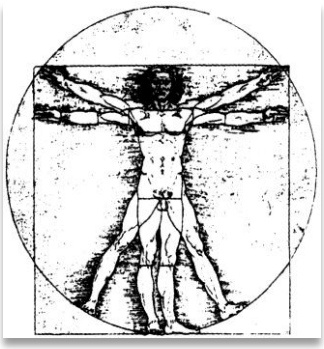
UDC: 61 **ISSN 1409-9837**

ЗАММ



MAAM

ACTA MORPHOLOGICA

INTERNATIONAL JOURNAL OF MACEDONIAN ASSOCIATION OF ANATOMISTS AND MORPHOLOGISTS

Vol. 12 (2) 2015

ACTA MORPHOLOGICA
International journal
of
Macedonian Association of Anatomists and Morphologists (MAAM)

Published
Twice a year

Corresponding address
Institute of Anatomy, Medical Faculty, 50 Divizija 6, Skopje, R.Macedonia
Tel/fax:++389 2 3125304
e-mail:acta_morphologica@yahoo.com

C O N T E N T


WEBDOCTOR.com.mk

UDC:572.524(497.7)

PLANTAR DERMATOGLYPHICS IN MACEDONIAN POPULATION

Chadikovska Elizabeta, Matveeva N, Zafirova B, Trpkovska B, Bojadzieva B
Institute of Anatomy, Medical Faculty, Skopje

ABSTRACT

The aim of the study was to establish and analyze individual dermatoglyphic features in healthy examinees of Macedonian nationality.

In the population study some dermatoglyphic parameters were read and classified on 400 right and left sole prints obtained from healthy individuals of Macedonian ethnic origin, 100 males and 100 females. Sole prints were taken using Cummins and Midlo's ink method. Dermatoglyphics were classified and comparison was made with different ethnic groups.

The arches' frequency decreases from the fifth to the first digit; the opposite was for the fibular loops, they increase from the fifth to the first digit for both sexes. Double whorls on the third digit were present as complex patterns. Found triradii were c=d>b>a>pm>p>e>p''>p' in males and c=d>a>b>pm>p>e>p'>p'' in females. Ridge count for the toe was 9.10 ridges in males and 7.93 in females. In the halucal, thenar distal and first interdigital space patterns ridge count was 15.08 in males and 14.39 in females. A-b ridge count was 15 ridges in males and 14 in females.

In conclusion, dermatoglyphic features on the sole in the Macedonian ethnic group are presented. We have made a comparison with other ethnic groups and we discussted the differences between them. The results provide possibilities for further biological investigation of the dermatoglyphics.

***Key words*:** dermatoglyphics, ink method, soles, Macedonian

INTRODUCTION

Dermatoglyphics is used to denote the pattern of lines on palms, fingertips, soles and toes. These patterns can be used in anthropology for determining the ethnic group differences, inherited characteristics or even as early markers for detection of some diseases. In 1926 for the first time Cummnis coined the term dermatoglyphics in the field of science. It has been adopted and accepted internationally. Etymologically this term connects two words *derma, skin*; *glyphe, carve*. It gives the impression that something has been carved out of the skin [1]. Dermatoglyphics are formed during the first and the second trimester of the developing embryo and once formed remain permanent and never change throughout the life except in the dimension in commensurate to the growth of an individual [2]. The classification of dermatoglyphics on the human sole reported in 1969 by Penrose and Loesch offered new possibilities for comprehensive analysis of sole and toes patterns. From simple looking at prints of the sole it is obvious that the interpretation of patterns is more difficult compared to the patterns analyzed on the hands. They are most often extra-limited, situated on the extreme fibular or tibial borders of the soles; they vary greatly in their size and appearance and are not limited to one particular configurational area. The toes are short with limited movements [3]. The aim of this paper was to present a variety of true patterns occurring on the sole in Macedonian ethnic group. The group consisted of two hundred examinees, the inclusion factor randomly chosen to be Macedonian nationality.

MATERIAL AND METHOD

In this paper we present a statistical analysis of plantar pattern frequencies and ridge count of the soles in 200 Macedonian (100 males and 100 females). All prints were taken by Cummnis and Midlo's ink method. The prints were taken from healthy examinees of Macedonian nationality from Butel settlement and students of the "Ss. Cyril and Methodius University" in Skopje. Plantar configurational types were formulated as: whorls of three types - concentric (Wc), elliptical (We) and spiral (Ws). Loops open in three directions - tibial (Lt), fibular (Lf) and distal (Ld). Arches are patterns without triradii, whorls have two and loops one triradii, Fig.1. We also detected complex patterns named double loops (Wd). Ridge count is the number of ridges which crosses the line between the triradii and the core of the pattern. In complex patterns with two triradii we count both sides and we name this as absolute ridge count. Ridge count was counted for the first toe, patterns present in hallucal region and plantar a-b ridge count between the two triradii a and b. Triradii are the center in which the line from three different regions meet and the angle between them is higher than 90 degrees. Triradii were divided into basic (a,b,c,d,e,p,pm) and additional ones with the apostrophe (p', p'').

Configurational areas on the sole are:
Hallucal (distal thenar and first interdigital)
Second interdigital configurational area
Third interdigital configurational area
Fourth interdigital configurational area
Thenar proximal, hypotenar distal and proximal
Calcar

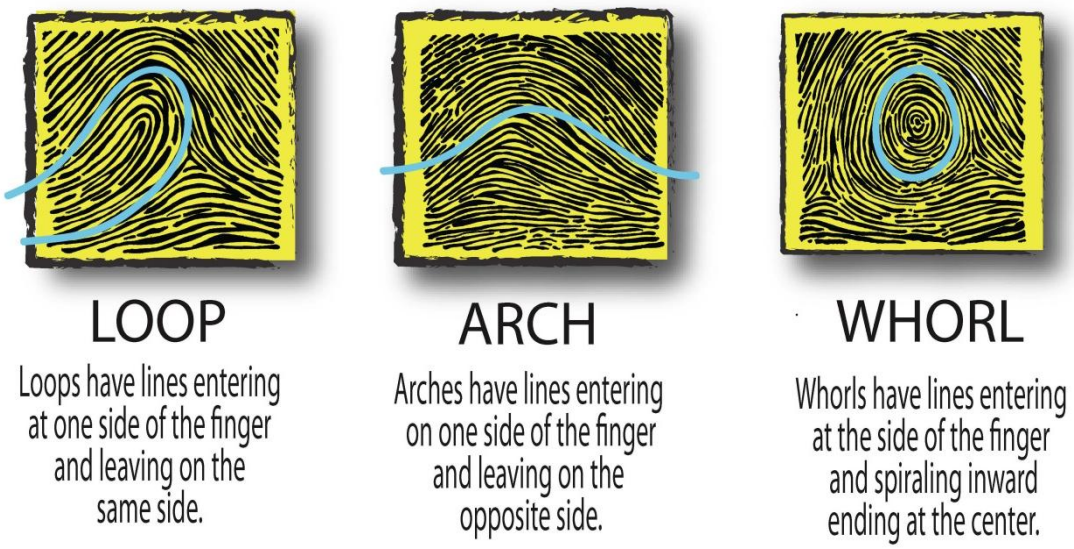


Fig. 1. Types of finger patterns

RESULTS

The results of the study for the found patterns of the digit area are shown on Charts 1 and 2. The arches' frequency decreases from the fifth to the first digit; the opposite was for the fibular loops, they increase from the fifth to the first digit for both sexes. Fibular loops were more abundant in males on the second, first, third and fourth digit; the first, second, third and fourth in females. Whorls (concentric ones) were present on the first digit in a larger number in both sexes. Wd-double loop, which represents complex patterns, was present in small number on the third digit in both sexes.

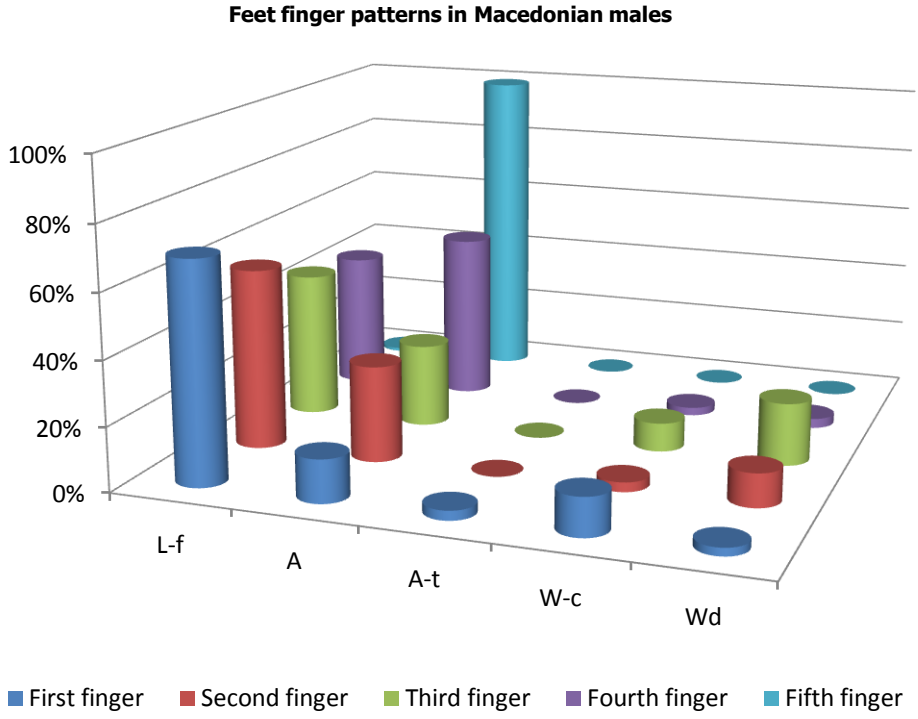


Fig. 2. Feet finger patterns in Macedonian males

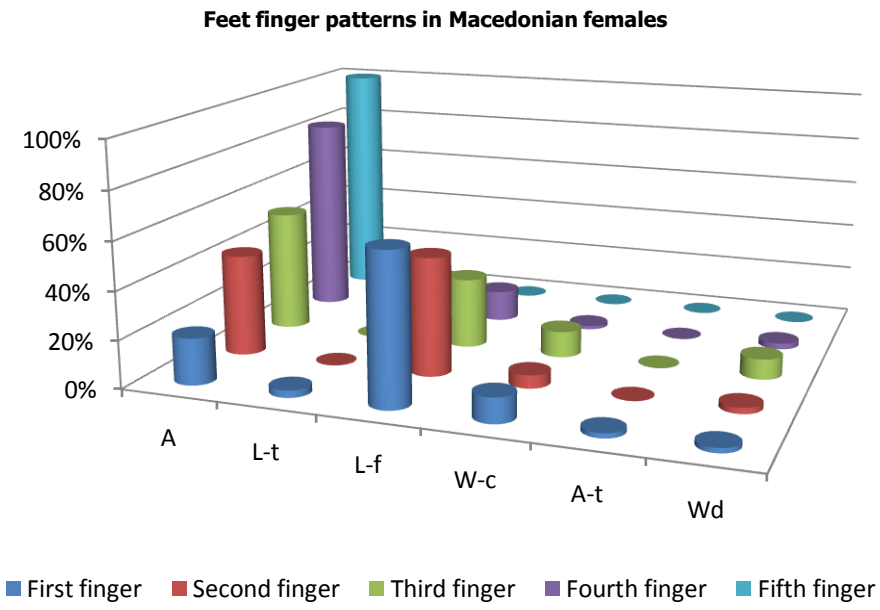


Fig. 3. Feet finger patterns in Macedonian females

The results of the basic triradii for males and for females are shown with the following formulas: c=d>b>a>pm>p>e>p''>p' in males and c=d>a>b>pm>p>e>p'>p'' in females.

The most common patterns on the sole regions in males were distal loops-Ld, and the same number was in females but in a lower percentage than whorls and arches. Tibial loops were present in a small percentage in females.

Total number of ridges (TRC) was 9.10 in males and 7.93 ridges in females for the patterns on the big toe. ATRC for the hallux was 10.40 in males and 8.97 in females.In the halucal, thenar distal and first interdigital space patterns ridge count was 15.08 in males and 14.39 in females.There were 15 ridges between the a-b triradii in males and 14 in females.

DISCUSSION

The results have shown dermatoglyphic quantitative and qualitative plantar characteristics in the Macedonian population; therefore, they are of significance for the Macedonian population in Macedonia.

When compared to other author's findings we can notice that in Loesch and Skrinjaric study including 219 children of Croatian and 63 of Polish origin there were more fibular loops than in the Roma people; the triradii were not specific and different enough to mark the chosen nationality. Precise definition of the plantar loops gives us possibility for further examination and exploring their biological and genetic significance according to the two authors [3].

The results of plantar prints of 500 people from Egypt were consistent with our findings for the patterns on the sole regions and toes. The most prevalent were fibular loops, arches on the fourth and fifth digit; arches were more frequently present than fibular loops. Tibial loops were rare, which is similar to our results. Distal loops prevailed on the sole region [4].

In healthy examinees in a tribe from Malawi, there were fibular loops on the four digits except on the fifth one. Arches were found in 100% on the fifth digit as was the case in our study, too. PII was higher than in our study [5].

Siemens' study included 310 Jewish and 124 non-Jewish whites. Jews showed higher PII with more dermatoglyphic patterns present; there were whorls on the third interdigital region, which does not coincide with our findings. Distal loops and whorls present in the second interdigital space was the same as in our study although in smaller percentage in the Macedonian people. In Jews' hallucal and hypothenar region whorls, tibial loops, and triradii were more abundant. Jews showed more ridges in transverse direction compared to the Macedonian and non-Jewish whites [6].

In conclusion, this paper has shown classification of the plantar dermatoglyphic traits present in the Macedonian population. The arches' frequency decreases from the fifth to the first digit; the opposite was for the fibular loops, they increase from the fifth to the first digit for both sexes. Double whorls on the third digit were present as complex patterns. Found triradii were c=d>b>a>pm>p>e>p''>p' in males and c=d>a>b>pm>p>e>p'>p'' in females. Ridge count for the toe was 9.10 ridges in males and 7.93 in females. In the halucal, thenar distal and first interdigital space patterns ridge count was 15.08 in males and 14.39 in females. A-b ridge count was 15 ridges in males and 14 in females.

Dermatoglyphic traits have been used to assess population affinities and structure [7]. There are differences compared to the plantar dermatoglyphics in other nationalities and hence they can be interpreted as markings of the Macedonian ethnic group, which is the main objective of anthropological studies. Exploration of dermatoglyphics gives us information about clinical traits of different nationalities. Specific morphological peculiarities might be useful in clinical and genetic studies. These results attest also to the usefulness of dermatoglyphics in resolving various evolutionary questions [8].

It is hoped that further studies in dermatoglyphics will broaden some new possible applications of skin ridge patterns and enable comparison with other nationalities.

REFERENCES

1. Kumbnani HK. Dermatoglyphics: A Review. Anthropol Special. 2007; 3:285-95.
2. Cummnis H, Midlo C. Palmar and plantar epidermal ridge configurations (dermatoglyphics) in European-Americans. Am J Phys Anthropol. 1926;9:471-502.
3. Loesch D, Skrinjaric I. Classification of dermal patterns on the proximal sole. Ann Hum Biol. 1980;7(6):529-45.
4. Hassan FZ, Sayed A, Shefaa M, Gawish M, Samea AR. Toe and Plantar Dermatoglyphics in Upper Egyptians. Assiut Med. J. 2000; 24(3):491-95.
5. Igbigbi PS, Adeloye A. Dermatoglyphics of mothers of Malawian children with spina bifida cystica: a comparative study with female controls. West Afr J Med. 2005;24(1):58-67.
6. Siemens GJ. Dermatoglyphic traits of Jewish and non-Jewish whites. Ohio J Sci. 1954;54(1):15-26.
7. Craford MH, Duggirala R. Digital dermatoglyphic patterns of Eskimo and Amerindian populations:relationships between geographic, dermatoglyphic, genetic and linguistic distances.Hum Biol. 1992;64(5):683-704.
8. Kamali MS, Mavalwala J, Khaneqah AA, Bhanu BV.Qualitative dermatoglyphic traits as measures of population distance.Am J Phys Anthropol. 1991;85(4):429-50.

UDC:572.087.1-053.5

SEX AND AGE SPECIFIC DIFFERENCES OF ANTHROPOMETRIC PARAMETERS IN MACEDONIAN ADOLESCENTS

Bojadzieva Stojanoska Biljana, Janevska Nakeva N, Matveeva N, Zafirova B, Chadikovska E
Institute of Anatomy, Medical Faculty, "Ss. Cyril and Methodius" University, Skopje, Macedonia

ABSTRACT

The aim of this study was to evaluate sex and age-specific differences of anthropometric parameters in Macedonian adolescents.

This study included 1183 adolescent students (622 males and 561 females) at the age of 15 to 18 years. Anthropometric parameters, which define longitudinal and transversal measures of skeleton, were measured using standard equipment and measurement technique. BMI was calculated from body height and weight (kg/m^2). Two skin-folds were measured: triceps and subscapular.

We found a significant sex-specific difference for body height, weight, elbow and knee diameter at the age of 15 to 18 years in favour of boys. Height, weight and two transversal parameters had higher values in boys. Triceps and subscapular skin-folds as indicators for subcutaneous fat component and a valuable technique for evaluating the nutritional status had higher values in females in all age groups. Sex-specific differences were registered for triceps skin-fold at the age of 15 to 18 years, and for subscapular skin-fold at the age from 15 to 17 years in favor of females.

Populations share their own genetic background and environmental factors, so anthropometric characteristics of the body of Macedonian adolescents can be used as criteria for the assessment of growth and nutrition within this group.

Key words: adolescents, nutritional anthropometry, growth parameters

INTRODUCTION

Linear growth and physical development are dynamic processes that include changes on molecular, celular and somatic level. The process of human growth means enlargment of body size or some of its parts and development means increasment of functional ability of organs and adaptation to enviorment. In postnatal period human organism goes through distinct periods of development. Beside childhood, adolescence is the most vulnerable period of growth and development which takes place between childhood and adulthood [1]. It is divided in early adolescence (10 to 14 years) and late adolescence (15 to 18 years) and the changes that occur in this period are noted with the beginning of puberty. Many clinical observations have stated that both sex and growth hormones have a key role in normal growth in puberty. If the secretion of sex hormones is disturbed or inhibited in this period of growth and development, the puberty will delay and the process of growth in height will be interrupted [2, 3]. For this reason it is very important to monitor growth and development of adolescents, by frequent anthropometric measurments, to follow the quality of health and eventually to take care of individuals or entire population [4, 5, 6]. Although the growth process is complex the adolescent growth spurt is a constant phenomenon and occurs in all children, though it varies in intensity and duration from one child to another [7].

In our study we measured several anthropometric parameters in 15 to 18-year-old adolescents from urban cities in R. Macedonia. The aim of this study was to assess the growth of anthropometric parameters in some longitudinal and transversal dimensions, and assess the two skin-folds as indicators of subcutaneous fat.

SUBJECTS

Data were obtained from a cross-sectional sample of students of primary schools in three different urban cities in Macedonia (Skopje, Strumica and Kumanovo). The sample included 1183 healthy adolescent students (622 males and 561 females) at the age of 15 to 18 years, from selected schools and classes. Adolescents' parents gave their written consent for participation in the study. In order to avoid errors in the selection of the sample, volunteer students were not included. Subjects were grouped according to sex and age. The Human Research Ethics Committee of the Medical Faculty - University "Ss. Cyril and Methodius" in Skopje, approved the experimental protocols.

ANTHROPOMETRY

Anthropometric variables were selected and measured according to the International Biological Program (IBP) for assessment of longitudinal, transversal and circular skeleton dimensionality: body height (cm), elbow diameter (cm) and knee diameter (cm), body weight (kg). Two skin-folds were measured: triceps and subscapular (mm). Measurements were made during school hours, not interrupting the lessons. The following standard anthropometric instruments were used: the anthropometer by Martin, with 1 mm reading accuracy for measuring body height; "John Bull" caliper square for determination of skin-folds with pressure of 10 g/cm^2 and precision of 0.1 mm; weight scale and caliper square for measuring of diameters with reading precision of 1 mm, and flexible metal tape for girth measurement. Body mass index was calculated from body weight (kg) divided by the square of body height (m).

STATISTICS

The data were analyzed with descriptive statistics represented by measures of central tendency and its deviation (arithmetic mean value and standard deviation). Testing of sex and age-specific differences was done with analysis of variance (ANOVA). Differences for $p < 0.05$ were considered to be statistically significant. The statistical package for the social sciences (version 20.0, SPSS Inc, Chicago, IL) was used for all statistical analyses.

RESULTS

In this transversal study 1183 adolescent students (622 males and 561 females) at the age of 15 to 18 years were included. Mean values and standard deviations of body height, body weight and body mass index of 15 to 18-year-old adolescents and their age and sex-specific differences are presented in Table 1.

The mean values of body height (cm) and body weight (kg) were higher in males in all age groups. The 15-year-old Macedonian boys had body height of 173.66±7.06 cm, weight 67.66±13.63 kg and BMI 22.40±4.23 kg/m^2. In females at the same age the values were: 163.16±5.91 cm, 56.29±9.18 kg and 21.12±3.23 kg/m^2 for body height, weight and BMI. In 16-years-old males the mean values were: 175.55±6.35 cm for body height, 69.26±11.81 kg for weight and 22.49±3.90 kg/m^2 for BMI, compared to values of females at the same age: 163.33±6.34 cm for body height, 56.29±8.21 kg for weight and 21.08±2.71 kg/m^2 for BMI. At the age of 17 years the mean values for height, weight and BMI in males were: 177.89±7.08 cm for body height, 71.74±13.72 kg for weight and 22.62±3.87 kg/m^2 for BMI, compared to values of females at the same age: 163.45±5.53 cm for body height, 58.72±9.33 kg for weight and 21.93±3.15 kg/m^2 for BMI and in 18-year-old males the mean values were: 179.35±7 cm for body height, 75.28±11.52 kg for weight and 23.34±3.13 kg/m^2 for BMI, compared to values of females at the same age: 164.78±6.02 cm for body height, 57.52±7.84 kg for weight and 21.19±2.76 kg/m^2 for BMI. Sex-specific differences for body height and weight were found at the age of 15 to 18 years in favour of males ($p<0.01$). Age-specific differences for body height and body weight were not found. The mean values of body mass index used as an indicator of nutritional status were higher in all age groups in males compared to females. Sex-specific differences were found in favour of males at the age of 15,16 and 18 years, and age-specific differences were not found.

The mean values as well as sex and age-specific differences of transversal parameters, diameter of elbow and knee, in males and females at the age of 15 to 18 years are shown in Table 2. The mean values of elbow and knee diameter increased with age but not significantly, except at the age of 16 years in males where we found age-specific differences for knee diameter ($p<0.05$). In all age groups males had higher values for both transversal parameters. Sex-specific differences for elbow and knee diameter were found in all age groups in favour of males ($p<0.05$).

Table 3 presents values (mean and standard deviation), as well as sex and age-differences of triceps and subscapular skin-folds. The values for triceps skin-fold in males had tendency to decrease with age, opposite of subscapular skin-fold values which had tendency to increase. In females both skin-folds increased their values at the age of 15 to 17 years. Still we had not found age-specific differences. Triceps and subscapular skin-folds as indicators for subcutaneous fat component had higher values in females and we found sex-specific differences in all age groups in favour of females except at the age of 18 years (no significant difference between two sexes in subscapular skin-fold mean value).

