to scientific meetings. The Editors will consider both invited and uninvited review articles. Authors should detail how their work differs from existing reviews on subject in cover letter.

Manuscripts/General Guidelines
The manuscript should conform the guidelines set forth in the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals", 5th edition, New Engl J Med 1997; 336 (4): 309–315.

Manuscript must contain no more than 5000 words. A cover letter signed by all authors should identify the person (post address, telephone number, and e-mail address) responsible for negotiations. Each author must sign a statement attesting that he or she fulfills the authorship criteria of the Uniform Requirements. Each author must significantly contribute to the submitted work.

Form of Manuscript
Three copies of each manuscript, along with a disk (see "Instructions for Electronic Manuscript Submission"), must be submitted in English, in double-spaced typewritten form with a 5-cm (2-inch) left margin. (Do not use "erasable" bond.) The text should be written in following sequence: Introduction, Methods, Results, Discussion, Acknowledgement, References, Tables, Illustrations and Figure Legends, Structured Abstract with key words and Condensed Abstract.

Page 1 should bear an article title, name(s) of the author(s) and institution where the work was done and a person whom proofs and reprint request should be sent, with complete address (including postal codes), telephone number and email address (address for correspondence).

Tables should be typed neatly, each on a separate sheet, with title above and any notes below. All abbreviations should be explained. Do not provide duplicite information in tables and figures.

Illustrations should be submitted as clear glossy prints (two duplicate sets may be photocopied), with lettering large enough to be legible if reduced. The maximal final size of any figure in the printed journal will be 20 by 28 cm (8.25x11inch). On the back of each figure, the name of author and the figure number should be writen, with the top indicated by an arrow. Each figure should have a separate, fully explicit legend; all parts of the figure and all abbreviations and symbols should be clearly defined. Figure legends should be typed on separate pages; figure numbers must follow their reference in text.
Drug names. Generic names should be used; trade names may be given in parentheses in the first mention, and generic names should be used thereafter.

Abbreviations. The list of abbreviations given in "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" (section References) should be followed. For additional abbreviations, consult the CBE Style Manual (available from the Council of Biology Editors, 9650 Rockville Pike, Bethesda, Maryland 20814, U.S.A.) or other standard sources.

References
The journal complies with the reference style given in "Uniform Requirements for Manuscripts Submitted to Biomedical Journals". References should be cited in text by number and numbered in order they are cited. The reference should by written in double-spaced form at the end of the text, following the sample formats given below. For the abbreviations of journal names, refer to the List of Journals Indexed in Index Medicus (available from the Superintendent of Documents, U.S. Government Printing Office, Washington, D.C. 20402, U.S.A., DHEW Publication No. NIH 83-267; ISSN 0093-3821). Provide all names of authors when fewer than seven: when seven or more, list the first three and add et al. Provide article titles and inclusive pages. The author is responsible for the accuracy of reference data.

Article:
1. Greenblatt DJ, Abernethy DR, Shader Jr RI. Pharmacokinetic aspects of drug therapy in the elderly (commentary).\ Ther Drug Monit. 1986;8(6):249-55.

Book:
2.Mitchell JR, Horning MG, (editors). Drug metabolism and drug toxicity. 2nd ed. New York: Raven Press; 1984.

Chapter of book:
3. Kutt H, Pippenberg CE et al. Plasma clearance of nor-methsuximide in a uremic patient. 223-226. In: Levy RH, Pitlick WH, Meijer J, (editorss). Metabolism of antiepileptic drugs. New York: Raven Press; 1984. pp-1-25.

Internet:
Greenblatt DJ, Abernethy DR, Shader Jr RI. Pharmacokinetic aspects of drug therapy in the elderly (commentary). Ther Drug Monit 1986; 8 (6): 249-55. Available from:http://www.med.monash.edu.au/medical

Structured Abstract
A structured abstract should be provided on a separate page with no more than 250 words, presenting essential data in five paragraphs introduced by separate headings in following order: Objectives, Background, Methods, Results, Conclusion. Complete sentences should be used. All data in the structured abstract must be present also in the submitted text or tables. Three to five key words should be added. Terms from Index Medicus should be used.

Condensed Abstract (for table of contents)
A condensed abstract of no more than 50 words should be provided for the expanded table of contents, stressing clinical implications. Do not include data which are not present in the text or tables.

Proofs
Proof must be returned within 3 days; late return may cause a delay in publication. Please check text, tables, legends, and references carefully.

Instructions for Electronic Manuscript Submission

The preferred storage medium is a 3.5 inch disk in MS-DOS compatible format. Each submitted disk must be clearly labeled with the name of the author, article title, journal title, type of the equipment used to generate the disk, word processing program (including version number), and filenames.

The manuscript submitted on a disk must be in the final corrected version and must agree with the final accepted version of the submitted paper manuscript. The submitted disk should contain only the final version of the manuscript. Delete all other material from the disk. Please follow the general instructions on style/arrangement and, in particular, the reference style as given in "Instruction to Authors".