Table 1. Analysis of variance of differences in body height (cm), body weight (kg) and BMI (kg/m^2) in Macedonian adolescents at the age of 15 to18 years

Age	Males		Females	
	Body height			
	n	$\bar{x}$±sd	n	$\bar{x}$±sd
15	164	173.66±7.06**	148	163.16±5.91
16	161	175.55±6.31**	150	163.33±6.34
17	160	177.89±7.08**	131	163.45±5.53
18	137	179.35±7**	132	164.78±6.02
Body weight				
15	164	67.66±13.63**	148	56.29±9.18
16	161	69.26±11.81**	150	56.29±8.21
17	160	71.74±13.72**	131	58.72±9.33
18	137	75.28±11.52**	132	57.52±7.84
Body mass index				
15	164	22.40±4.23*	148	21.12±3.23
16	161	22.49±3.90**	150	21.08±2.71
17	160	22.62±3.87	131	21.93±3.15
18	137	23.34±3.13**	132	21.19±2.76

Significance * $p<0.05$, ** $p<0.01$(sex difference) $^b p<0.05$ (age difference) (ANOVA)

Table 2. Analysis of variance of differences in elbow and knee diameter (cm**)** in Macedonian adolescents at the age of 15 to18 years

Age	Males		Females	
		Elbow diameter		
	n	$\bar{x}$±sd	n	$\bar{x}$±sd
15	164	6.55±0.47**	148	5.72±0.41
16	161	6.58±0.44**	150	5.77±0.40
17	160	6.70±0.41**	131	5.72±0.39
18	137	6.87±0.44**	132	5.80±8.59
		Knee diameter		
15	164	9.30±0.72**	148	8.62±0.60
16	161	9.60±0.63**b	150	8.56±0.50
17	160	9.65±0.76**	131	8.70±0.65
18	137	9.57±0.65**	132	8.59±0.47

Significance *p<0.05, **p<0.01(sex difference) [b]p<0.05 (age difference) (ANOVA)

Table 3. Analysis of variance of differences in triceps and subscapular skin-folds (mm) in Macedonian adolescents at the age of 15 to18 years

Age	Males		Females	
		Triceps skin-fold		
	n	$\bar{x}$±sd	n	$\bar{x}$±sd
15	164	12.88±7.44	148	17.51±5.32**
16	161	12.15±5.85	150	16.87±4.88**
17	160	12.45±7.36	131	17.75±5.78**
18	137	12.13±4.87	132	16.18±4.65**
		Subscapular skin-fold		
15	164	10.75±6.01	148	13.01±4.71**
16	161	10.66±4.39	150	12.77±4.35**
17	160	11.55±5.49	131	14.21±5.26**
18	137	11.86±4.25	132	12.63±4.46

Significance *p<0.05, **p<0.01(sex difference) [b]p<0.05 (age difference) (ANOVA)

DISCUSSION

Although the growth process is complex the adolescents growth spurt is a constant phenomenon and occurs in all children, though it varies in intensity and duration from one child to another [7]. According to Tanner the peak velocity of growth in height averages about 10 cm a year in boys, and slightly less in girls at the age between 11 and 13 years, and after this period males continue to grow in height and females height is stabilized [8]. At the age of 11 and 12 there are no great differences in height between males and females, but after the age of twelve males grow faster and are taller than females [4, 9]. Most girls have completed the physical changes related to puberty by the age of 15, while boys are still maturing and gaining strength, muscle mass, and height and are completing the development of sexual traits. In our study we measured several anthropometric parameters in 15 to 18-year-old adolescents from urban cities in R. Macedonia. The aim of this study was to assess the growth of anthropometric parameters in some longitudinal and transversal dimensions, and assess two skin-folds as indicators of subcutaneous fat.

Body height and weight in our examinees had tendency to grow in males with the age, while body height in females remained stable, which is in agreement with the data from other studies [10, 11, 12, 13]. We compared the results of our study with similar data for adolescents from other countries and we noticed some population's differences. We compared our results with the data from the transversal study of growth in children from Sombor, Republic of Serbia at the age of 16 to 18 years. We found that our male adolescents had lower values for body height (175.55±6.31 cm, 177.89±7.08 cm, 179.35±7 cm) than their peers from Sombor (179.9±7.6 sm, 181.6±6.6 cm, 181.2±6.8 cm). Macedonian female adolescents had also lower values for body height (163.33±6.34 cm, 163.45±5.53 cm, 164.78±6.02 cm) than their peers from Sombor (166.7±6 cm, 168.6±5.6 cm, 167.66±5.8 cm). Macedonian adolescents had lower values for body weight compared to their peers from Sombor [14]. We compared our values with the NCHS reference values of body weight and height in American adolescents and we found that our male and female examinees had higher values for height and lower values for body weight.

Body height had tendency to increase with the age and at the age of 18 according to NCHS mean value for body height in the American male adolescents was 176.4 cm and in females 163.1 cm, compared with the mean values of height in our examinees (body height was 179.35 in males and 164.78 cm in females). At the age of 18 years the mean value for body weight in American male adolescents was 81.4 kg, and in females 65 kg, compared to Macedonian adolescents who had lower values for body weight (75.28 kg in males and 57.52 kg females) [15].

Transversal body parameters, diameter of elbow and knee, give information on bone growth in width. Zivicnjak M. suggests that the optimal bone growth in width in the period of childhood and adolescence is important for the body statics and prevention of fractures later in life. In his study males in all age groups from 2 to 18 years had higher values for diameters of elbow and knee, which is in agreement with our results. At the age of 15 to 18 years our examinees had slightly lower mean values for elbow diameter compared to their peers from Zagreb at the same age, and there were no differences in diameter of knee between our examinees and peers from Zagreb [16]. We compared our results with the data from the study of growth in children from Zaria, Nigeria and we concluded that our male examinees had higher values for both measured transversal parameters while Macedonian females had lower values compared to their peers from Nigeria [17].

Distribution of fat tissue and the main changes that occur in body during the childhood and adolescence are of great interest for researchers. Beside body mass index, the thickness of skin-fold is treated as a useful parameter for evaluation of fat structural component. It is necessary to have the unique reference for screening the body growth in children and adolescents. Other countries implement the standards for growth of children under 5 years of age, but there is a gap between these standards and the existing growth reference for older children. Now it is widely accepted to use descriptive samples of population that reflect a secular trend towards overweight and obesity. It is necessary to construct growth references so that obesity can be recognized [18]. Marshall JD and Zemel BS suggest that these two skin-folds are the most valid indicators of body fat mass [19, 20]. In our study we measured two skin-folds: triceps and subscapular, and we compared our data with the data from other studies. Macedonian females have higher mean values for both measured skin-folds than males. American adolescents of both sexes according to the reference values of Centers for disease control and prevention – NCHS, have higher values for triceps skin-fold than our examinees at the age of 16 to 18 years [20]. At the age of 18 years mean value of triceps skin-fold in American adolescents was 14 mm, in comparison with the mean value of triceps skin-fold in our examinees which was 12.13 mm.

Comparing the mean values of triceps and scapula skin-folds in our examinees and their Macedonian peers from the previous studies of Nakeva Natasa (1995) and Todorovska Lidija (1997), our male and female examinees have higher values, thus we can conclude that today school adolescents are more obese than their peers two decades ago.

CONCLUSION

The dimorphism differences in some anthropological variables in Macedonian adolescent population were investigated with males having higher values than females, except for the measured skin-folds that had higher values in females. The results obtained in our study coincide with the results obtained in earlier studies that the growth spurt in girls stabilizes at the age of 15 and in boys their body height continues to increase until the age of 18 years. By comparing the results from anthropometric measurements of body parameters for assessment of longitudinal and transversal dimensions in Macedonian population with a representative sample of other populations at the same age we can conclude that anthropometric characteristics of the body are frequently distinctive within a group between different populations depending on their own genetic background and environmental factors. The values found in our study are recommended to be applied for evaluation of deviations in growth in 15 to 18-year-old Macedonian adolescents.

REFERENCES

1.
2. World Health Organization. Physical status: The use and interpretation of anthropometry. WHO Technical Report Series No.854. Geneva: WHO; 1995. p.263-311.
3. Root AW, Powers PS. Anorexia nervosa presenting as growth retardation in adolescence. J Adolesc Health Care. 1983;4:25-30.
4. Nussbaum M, Baird D, Sonnenblick M, et al. Short stature in anorexia nervosa patients. J Adolesc Health Care. 1985;6:453-5.
5. McDowell M, Fryar CD, Ogden CL, Flegal KM. Anthropometric Reference Data for Children and Adults: United States, 2003–2006. National Health Statistics Reports. 2008.
6. Tanner JM. Obituary 1920-2010. Ann Hum Biol. 2011;38(3):243-6.
7. Rogol AD, Roemmich JN, Clark PA. Growth at puberty. J Adolesc Health. 2002;31:192-200.
8. Tanner JM. Growth and maturation during adolescence. Nutrition reviews. 1981; 39(2): 43-55.
9. Tanner JM, Whitehouse RH, Takaiski M. Standards from birth to maturity for height, weight, height velocity and weight velocity. Arch Dis Child. 1966;41:454-71.

10. Bojadzieva Stojanoska Biljana, Janevska Nakeva N, Zafirova B, Matveeva N, Chadikovska E, Gontarev S. Sex and age specific differences of some anthropometric parameters and BMI in Macedonian adolescents. Acta morphol. 2014; 11(2):10-14.
11. Janevska-Nakeva N. Morfoloska struktura i strukturni komponenti na teloto kaj ucilisnata mladina od makedonska nacionalnost. Doktorska disertacija, Medicinski fakultet, Skopje, 1995.
12. McDowell M, Fryar CD, Ogden CL, Flegal KM. Anthropometric Reference Data for Children and Adults: United States, 2003–2006. National Health Statistics Reports. 2008.
13. Zafirova B, Todorova L. Anthropometric parameters of growth and nutritional status in children aged 6 to 7 years in R. Macedonia. Adv Med Sci. 2009; l54(2): 289-95.
14. Todorovska L, Zivkovic V, Nikolic S, Pluncevic J, Sivevska E, Handziska,E, Karadjozova I. Anthropometric characteristics of Macedonian children at the age of 9-14 years. PESH. 2012; 1:11-9.
15. Rakic R, Bozic-Krstic V, Pavlica T. Stanje uhranjenosti adolescenata u Somboru. J Anthropol Soc Serb. 2008;43: 336-41.
16. Fryar CD, Gu Q, Ogden CL. Anthropometric reference data for children and adults: United States, 2007–2010. National Center for Health Statistics. Vital Health Stat. 11. 2012;(252):1-48.
17. Zivicnjak M, Narancic NS, Szirovicza L, Franke D, Hrenovic J, Bisof V, Tomas Z, Skaric-Juric T. Gender-Specific Growth Patterns of Transversal Body Dimensions in Croatian Children and Youth (2 to 18 Years of Age). Coll Antropol. 2008;32(2):419–31.
18. Oyewale AA, Ojo SA, Adebisi SS, Danborno SB. The study of anthropometric variables on growth and development of school children in Zaria, Nigeria. Asian J Med Sci. 2010; 2(4):185-9.
19. Djalma RR, Soares de Araujo C.G. Body mass index: A scientific evidence –based inquiry. Arq Bras Cardiol. 2002; 79(1):70-8.
20. Marshall JD, Hazlett CB, Spady DW, Conger PR, Quinney HÁ. Validity of convenient indicators of obesity. Hum Biol. 1991; 63:137-53.
21. Zemel BS, Riley EM, Stallings VA. Evaluation of methodology for nutritional assessment in children: anthropometry, body composition and energy expenditure. Ann Rev Nutr. 1997;17:211-35.

UDC:615.252.44.036.8:611.44.086]:599.323.4

EXPERIMENTAL STUDY OF THE EFFECTS OF ONE MONTH APPLICATION OF TWOFOLD THERAPEUTIC DOSE OF METHIMAZOLE ON THYROID GLAND TISSUE STRUCTURES IN ADULT WISTAR RATS

Mitevska Elida, Kiroska G
Institute of Medical, Experimental and Applied Histology and Embryology, Medical Faculty, Ss Cyril and Methodius University, Skopje, Republic of Macedonia

ABSTRACT

Aim: To determine the histological characteristics of the thyroid gland of adult male Wistar rats after one month application of a twofold dose of antithyroid drug, methimazole.

Material and methods: A total of 20 Wistar rats were divided into two groups. Control group of rats received drinking water and experimental rats received 0.05% aqueous solution of methimazole in the course of 30 days. The paraffin sections were stained according to hematoxylin-eosin, Azan and Florentin methods.

Results: Qualitative histological analysis demonstrated that methimazole caused dilation of thyroid follicles lumen, especially in the peripheral parts of the gland; activation of follicular epithelium that was extremely high, with numerous cellular mitoses and appearance of papillae that were prominent in the follicular colloid, consisting of interstitial connective tissue and follicular epithelium.

Conclusion: Our results have shown that one month application of a twofold dose of methimazole resulted in characteristic histological changes of the thyroid gland structures in terms of occurrence of hypothyroidism.

Key words: thyroid gland, adult male Wistar rats, methimazole, histological analysis

INTRODUCTION

Methimazole is one of the most used drugs for treatment of an overactive thyroid gland (hyperthyroidism), due to its efficacy and rarity of serious toxicity [1, 2].

An enzyme (peroxidase) produces thyroid hormones, i.e., thyroxine (T4) and triiodothyronine (T3), by combining iodine with a protein called thyroglobulin. Methimazole (Favistan®) like the most antithyroid drugs prevents iodine and peroxidase from their normal interactions with thyroglobulin to form T4 and T3. This action decreases thyroid hormone production and reduces the level of thyroid hormones [3]. Thus, methimazole is considered to be an efficient and useful antithyroid agent.

However, this drug causes some adverse effects in humans such as agranulocytosis [4], arthritis [5] and hepatotoxicity with cholestatic jaundice [6]. Most of the side effects have been observed in some experimental animals, too.

Experimental studies have shown that the effects of prolonged treatment with this drug caused retarded growth in Harlan rats [7] and significant increase of rat thyroid gland mass [8].

The aim of our study was to determine the histological characteristics of the thyroid gland and eventual changes in its structures in adult male Wistar rats, after one month application of a twofold dose of methimazole.

MATERIAL AND METHODS

The experiment included a total of 20 male adult Wistar rats, divided into two groups, each one consisting of 10 animals. The first one, the control group, received drinking water and the second one, the experimental group, received 0.05% aqueous solution of methimazole, which was twofold higher than the therapeutic dose given to humans. Methimazole (Favistan®) used in the experiment was a product of "Bosnalijek" from Sarajevo. We defined the dose and adjusted it in percentages in the solution given ad libitum during a period of 30 days. The quantity of the water was determined prior to administration. Minimum quantity of water where the drug was dissolved had to remain for each next day.

The animals were acclimatized to a room temperature of 18 - 22ºC, submitted to a regimen of 12 hours of light and 12 hours of darkness and fed with standard food for laboratory Wistar rats. All the procedures concerning manipulation with the animals were in accordance with the legal requirements for experimental work with laboratory animals.

After the treatment period, the rats were sacrificed under anesthesia with ether. Thyroid glands were removed with blunt tweezers and measured with precision electronic scales. The paraffin sections were prepared by the usual staining methods for light microscopy analysis: hematoxylin-eosin, Azan and Florentin.

RESULTS

Histological analysis of the thyroid gland in the control group of rats has shown spheroid follicles with variable size. Smaller follicles lined by tall cuboidal or columnar cells were located in the central gland parts and larger follicles lined by lower epithelium containing large amounts of stored colloid, were observed in the peripheral gland zone. Rare and small resorptive vacuoles were present in the follicular colloid. The interstitial connective tissue consisted of fine collagenous septa (Fig. 1). This cytological architecture is characteristic for normal activity of the thyroid gland.

Histological analysis of treated group of rats showed that the methimazole application changed the histological architecture of the thyroid gland in terms of nodular structuring of the thyroid follicles (Fig. 2). Irregular follicles were also observed in both, peripheral and central parts of the gland. Some of them were dilated and deformed in terms of loss of their usual round or oval shape (Fig. 3). In most experimental animals cystic expansion of peripheral follicles was observed accompanied by a papillary infolding inside the coloid.

Papillae were structured by connective tissue base coated with follicular epithelium (Fig. 4). The height of follicular epithelial cells was markedly increased by the presence of frequent mitoses with maximum resorbed colloidal content. These changes were especially visible in the central parts of the gland (Fig. 5). Detachment of epithelial cells from follicular epithelial line was registered. Detached epithelial cells were floating in the colloid. The described changes were accompanied by the appearance of numerous resorptive vacuoles (Fig. 6).

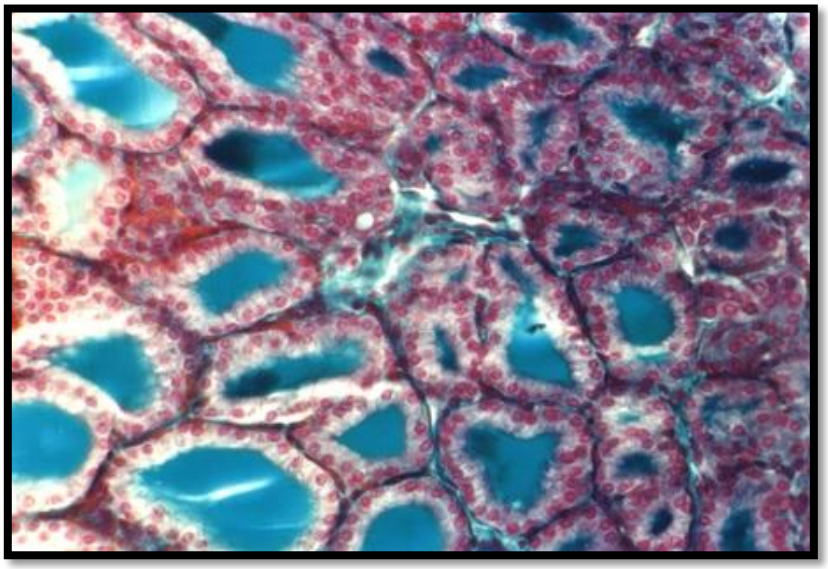


Fig. 1. Control group: normally structured thyroid gland; Florentin (10 X 40)

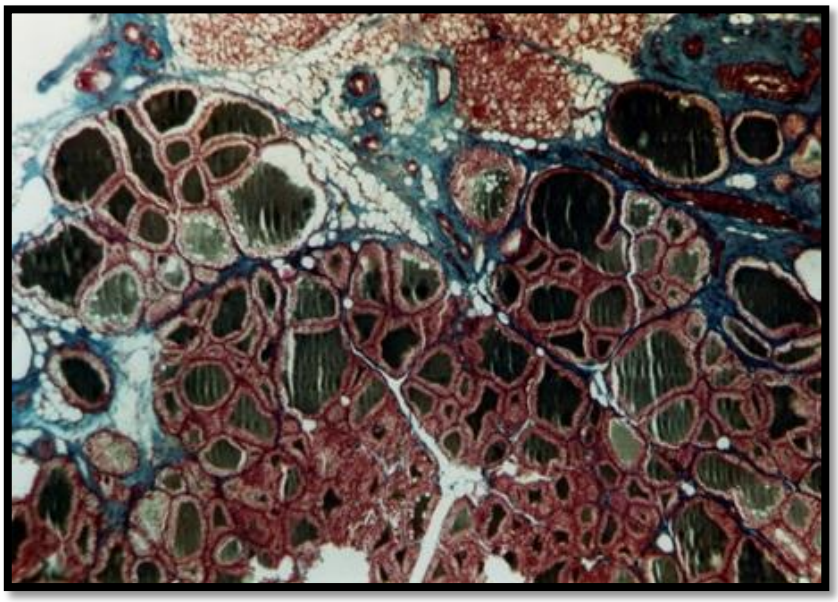


Fig. 2. Experimental group: nodular structured thyroid gland follicles; Azan (10 X 10)

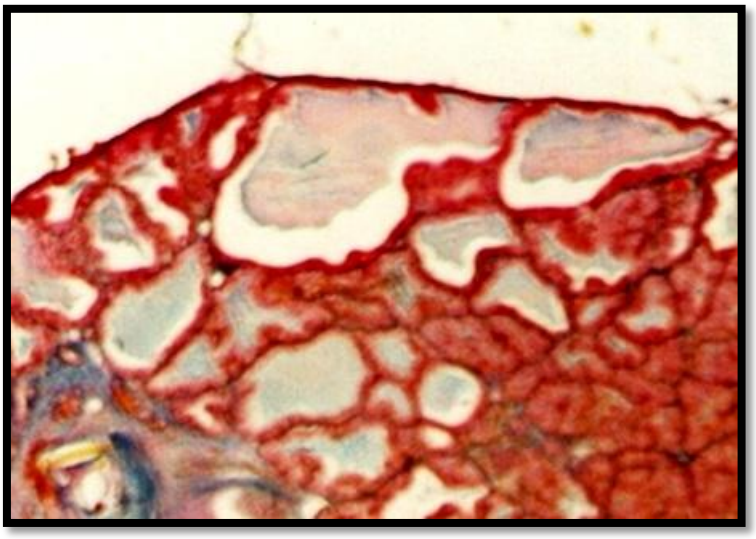


Fig. 3. Experimental group: dilated follicles with different size and form, irregular shaped in peripheral and central parts of the gland; H&E (10 X 10)

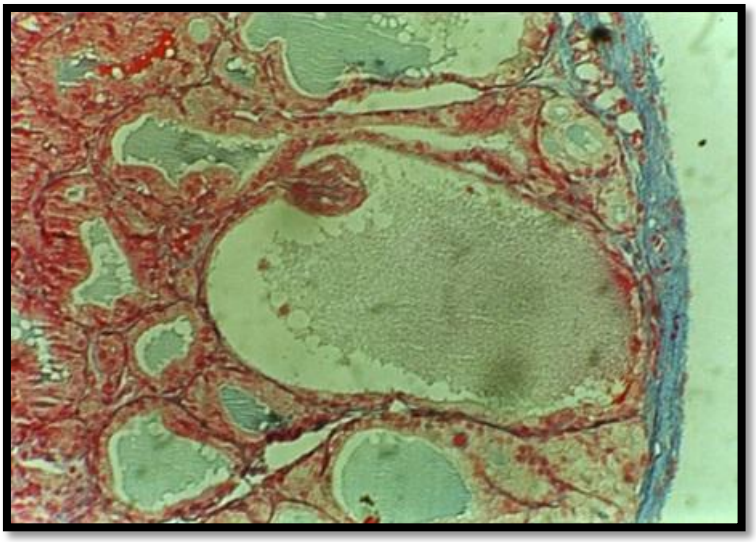


Fig. 4. Experimental group: cyclically structured follicles and papillary infolding of activated follicular epithelium in the colloid; Azan (10 X 10)

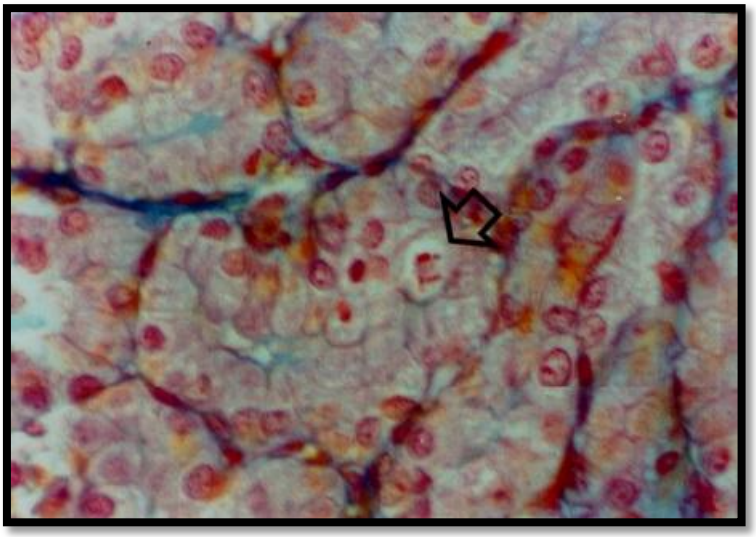


Fig. 5. Experimental group: the presence of frequent mitoses in the follicular epithelium; Azan (10 X 100)

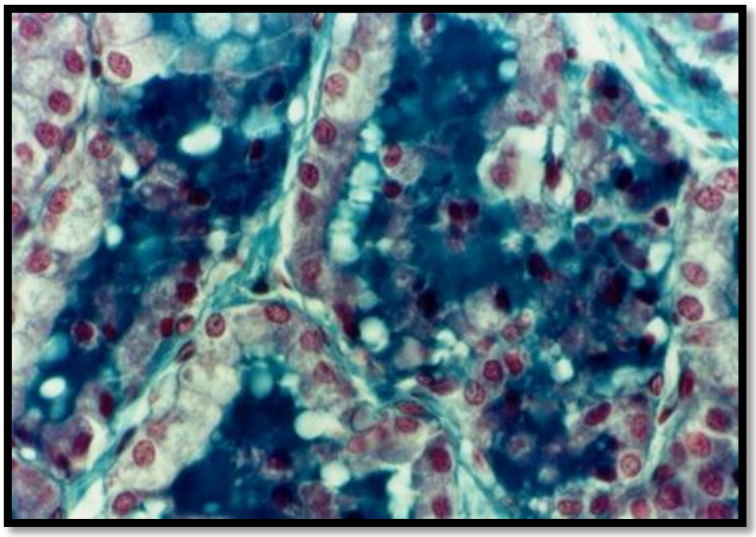


Fig. 6. Experimental group: detachment of superficial follicular epithelial cells inside the colloid; Florentin (10 X 100)

DISCUSSION

One of the most commonly applied drugs in the treatment of hyperthyroidism is the antithyroid drug methimazol. Its main mechanism of action is to prevent iodine and peroxidase from their normal interactions with thyroglobulin to form T4 and T3. This action decreases thyroid hormone production. Methimazole also interferes with the conversion of T4 to T3. Since T3 is more potent than T4, this also reduces the activity of thyroid hormones.

From a clinical point of view there is a marked variability of methimazole side effects ranging from mild to pronounced toxic effects [9]. Fortunately, minor side effects such as skin reactions and arthralgias are encountered more frequently in comparison with the major ones such as agranulocytosis, hepatitis and polyarthritis [10]. Depending on the drug, the side effects may be dose-related [11].

In addition to the above-mentioned side effects that are manifested on the skin, the joints, liver and blood cell population, antithyroid drug therapy may result in thyroid tissue adverse effects. Cases of diffusely enlarged thyroid gland have been described [5]. Inadequate dosing of antithyroid medications sometimes results in changes of the thyroid gland in favor of occurrence of hypothyroidism.

In our study we analyzed the histological changes of the thyroid gland as a result of the methimazole usage. The results showed changes such as distended cystic follicles, follicular epithelium with increased height and its papillary infolding inside the colloid, presence of frequent mitoses in follicular epithelial cells and maximum resorbed colloidal content with presence of numerous resorptive vacuoles. Thus, methimazole side effects in the structural components of the thyroid gland may have caused medically induced state of hypothyroidism. In the literature similar histological findings are reported where the thyroid gland has follicles lined by activated, markedly high follicular epithelium and minimum colloid, a condition that results in decreased levels of thyroid hormones [5, 12, 13]. Distended cystic follicles may be due to disproportion between the synthesis and excretion of thyroglobulin in thyroid follicles and its resorption [14].

Our results are similar to previously published morphological findings such as the presence of hypertrophic columnar follicular cells [15, 16], increased number and a pronounced growth activation of the follicular epithelial cells with frequent mitoses, accompanied with improved vascularisation [17, 18].

Our histological results have shown that one month application of a twofold dose of methimazole causes reactive changes of thyroid gland structure components under our experimental conditions.

CONCLUSION

The results obtained by analyzing histological parameters under experimental conditions have indicated that one month application of a twofold dose of methimazole provoked adverse effects on thyroid gland structure in adult Wistar rats. The established effects of the application of this antithyroid drug on the histological structure of the thyroid gland have suggested maximal precaution and regular control when prescribing the drug in clinical practice.

REFERENCES

1. Moreno C, Alves M, Paiva I, Rodrigues D, Ruas L, Saraiva J, Guelho D, Vicente N, Cardoso L, Carrilho F. Severe side effects of methimazole in the treatment of hyperthyroidism. Endocrine Abstracts. 2014; 35:1009.
2. Franklyn JA. The management of hyperthyroidism. N Engl J Med 1994; 330:1731.
3. Nakamura M, Yamazaki I, Nakagawa H, Ohtaki S, Ui N. Iodination and oxidation of thyroglobulin catalyzed by thyroid peroxidase. J Biol Chem. 1984; 259(1):359-64.
4. Tajiri J, Noguchi S. Antithyroid drug induced agranulocytosis: special reference to normal white blood cell count agranulocytosis. Thyroid. 2004; 14(6):459-62.
5. Ploegstra MW, Boontje PR, Kamps WAA. Arthritis associated with antithyroid therapy in a 15-year-old girl. J Pediatr Pharmacol Ther. 2011; 16(2):98-101.
6. Miljic D, Stojanovic M, Jesic R, Bogdanovic G, Popovic V. Role of plasma exchange in autoimmune hyperthyroidism complicated by severe tiamazol-induced cholestatic jaundice. Transfus Apher Sci. 2013; 49(2):354-6.
7. Potkonjak D. Farmakolosko mislenje za obnovu registracije leka Favistan® (Methimazol). 1988.
8. Krusius FE, Peltola P. The effect of prolonged treatment with mercazole on the thyroid in rats. Ann Med Eksp Fenn. 1963;41:3.
9. Cooper DS. Antithyroid drugs. N Engl J Med. 2005; 352(9):905-17.
10. Cooper DS. The side effects of antithyroid drugs. Endocrinologist. 1999; 9(6):457-76.
11. Cooper DS. Antithyroid drugs in the management of patients with Graves'disease: an evidence approach to therapeutic controversies. J Clin Endocrinol Metab. 2003;88(8):3474-81.
12. Roti E, Gardini E, Minelli R, Bianconi L, Braverman LE. Sodium ipodate and methimazole in the long term treatment of hyperthyroid Graves's disease. Metab Clin Exp. 1993;42(4):403-8.
13. Isosaki O, Tsushima T, Emoto N, Saji M, Tsuschiya Y, Demura H, Sato Y, Shizume K, Kimura S, Kohn LD. Methimazole regulation of thyroglobulin biosynthesis and gene transcription in rat FRTZ-5 thyroid cells. Endocrinology. 1991;128(6):3113-21.
14. Logan A, Smith C, Becks GP, Gonzales AM, Philips IP, Hill DJ. Enhanced expression of transforming growth factor – beta I during thyroid hyperplasia in rats. J Endocrinol. 1994;141(1):45-57.
15. Hornung WM, Degitz JS, Korte ML, Olson MJ, Kosian AP, Linnum LA, Tietge EJ. Inhibition of thyroid hormone release from cultured amphibian thyroid glands by methimazole, 6-propylthiouracil, and perchlorate. Toxicol Sci. 2010;118(1):42-51.
16. Tsuijo M, Watahiki Y, Yoshioka K, Mutoh K. Morphology of thyroid follicular cells of methimazole-treated rats. Anat Histol Embriol. 2007;36(4):290-4.
17. Sur D. Methimazole-induced hypothyroidism in rats: effect of methimazole-induced cellular damage on heart, lung and ovary. IJANS. 2014;3(4):21-8.
18. Cakic-Milosevic M, Korac A, Davidovic V. Methimazole-induced hypothyroidism in rats: effects on body weight and histological characteristics of thyroid gland. Jugoslav Med Biohem. 2004;23(2):14

UDC:616.441-006.327.04

MALIGNANT FIBROUS HISTIOCYTOMA ON THE THYROID GLAND

Majstorov Venjamin[1], Petrushevska G[2]

[1]Institute of Pathophysiology and Nuclear Medicine, [2]Institute of Pathology, Medical Faculty, University „Ss. Cyril and Methodius“, Skopje, Republic of Macedonia

ABSTRACT

We report an extremely rare case of malignant fibrous histiocytoma on the thyroid gland in a 67-year-old man. Only several cases of primary malignant fibrous histiocytoma on this location has been noted and described in the literature. The patient presented with one and a half month history of progressively growing mass in the right middle of the neck and thyroid bed after previous episode of common cold. During the work-up thyroid and neck ultrasound, thyroid scan, CT scan of the neck, fine needle aspiration cytology and incisional biopsy were done. Pathologic diagnosis of malignant fibrous histiocytoma was finally made and the sub-type of histiocytoma was storiform-pleomorhic, one that is most frequently encountered. The tumor was rendered unresectable and patient was referred to an oncologist for further treatment. The prognosis was poor.

Key words: malignant fibrous histiocytoma, sarcoma, neck mass, thyroid gland

INTRODUCTION

Malignant fibrous histiocytoma belongs to the group of soft tissue sarcomas and as a distinct pathologic entity for the first time was described in the beginning of the sixties of the previous century [1]. The most affected sites are extremities in 70-75%, especially lower extremities (in as much as 60% of cases) [2]. Localisation in head and neck is uncommon and it has been reported in only 3-10% of cases [3]. Primary involvement of the thyroid gland is exceptionally rare and just few cases have been described in the literature [4, 5]. We describe a case of malignant fibrous histiocytoma on the thyroid gland from our department.

CASE REPORT

A 67-year-old man presented at our outpatients' department with one and a half month history of rapidly growing swelling in the right middle of the neck and stridor after having previous episode of common cold. Physical examination revealed large, tense and hard mass in the right middle of the neck and thyroid bed that was painless and fixed to the underneath structures. No cervical lymph nodes were detected on palpation. Vital signs were normal. Neck and thyroid ultrasound showed infiltration of right thyroid lobe with large hypoechoic formation and some isoechoic parts, with dimension >50 mm. Left thyroid lobe and isthmus were sonographically normal and no enlarged neck lymph nodes were seen. It was not possible to differentiate whether the mass originated from thyroid or from adjacent soft tissue and involved the thyroid by ultrasonography alone.

The general laboratory tests were within the normal range. Patient's clinical and thyroid hormonal status was euthyroid (FT4=16.7pmol/l, TSH=0.96mIU/ml). Thyroid technetium-99m scan was "cold", showing no accumulation of the tracer in the entire right thyroid lobe (Fig.1). Fine needle aspiration cytology was done and the findings were in favor of metastatic deposit from malignant epithelial neoplasm. Thyroglobulin measurements in the aspirate were rather low on several occasions (9.63 ng/ml; 9.05 ng/ml; 11.3 ng/ml and 13.8 ng/ml). Neck CT imaging depicted relatively hypodense soft tissue mass in projection of the right thyroid bed, which was spreading distally to upper mediastinum and pushing trachea to the left. Again no enlarged neck lymph nodes were present.

Finally, an incisional biopsy of the mass was performed and pathologic diagnosis of malignant fibrous histiocytoma (storiform-pleomorphic type) was made. Predominant were spindle cells with hyperchromatic, elongated nuclei and sparse cytoplasm (Fig. 2 and 3). Part of the cells had bizarre nuclei and cell margins were rather hard to be differentiated. On the periphery malignant giant cells with multiple nuclei were also present. In the invasive front of the neoplasm the stroma was partly hyalinized with medium rich inflammatory infiltrate, mostly consisting of lymphocytes. The neoplasm extended to surrounding muscles and on the periphery displaced thyroid tissue was also observed. Immunohistochemically malignant cells were positive for vimentin, CD68, neuron specific enolase and cytokeratin 19.

As soon as the diagnosis was made and tumor was rendered to be unresectable, the patient was referred to an oncologist for further treatment, but he refused any therapy and was lost from follow-up. In oral communication with the family we learned that he died at home 8 months later.

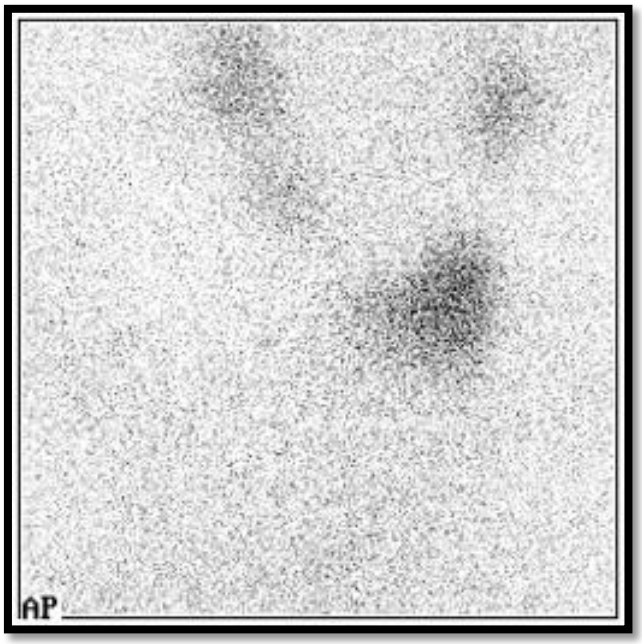


Fig. 1. Thyroid technetium-99m scan showing no accumulation of the tracer in the right thyroid lobe

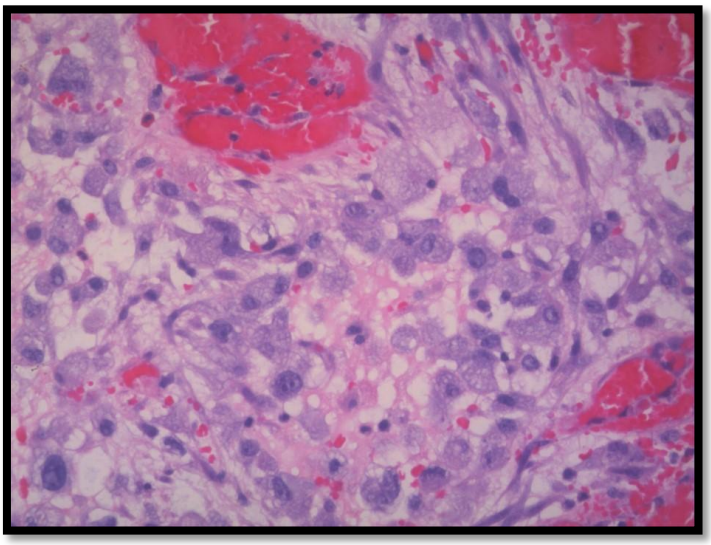


Fig. 2. Malignant fibrous histiocytoma of the thyroid: storiform pattern

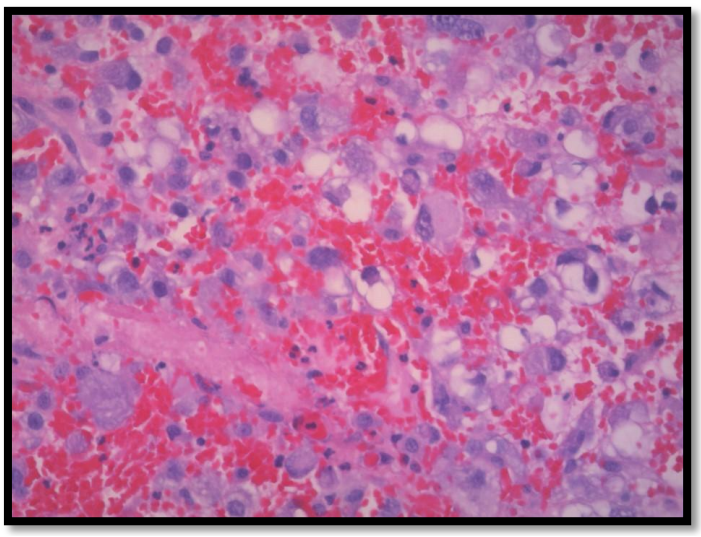


Fig. 3. Malignant fibrous histiocytoma of the thyroid: stromal collagenisation

DISCUSSION

Although the origin of malignant fibrous histiocytoma is still a matter of debate, the most accepted view is that it derives from primitive mesenchymal cells of soft tissue, which later on transfom into fibroblasts and histiocytes [6, 7, 8]. It arises from deep fasciae or skeletal muscles and presents as rapidly growing mass, usually painless. In our case it was not possible to discriminate accurately whether it started in fasciae beneath the thyroid or was derived from structures just adjacent to the thyroid and subsequently infiltrated the gland. Either could hold true, as by means of physical examination and performed imaging studies we were not able to find out with certainty where the primary descent of the tumor was. Men are more affected and in those aged over 40 malignant fibrous histocytoma is most frequently encountered soft tissue sarcoma [9]. There are five known sub-types at present: storiform-pleomorhic, myxoid, giant cell, inflammatory and angiomatoid, dependent on predominant type of cells found, although in all there are fibroblastas and histocytes in various ratios. Up to 60% of all malignant fibrous histocytomas belong to the storifom-pleomorhic sub-type, as it was the case with our patient.

According to the etiology, malignant fibrous histiocytoma like other soft tissue sarcoma can be primary or idiopathic and secondary. Primary tumors are the most common type of malignancy and account for about 80% of the cases. For the secondary form exposition to radiation has been the most important known cause for the development of tumors and they are termed radiation- induced sarcomas [10]. In our case we could not identify radiation exposure, but from the history of the patient we found out that he caught cold before the onset of the swelling in the neck. Therefore, we could hypothesize that some viral infection might be the trigger for carcinogenesis.

Treatment of malignant fibrous histiocytoma in head and neck could be surgical, with radiotherapy and chemotherapy, but generally it is a combination of these three [9]. The best results are achieved if tumor is resectable and wide excision with clean margins is performed [11]. In most instances this is not a case and only incomplete resection can be done followed by post-operative adjuvant therapy, usually radiotherapy or combination of radiotherapy and chemotherapy. In some patients reduction of the tumor mass with chemotherapy prior to surgery could be effective. Patients with unresectable tumor have the worst prognosis, and this was the situation in our case. There is no standard treatment in such circumstances. Primary sites of metastasis are lungs, followed by liver and bone due to the predominant hematogenous spread of the cancer. Lymph node metastases are rear and we did not detect any. The poor prognosis of our patient was probably a result of occurrence of lung metastasis.

CONCLUSIONS

We would like to indicate on several important points as conclusions derived from this case. First, malignant fibrous histiocytoma is a rare, but aggressive tumor. Primary thyroid histiocytomas are extremely rare and only several cases have been described in the literature. We should think of this malignancy whenever we have a patient with progressively growing neck mass that is usually painless. Second, final diagnosis is not easy to be made, sometimes even pathohistology is not straightforward and additional immunohistochemical stainings are required to make the diagnosis. Third, treatment is mostly dependent on the local status of the tumor. Aggressive surgical treatment and complete excision of the tumor with clear margins, when it is feasible, is the best prognostic option for the patient.

REFERENCES

1. O'Brien JE, Stout AP. Malignant fibrous xantomas. Cancer 1964;17:1445-58.
2. Mohan RPS, et al. BMJ Case Rep 2013. doi:10.1136/bcr-2013-008875.
3. Mevio E, Sbrocca M, Gorini E, et al. Malignant fibrous histiocytoma of the pharynx. Acta Otorinilaryngolol Belg. 2003;57(1):79-81.
4. Malandrinou F, Tseleni-Balafouta S, Kakaviatos N, Singhellakis P. Primary malignant fibrous histiocytoma on the thyroid. Hormones(Athens). 2002; 1(4):255-9.
5. Hsu KF, Lin YS, Hsieh CB, et al. Primary malignant fibrous histiocytoma of the thyroid: review of the literature with two new cases. Thyroid 2008;18(1):51-5.
6. Fu YS, Gabbiani G, Kaye G, Latters R. Malignant soft tissue tumors of probable histiocyte origina (malignant fibrous histiocytomas): General considerations and electron microscopic and tissue culture studies. Cancer 1975; 35:176-98.
7. Merkow LP, Frich JC, Sliekin M, Kyreages CG, Pardo M. Ultrastructure of fibroxanthosarcoma (malignant fibroxantoma). Cancer 1971; 28:372-83.
8. Taxy J, Battifora H. Malignant fibrous histiocytoma: A clinicopathologic and ultrastructural study. Cancer 1977; 40:254-67.
9. Aljabab AS, Nason RW, Kazi R, Pathak KA. Head and neck soft tissue sarcoma. Indian J Surg Oncol 2011; 2(4):286-90.
10. Thiagarajan A, Gopalakrishna Iyer N. Radiation-induced sarcomas of the head and neck. World J Clin Oncol 2014;5(5):973-81.
11. Hardison SA, Davis PL 3rd, Browne JD. Malignant fibrous histiocytoma of the head and neck: a case series. Am J Otolaryngol 2013;34(1):10-5.

UDC:616.24-022.7-085.33

GASTROINTESTINAL TRACT - RESERVOIR OF EXTENDED SPECTRUM B LACTAMASES PRODUCING STRAINS COLONIZING RESPIRATORY TRACT IN INFANTS

Popova Gorica[1], Tasik V[2], Boskovska K[1], IlievskaT[1]
[1]Institut for respiratory diseases in children Kozle, Skopje; [2]Clinic of Pediatric Diseases, Skopje, R. Macedonia

ABSTRACT

Introduction The useage of broad spectrum antibiotics leads to emergence of ESBL (extended spectrum β lactamases) gram - negative strains. Members of the normal gastrointestinal (GI) tract flora may cause endogenous disease if they reach tissues where they can not be tolerated.

Objectives The aims of this study are: to elaborate the prevalence of ESBL – producing strains obtained from patients with respiratory tract infections (RTIs), to investigate gastrointestinal colonization and possible endogenous reservoirs of infection, and to elaborate some epidemiological features of patients harboring ESBLs.

Methods Standard microbiological procedures were used for detecting bacteria, modificate triple disk diffusion test for detecting ESBLs, and disc diffusion test for measurement of antibiotic susceptibility

From 20 randomly selected patients with ESBLs cultivated from respiratory tract, stool samples and gastric contents were taken for detecting GIT colonization at the same time.

Results Of all enterobacteriaceae, ESBLs producers were 62.21% (214/344). Of all stool samples, 100% (20/20) were with predominant ESBLs gram - negative flora, and of all gastric samples in 90% (18/20) ESBLs gram- negatives were isolated, of which 94.4% (17/18) identified to the species level were identical to those isolated from respiratory tract.

Conclusion The prevalence of ESBLs isolated from respiratory tract in young patients is increasing. It seems that gram-negative ESBL-producers isolated from respiratory tract were indigenous gastrointestinal tract flora. Some epidemiological findings suggest possible risk factor for translocation of microbiological flora.

Keywords: ESBL strains, indigenous microbiological flora, translocation

INTRODUCTION

Beta-lactams are a huge group of antibiotics which includes: penicillins, cephalosporins, monobactams and carbapenems. The presence of beta – lactam ring in their structure is common for these antibiotics [1, 2].

Members of the family *Enterobacteriaceae* usually produce plasmid coded enzymes known as beta lactamases (for example TEM-1, TEM-2 and SHV-1). The beta lactamases induce resistance to penicillins, in lower percentage to first generation cephalosprins, but they do not induce resistance to third generation cephalosporins [3]. More than 90% of ampicillin resistance among *E.coli* is because of TEM-1 production [3].

In the middle of the 1980s, a noval group of enzymes, called *e*xtended *s*pectrum *b*eta lactamases (ESBL), was detected. These enzymes evolved by mutation of parenteral genes for TEM-1, TEM-2 and SHV-1. These mutations lead to amino-acid changes near the active center of the enzymes, which enables them to hydrolyze the oxyimono-cephalosporins (cefotaxime, ceftriaxone, ceftazidime) and monobactams (aztreonam) [3, 4, 5]. According to Bush, Jakoby and Medeiros classification, ESBL enzymes are defined as beta-lactamases capable of hydrolyzing oxyimono-cephalosporins (third generation cephalosporins) and they are inhibited by beta-lactamases inhibitors, for example clavulanic acid. [4, 5, 6].