Note, that while the paper version of the manuscript must be presented in the traditional double spaced format, the electronic version will be typeset and should not contain extraneous formatting instructions. Do **not** use tabs or extra space at the beginning of a paragraph or for list entries. Do **not** indent runover lines in references. **Turn off** line spacing. Do **not** specify page breaks, page numbers, or headers. Do **not** specify typeface.

Take care to enter "one" (1) and lower case "el" (1)", as well as "zero" (0) and capital "oh" (O) correctly.

Please note the following conventions on dashes: Use a single hyphen with space before it for a minus sign, use a double hyphen (with space before and after) to indicate a "long dash" in text, and a triple hyphen (with no extra space) to indicate a range of numbers (e.g. "23–45").

Non-standard characters (Greek letters, mathematical symbols, etc.) should be coded consistently throughout the text. Please make a list and provide a listing of the used codes.

Authors agree to execute copyright transfer forms as requested. Authors should express all measurements in conventional units, with Systéme International (SI) units given in parentheses throughout the text. Conventional units should be used in figures and tables, with conversion factors given in legends or footnotes.

In electronic manuscript submission **text editor Word 6 or higher** is recommended (editor T602 is possible). Text should be **aligned left (not justified), without hyphenation, without bullets, numbering and underlines**, without extra hard returns at the end of line (only at the end of paragraphs). **One type of Word paragraph** should be used throughout the text. Word graphic experiments should not be used.

Word tables: do not use vertical lines, unless it is necessary. Provide tables as a separate file (do not place in text).

Excel graphs: provide as Excel file.

Word graphs: provide as a separate Word file (**do not place in text!)**

Table and graph legends should be provided separately at the end of the text.
Graphs should be processed for black and white print. **Graphs printed on laser or ink printers could not serve as templates– always provide original electronic files**!

Figures: provide original or scan. Scan to **600-800 dpi!** – set to B/W or line art.

Figures – black and white photos – provide high-quality original or scan to 350 dpi !
Figures – color photos — provide high-quality original or scan to **350 dpi!**
Figures scanned to 72 or 96 dpi are not suitable for print!

On principle, **do not place scans in text!** Always provide original figures in tif or jpg format (with minimal compression). Placing scan in Word text causes a loss of quality!

Figure legends should be provided as a separate text file.

Do not place figures in PowerPoint – this application is meant for presentations and it is not possible to use it as a template for print!

Figures from digital camera should not be placed in text. Provide them in **tif** or **jpg** format (with minimal compression)! Transciption of Macedonian Cyrillic Alphabet into English Latin On the basis of ISO Recomandation R-9-1968 International List of Periodical Title Abbreviations (1970).

Transciption of Macedonian Cyrillic Alphabet into English Latin

A a	A a	N n	N n
B b	B b	W w	Nj nj
V v	V v	O o	O o
G g	G g	P p	P p
D d	D d	R r	R r
\ |	G g	S s	S s
E e	E e	T t	T t
@ ‘	Zh zh	] }	K k
Z z	Z z	U u	U u
Y y	Dz dz	F f	F f
I i	I I	H h	Kh kh
J j	J j	C c	Ts ts
K k	K k	^ ~	Ch ch
L l	L l	X x	Dzh dzh
Q q	Lj Lj	[{	Sh sh
M m	M m		

On the basis of ISO Recomandation R-9-1968 International List of Periodical Title Abbreviations (1970)

ЕКСКЛУЗИВНА ИЗЈАВА ЗА ОБЈАВУВАЊЕ НА АВТОРИТЕ КОИ ПОДНЕСУВААТ ТРУД
AN EXCLUSIVE STATEMENT FOR PUBLICATION IS NESSESARY WHEN SUBMITTING AN ARTICLE FOR PUBLICATION

Потврдувам дека ниту еден материјал од овој ракопис не е претходно објавен или даден за објавување во било кој вид, освен извадок (апстракт) од 400 збора или помалку.

I hereby confirm that the materials of this manuscript have neither been previously published nor handed for publishing, except the abstract of 400 words or less.

СОГЛАСНОСТ ЗА ПРЕНОС НА ПЕЧАТАРСКИ ПРАВА
TRANSFER OF COPYRIGHT AGREEMENT

Печатарски права на трудот со наслов:
Copyright to the article entitled:

_____

_____

кој ќе се објави во списанието Acta Morphologica, се пренесуваат на Acta Morphologica, но авторите го задржуваат следново:

to be published in the journal Acta Morphologica is hereby transferred to the Acta Morphologica, but this authors reserve the following:

1. Сите права на сопственост освен печатарските, како правото на патент
   All proprietary rights other than copyright, such as the patent right.

2. Правото за употреба на дел или сите делови од овој труд за своја лична работа
   The right to use all of the parts of the article in future works of their own.

Име и презиме
First and last name

_____
_____
_____

Потпис
signature

ВАЖИ САМО ПО ПРИФАЌАЊЕ НА ТРУДОТ
VALID ONLY AFTER THE ACCEPTANCE OF THE ARTICLE