CTX – M is a recently described group of ESBL enzymes [7, 8, 9]. These enzymes successfully hydrolyse cefotaxime, but are less successful in hydrolyzing cefatzidime (the reason behind its given name) [7, 8]. CTX - M genes are located on plasmids with size from 7 to 260 kb [7]. These genes are derived from the *Kluyvera spp*. chromosome which is a saprophytic species [10], an example for horizontal gene transfer. CTX – M are reported from different parts of the world and it seems that these are the most prevalent ESBL enzymes nowadays.

Plasmids are extarchromosomal gene material and they are replicated independently of the chromosome. Besides ESBL genes, other genese responsible for the resistance to other groups of antibiotics (for example aminoglycosides) is located on the plasmids and because of these antibiotic choices for ESBL infection treatment sometimes is extremely limited [5]. These genetic elements can easily be transmitted by conjugation from one to another bacterial cell and this process overcomes the frame of bacterial species [5].

Gastrointestinal tract (GUT) is a complex eco - system which includes aproximatly 800-1000 different bacterial species [11]. In GUT this majority of bacterial species function together in many complex and different physiological processes. Furthermore, commensal GUT flora in the same time interacts with the host immune cells and host enterocele [11].

The diversity of bacterial eco – system is relatively poor in infancy, but later it reaches complexity and it stays relatively stabile during the life [12, 13, 14, 15]. Ecological balance between commensal GUT flora and human as a host may be destrubed by many factors. The most dramatic disturbance is a result of antimicrobial administration [16, 17, 18]. Antibiotic therapy is given to destroy bacteria cousing illness, but in the same time it effects commensal GUT flora. This is a side effect and it depends of many factors such as: type of antibiotic, its spectrum of activity, dose of antibiotic, length of antibiotic treatment and antibiotics pharmacokinetics and pharmacodynamics propartis. If the parenteraly given antibiotic is secreted in greater amount through intestinal mucosa, or if the antibiotic is excreted in active form through billiary system its inpact on commensal GUT flora will be greater [17, 19, 20]. Mainly this impact is on density of normal GUT flora and occurs of resistant strains [17]. This disturbace of normal GUT flora may lead to many pathological conditions in the human host [21, 22].

Members of the normal GUT flora may cause endogenous disease if they reach tissues where they can not be tolerated [23,24].

Objectives

The objectives of this study was to elaborate the prevalence of ESBL producing strains isolated from patients with respiratory tract infections. To elaborate gastrointestinal tract colonization as endogenous rezervour of infection. To elaborate some epidemiological features of patients harboring ESBL producing strain.

MATERIALS AND METHODS

For this study, samples from hospitalized patients in period from Januray 2014 to November 2014 were used. All patients were aged from 0 to 18 years and hospitalized in The Institute for respiratory deseases in children in Skopje, because of severity of infections of the respiratory tract. 268 strains of *Escherichia coli* and 76 *Klebsiella pneumoniae* were isolated and identificated from samples as sputum and/or tracheal aspirat, using standard microbiological procedures. For isolation and identification of bacteria, blood agar plate (Oxoid, UK), chromogenic agar plate – UTI (Oxoid, UK), ChromoID ESBL agar (bioMerieux, France) – which is chromogenic selective medium for ESBL scrinning were used. For investigating of biochemical activity and mobility of bacteria IMVC (indol, metil rot, Voges-Proskauer, citrate) mediums were used. They were home prepared from dry supstances made in Merck, Germany.

Disc diffusion test was used for measurement of antimicrobial susceptibility. For this purpose bacterial suspensions with turbidity of 0.5 McFarlands were prepeard and than inoculated on Mueller-Hintons agars wich were 4 mm in depth. On the surface of the plates, antimicrobial discs were placed manually and the distance between the discs was approximatly 3cm. The following discs (Oxid, UK) were used: amoxicillin/clavulanic acid (20/10μg), cefotaxime (30μg), ceftazidime (30μg), cefoxitin (30μg), cefepime (30μg), imipenem (10μg), ciprofloxacin (5μg), trimethoprim-sulfamethoxazole (1.25/23.75μg), amikacin (30μg), gentamicin (10μg). The plates were incubated at aerobic conditions, at temperature of 37^{o}C, during 18 – 24 hours. The interpretation of the tests was according CLSI (Clinical Laboratory Standard Institute) recommendations, measuring the zone of inhibition around the antibiotic discs. Strains wich were resistant towards some of the third generation cephalosporins were selected as suspect ESBL producers. These strains were ongoing for phenotypic testing for ESBL, so, modificated triple disc diffusion test (suggested by Jarlier et al.) was performed. This test is based on synergy acting between third generation cepfalosporins and cavulanic acid. The susceptibility disc, containing amoxicillin-clavulanate, was placed in proximity to discs containing ceftazidim (CAZ) and cefotaxim (CTX). Enhancement of the zone of inhibition of the CAZ and CTX caused by the synergy of the clavulanate in the amoxicillin-clavulanate disc indicates the presence of Bush group 2be enzyme. Using this method, 214 ESBL positive strains were selected of wich *Escherichia coli* - 190 and *Klebsiella pneumoniae* - 24. All selected ESBL-positive strains were sussceptible to cefoxitin (30 μg) and imipenem (10 μg). This excluded the presence of AmpC – beta – lactamases (wich are capable of hydrolyzing cefoxitin and they are not inhibited by clavulanic acid), and the presens of carbapenemases (imipenem is supstrat of carbapenemases acting).

Gastric lavation and feces from 20 randomly selected patients with ESBL strain isolated from sputum and/or tracheal aspirat were taken for microbiological analyses at the same time. The samples from the digestive system were seeded on blood agar plate (Oxoid) and incubated aerobicly at 37^{o}C during 18-24 hours. After the incubation time for futher analyses only the Gram – negative strains which were dominant on the plate were undertaken. The same principle (previously explained) was used for detection of ESBL ensymes. Resistotypization was done by using the same antibiotic discks in disc diffusion tests for isolates from respiratory tract and from digestive system.

In purpose to identify the possible risk factors for infection or colonisation with ESBL positive strains, retrogradely 50 records fom patients with ESBL isolates from sputum or tracheal aspirat were analised.

RESULTS

In a period from Januray 2014 to November 2014, of all Grams - negatives isolated from sputum/tracheal aspirat, ESBL producers were 62.21% (214/344). Of all isolated *Escherichia coli*, ESBL - positive were 70.89% (190/268), and of all isolated *Klebsiella pneumoniae*, ESBL- positive were 37.58% (24/76). Of (n=20) patients with ESBL- strain isolated from sputum / tracheal aspirat, samples from digestive system (feces and gastric lavat) were taken for microbiological analysis. In 100% (20/20) ESBL – positive Gram – negative strains (as dominant flora) were isolated from aerobicly cultivated feceses (fig. 1.).

In 90% (18/20) ESBL – positive Gram – negative strains were isolated from aerobicly cultivated gastric lavations.

In those (n=17) cases where the same strain (*Escherichia coli*) was isolated from sputum and gastric lavat, resistotypization was done and in 88.2% (15/17) the same resistotype was identified (fig. 2, fig. 3).

Retrogradely analised records of hospitalised patients (n=50) with ESBL – positive strain isolated from respiratory tract, showed that middle age of the patients was 5,6 months (from 1 to 10 months). In 40% (20/50) there was a previous hospitalization (in the last four mounts).

Before hospitalisation, 38% (19/50) had been treated with perorally given cephalosporins (cefixime, cefaclor, cefuroxime, cefpodoxime). All patients (n=50) during hospiatlisation were treated with parenteral cephalosporins and middle time of antibiotic treatmen before the samples for microbiological analysis was taken was 2.58 days.

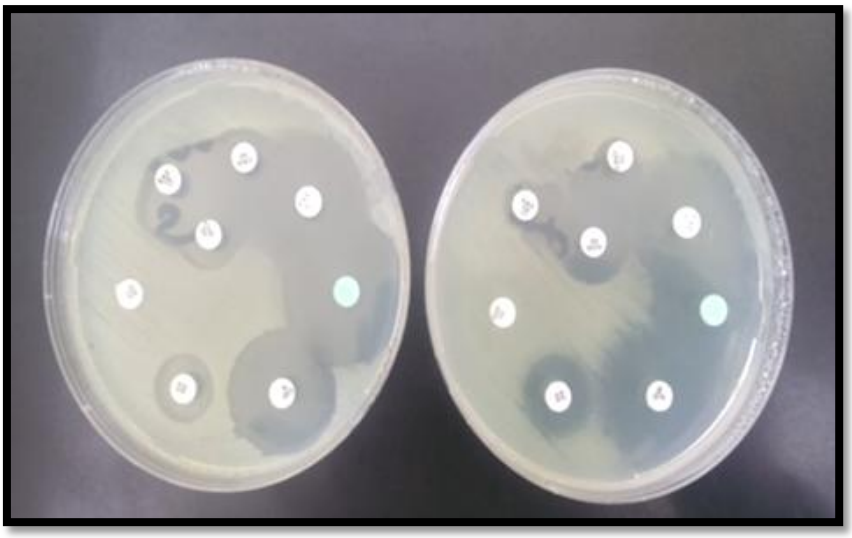


Fig. 1. Resistotyping of two strains (*Escherichia coli*) isolated from sputum and feces in the same patient

Fig. 2. Resistotyping of two strains (*Escherichia coli*) isolated from sputum and gastric lavat in the same patient

Fig. 3. Comparison between resistotypes isolated from sputum, feces and gastric lavat in the same patient

DISCUSSION

The intoduction of 3rd generation cephalosporins in clinical practice in the early 80s means a successful fight agains bacterial resistance conditioned by production of beta lactamases.

In 1983 the first plasmid-coded beta- lacatamase capeble to hydrolyse the 3rd generation cephalosporins was puplished [23]. Since then more than 220 TEM and 180 SHV types of beta –lactamases with caracteristics of ESBL have been detected. World wide, new types of beta-lactamases continually are detected and they are registrated in few databases [24, 25].

Since their first appearance, ESBL producing strains have dramatically spread [26, 27, 28, 29, 30].

There are more relevant studies about the inpact of parenteral given antibiotics on commensal GUT flora [31, 32, 33, 34, 35]. One of the unwanted effects is appearance of resistant strains, including ESBL producing gram negatives. This aspect is proven by many clinical studies and many experimental studies on animal models [36, 37, 38, 39].

On the other hand, there are studies which attest the preventive role of orraly given beta lactamases, capable to hydrolyse the part of parenteraly given antibiotic which is secreted in the GUT [36, 37, 39].

These findings are hipoteticly very interesting in purpose to prevent GUT flora by the acting of pareteraly given antibiotics.

In our study as a risck factor for colonization/infection of respiratory tract with gram- negatives, including ESBL starains is a small age (under the age of one).

Acid gastric content is a huge barrier for bacteria, but according Mitchella's and her coworkers's research, gastric content in infancy has different biochemical characteristics [41]. Milk is the basis of infancy feeding and milk has near neutral pH with little changes during lactation. Besides the pH value, milk has significantly specified puffer capacity [42].

Mitchella and coworkers were surprised because of the small reflux index measured in infants with clinical manifestation of reflux diseases. The explanation is that a long period of time during pH monitoring, the gastric content had pH >4 (with these pH values reflux can not be detected) and this was connected with the feeding of milk.

Besides the pH values of gastric content, the length of oesophagus, immaturity of gastroesophageal sphincter and horizontal position are possible risk factors for bacterial translocation.

Possibility of bacterial translocation is supported by our microbilogical findings with high percent of identical strains and resistotypes isolated from both – GUT and respiratory tract.

There is a need for future molecular researches in order to detect wich is the molecular similarity between the strains isolated from both – GUT and respiratory tract in the same patient [43, 44, 45].

CONCLUSION

There is a high prevalence of ESBL producing starins isolated from respiratory tract in small children (under the age of one). Using 3rd generation cephalosprins is a major risk factor for appearance of ESBL producing strains It seems that gram-negative ESBL-producers isolated from respiratory tract were indigenous gastrointestinal tract flora and GUT is their main reservoir . Small age is a risik factor for translocation of bacteria, enabling the colonisation of the respiratory tract.

REFERENCES:

1. Jawetz E, Melnick JL, Adelberg AE. Medicinska mikrobiologija. 20 izd. Belgrad: Savremena administracija, 1998.
2. Cazzola M, Blasi F, Ewing S. Antibiotics and the lung. Eur Respir Mon. 28th ed. Sheffield: Europian Respiratory Society Journals Ltd, 2004.
3. Bredford PA. Extended spectrum ß lactamases in the 21st century: characterization, epidemiology and detection of this important resistance threat. Clin Microbiol Rev 2001; 48:933-51.
4. Jarlier V, Nicolas MH, Fournier G, Philippon A. Extended broad spectrum beta lactamases conferring transferable resistance to newer beta lactam agents in *Enterobacteriaceae*: hospital prevalence and susceptibility patterns. Rev Infect Dis 1988; 10:867-76.
5. Paterson LD, Bonomo AR. Extended spectrum beta lactamases: a clinical update. Cli Microbiol Rev 2005; 18(4):657-86.
6. Bush K, Jacoby GA. Update functional classification sheme of β – lactamases. Antimicrob Agents Chemother 2010; 54(3):969-976.
7. Bonnet R. Growing group of extended spectrum: the CTX-M enzymes. Antimicrob Agent Chemother 2004; 48:1-14.
8. Lewis JS, Herrera M, Wickes B, Patterson JE, Jorgensen JH. First Report of the emergence of CTX-M-type extended-spectrum β-Lactamases (ESBLs) as the predominant ESBL isolated in a U.S. health care system. Antimicrob Agents Chemother. 2008; 52(2): 810
9. Alobwede I, Mzali FH, Livermore DM, Hentige J, Todd N, Hawkey PM. CTX-M extended-spectrum beta-lactamases arrives in UK. J Antimicrob Chemother 2003; 51: 470-1.
10. Humeniuk C, Arlet G, Gautier V, Grimont P, Labia R, Philippon A. Beta-lactamases of Kluyvere ascorbata probabl progenitors of some plasmid – encoded CTX-M types. Antimicrob Agent Chemother 2002;46:3045-49.
11. Backhed F, Ley RE, Sonnenburg JL, Peterson DA, Gordon JI. Host bacterial mutualism in the human intestine. Science 2005;307:1915-20.
12. Penderes J, Thijs C, Vink C et al. Factors influencing the composition of the intestinal microbiota in early infancy. Pediatrics 2006;118(2):511-21.
13. Jernberg C, Lofmark S, Edlund C, Jansson JK. Long – term ecological impacts of antibiotic administration on the human intestinal microbiota. Microbiology 2010;156:3216-23.
14. Zoeteudal EG, Akkermans AD, De Vos WN. Temperature gradient gel electrophoresis analysis of 16SrRNA from human fecal samples reveals stable and host – specific communities of active bacteria. Appl Environ Microbiol 2009;64:3854 - 9.
15. Qin J, Zi R, Raes J et al. A human gat microbial gene cataloque established by metagenomic sequencing. Nature 2010;464:59-65.
16. Rolan JM. Food and human gut as reservoirs of transferable antibiotic resistance encoding genes. Front Microbiol 2013; 4:173
17. 17.de la Cochetiere MF, Durand T, Lepage P, Bourreille A, Galmiche JP, Dore J. Resilience of the dominant human fecal microbiota upon short-course antibiotic challenge. J Clin Microbiol 2005;43:5588-92.
18. Perez-Cobas AE, Gosalbes MJ, Friedrichs A et al. Gut microbiota disturbance during antibiotic therapy: a multi-omic approach. Gut 2013;62:1591-601.
19. Sullivan A, Edlund C, Nord CE. Effect of antimicrobial agents on the ecological balance of human microflora. Lancet Infect Dis 2001;1:101-14.
20. Karami N, Martner A, Enne VI et al.Transfer of ampicillin resistance gene between two *Escherichia coli* strains in the bowel microbiota of an infant treated with antibiotics. J Antimicrob Chemother 2007; 60: 1142-5.
21. Young VB, Schmidt TM. Antibiotic-associated diarrhea accompanied by large-scale alterations in the composition of the fecal microbiota. J Clin Microbiol 2004;42:1203–6.
22. Tlaskalova-Hogenova H, Stepankova R, Hudcovic T, et al. Commensal bacteria (normal microflora), mucosal immunity and chronic inflammatory and autoimmune diseases. Immunol Lett. 2004;93(2-3):97-108.
23. Knothe H, Shah P, Krcmery V, Antal M, Mitsuhashi S. Transferable resistance to cefotaxime, cefoxitin, cefamandole and cefuroxime in clinical isolates of *Klebsiella pneumoniae* and *Serratia marcescens*. Infection. 1983;11**:**315-7.

24. Bush K, Jacoby GA. Beta – lactamase classification and amino acid sequences for TEM, SHV and OXA extended spectrum and inhibitor resistant enzymes. http://www.lahey.org/studies.
25. Abhishikha Srivastava, Neelja Singhal, Manisha Goel, Jugsharan Singh Virdi, Manish Kumar. CBMAR: a comprehensive β-lactamase molecular annotation resource. Database (Oxford). 2014; 2014: bau111 http://14.139.227.92/mkumar/lactamasedb.
26. Albertini MT, Benoit C, Berardi L, et all. Surveillance of methicillin-resistant *Staphylococcus aureus* (MRSA) and *Enterobacteriaceae* producing extended-spectrum beta-lactamase (ESBLE) in Northern France: a five-year multicentre incidence study. J Hosp Infect 2002;52**:**107-13.
27. Fedler KA, Biedenbach DJ, Jones RN. Assessment of pathogen frequency and resistance patterns among pediatric patient isolates: report from the 2004 SENTRY Antimicrobial Surveillance Program on 3 continents. Diagn Microbiol Infect Dis. 2006;56(4):427-36.
28. Chandramohan L, Revell PA. Prevalence and Molecular Characterization of Extended-Spectrum-β-lactamase producing Enterobacteriaceae in a pediatric patient population. Antimicrob Agents Chemother. 2012;56(9):4765-70.
29. Blaschke AJ, Korgenski K, Daly JA et al. Extended-spectrum beta-lactamase-producing pathogens in a children ˜s hospital: a five-year experience. Am J Infect Control 2009;37(6):435-41.
30. Gupt A, Ampofo K, Rubenstein D, Saiman L. Extended spectrum beta lactamase producing *Klebsiella pneumoniae* infections: a review of the literature. Journal of Perinatology 2003;23:439-43.
31. Christiaens G, Ciccarell Y, Damas P, Hayette MP, Melin P, Nys M, De Mol P. Prospective survey of digestive tract colonization with Enterobacteriaceae that produce extended-spectrum β lactamases in intensive care units. J. Hosp. Infect 2006;62:386-8.
32. Lindgren M, Lofmark S, Edlund C, Huovinen P, Jalava J. Prolonged impact of a one-week course of clindamycin on *Enterococcus* spp. in human normal microbiota. Scand J Infect Dis 2009;41:215–9.
33. Nyberg S D, Osterblad M, Hakanen A J, Lofmark S, Edlund C, Huovinen P, Jalava J. Long-term antimicrobial resistance in *Escherichia coli* from human intestinal microbiota after administration of clindamycin. Scand J Infect Dis 2007;39:514–20.
34. Valverde A, Coque TM, Sanches-Moreno MP, et al. Dramatic increase in prevalence of fecal carriage of extended spectrum beta lactamase producing *Enterobacteriaceae* during non outbreak situations in Spain. J Clin Microbiol 2004;42(10):4769-75.
35. Quigley EM, Quera R. Small intestinal bacterial overgrowth:roles of antibiotics, prebiotics and probiotics. Gastroenterology 2006;130:S79-S90.
36. Harmoinen J, Mentula S, Mentula S, et al. Orally administered targeted recombinant β-lactamase prevents ampicillin-induced selective pressure on the gut microbiota: a novel approach to reducing antimicrobial resistance. Antimicrob Agents Chemother 2004;48:75-9.
37. Tarkkanen AM, Heinonen T, Jog R, et all. P1A recombinant β-Lactamase prevents emergence of antimicrobial resistance in Gut microflora of healthy subjects during intravenous administration of Ampicillin Antimicrob. Agents Chemother. 2009; 53:6
38. Nicole JP, Usha S, Curtis JD. Effects of daptomycin, linezolid, and vancomycin on establishment of intestinal colonization with vancomycin-resistant enterococci and extended-spectrum beta-lactamase producing Klebsiella pneumoniae in Mice Antimicrob. Agents Chemother 2005; 49(8): 3513-6.
39. Stiefel U, Nerandzic MM, Koski P, Curtis J. Donskey. Orally administered beta-lactamase enzymes represent a novel strategy to prevent colonization by Clostridium difficile. J Antimicrob Chemother 2008; 62 (5):1105-8.
40. Curtis J. Donskey. The role of the intestinal tract as a reservoir and source for transmission of nosocomial pathogens. Clinical Infectious Diseases 2004;39(2): 219-26.
41. Mitchella DJ, McClurea BG, Tubmanb TRJ. Simultaneous monitoring of gastric and oesophageal pH reveals limitations of conventional oesophageal pH monitoring in milk fed infants. Arch Dis Child 2001; 84:273-6.
42. Morriss H F, Brewer DE, Spedale BS et al. Relationship of human milk pH during course of lactation to concentrations of citrate and fatty acids. Pediatrics 1986;78:458-464.
43. Healy M, Houn J, Lupski JR. Mycrobial DNA typing by automasted repetitive swquence based PSR. J Clin Microbiol 2005;43(1):199–207.
44. 44. Nemoy LL, Kotetishvili M, Tigno J, et al. Multilocus sequence typing versus pulsed-field gel lectrophoresis for characterization of extended-spectrum beta-lactamase-producing Escherichia coli isolates. J Clin Microbiol 2005;43(4):1776-81.
45. 45.Brolund A, Hæggman S, Edquist PJ, Gezelius L, Olsson-Liljequist B, Wisell KT, Giske CG. The DiversiLab system versus pulsed-field gel electrophoresis: characterisation of extended spectrum β-lactamase producing Escherichia coli and Klebsiella pneumoniae. J Microbiol Methods 2010;83(2):224-30.

UDC:618.15-002-022.7:616.39-084

BACTERIAL VAGINOSIS IN ETIOLOGY OF PRETERM LABOUR

Lazarova Aleksandra[1], Jankoska G[2], Stojovski M[1]
[1]University Clinic of Obstetrics and Gynecology, [2]Institute of Microbiology and Parasitology,
Medical Faculty, Skopje, R.Macedonia

ABSTRACT

Bacterial vaginosis (BV) is condition characterized by a polymicrobial disorder, with an overgrowth of several anaerobic or facultative bacteria and with a reduction or absence of lactobacillus colonisation.

The prevalence of BV ranges from 4-64%, depending on the racial, geographic and clinical characteristics of the study population. Although BV is associated with several adverse outcomes, such as PID, endometritis, preterm labour and low birth weight, many questions regarding the pathogenesis of BV remain unanswered. Mucosal immune system activation may represent a critical determinant of adverse consequences associated with BV. An unequal risk for BV acquisition and/or recurrence could derive from different mucosal immune host abilities and/or capability of invading microbes to produce factors that inactivate the local immune response. BV is associated with a two-fold increased risk of preterm labour, with the greatest risk when BV is present before 16 weeks of gestation. The results of treatment trials for pregnant women with BV have been heterogeneous, with anywhere from an 80 % reduction to a two-fold increase in preterm labour among women who received treatment. For this reason, in current clinical practice significant controversy surrounds determining not only who and when to screen, but also who and how to treat. Recent evidence shows that individual genetic backgrounds can affect chemokine production. This is an interesting area for future research and could lead to trials of treatment only for women genetically predisposed to preterm birth.

Key words: Bacterial vaginosis, preterm labour, risk factors**,** vaginal pH

INTRODUCTION

Bacterial vaginosis is the most common lower genital tract disorder among women of reproductive age. It is not a classical infection caused by a single pathogen, but is rather a complex alteration of the vaginal ecosystem, where the physiologic lactobacilli-dominant flora is replaced by an overgrowth of mixed flora, with a high concentration of anaerobic bacteria, normally present in the vagina in fewer numbers.

Although the normal vaginal milieu is characterized by the presence of a large number of bacterial species, under physiologic conditions H2O2-producing lactobacilli account for 95% of these and act against the proliferation of other microorganisms by maintaining an acidic vaginal pH. In normal conditions, the anaerobe to aerobe ratio is normally kept between 2:1 and 5:1. In the presence of BV, the quantity and quality of lactobacilli decreases, the vaginal pH increases, and the anaerobe-aerobe ratio changes between 100:1 and 1000:1. The same bacteria found in the healthy vaginal environment are also found in BV, the difference being in the quantity of the microorganisms present. It isn't possible to identify any single species as the cause of BV, although Gardnerella vaginalis, Prevotella spp., Bacteroides spp, Mobiluncus spp, Mycoplasma spp. are the most commonly found in association.

The reason why this shift in the microbal flora occurs remains unknown. BV does not seem to be associated with signs of local inflammation. Leucocytes can't be found in the vaginal fluid of women with BV, so the term vaginosis has been used rather than vaginitis. More than 50 % of women with BV are asymptomatic and when symptoms do occur they are generally mild, with the presence of malodorous vaginal discharge.

BV is a very common condition. The prevalence observed in the literature ranges between 4-60%. The highest prevalence is found in women with sexually transmitted disease. Epidemiological differences in the population studied can explain this wide range in the prevalence. Black race, smoking, sexual activity, contraceptive practice and the use of vaginal douching have all been found to be associated with increased prevalence.

MATERIAL AND METHODS

Thirty pregnant symptomatic women were included in the study at the University Clinic of Gynecology and Obstetrics, Skopje. They had symptoms of preterm labor and were at 22-34 weeks of gestation. The symptoms suggestive of preterm labor included uterine contractions, lower abdominal and back pain, and pelvic pressure. Recruited patients had intact amniotic membranes determined by vaginal pH and speculum examination. The patients were less than 3 cm dilated. Women were excluded from the study if they had multiple pregnancy, cervical operations (conisation or cerclage), active labor, ruptured membranes or chorioamnionitis. Patients were placed in dorsal lithotomy position. An ultrasound probe was inserted in the vaginal interior fornix. The cervical length was measured (external to internal os). In these women microbiological class (vagina, cervix, Mycoplasma and Ureaplasma urealiticum) was detected. Also fetal fibronectin was measured (glicoprotein that lies between chorion and decidua detectable till 22 weeks of gestation) because it has the best negative predictive value(more then 95%).

DIAGNOSIS OF BV

One factor that may influence the detection of BV is the method used for the diagnosis. Since BV isn't an infection caused by a single pathogen, but rather an imbalance in the proportions of the normal vaginal microflora, the microbiological culture, classically considered as the gold standard in the diagnosis of any infection, can't reliably predict the presence of BV. Different diagnostic techniques have been developed to test for the condition. The two most widely used are clinical criteria and Gram staining of vaginal secretions.

The clinical diagnosis of BV can be made in the presence of three of the following four signs first described by Amsel:

- the presence of an adherent and homogeneous vaginal discharge
- a vaginal pH>4.5
- detection of "clue cells" on saline wet mount
- an amine odor (positive whiff test) after addition of the amine potassium hydroxide (10 %) to the vaginal secretions.

These criteria have some limitations, being subjective, not reproducible and unpleasant. As a consequence, the Gram staining of an air-dried smear of vaginal secretions has become the most widely used diagnostic method. Two systems have been developed to interpret and score the Gram stain. The first, proposed by Spiegel is less commonly used and the second, called Nugent's is the most widely used. In the Nugent's score system, each microbial morphotype is quantified from 1+ to 4+ based on the number of morphotypes per oil immersion field and a corresponding total score is assigned. The diagnose of BV can be made when the score is 7 or more. A score 0-3 is considered normal and a score of 4-6 is intermediate. In addition, a good correlation between Gram stain scores and clinical signs of BV has been shown.

TREATMENT

Treatment of asymptomatic BV in pregnancy is controversial, with the possible exception of women with a history of preterm birth. Most studies have shown that treating asymptomatic patients with BV doesn't markedly prolong pregnancy, nor increases birth weight.

The question of why BV is associated with preterm birth in some women, but not in others remains unanswered, and the exact mechanism by which the organisms associated with BV may affect the initiation of spontaneous preterm labour remains unclear.

Twelve studies have investigated the potential to reduce the incidence of spontaneous preterm labour through treating women with BV. The results are contradictory. Four studies indicate a reduced risk of spontaneous preterm labour and preterm birth with treatment, while others do not. Most of the studies have proved that low risk women with BV found no benefit after antibiotic treatment and it isn't correlated with lower incidence of preterm birth (especially < 32 gestational weeks). One possible explanation for these conflicting results is that therapy was introduced relatively late in pregnancy (gestational age week 20).Women symptomatic for BV were generally excluded from many trials because they are treated, and these women may be at high risk of adverse pregnancy outcome.

Studies involving screening and treating BV have been published not so long ago. The results of the analysis suggest a reduction in the incidence of preterm birth in low risk women, but not in high risk women. These results could be explained by several factors including the differences in antibiotic sensitivity between high and low –risk groups, and that preterm birth in high and low-risk pregnant women are different entities and don't belong to the same syndrome.

One consideration emerging in recent years is the possibility that the conflicting results of these studies could be related to antimicrobial resistance associated with treatment of BV. Despite clinical equivalence in efficacy between intravaginal metronidazole and clindamycin, the two agents produce different antibiotic resistance profiles among anaerobic bacteria present in the vagina after treatment for BV. Intravaginal clindamycin use was associated with significant evidence for anaerobic resistance after treatment, whereas intravaginal metronidazole use was not associated with significant antibiotic resistance. But, four studies in low-risk women have shown a statistically significant reduction in the incidence of preterm birth when BV was treated with clindamycin in early pregnancy.

So, the best time for treating BV is the first trimester or till 16-th weeks of gestation. The antibiotics used for treatment are metronidazole or clindamycin, used either orally or intravaginally.

RESULTS

Fetal fibronectin was negative in 21 patients,so they didn't delivered in the next 7-14 days.Vaginal pH was > 4.5 in 25 patients and positive micribiological specimen was detected in 17 of them. The cervical length in 27 patients was > 20 mm. Most detected specimens were Gardnerella vaginalis in combination with Ureaplasma urealiticum or Mycoplasma spp.

So,the most of the women with symptomatic preterm labor didn't delivered in the next 7-14 days. But, almost all of them had positive micribiological specimen and vaginal pH > 4.5. The most reliable results were made with combination of fetal fibronectin, vaginal pH, microbiological class and cervical length measurement. Most important is the gestational age of the woman with bacterial vaginosis.

We'll have a poor prognosis if the infection is detected earlier (before 20 gestational weeks). In this study those women were excluded, so we had good prognosis. The only reliable risk factor will be prior preterm delivery and those women should be treated as high-risk patients. Treatment should be given to all of them, especially symptomatic patients with shorter cervix.

DISCUSSION

BV has long been taken as an inconvenient, but not a pathological problem. Increasingly, BV has attracted interest because of an observed association between its presence and a number of significant obstetric and gynecological complications, such as preterm birth, preterm prelabour rupture of the membranes (PPROM), chorioamnionitis, postpartum endometritis, postsurgical infection and pelvic inflammatory disease.

The association between BV and preterm birth has been a subject of a large number of prospective studies in the past 20 years, the majority of which have shown a significant association. In 2013, studies involving 20 230 women showed that BV doubles the risk of preterm delivery at less than 37 weeks of gestational age. The studies included different screening methods in the evaluated women, (low-risk, high-risk and mixed-low and high-risk, single or twin pregnancies, women with symptoms of spontaneous preterm labour), different gestational age at screening, which may partly explain the heterogeneity in the statistical analysis. A subgroup analysis of the included studies suggests that the risk of preterm birth is further increased when the diagnosis is made early in pregnancy. The rate of preterm birth is seven-fold higher if BV is diagnosed before 16 weeks of gestation and four-fold higher in women with BV diagnosed before 20 weeks.

Despite the voluminous literature evaluating the association of BV with spontaneous preterm labour and preterm birth and the demonstration of the presence of a significant association, many doubts remain about the optimal time for diagnosis, the role of BV in cases of very preterm birth, its association with the most serious neonatal sequence, and the different strength observed in different populations. What is surprising about BV is why such a prevalent condition, caused by relatively low virulence microorganisms,which is often asymptomatic and without severe inflammatory symptoms, can be responsible for serious adverse sequels.

Maybe, some of these contradictions can be explained by shifting the concept of BV as a disease to the more intriguing idea of BV as a microbal/mucosal immunity disorder. BV must be considered a heterogeneous condition, where the most serious complications involve only a limited subgroup of women predisposed to the progression of the disease. The factors causing this variable expression of BV are still largely unknown. It is possible that the difference in the immune response mounted by the host against the infection can determine the severity of the condition. Recently, in many studies evaluated there is a presence of a subgroup of women with BV who show a higher risk of preterm birth, characterized by a presence of an impaired immune response against the infection. These women show a low or negative immunoglobulin A (IgA) response against the haemolytic Gardnerella vaginalis toxin associated with a high level of sialidase and/or prolidase activity in vaginal secretion. Both enzymes are capable of impairing the host immune defense by inactivating mucosal factors involved in innate and acquired immune mechanisms. In 2002, it has been shown that elevated levels of interleukin-1 beta (IL-1B), a crucial proinflammatory chemokine, can be found in 30 % of BV-positive women, and these women show a high anti-IgA response and a high number of leucocytes. It is possible that women exposed to anaerobic mucosal invasion who do not mount a strong innate and adaptive immune response may be more prone to poor outcomes. In other studies are found that an elevated IL-1B concentration not balanced by a presence of similarly high level of IL-1 receptor antagonist, which is normally produced to counteract the proinflamatory effect of IL-1,can identify women at higher risk of preterm birth. In these women, the immune response is suboptimal, with an excessive inflammatory response mounted against the infection. It would appear that together with environmental factors, the individual genetic background could play the most important role in preterm birth.

In third group of studies, the presence of BV was associated with an increased risk of preterm birth and women carrying the tumor necrosis factor-alpha (TNF-A) allele 2(which leads to overproduction of TNF) also had an increased incidence of preterm birth. When both conditions (BV and presence of TNF-A allele 2) are present in the same women, the risk of preterm birth is 6. 1 fold-higher, suggesting that a gene-environment interaction can confer a higher risk.

CONCLUSIONS

In this paper, we have outlined the conflicting results from several clinical trials of BV in pregnancy, and considered a number of possible reasons for these discrepancies (gestational age at diagnosis, history of preterm birth, choice of antimicrobial agents etc.) The most likely scenario is that only a subgroup of women is truly at a risk for infection-associated preterm birth. Infectious diseases and their consequences result not only from the microbal invasion, but also the nature of host response. The maternal immune response is crucial in the different outcome of pregnancy. Immunologicaly hyperresponsive women may not be able to control the microbial burden, and this in turn may predispose to ascending intrauterine infection and clinical chorioamnionitis.

In contrast, hyperresponders would develop an excessive local inflammatory response, clinical symptoms of vaginitis and be at risk for preterm birth if microorganisms gain access to the deciduas.

The optimal host is capable of mounting a measured and proportionate inflammatory response, which can deal with changes in the vaginal ecosystem without paying the price of adverse pregnancy outcome. The genetic profile of these women, the gene/environment interaction and the relationship between the changes in the vaginal ecosystem/vaginal inflammation and host cells are interesting areas for future research.

REFERENCES

1. Amsel R, Totten PA, Spiegel CA, Chen KC, Eschenbach D, Holmes KK. Nonspecific vaginitis. Diagnostic criteria and microbial and epidemiologic associations. Am J Med 2003;74:14-22.
2. Spiegel CA, Amsel R, Holmes KK. Diagnosis of bacterial vaginosis by direct gram stain of vaginal fluid. J Clin Microbiol1993;18:170-7.
3. Nugent RP, Krohn MA, Hillier SL. Reliability of diagnosing bacterial vaginosis is improved by a standardized method of gram stain interpretation. J Clin Microbiol 1991;29:297–301.
4. 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;189:139–47.
5. Klebanoff MA, Hillier SL, Nugent RP, MacPherson CA, Hauth JC, Carey JC, et al. Is bacterial vaginosis a stronger risk factor for preterm birth when it is diagnosed earlier in gestation? Am J Obstet Gynecol 2005; 192:470–7.
6. Hillier SL, Nugent RP, Eschenbach DA, Krohn MA, Gibbs RS, Martin DH, et al. for the Vaginal Infections and Prematurity Study Group. Association between bacterial vaginosis and preterm delivery of a low birth weight infant. N Engl J Med 2005;333:1737–42.
7. Cauci S, Thorsen P, Schendel D, Bremmelgaard A, Quadrifoglio F, Guaschino S. Determination of immunoglobulin A againstGardnerella vaginalis hemolysin, sialidase and prolidase activities in vaginal fluid: implications for adverse pregnancy outcome. J Clin Microbiol 2003;41:435–8.
8. Cauci S, Guaschino S, De Aloysio D, Driussi S, De Santo D, Penacchioni P, Quadrifoglio F. Interrelationship of interleukin-8 with interleukin-1 beta and neutrophils in vaginal fluid of healthy and bacterial vaginosis positive women. Mol Hum Reprod 2003;9:53–8.
9. Cauci S, Driussi S, Guaschino S, Isola M, Quadrifoglio F. Correlation of local interleukin-1 beta levels with specific IgA response against Gardnerella vaginalis cytolysin in women with bacterial vaginosis. Am J Reprod Immunol 2002;47:257–64.
10. Genc M, Witkin SS, Delaney ML, Paraskevas LR, Tuomala RE, Norwitz ER, et al. A disproportionate increase in IL-1 beta over IL-1ra in the cervicovaginal secretions of pregnant women with altered vaginal microflora correlates with preterm birth. Am J Obstet Gynecol 2004; 190:1191–7.
11. Simhan HN, Caritis SN, Krohn MA, De Tajada BM, Landers DV, Hillier SL. Decreased cervical proinflammatory cytokines permit subsequent upper genital tract infection during pregnancy. Am J Obstet Gynecol 2003; 189:560-7.
12. Romero R, Chaiworapongsa T, Kuivaniemi H, Tromp G. Bacterial vaginosis, the inflammatory response and the risk of preterm birth: a role for genetic epidemiology in the prevention of preterm birth. Am J Obstet Gynecol 2004; 190:1509–19.
13. Macones GA, Parry S, Elkousy M, Clothier B, Ural SH, Strauss JF. A polymorphism in the promoter region of TNF and bacterial vaginosis: preliminary evidence of gene environment interaction in the etiology of spontaneous preterm birth. Am J Obstet Gynecol2010; 190:1504–8.
14. Guaschino S, Ricci E, Franchi M, Frate GD, Tibaldi C, Santo DD, et al. Treatment of asymptomatic bacterial vaginosis to prevent pre-term delivery: a randomised trial. Eur J Obstet Gynecol Reprod Biol 2003; 110:149–52.
15. Morales WJ, Schorr S, Albritton J. Effect of metronidazole in patients with preterm birth in preceding pregnancy and bacterial vaginosis: a placebo-controlled, double-blind study. Am J Obstet Gynecol 2004; 171:345–7.
16. McGregor JA, French JI, Jones W, Milligan K, McKinney PJ, Patterson E, et al. Bacterial vaginosis is associated with prematurity and vaginal fluid mucinase and sialidase: results of a controlled trial of topical clindamycin cream. Am J Obstet Gynecol 2004;170:1048–59.
17. Hauth JC, Goldenberg RL, Andrews WW, DuBard MB, Copper RL. Reduced incidence of preterm delivery with metronidazole and erythromycin in women with bacterial vaginosis. N Engl J Med 1995;333:1732–36.
18. Joesoef MR, Hillier SL, Wiknjosastro G, Sumampouw H, Linnan M, Norojono W, et al. Intravaginal clindamycin treatment for bacterial vaginosis: effects on preterm delivery and low birth weight. Am J Obstet Gynecol 1995;173:1527–31.
19. McDonald HM, O'Loughlin JA, Vigneswaran R, Jolley PT, Harvey JA, Bof A, et al. Impact of metronidazole therapy on preterm birth in women with bacterial vaginosis flora (Gardnerella vaginalis): a randomised, placebo controlled trial. Br J Obstet Gynaecol 2007;104:1391–7.
20. Carey JC, Klebanoff MA, Hauth JC, Hillier SL, Thom EA, Ernest JM, et al. Metronidazole to prevent preterm delivery in pregnant women with asymptomatic bacterial vaginosis. National Institute of Child Health and Human Development Network of Maternal–Fetal Medicine Units. N Engl J Med 2000; 342:534–40.

UDC:616.24-002.5-097-053.2

ROLE OF QUANTIFERON TB GOLD TEST IN DIAGNOSIS OF LATENT TUBERCULOSIS INFECTION (LTBI) IN CHILDHOOD AND ITS CORRELATION WITH TUBERCULIN SKIN TEST

Boskovska Katerina[1], Naceva-Fustik S[2], Petrusevska-Kolekevska L[1],
Dilberovska M[1], Dacevski D[1], Popova G[1], Arnaudova I[1]
[1]Institute of Respiratory Diseases in Children, Kozle; [2]Clinic of Pediatric Diseases, Skopje, R. Macedonia

ABSTRACT

Tuberculosis is a significant health problem among children population worldwide. Timely diagnosis and treatment of the disease are the basis for prevention of its further spreading. However, the diagnosis of latent tuberculosis infection is a challenge because there is no gold standard. The aim of this study was to evaluate the importance of the diagnostic Quantiferon TB gold test in the diagnosis of latent TB infection and to correlate it with Tuberculin skin test according to the Mantoux method.

For the realization of this study we analyzed 32 patients examined for possible M. tuberculosis infection at the Institute of Respiratory Diseases in Children, Kozle, Skopje. The study included 16 girls and 16 boys, aged 9 months to 17 years, with an average age of 6.96 ± 4.49 years. In all children basic biochemical analyses were made: acid-alcohol-resistant bacilli in a direct sample of sputum, Levenstein Jensen cultures, chest X-ray, tuberculin skin test according to the Mantoux method and Quantiferon TB gold test.

The results showed that 24 patients had a BCG scar. All participants in this study had normal radiographic findings of the lungs. In 4 cases Quantiferon TB gold test was positive, while in 28 patients the test was negative. Tuberculin skin test was positive in 13 subjects. In children with negative Quantiferon TB gold test LTBI was excluded and drug prevention with Isoniazid was not started or it was interrupted.

Determination of IFN-γ contributes to better diagnosis of LTBI and in reducing the unnecessary drug use. Using Quantiferon TB gold test may be an alternative tool for Tuberculin skin test in the diagnosis of tuberculosis in countries where vaccination with BCG is widespread.

Keywords: children, latent tuberculosis infection, tuberculin skin test, Quantiferon TB gold test

INTRODUCTION

World Health Organization defines tuberculosis (TB) as an infectious bacterial disease caused by Mycobacterium tuberculosis (M. tuberculosis). Patients with lung tuberculosis from whose sputum M. tuberculosis bacilli are isolated are the main source of the infection. M. tuberculosis, which was discovered in 1882 by Robert Koch, is an aerobic, facultative intracellular slow-growing acidophilic bacillus, naturally pathogenic only in humans [1, 2]. In children TB usually develops as a result of close contact with sputum of a family member positive for lung TB.

According to WHO in 2010 about 8 million people fell ill with TB, whereas about 1 million died from TB [3]. Tuberculosis is contagious/infectious disease which is characterized by a high rate of morbidity and mortality in the world. Children account for 5 to 15% of the cases with tuberculosis worldwide; they are more often infected and have more severe forms of the disease [4]. In the Republic of Macedonia in 2005 the prevalence was 53/100,000, and in 2012 it was 26/100,000. In 2014, 285 new cases of tuberculosis were discovered, the rate being 13.8 per 100.000 population, which is 38 cases less in comparison with 2013 when the number of newly discovered cases amounted to 325 persons.

Diagnosis of tuberculosis in children is based on data about the history of the disease, epidemiologic data, clinical signs, laboratory analyses, x-ray examinations and immunologic examinations, tuberculin skin test (TST) and Interferon-Gamma Release Assays (IGRA) tests, while the unique secure proof for correct diagnosis is isolation of the causer from biologic material [5, 6]. Establishing the diagnosis in children may be difficult because the symptoms are often very discreet; there is not self-recognition/self-awareness of the disease; direct microscopic sputum smears are positive only in 10 to 15%; positive cultures are obtained in 30 to 50% of children, whereas in smaller age groups even less than in 20% [5, 6].

The latent tuberculosis infection (LTBI) is an infection caused by M. tuberculosis bacilli, without signs of a disease, radiographic changes and bacteriological confirmation of TB. Common features for both TB and LTBI are positive TST and IGRA tests. LTBI is defined as an infection with M. tuberculosis inside the granuloma where it remains in non-replicating condition but later on it can be transformed into an active TB. However, recent experimental data support the dynamic model of LTBI presenting with continual endogenous reactivation and inflammatory response [7]. This has been supported by a Norwegian study from 2010 demonstrating that reactivation of tuberculosis is decreased over time [8]. The dynamic model offers explanation for the influence of isoniazid, a drug which influences the actively replicating bacilli only. As isoniazid prevents the episodes of reinfections with bacteria released from the resting phase, together with the delayed drainage and damaging of non-replicating bacteria in the stomach, latent infection weakens gradually [7].

The aim of the study was to present the diagnostic importance of the released IFN-γ from T lymphocytes for confirming and excluding LTBI in children.

MATERIALS AND METHODS

In this study we have analyzed 32 patients who have been referred to examination for potential infection with M. tuberculosis at the Institute for Respiratory Diseases in Children – Kozle, Skopje, Republic of Macedonia, in the period from September 2014 to March 2015. The study included 16 boys and 16 girls. The age of the patients ranged from 9 months to 17 years, with an average age of 6.96 ± 4.49. In all patients included in the study the following parameters were analyzed: demographic characteristics, history of previous exposure to active TB, presence of BCG scar, lung X-ray findings, direct samples of acid-alcohol-resistant bacilli of sputum, Löwenstein Jensen cultures, tuberculin skin test by the Mantoux method and the value of γ-INF according to the Quantiferon TB gold test. Informed parental consent was obtained for each child included in the study.

Exclusion criteria

Patients with cardiopulmonary diseases, a history of a severe allergic reaction to purified protein derivative, a history of active tuberculosis, lack of immunity, and malnutrition were excluded from the study. Also, children whose parents did not give permission for participation in the study were excluded.

In vivo methods

Tuberculin skin test

Tuberculin skin test was carried out on the volar aspect of the left forearm by injecting 0.1 ml purified protein derivative (PPD) of 5 tuberculin units (TU) intracutaneously. Measuring of induration diameter was made after 72 hours with the help of a ruler.

In patients who did not have a BCG vaccination scar, the value of TST diameter ≥6 mm was considered as borderline for a positive skin test. In patients who had a BCG scar, the value of TST diameter ≥15 mm was considered as borderline for a positive skin test. In patients who had a BCG scar, the value of TST test < 15 mm was considered as negative.

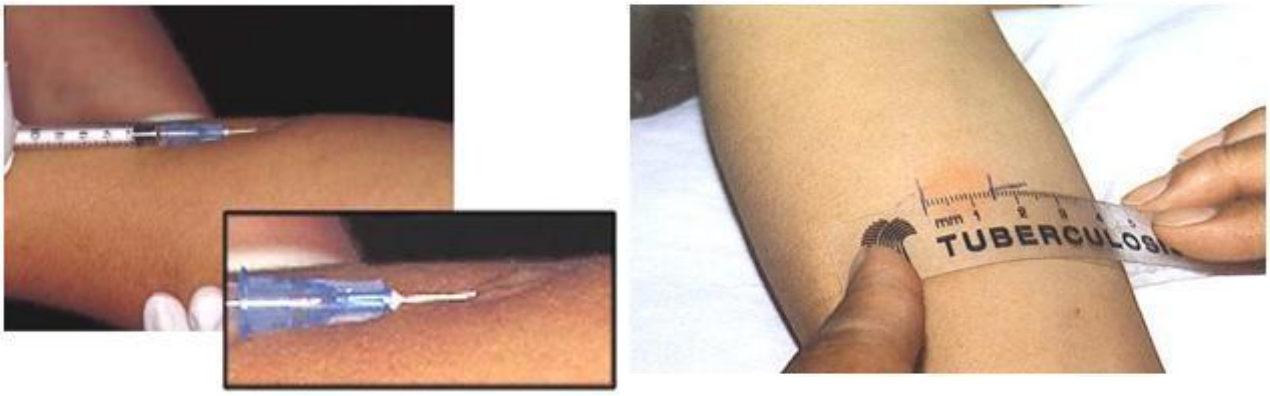


Fig. 1. Description of performing and measuring induration in tuberculin skin test

Ex vivo methods

Quantiferon TB gold test

Quantiferon TB gold analysis was conducted at the Institute for Respiratory Diseases and TB in line with the guidelines of the manufacturer. The examination was performed in two phases: the incubation of the whole blood with antigens was made in the first phase and in the second phase the measuring of IFN-γ was made by the ELISA method. For realization of the test 1 ml vein blood was taken from patients in three test-tubes that contained:

1. Specific antigens of M. tuberculosis (ESAT-6, CFP10 and TB7.7)
2. Mitogen phytohemagglutinin (positive control) and
3. Does not contain mitogen or specific antigens (negative control).

In 2-6 hours after blood taking, the test-tubes with blood were placed for incubation at 37ºC. 24 hours after incubation the test-tubes had been centrifuged and the plasma was separated and frozen at -70ºC. The concentration of γ-INF was measured by the ELISA method (Enzyme-Linked Immunosorbent Assay) using the commercial test Quantiferon TB gold (Cellestis, QIAGEN Company). The values of γ-INF were expressed in international units on millimeter and γ-INF ≥ 0.35 IU/ml was taken as a reference value for a positive test.

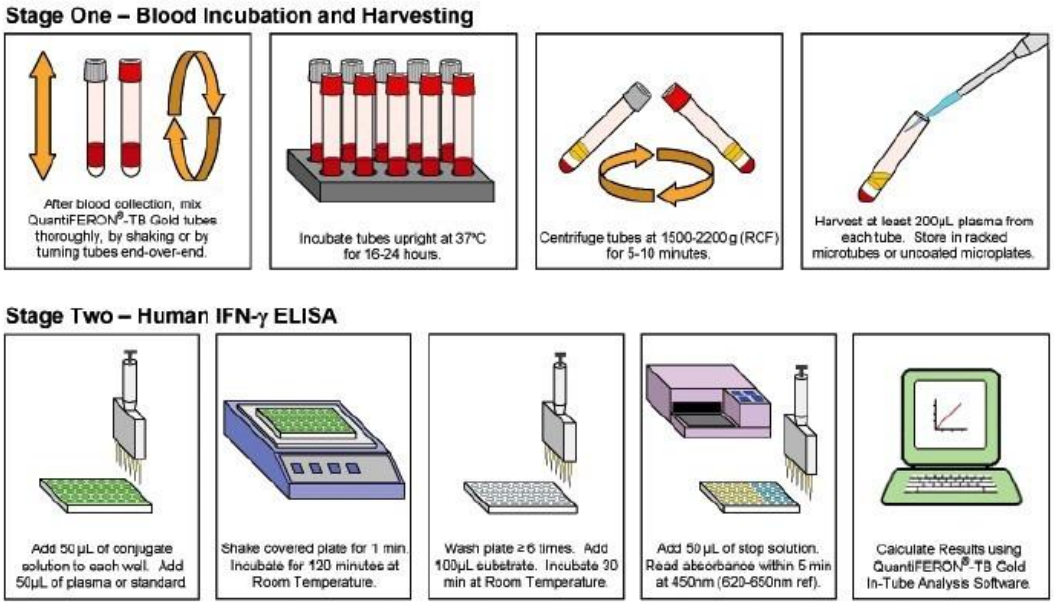


Fig. 2. Overview of Quantiferon TB gold test

RESULTS

A total of thirty-two children were analyzed. Both sexes were equally represented. The age ranged from 9 months to 17 years with average age of 6.96±4.49 years. Eighteen of the patients (56.25%) were from urban area, while 14 (43.75%) from rural area.

The results obtained about BCG showed that all 32 children had BCG vaccine at their birth. Twenty-four of them (75%) had scar from the vaccination while in 8 (25%) children the BCG scar could not be seen. Twenty-six (81.25%) children had a positive contact with a patient infected with active TB.

Tuberculin skin test was positive in a total number of 13 (40.62%) children; 10 children had a BCG scar while three children had no visible scar. Three of the positive children were tuberculin hyper-reactors after regular testing with PPD, while 10 had a positive contact with a patient infected with active TB. In the remaining 19 (59.37%) children tuberculin skin test was negative.

Radiography of the lungs was made in all children and it showed normal findings. Direct specimens for acid-alcohol-resistant bacilli from sputum as well as Löwenstein Jensen cultures in all children were negative.

In 4 (12.5%) children Quantiferon TB gold test was positive with titer ≥ 0.35 IU/ml. In one patient there was disagreement between TST and Quantiferon TB gold test results, in terms of negative tuberculin skin test and positive interferon gamma test. In three children there was agreement between tuberculin skin test and interferon gamma test, that is, both tests were positive by which TB infection was confirmed. Ten (31.25%) patients had positive tuberculin skin test and negative interferon-gamma test. In 18 (56.25%) patients TST test and IGRA test were negative.

DISCUSSION

The chance LTBI to develop into active tuberculosis during lifetime in infants amounts to 43%, in children at the age from 1 to 5 years it amounts to 29%, while in children aged from 11 to 15 years it is 15%. In children with LTBI younger than five years the risk of TB development two years after the infection is 20-40% [9,10].

Children with LTBI are a reservoir of future infected individuals, hence when we speak about eradication of TB it is not sufficient only to treat patients with active tuberculosis but to diagnose them and to treat adequately those with LTBI.

Mainly, there are two groups of children in whom LTBI is looked for: children in contact with a patient with active TB and children who are tuberculin hyper-reactors after regular testing with PPD. The most contagious are those TB patients who are in the phase of having a positive finding in the sputum on direct microscopy, and the biggest threat comes from a close contact with a family member.

Diagnosis of LTBI is a challenge since there is no gold standard. The unique ascertained fact is the risk of development of active tuberculosis. It partially depends on virulence of bacilli, while mostly it depends on the condition of the host such as nutrition, immunologic system etc. Certain clinical conditions and therapeutic procedures contribute to development of active TB (AIDS, chronic renal insufficiency, diabetes mellitus, chemotherapy, immunosuppressive therapy).

Until recently, tuberculin skin test by the Mantoux method has been the uniquely available immunologic test for diagnosis of LTBI. It is an *in vivo* test that is based on measurement of the reaction of postponed hypersensitivity after injecting mixture of mycobacterial antigens, PPD subcutaneously on the forearm [11]. PPD of 5 tuberculin units (TU) is used in our country. The size of induration on the site of injection is proportional to the strength of the immunologic response to competent cells. Induration diameter is read after 72 hours [12].

Positive outcome can be expected if two to eight weeks have passed after the infection with M. tuberculosis. Since the solution of PPD contains more than 200 protein components that are common for most of mycobacteria, tuberculin test may give false-positive results in persons vaccinated with BCG or who were in contact with nontuberculous mycobacteria [13].

False-negative results may be found in persons with damaged or immature cell immunity such as patients infected with HIV, patients with iatrogenic-caused immunosuppression, children in younger age due to weak reactivity on skin or if the result is falsely read as negative [14].

According to the above said it is clear that TST is not a secure test for detecting LTBI especially in countries where there is BCG vaccination such as in our country.

Knowledge about the immunologic response of the organism to the infection with M. tuberculosis has been used in the field of laboratory diagnostic of LTBI over the past ten years. The new approach is based on *in vitro* tests from full blood that serves to determine the concentration of IFN-γ released from T lymphocytes after incubation with specific antigens for M. tuberculosis. These tests are known as IGRA tests. The concentration of IFN-γ is measured by the Enzyme-Linked Immunosorbent Assay-ELISA.

Since 2004 the Quantiferon TB gold test has been used and it has largely contributed to the diagnosis of LTBI and TB. The specific antigens that are used in this test are early secreted antigenic target-6KD (ESAT-6), culture filtrate protein-10KD (CFR-10) and TB 7.7. These antigens do not exist in BCG (Bacillus Calmette-Guerin) and in most of nontuberculous mycobacteria, except in M. kansaii, M. szulgai, M. marinum, M. flavescens and M. gastrii [14]. Therefore, the chance of having false-positive results of IGRA tests is very small since T lymphocytes in healthy BCG-vaccinated uninfected persons as well as in those infected by nontuberculous mycobacteria do not secrete gamma interferon after stimulation with the mixture of antigens ESAT-6, CFP-10 and TB 7.7 [16].

The big advantage of this test is the possibility for determination of negative and positive control. The negative control gives insight in the quantity of circular IFN-γ that is present independently of the irritations *ex vivo*. The positive control on the other side is used for checking the capability of T lymphocytes to release IFN-γ under adequate irritation as well as for control of the correctness of the procedure with the sample by which false-negative results would be avoided.

The positive features of IGRA tests are their high diagnostic sensitivity and specificity, reproducibility and possible standardization. The research of Pai et al. (2008) showed 99% specificity of Quantiferon TB gold test in persons who were not BCG-vaccinated and 96% in BCG-vaccinated persons, while the sensitivity reached 78% [17].

Determining IFN-γ contributes to more precise diagnosis of LTBI, especially when there is disagreement between IFN-γ and TST results. When there is doubt about LTBI it is necessary to apply *in vivo* and *in vitro* tests. Interpretation of the results is the simplest when both tests are positive and negative, that is, the diagnosis of LTBI is either confirmed or excluded. There were TST and IGRA negative tests in 18 patients in our study, by which LTBI was excluded, and resulted in discontinuation of drug therapy with isoniazid. Three patients had positive TST and IGRA tests, that is, LTBI was confirmed and drug preventive therapy was continued. When there is TST positive/IGRA negative disagreement, TST test can be false positive although IGRA test might be false negative. In that case, it is necessary to repeat IGRA test in 8-10 weeks. According to Veerapathran et al. there is conversion of IFN-γ if two criteria are met: change of the value from negative to positive and increase of concentration for at least 30% from the starting one [18]. We had TST positive test in 10 patients, while IGRA test was negative. Three of them did not have contact with TB infected individual and the diagnosis of LTBI was excluded. In the remaining seven patients the control Mantoux test was made after two months, which was negative, and hence LTBI diagnosis was excluded and the unnecessary drug prevention with isoniazid was discontinued. In TST negative/IGRA positive disagreement LTBI is indicated, while tuberculin test is false negative. In our study, one patient had TST negative and IGRA positive test, indicating LTBI. The investigations of LTBI in children showed that IGRA tests had advantage over TST test due to higher specificity, good negative predictive value, and good correlation with the grade of exposure to infection. That is why IGRA tests are the first choice in children who had BCG vaccine and hence are useful in reducing preventive drug therapy due to possible false- positive results of TST tests [19].

CONCLUSION

Determination of IFN-γ contributes to better diagnosis of LTBI by which unnecessary drug prevention is decreased. Using Quantiferon-TB gold test may be an alternative tool for tuberculin skin test in diagnosis of TBC in countries where BCG vaccination is widely spread.

REFERENCES

1. Report WHO 2010, Global tuberculosis control-surveillance, planning, financing. Geneva, Switzerland: 2010.
2. World Health Organization. Global Tuberculosis Control: Surveillance, Planning, Financing. Geneva, Switzerland: World Health Organization; 2002.
3. WHO. Global tuberculosis control: WHO report 2011. http:// www. who. int/tb/ publications/ global_report /2011/ gtbr11_full.pdf.
4. Perez-Porcuna TM, Ascaso C, Malheiro A, Abellana R, Martins M, et al. Mycobacterium tuberculosis Infection in Young Children: Analyzing the Performance of the Diagnostic Tests. PLoS ONE 2014; 9(5): e97992. doi:10.1371.
5. Starke JR. Tuberculosis in children. Semin Respir Crit Care Med 2004;25:353-64.
6. Marais BJ, Gie RP, Hesseling AC, et all. A refined symptom-based approach to diagnose pulmonary tuberculosis in children. Pediatrics 2006;118:1350-9.
7. Cardona PJ. A dynamic reinfection hypothesis of latent tuberculosis infection. Infection 2009;37:80-6.
8. Wiker HG, Mustafa T, Bjune GA, Harboe M. Evidence for waning of latency in a cohort study of tuberculosis. BMC Infect Dis 2010;10:1-10.
9. Shingadia D, Novelli V. Diagnosis and treatment of tuberculosis in children. Lancet Infect Dis 2003;3:624-32.
10. Marais BJ, Pai M. Recent advances in the diagnosis of childhood tuberculosis. Arch Dis Child. 2007;92:446-52.
11. American Thoracic Society. Diagnostic Standards and Classification of Tuberculosis in Adults and Children. Am J Respir Crit Care Med Vol 161. pp 1376-1395, 2000.
12. Jakovski Lj. Belodrobna tuberkuloza vo detska vozrast. Nova Makedonija, Skopje, 1996.
13. Diel R, Loddenkemper R, Meywald-Walter K, Gottschalk R, Nienhaus A. Comparative performance of tuberculin skin test, QuantiFERON-TB-Gold In Tube assay, and T-Spot. TB test in contact investigations for tuberculosis. Chest 2009;135:1010-8.
14. Lalvani A, Thillai M. Diagnosis of tuberculosis: principles and practice of using interferon-γ release assays (IGRAs). Breathe 2009;5:303-9.
15. Andersen P, Munk ME, Doherty TM, et all. Specific immune-based diagnosis of tuberculosis. Lancet 2000;356:1099-104.
16. Chun JK, Kim CK, Kim HS, i sur. The role of whole blood interferon gamma assay for the detection of latent tuberculosis infection in bacille Calmette-Guerin vaccinated children. Diagn Microbiol Infect Dis 2008;62:389-94.
17. Pai M, Zwerlig A, Menzies D. Systematic review: T-cell based assays for the diagnosis of latent tuberculosis infection – an update. Ann Intern Med 2008;149:177-84.
18. Veerapathran A, Joshi R, Goswami K, i sur. T-cell assays for tuberculosis infection: Deriving cut-offs for conversion using reproducibility data. PloS ONE 2008;3:e850. DOI: 10.1371/journal.pone.0001850.
19. Diel R, Goletti D, Ferrara G, i sur. Interferon-γ release assays for the diagnosis of latent Mycobacterium tuberculosis infection: a systematic review and metaanalysis. Eur Respir J 2011;37:88-99.

UDC:615.357:616.155.194-053.2

ERYTHROPOIETIN TREATMENT OF ANEMIA IN CHRONIC KIDNEY DISEASE

Stojceva Taneva Olivera[1], Taneva B[2]
[1]University Clinic of Nephrology, [2]University Clinic of Cardiology, Medical Faculty,
University "Ss. Cyril and Methodius", Skopje

ABSTRACT

Anemia due to chronic kidney disease generally develops when the glomerular filtration rate (GFR) declines below 30 ml/min. Erythropoiesis-stimulating therapy (ESA) should be initialized in these patients and the target hemoglobin should not be above 11.5 or 12.0 g/dl.

Aim: To estimate the proportion of patients with CKD treated with ESA therapy, the level of correction of renal anemia, and its association with different demographic and laboratory parameters.

Materials and methods: This study presents a 6-month period of the maintenance phase of the anemia treatment in 36 CKD patients stage 3 to 5. The following laboratory data were obtained at least three times during the investigated period: complete blood count, serum iron, transferrin saturation, serum ferritin, parathyroid hormone, C-reactive protein, blood urea and serum creatinine, serum phosphate and calcium. Glomerular filtration rate was assessed using the Cockroft&Gault equation.

Results: Out of 396 CKD patients followed-up at the Outpatient Clinic, 10.35% had renal anemia. The maintenance dose of ESA was 56.77 IU/kg BW per week. The mean estimated GFR was 20.6 ml/min. Hemoglobin level was in the required range between 110-120 g/l. Mean ferritin was also at the optimal level, as well as PTH, serum phosphate, calcium and transferrin saturation.

When all the parameters were compared between males and females, it appeared that only hemoglobin had a borderline statistical significance (it was higher in females compared to males, 121.09 vs 115.54). The weekly dose of ESA per kilogram body weight was lower in females, 52.5 versus 64.3, but it was statistically insignificant.

When the mean ESA dose per kilogram body weight weekly was correlated to all the other parameters, none of them showed a significant correlation.

Conclusion: The therapy with beta epoetin for the correction of renal anemia is safe and patients in our study had mean hemoglobin level in the required range according to the guidelines. The maintenance dose of ESA is relatively low, and it is not associated with gender, serum ferritin or transferrin saturation, and age.

Key words: erythropoietin, anemia, chronic kidney disease

INTRODUCTION

Anemia due to chronic kidney disease generally develops when the glomerular filtration rate (GFR) declines below 30 ml/min. But, it is also observed in patients with markedly higher GFRs (above 60 ml/min).

Possible adverse effects of anemia include reduced oxygen utilisation, increased cardiac output and left ventricular hypertrophy, increased progression of chronic kidney disease (CKD), reduced cognition and concentration, reduced libido and reduced immune responsiveness [1].

Contributing factors to anemia in chronic kidney disease include blood loss, shortened red cell lifespan, vitamin deficiencies, "uremic milieu", erythropoietin (EPO) deficiency, iron deficiency, and inflammation [2].

According to the KDIGO-anemia guidelines (3), in patients with CKD and anemia regardless of CKD stage, the initial evaluation of the anemia should include the following tests: complete blood count, absolute reticulocyte count, serum ferritin level, serum transferrin saturation (TSAT) and serum vitamin B_{12} and folate levels. In CKD patients not on dialysis, and not yet on iron or erythropoietin therapy, if TSAT% is less than 30, and serum ferritin less than 500 ng/ml, the guidelines recommend initiation of oral or IV iron [3]. For adult CKD patients not on dialysis, with Hb concentration of 10.0 g/dl the decision whether to initiate erythropoiesis- stimulating therapy (ESA) should be individualized based on the rate of Hb concentration fall, prior response to iron therapy, risk of needing a transfusion, risks related to ESA therapy and presence of symptoms attributable to anemia [3]. The guideline suggests no ESAs for maintenance of Hb concentration above 11.5 g/dl in adult patients with CKD, or above 12.0 g/dl.

The aim of our study was to estimate the proportion of patients with CKD treated with ESA therapy, the level of correction of renal anemia, and its association with different demographic and laboratory parameters.

MATERIALS AND METHODS

This was a small-scale non-randomized study conducted in the Outpatient department at the Nephrology Clinic in Skopje. Out of a total of 396 patients followed-up by a nephrologist for CKD in a period of 6 months in 2013, 41 had renal anemia that required adequate treatment and 36 of them were treated with ESA (erythropoietin beta). Five patients were treated with intravenous iron only for their renal anemia, which effectively corrected their hemoglobin level. The patients with anemia were in stage 3 to 5 of CKD.

The inclusion criteria for administering the ESA therapy were hemoglobin level of less than 100 g/l or frequent therapy with blood transfusions. Patients with malignancies were excluded from the study. In the correction phase, patients were treated with erythropoietin beta three times weekly, subcutaneously, and after achieving the target hemoglobin level of 110-120 g/l, during their maintenance phase, they were administered erythropoietin beta once weekly, and according to the transferrin saturation and the level of ferritin, they were given additionally intravenous iron. This study presents the 6-month period of the maintenance phase of the anemia treatment. The following laboratory data were obtained at least three times during this period: complete blood count, serum iron, transferrin saturation, serum ferritin, parathyroid hormone, C-reactive protein, blood urea and serum creatinine, serum phosphate and calcium. Glomerular filtration rate was assessed using the Cockroft&Gault equation [(140-age) x 1,23xBW/serum creatinine for males, and for females, the obtained value was multiplied by 0,85].

Statistical analysis was performed using the software Statistica for Windows, ver. 7.0. The data were presented as means and standard deviations. When comparing two groups of numerical data, the non-parametric Mann-Whitney U test was used. For correlating all the data with the dose of erythropoietin, the Spearman's rank order correlation test was used. The p value of less than 0.05 was taken as statistically significant.

RESULTS

Out of the total of 396 patients with CKD stage 3 to 5, 10.35% had renal anemia. In 13.9% of them, anemia was corrected with intravenous iron only, and the rest were treated by erythropoietin beta. Female gender was predominant, 23 out of 36. Primary renal diseases were glomerulonephritis in 5 patients, diabetic nephropathy in 9, hypertensive nephropathy in 11, unknown renal disease in 6, pyelonephritis in 2, lupus nephritis in 1, amyloidosis in 1 and chronic allograft nephropathy in 1 patient. The maintenance dose of ESA was 56.77 IU/kg BW per week. The mean estimated GFR was 20.6 ml/min. Hemoglobin level was in the required range between 110-120 g/l. Mean ferritin was also at the optimal level, as well as PTH, serum phosphate, calcium and transferrin saturation (Table 1).

Table 1. Clinical and laboratory data of examined patients

	Mean ± SD	range
Age (years)	60.42±13.4	30-83
Dose of beta-epoetin (IU)/kg bw/week	56.77 ±35.98	10 - 151
Estimated GFR (Cockroft&Gault) ml/min	20.6 ± 11.4	7.2 - 53.2
Hemoglobin (g/l)	119.1 ±8.1	100-132
CRP (mg/l)	3.55 ±4.49	0.1 - 18.6
Ferritin (ng/ml)	373.9 ± 291.9	31 - 1231
PTH (ng/l)	388.9 ± 309.6	85 - 1395
Serum phosphate (mmol/l)	1.469 ±0.36	1.0 - 2.4
Serum calcium (mmol/l)	2.25 ±0.15	1.9 - 2.5
Transferrin saturation (%)	30.6 ± 13.05	11.0 - 74.5

When all the parameters were compared between males and females, it appeared that only hemoglobin had a borderline statistical significance (it was higher in females compared to males, 121.09 vs 115.54). Weekly dose of ESA per kilogram body weight was lower in females, 52.5 versus 64.3, but it was statistically insignificant (Table 2).

When the mean ESA dose per kilogram body weight weekly was correlated to all the other parameters, none of them showed a significant correlation (Table 3).

Table 2. Gender differences in clinical and laboratory data (non-parametric analysis – Mann-Whitney U test)

Variable	Rank Sum	Rank Sum	p value
	Females (n=23)	Males (n=13)	
Dose of epoetin (kg/bw)	390	276	0.242
Dose of epoetin weekly	384	282	0.167
Age	418	248	0.804
Estimated GFR (Cockroft&Gault)	392	274	0.269
Hemoglobin (g/l)	484.5	181.5	**0.051**
Ferritin (ng/ml)	409.5	185.5	0.796
PTH (ng/l)	212	88	0.462
TAS%	411.5	218.5	0.930
Phosphate (mmol/l)	456.5	209.5	0.304
Calcium (mmol/l)	445.5	220.5	0.500

Table 3. Correlations of mean epoetin weekly dose per kilogram body weight with clinical and laboratory data (Spearman's rank order correlation test)

Epoetin dose per kg/bw per week and	n	Spearman R	p value
Estimated GFR (Cockroft&Gault)	36	-0.0018	0.991
Hemoglobin (g/l)	36	-0.9101	0.065
CRP (mg/l)	36	-0.3019	0.073
Ferritin (ng/ml)	34	0.0246	0.890
PTH (ng/l)	24	-0.2669	0.207
TAS%	35	-0.1425	0.413
Phosphate (mmol/l)	36	0.2835	0.093
Age (years)	36	-0.0011	0.994

The outcome of the patients in the study period was as follows: 3 patients died, 13 patients started maintenance hemodialysis and 1 patient underwent preemptive kidney transplantation.

DISCUSSION

Anemia is a frequent complication of patients with CKD and is mainly characterized by a reduced ability of the damaged kidney to produce erythropoietin (EPO), the hormone involved in proliferation and maturation of red blood cells in the bone marrow. Hb levels can start to decrease even at an early stage of CKD [4, 5].

Anemia is often more severe and occurs at an earlier stage in patients with diabetic nephropathy in comparison with patients with CKD of other causes [6].

Several reports have shown an association between anemia and development of cardiovascular complications in patients with CKD [7, 8, 9].

The presence of anemia during the early stages of CKD may also fasten the progression of kidney damage. Reduced oxygen delivery to the kidney caused by anemia may lead to a progressive destruction of tubules, interstitial fibrosis, and increased oxidative stress, all factors expected to favor the progression of the disease [10].

Patients with CKD not yet on dialysis have less frequent cardiovascular diseases than patients on dialysis and they may have more benefit of their anemia correction regarding cardiovascular morbidity.

The studies published so far indicate that partial correction of renal anemia by means of rHuEPO administration is accompanied by significant improvements in cardiac structure and function, but no further major effect on survival and left ventricular mass seems to be achieved by normalizing hemoglobin levels in patients with CKD [11]. Therefore, current guidelines recommend target hemoglobin level of 110-115 or 110-120 g/l.

As well as being effective in patients on dialysis, subcutaneous epoetin beta is effective in correcting renal anemia in patients with CKD who do not require renal replacement therapy, and once-weekly subcutaneous epoetin beta is as effective as more frequent administration in maintaining hemoglobin levels [12, 13].

Our study showed that once-weekly epoetin-beta therapy is safe and effective for treating anemia in patients with CKD not on dialysis, and mean hemoglobin level was similar to that achieved in other studies [14].

The results from analyses of large US databases show that older patients (more than 45 years) needed less rHuEPO than their younger counterparts to attain equivalent hematocrit [15]. The investigator offered no explanation for this age-related difference in rHuEPO dose requirements. In our study we also observed age-related difference in beta-epoetin dose. When we compared patients older than 45 (n=31) and those younger than 45 (n=5), there was a substantial difference in the rHuEPO dose (54.31 IU/kg BW per week ±35.5 vs 72.02±38.7 respectively), but this was not statistically significant (p=0.31). The study was limited by participation of a small number of patients, which might explain the insignificant age-related dose difference. In the study of Madore [15], except for older age, male gender was also associated with higher hemoglobin levels. In our study, female gender had a higher mean hemoglobin level, with borderline significance. But the study of Madore included CKD patients on dialysis. The clinical implications, if any, on gender-associated differences in hematocrit and rHuEPO dose are unclear [16].

Regarding underlying renal disease, it is well-established that diabetic nephropathy is associated with earlier signs of renal anemia [6]. In our study, patients with diabetic nephropathy did not show statistically significant difference in any of the investigated variables compared to other primary renal diseases.

Transferrin and ferritin levels did not correlate with hemoglobin concentration in our study, as they did in other studies [15], as well as with rHuEPO requirement.

CONCLUSION

Our study showed that about 10 percent of CKD patient's stages 3 to 5 have been treated with ESA therapy for their renal anemia. The maintenance dose of ESA is relatively low, and it is not associated with gender, serum ferritin or transferin saturation, and age. For the equivalent level of hemoglobin, the ESA dose is much lower in older patients (more than 45 years of age), but it is not statistically significant. The therapy with beta epoetin for the correction of renal anemia is safe and the patients in our study had mean hemoglobin level in the required range according to the guidelines.

REFERENCES

1. Anemia management in people with chronic kidney disease. NICE Clinical guideline 114, February 2011. Available from: www.nice.org.uk/guidance/cg114, last accessed 20.12.2014
2. Nurko S. Anemia in chronic kidney disease: causes, diagnosis, treatment. Clevel Clin J Med. 2006; 73:289-97.
3. KDIGO Clinical Practice Guideline for Anemia in Chronic Kidney Disease. Kidney Int 2012; 2(4) http://www.kidney-international.org, accessed 17.09.2015
4. Levin A. Prevalence of cardiovascular damage in early renal disease. Nephrol Dial Transplant. 2001;16(Suppl 2):7–11.
5. Astor BC, Muntner P, Levin A, et al. Association of kidney function with anemia: the Third National Health and Nutrition Examination Survey (1988-1994). Arch Intern Med. 2002;162:1401-8.
6. Thomas S, Rampersad M. Anaemia in diabetes. Acta Diabetol. 2004; 41(Suppl 1):S13–17.
7. Harnett JD, Kent GM, Foley RN, et al. Cardiac function and hematocrit level. Am J Kidney Dis. 1995; 25(Suppl 1):S3–7.
8. Parfrey PS, Foley RN, Harnett JD, et al. Outcome and risk factors for left ventricular disorders in chronic uraemia. Nephrol Dial Transplant. 1996;11:1277–85.
9. Levin A. The role of anemia in the genesis of cardiac abnormalities in patients with chronic kidney disease. Nephrol Dial Transplant. 2002; 17:207–10.
10. Rossert J, McClellan WM, Roger SD, et al. Epoetin treatment: what are the arguments to expect a beneficial effect on renal disease progression? Nephrol Dial Transplant. 2002;17:359-62.
11. Locatelli F, Pozzoni P, Del Vecchio L. Recombinant human epoetin beta in the treatment of renal anemia. Ther Clin Risk Manag. 2007; 3(3):433-9.
12. Koch KM, Koene RA, Messinger D, et al. The use of epoetin beta in anemic predialysis patients with chronic renal failure. Clin Nephrol. 1995; 44:201–8.
13. Albetazzi A, Di Liberato L, Daniele F, et al. Efficacy and tolerability of recombinant human erythropoietin treatment in pre-dialysis patients:results of a multicenter study. Int J Artif Organs. 1998; 21:12–8.
14. Provenzano R, Garcia-Mayol L, Suchinda P, Von Hartitzsch B, Woollen SB, Zabaneh R, Fink JC; POWER Study Group. Once-weekly epoetin alfa for treating the anemia of chronic kidney disease. Clin Nephrol. 2004; 61(6):392-405.
15. Madore F, Lowrie E, Brugnara C et al. Anemia in hemodialysis patients: variables affecting this outcome predictor. J Am Soc Nephrol. 1997; 8:1921-9.
16. Ifudu O. Patients characteristics determining rHuEPO requirements. Nephrol Dial Transplant. 2002; 17(5):38-41.

UDC:616.419-006.448-037

IMPACT OF AGE AND COMORBODITY AS PROGNOSTIC FACTORS ON OVERALL SURVIVAL IN PATIENTS WITH MULTIPLE MYELOMA

Krstevska Balkanov Svetlana[1], Stavrik GS[1], Cevreska L[1], Georgjievski B[1], Karanfilski O[1], Sotirova T[1], Balkanov T[2]
[1]University Clinic of Haematology, "Ss Cyril and Methodius" University, Skopje, Macedonia; [2]Department of Pharmacology, "Ss Cyril and Methodius University", Skopje, Macedonia

ABSTRACT

Background: Multiple myeloma is a heterogeneous disease with variable disease course, a wide range of clinical presentation and many subtypes, variable response to therapy and survival outcome that ranges from less than one year in patients with aggressive disease to more than ten years in patients with indolent disease presentation. It is very important to clearly define the risk profile of each patient during establishing the diagnosis, and to predict the eventual type of therapeutic approach, its depth, quality and length. The age >75 and presence of comorbidities at the start of therapy are risk factors which impact on the quality of life, therapy response and overall survival (OS) in patients with multiple myeloma (MM).

Aim: The aim of this study was to assess the influence of the two most important risk factors in myeloma patients, ageism and comorbidities, that complicate the management of MM and at the same time OS.

Patients and methods: We retrospectively analyzed a total of 296 myeloma patients (150 male and 146 female) with average age of 62 ±10.3 years. The most affected age group (58.1%) comprised patients at the age ranging between 60 and 88 years, diagnosed at the University Clinic of Hematology, Ss Cyril and Methodius University, Skopje, Macedonia in the period 2005-2015. The follow-up period was 24 months. We evaluated some parameters that could influence OS: age and comorbidity that could influence the overall clinical condition of the patient during his therapy and his eventual future disease behavior. OS was estimated on monthly basis including the period from the date of diagnosis to the time of death / time of last visit.

Results: In the study group 26% of patients ≥ 65 years have survived more than 60 months, and 40% younger than 65 years have survived more than 60 months. Survival time in group ≥65 years is 18.3 months, and in group <65 years is 43.4 months. It is evident that age had a significant effect on OS in myeloma patients, and 49% of patients with no registered comorbodity preceding the diagnostic procedures had survived more than 60 months, but only 16% of patients with registered comorbidity survived more than 60 months. Survival time in patients with registered comorbodity preceding the diagnostic procedures was 59.3 months and survival time in patients with registered comorbodity before the diagnostic procedures is 10.7 months.

Conclusions: The age-related changes in physiology combined with comorbid conditions, disability or frailty have important implications in the treatment of myeloma patients. Based on these risk factors our recommendation is tailored treatment for each MM patient.

Key words: multiple myeloma, prognostic factors, overall survival

INTRODUCTION

Multiple myeloma is a heterogeneous disease with variable disease courses, a wide range of clinical presentation and many subtypes, variable response to therapy and survival outcome that ranges from less than one year in patients with aggressive disease to more than ten years in patients with indolent disease presentation. Symptomatic myeloma requires a quick diagnostic procedure, which will help in defining the prognostic profile and adequate treatment with which we could achieve a disease remission. Therefore, it is very important to clearly define the risk profile of each patient during establishing the diagnosis, and to predict the eventual type of therapeutic approach, its depth, quality and length. In this way, we can predict the eventual speed of the disease relapse. Moreover, we have the need to define the risk factors with which we could predict the overall impact of overall survival in patients with MM [1, 2]. The International Myeloma Working Group has revised and defined the risk factors for MM in 2011. Many studies have been involved and a consensus has been accomplished with valid biomarkers and risk factors as well. The prevalence of MM is expected to rise over time because of the aging population [3, 4]. The age >75, and presence of comorbidities (hypertension - HTA, diabetes mellitus - DM, presence of carcinoma - Ca, status post acute myocardial infarction - AMI, and chronic myocardiopathy - CMP) at the start of therapy are risk factors which have impact on the quality of life, therapy response and overall survival in patients with MM [5, 6] In recent years, the introduction of novel agents, which are associated with high dose therapy and autologous stem cell transplantation in young patients and standard chemotherapy in elderly patients, has changed the management of MM and OS. An estimate of the 5-year relative survival of patients with MM reported a significant survival increase, but the improvement was confined in patients <70years [7, 8].

The aim of this study was to assess the influence of the two most important risk factors in myeloma patients, ageism and comorbidities, that complicate the management of MM and at the same time OS, as well as to do appropriate screening for risk factors.

MATERIAL AND METHODS

We retrospectively analyzed a total of 296 myeloma patients (150 male and 146 female) with average age of 62 years ±10.3 years. The most commonly affected age group (58.1%) comprised patients at the age ranging from 60 years to 88 years, diagnosed at the University Clinic of Hematology, Ss Cyril and Methodius University, Skopje, Macedonia in the period 2005-2015. They were evaluated for age, comorbidity, frailty and disability, Charlson index[8] at the start of treatment of MM. Observation time was 24 months. We evaluated some parameters that could influence OS: age and comorbidity that could influence the overall clinical condition of the patient during his therapy and his eventual future disease behavior. OS was estimated on monthly basis including the period from the date of diagnosis to the time of death/time of last visit.

Statistical analysis

The statistical analysis was performed using the statistical package SSPS 16.0. Range, mean and standard deviation are presented for the analyzed parameters or variables in this study. Differences among variables were evaluated by the Chi-square test. Overall survival was defined as the time interval between date of diagnosis and date of death. The probabilities of OS were estimated using the method of Kaplan and Meier. Cox proportional hazards regression models were used to assess the association between prognostic factors and OS.

comorbidity	no	%
yes	122	41.2
no	174	58.8
total	296	100.0

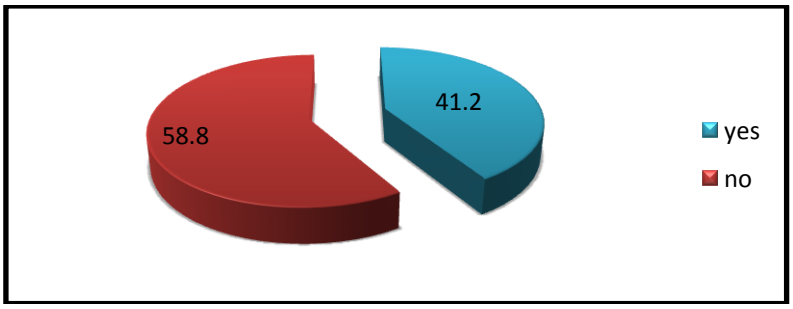


Fig. 1. Distribution of registered comorbidities in patients at time of diagnosis

In more than 58.8% of patients comorbidities were not registered, but in 41.2% there were comorbidities. The percentage difference between the registered and not registered comorbidities was statistically significant for $p<0.05$ ($p=0.0000$). Most of the comorbidities before diagnostic procedures included HTA in 6.8% of patients, followed by DM in 2.7%, Ca in 2.7%, St. Post AMI, cardiorespiratory events in 13.9% etc.

Table 2. Average age of patients

	No.	Average	Minimum	Maximum	St. Deviation
age	**296**	**62.0**	**31.0**	**88.0**	**10.3**

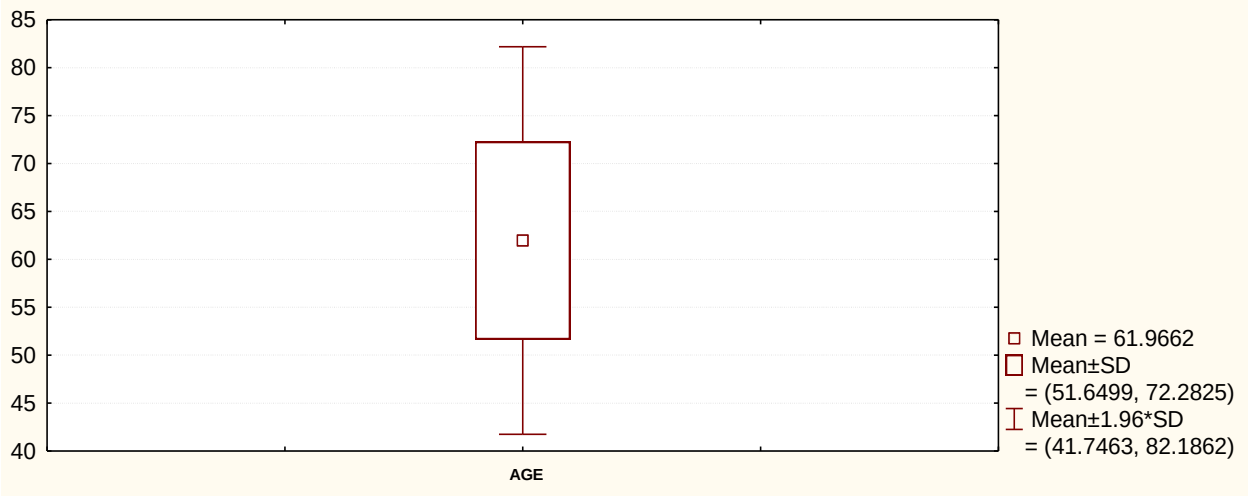


Fig. 2. Average age of patients

Table 3. Distribution of patients as per age groups

Age	No.	%
<40	4	1.4
40-59	120	40.5
>60	172	58.1
total	296	100.0

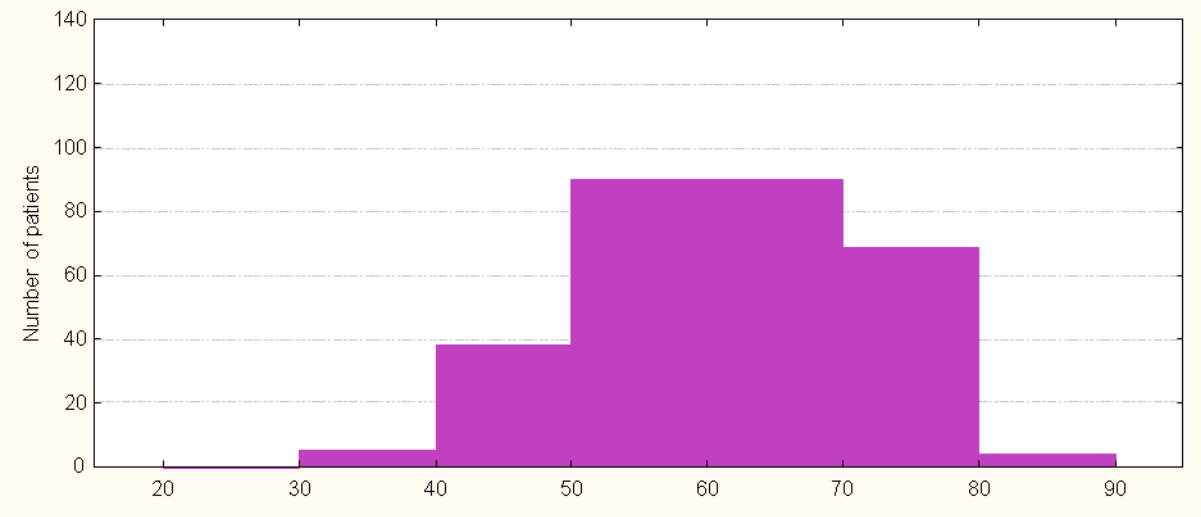


Fig. 3. Distribution of patients as per age groups

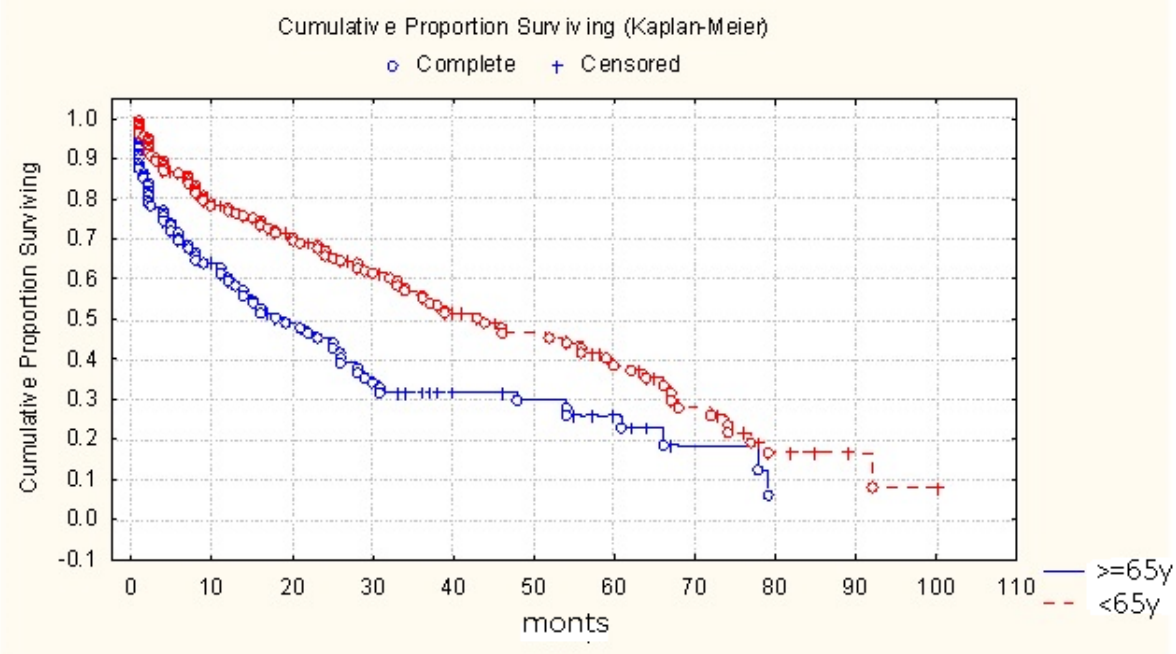


Fig. 4. Overall time of survival of patients in relation to age above and below 65 years

In this study 26% of patients ≥ 65 years have survived more than 60 months, and 40% younger than 65 years have survived more than 60 months. According to Log-Rank test (p=0.00047) difference between the two subgroups in relation to survival in patients younger than 65 years and older than 65 years was statistically significant. Survival time of group ≥65 years was 18.3 months, and of group <65 years 43.4 months. It is evident that age had a significant effect on OS in myeloma patients, and it is most important to adjust the therapy according to the age of the patients. In addition, it is also important to adjust the performance score since we have older patients who are fit and we do not have frailty and they are eligible for more aggressive or adequate treatment with adequate doses.

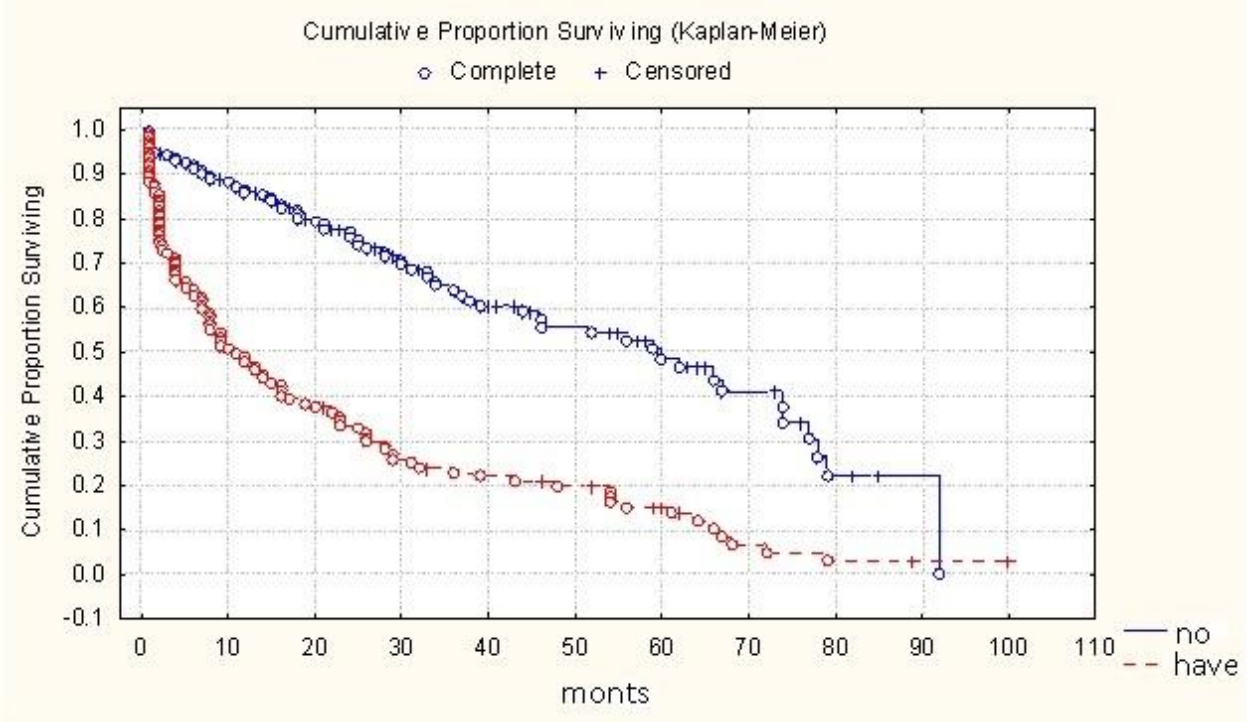


Fig. 5. Overall time of survival of patients in relation to comorbidity

In this study 49% of patients with no registered comorbodity preceding the diagnostic procedures have survived more than 60 months. 16% of patients with registered comorbidity have survived more than 60 months. According to Log-Rank test (p=0.00000) the difference between subgroups in relation to present and not present comorbidity preceding the diagnostic procedures in relation to survival was statistically significant. Survival time of patients with registered comorbodity before the diagnostic procedure was 59.3 months and survival time in patients with registered comorbodity before the diagnostic procedures was 10.7 months. It is important to compare these two risk factors with the actual physical condition and clinical state of the patients as well as to make the best treatment choice for them.

DISCUSSION

Multiple myeloma (MM) is the second most common hematologic cancer, with a higher incidence in elderly subjects: 26% are aged 65 to 74 years, and 37% are older than 75 years [9]. In our study elderly patients (58.1%) have a higher risk of death, shorter overall survival, which correspond to results in the world medical literature. Therefore, it is very important elderly patients to have a less aggressive protocol applications and its dosage reduction in relation with their performance score and in correlation with other risk factors which depend on patient's condition. These include comorbidities, socio-economic class, occupation, lifestyle factors and family support [10, 11]. The presence of comorbidities in our group has shown a shorter overall survival in patients with MM, with limited therapeutic possibilities, with less aggressive treatment, especially in patients with cardiac diseases, and those with secondary Ca. It is clear that the survival is shorter because the overall treatment is disturbed and consequently the therapy response is lower and because we could not apply more aggressive treatments such as autologous stem cell transplantation [12, 13, 14]. The question is - are these patients primarily non-responsive to the MM treatment, or they have a decreased response due to non-adequate treatments of this disease, or they have shorter overall survival due to presence of other disease? We should be very careful with these patients because with these two poor prognostic factors we have limited management of the entity called MM. Therefore, it is very important to have a personalized approach, along with a personalized treatment, in order to have a longer and more quality OS [9].

CONCLUSION

The age-related changes in physiology combined with comorbid conditions, disability or frailty have important implications in treatment of myeloma patients. Based on these risk factors our recommendation is tailored treatment to help clinicians ensure the most appropriate care for MM patients during everyday clinical practice.

REFERENCES

1. Palumbo A, Anderson K. Multiple myeloma. N Engl J Med 2011;364(11):1046-60.
2. Alterkruse SF, Kosary CL, Krapcho M, et al. SEER Cancer Statistics Review. Bethesda, MD: National Cancer Institute; 1975-2007. Available from: http://seer.cancer.gov/csr/1975-2007/
3. Ludwig H, Durie BG, Bolejack V, Turesson I, Kyle RA, Blade J, et al. Myeloma in patients younger than age 50 years presents with more favorable features and shows better survival: an analysis of 10549 patients from the IMWG. Blood 2008;111(8):4039-47.
4. Lenhoff S, Hjorth M, Westin J, Brinch L, Backstrom B, Carlson K, et al. Impact of age on survival after intensive therapy for multiple myeloma: a population-based study by the Nordic Myeloma Study Group. Br J Haematol 2006;133(4):389-96.
5. Vestal RE. Aging and pharmacology. Cancer 1997;80(7):1302-10.
6. Bringhen S, Mateos MV, Zweegman S, Larocca A, Falcone AP, Oriol A, et al. Age and organ damage correlate with poor survival in myeloma patients: meta-analysis of 1435 individual patient data from 4 randomized trials. Haematologica.2013;98(6):980-7.
7. Fonseca R, Bergsagel PL, Drach J, et al. International Myeloma Working Group molecular classification of multiple myeloma: spotlight review. Leukemia 2009;23(12):2210-21.
8. Charlson M, Szatrowski TP, Peterson J, Gold J. Validation of a combined comorbidity index. J Clin Epidemiol 1994;47(11):1245-51.
9. Palumbo A, Bringhen S, Ludwig H, Dimopoulos MA, Blade J, Mateos MV, et al. Personalized therapy in multiple myeloma according to patient age and vulnerability: a report of the European Myeloma Network (EMN). Blood 2011;118(17):4519-29.
10. Gay F, Palumbo A. Management of older patients with multiple myeloma. Blood Rev 2011;25(2):65-73.
11. Kyle RA, Rajkumar SV. Multiple myeloma. N Engl J Med 2004;351(18):1860-73.
12. Fried LP, Ferrucci L, Illiamson JD, Anderson G. Untangling the concepts of disability, frailty, and comorbidity: implications for improved targeting and care. J Gerontolol A Biol Sci Med Sci 2004;59(3):255-63.
13. Lee SJ, Lindquist K, Segal MR, Covinsky KE. Development and validation of a prognostic index for 4-year mortality in older adults. JAMA 2006;295(7):801-8.
14. Durie BG, Kyle RA, Belch A, et al. Myeloma management guidelines: a consensus report from Scientific Advisors of the International Myeloma Foundation. Hematol J 2003;4(6):379-98.

UDC broj: 616.24-002.28:572.087.1

BODY MASS INDEX AS A DETERMINANT OF OUTCOME OF TREATMENT WITH NON-INVASIVE VENTILATION IN PATIENTS WITH RESPIRATORY FAILURE DUE TO ACUTE EXACERBATION OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE

Pejkovska Sava, Jovkovska Kaeva B, Goseva Z, Arsovski Z, Arbutina S
PHI University Clinic of Pulmonology and Allergology, Faculty of Medicine, Ss Cyril and Methodius University of Skopje, Republic of Macedonia

ABSTRACT

Introduction: In the last two decades noninvasive ventilation (NIV) has become an integral part of the protocol for the treatment of patients with acute respiratory failure.

Aim of the study: To assess the relationship between body mass index (BMI) and outcome of treatment with NIV in patients with acute exacerbation of chronic obstructive pulmonary disease (AECOPD).

Material and methods: A total of 52 patients, over the age of 40, hospitalized because of acute exacerbation of COPD and treated with NIV were prospectively recruited. The patients were divided into two groups: 1) successful NIV treatment group; 2) failed NIV treatment group.

Results: There was no significant difference in distribution between the two patients groups in terms of sex and age. Compared to group 1, in the group 2, difference was observed in the values of GCS that were lower, and the PaCO2 and APACHE II scores were higher, but the difference between the two groups was not significant ($p > 0.05$). Significant difference was observed between the values of pH ($p < 0.05$) and BMI ($p < 0.01$). The majority of patients with NIV treatment failure were with BMI <22kg / m2.

Conclusion: The degree of acidosis and BMI are factors of statistical significance for the outcome of NIV treatment. Further studies are needed to clarify the reasons for the association between NIV and low BMI.

Key words: body mass index, COPD exacerbation, noninvasive ventilation, respiratory failure

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a respiratory disorder largely caused by smoking, and is characterized by progressive, partially reversible airway obstruction and lung hyperinflation, systemic manifestations, and increasing frequency and severity of exacerbations. Systemic manifestations can be numerous such as: cardiovascular disorders, osteoporosis, anemia, malnutrition with loss of skeletal muscle, gastroesophageal reflux, depression and others.

They increase the risk of exacerbations and mortality in these patients. COPD gradually climbs to third place as a cause of death among the adult population in the world [1]. Forced expiratory volume in the first second (FEV1) is often taken as a factor that predicts mortality in patients with COPD [2,3]. However, there are other factors such as degree of hypoxia, hypercapnia [4,5], degree of dyspnea [6] and low body mass index (BMI) [7,8] that are often associated with an increased risk of death. In 2008 National UK COPD audit showed that 7.7% of patients hospitalized for exacerbations died during admission, and one third of those who had improvement and were released from hospital, were again hospitalized within 90 days [9]. The total percentage of patients hospitalized for acute respiratory failure due to exacerbation of chronic obstructive pulmonary disease (COPD) as a leading cause of hospitalization, has increased by about two thirds (from 1.8% to 3.0%) [10]. With worsening of the disease leading to the terminal stage, exacerbations, and therefore hospitalizations, are more common, making it possible to identify the reasons for the poor outcome of treatment [11].

In the last two decades noninvasive ventilation (NIV) has become an integral part of the protocol for the treatment of patients with acute respiratory failure. NIV is administration of ventilatory support through the upper airways of a patient using the mask without using the endotracheal intubation. It proved to be an effective treatment in acute exacerbations (AE) of COPD, which together with standard therapy has a series of advantages as opposed to invasive mechanical ventilation. NIV is an intermittent pattern of ventilatory support lasting several hours a day (usually 6-12 h). Important advantage of NIV, compared to invasive ventilation is that NIV allows the patient to eat, speak, and reduce the incidence of complications, especially pneumonia [12], the number of intubations (13,15,16) and mortality (13,14,16).

The proportion of patients who were treated with noninvasive ventilation (NIV) because of AECOPD was increased from 14% in 1998 to 50% in 2008 ($p < 0.0001$), along with a small but significant reduction in the use of invasive ventilation in this group of patients (62% in 1998. vs. 58% in 2008) [10]. NIV treatment is not always successful. The rate of failure is estimated between 5-40% [17]. The success of treatment with NIV depends on factors such as type of the device used, training of the staff, cooperation of the patient, synchronization between the device and the patient, etc.

Aim of the study: To assess the relationship between BMI and outcome of treatment with NIV in patients with AECOPD. Knowledge of the factors of treatment success with NIV helps in avoiding the risk of delayed endotracheal intubation and invasive mechanical ventilation.

MATERIAL AND METHODS

Between September, 2012 and December 2013, a total of 52 patients, over the age of 40, hospitalized because of acute exacerbation of COPD and treated with NIV, were prospectively recruited from the University Clinic of Pulmology and Allergy in Skopje. The COPD diagnosis was established according to the current guidelines [18].

-Inclusion criteria were as follows: severe dyspnea, or obtained score of 3 or 4 on mMRC scale, respiratory rate >25/min., hypoxemia: PaO2<7.3 kPa, hypercapnia: PaCO2>6.1 kPa, respiratory acidosis: pH<7.35.

-Exclusion criteria were as follows: respiratory or cardiac arrest, Glasgow coma scale (GCS)-score<10, hemodynamic instability, altered consciousness, confusion, agitation, facial or chest trauma, recent surgical intervention of the face, upper airways and upper gastrointestinal tract, fixed obstruction of the upper respiratory tract, vomiting, APACHE II score> 25, pregnancy.

Protocol for performing NIV

Firstly, patients were treated with medications and oxygen therapy using nasal cannula no more than one hour. Patients who did not improve in terms of acid-base status were initiated with NIV treatment.

NIV was carried out through a ventilatory support system (BiPAP ST/D, Respironiks). A patient was positioned in a semi-upright sitting position (45^o degrees) in order to minimize the risk of pulmonary aspiration. Oronasal or face mask was used. Expiratory pressure was at its minimum (4 cmH_2O), while the inspiratory pressure was at 10 cmH_2O. The inspiratory pressure was increased for 2 cmH_2O in all patients, until the patient showed signs of discomfort (dyspnea) or increased air leak out of the face mask occurred, or until the pressure of 20 cmH_2O was reached. Similarly, the expiratory pressure was raised, that is, until the appearance of discomfort or the pressure of 7 cmH_2O was reached. The oxygen was delivered until the saturation was 90%. The NIV treatment was carried out during three days or longer, depending of the clinical indications.

The treatment failure was defined as death or necessity of invasive mechanical ventilation. Its manifestations were worsening of the clinical picture that is, worsening of:

1) Ph<0.04 and PaCO2>0.08; 2) coma or seizure disorders; 3) hemodynamic instability (heart rate < 50 bpm/and or systolic blood pressure <70 mmHg; 4) agitation and inability to tolerate the mask.

The treatment success was defined as improvement in the acid-base, as well as clinical status and reversal of the condition at the level present before the exacerbation. Thus, the measured oxygen saturation was expected to be >85% without nasal cannula (i.e. >90% with nasal cannula and oxygen 1-2 L/min); pH>7.35; RR<25/min. without engagement of the accessory respiratory musculature. The commencement of weaning from the NIV was performed with greater pauses during the day or the NIV was used only during the night.

The following data were analyzed: demographic data (age, sex); body mass index (BMI), Glasgow Coma Scale (GCS); Acute Physiology and Chronic Health Evaluation (APACHE) II score; pH; partial pressure of oxygen (PaO_2); partial pressure of carbon dioxide ($PaCO_2$). Arterialized blood sample was drawn from the earlobe.

Statistical analysis

The two groups of patients were compared: group 1 - successful NIV treatment, and group 2 - unsuccessful NIV treatment. The following variables were compared between the two groups: age, sex, BMI, APACHE II score, pH, PaO2 and PaCO2. Chi-square test was used for analysis of the statistical significance of the differences between the two groups. Fisher's exact test was used to test the significance of the number of of male and female patients. Significant difference was considered for $p < 0.05$.

RESULTS

From a total of 52 patients who met the inclusion criteria, early success (in the first 48 hours) of NIV treatment was observed in 41 patients. Late failure after 48 h. of NIV treatment was registered in 4 patients. Total success rate was 71.1% (37 patients).

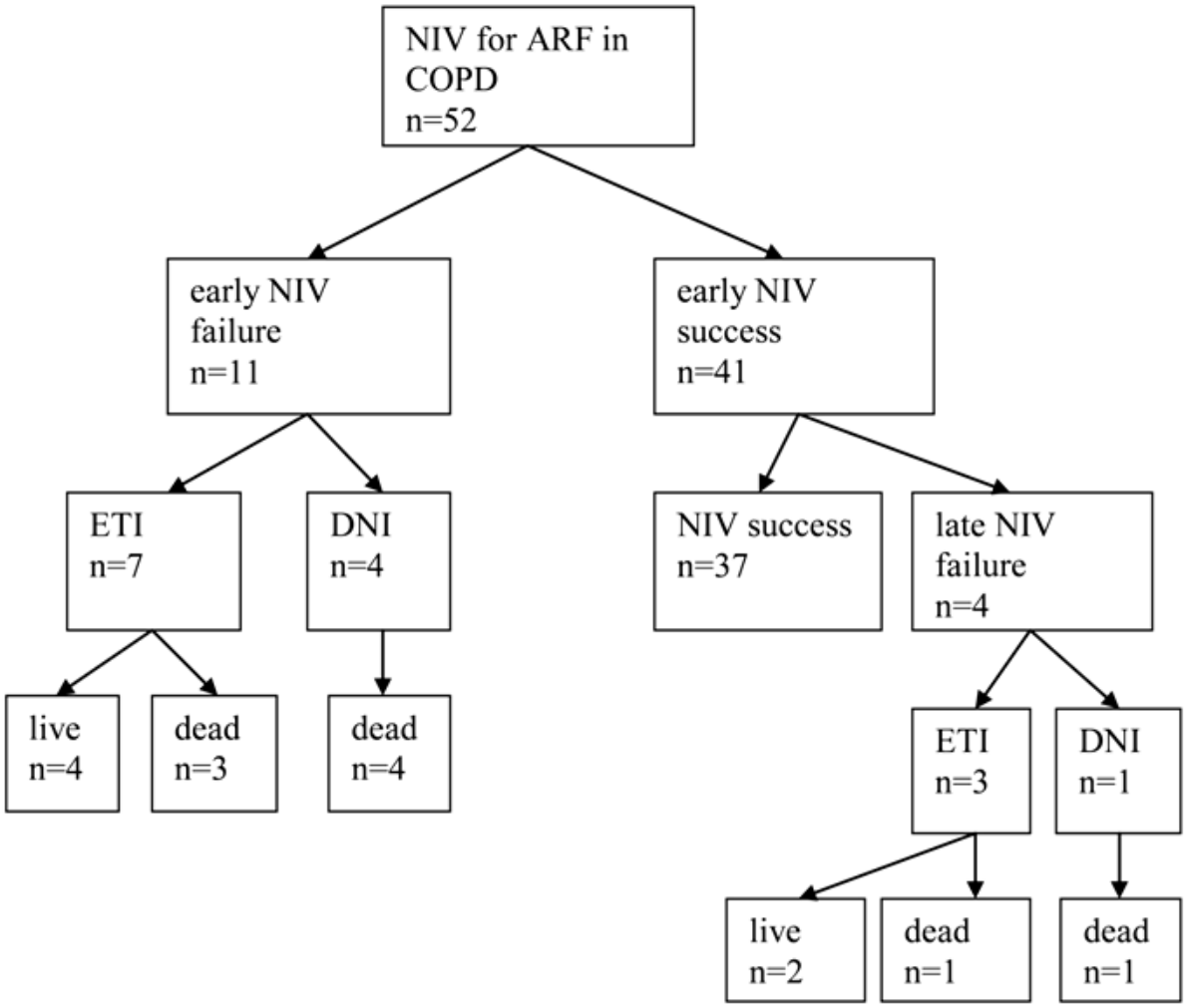


Fig. 1. Flow chart showing patient's outcomes according to early (within first 48 h.) or late (after 48 h.) NIV success or failure

Abbreviations: ETI= endotracheal intubation, DNI= do not intubate, NIV= noninvasive ventilation.

Table 1. Demographic and laboratory characteristics at admission

Total patients randomized	**52**
Age (mean±SD)	67.4±8
Sex (male) [n (%)]	39 (75%)
BMI* (kg/m^2) (mean±SD)	25.5±9
pH (mean±SD)	7.27 ±0.04
PaO_2kPa (mean±SD)	6.5±0.7
$PaCO_2$kPa (mean±SD)	8.5±0.7
APACHE II (mean±SD)**	18±4
GSC* (mean±SD)**	12±2

Abbreviations: BMI= body-mass index (body weight in kilograms divided by squared height in meters),
APACHE** = Acute Physiology and Chronic Health Evaluation,
GCS***Glasgow coma scale, NIV= Noninvasive ventilation.

Patients were divided into two groups: Group 1 – successful NIV treatment and Group 2 – failed NIV treatment.

Table 2. Comparison of the clinical and laboratory characteristics between both groups at the time of admission

Parameters	Group1 successful NIV (n=37)	Group 2 failed NIV (n=15)	p-value
Age	65±5	67±6.0	NS
BMI*	29±6	22±4	p<0.01
Ph	7.30± 0.03	7.24 ± 0.06	p<0.05
PaO_2kPa	6.3± 0.9	6.2± 1.0	NS
$PaCO_2$kPa	8.4±0.9	8.8± 0.8	NS
APACHE II**	18.2±4.0	19.3± 3.0	NS
GSC**	13.0± 1.0	12.6± 1.2	NS

Abbreviations: BMI*=body mass index (body weight in kilograms divided by squared height in meters), APACHE**= Acute Physiology and Chronic Health Evaluation, GCS*** = Glasgow coma scale, NIV= Noninvasive ventilation, NS=non-significant.

There was no significant difference in distribution between the two patients groups in terms of sex and age. Compared to group 1, in the group 2, with failure of NIV treatment, difference was observed in the values of GCS that were lower, and the PaCO2 and APACHE II score that were higher, but the difference between the two groups was not significant (p>0.05). Significant difference was observed between the values of pH (p<0.05) and BMI (p<0.01). The majority of patients with NIV treatment failure were with BMI <22kg/m2.

DISCUSSION

In recent years of increased use of NIV as an alternative to invasive mechanical ventilation, which is associated with a number of adverse effects, it is still unclear when to stop treatment with NIV, when to declare this treatment unsuccessful, and to start with invasive treatment. After analyzing the results obtained in our study, we concluded that the rate of success of treatment with NIV was 71.1%, which is consistent with other studies [19] while the percentage of mortality was 17.3%. As in many other similar studies, degree of acidosis was proved to be a crucial factor predicting the treatment outcome with NIV [20, 21, 22]. Our study included patients who were with similar values of APACHE II score, and GCS score, hence there was no great variation between included subjects, and there was no great difference in these variables between the two groups.

There is evidence that not only the lungs are involved in COPD, but there are many extrapulmonary manifestations of the disease, including malnutrition which is the central problem. In a large number of studies which topic of interest are the factors that predict the outcome of treatment with NIV, the role of BMI in the success of treatment with NIV has not been clarified enough. The results of our study showed that lower BMI, under 22kg/m^2, is associated with an increased risk of failure of treatment with NIV and with increased mortality. Celli *et al.* in their study, including outpatients, also concluded that patients with COPD malnutrition and lower BMI had a worse prognosis [23]. According to Raherison and Girodet, achieving optimal nutritional status can slow down the progression of COPD and reduce the risk of early mortality [24].

The mechanism of nutritional depletion in patients with COPD is connected with various reasons. Acquired muscle atrophy, which occurs in the terminal stage of the disease, is due to physical inactivity, as well as to disorders in the metabolism of proteins, especially NF-kB- activated ubiquitin/proteozomal pathway and apoptosis [25]. The resting energy consumption in COPD patients has been reported to be 15-20% above predicted values, due to the increased energy required for breathing [26]. The connection between COPD with insulin resistance and impairment of glucose metabolism is well-known. Data indicate that impaired glucose regulation is associated with impaired lung function [27]. Patients with COPD also have dyslipidemia associated with metabolic syndrome, as a result of disorder in the lipid metabolism [28]. It is known that hypoxia by stimulating the production of cytokines, stimulating the sympathetic nervous system, and by inducing systemic inflammation, including oxidative stress, is the cause of cachexia in COPD [29].

In patients with low BMI pronounced changes in the lung mechanics and the chest are observed, as follows: reduction of the surfactant, decrease in the elasticity of the lung parenchyma, altered structure of the diaphragm and intercostals muscles, leading to reduction in inspiratory muscle strength and reducing the maximum inspiratory pressure that can be generated [30]. The diaphragm, the most important inspiratory muscle, suffers from muscle degradation as a result of loss of contractile proteins [31]. Causal connection between the success of treatment with NIV in patients with exacerbation of COPD and BMI still remains an enigma. Perhaps patients with a lower BMI require application of a different approach and methodology for application of NIV.

CONCLUSION

Our study showed that the degree of acidosis and BMI are factors of statistical significance for the outcome of NIV treatment. Although PaCO2 and APACHE II score showed higher values in the group with failure of NIV, the values were not statistically significant. Mean BMI in the group that showed failure of NIV was 22 kg/m2, which is relatively less than normal value. Low muscle mass and poor nutritional status have been proven predictors of failure of NIV. NIV failure was the reason for a higher rate of endotracheal intubation and higher mortality. Further studies are needed to clarify the reasons for the association of NIV and low BMI.

REFERENCES:

1. Lozano R, Naghavi M, Foreman K, et al. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet 2012;380: 2095–128.
2. Pauwels RA, Buist AS, Calverley PM, Jenkins CR, Hurd SS. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: NHLBI/WHO Global Initiative for Chronic Obstructive Lung Disease (GOLD) Workshop summary. Am J Respir Crit Care Med. 2001;163:1256-76.
3. Definitions, epidemiology, pathophysiology, diagnosis, and staging. Am J Respir Crit Care Med. 1995;152:S78-S83.
4. Nocturnal Oxygen Therapy Trial Group. Continuous or nocturnal oxygen therapy in hypoxemic chronic obstructive pulmonary disease: a clinical trial. Ann Intern Med. 1980; 93:391-8.
5. Intermittent positive pressure breathing therapy of chronic obstructive pulmonary disease: a clinical trial. Ann Intern Med. 1983; 99:612-20.
6. Nishimura K, Izumi T, Tsukino M, Oga T. Dyspnea is a better predictor of 5-year survival than airway obstruction in patients with COPD. Chest. 2002;121:1434-40.
7. Schols AM, Slangen J, Volovics L, Wouters EF. Weight loss is a reversible factor in the prognosis of chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 1998;157:1791-7.
8. Landbo C, Prescott E, Lange P, Vestbo J, Almdal TP. Prognostic value of nutritional status in chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 1999;160:1856-61.
9. Royal College of Physicians, British Thoracic Society, British Lung Foundation. Report of the National Chronic Obstructive Pulmonary Disease Audit 2008: Clinical audit of COPD exacerbations admitted to acute NHS trusts across the UK London: Royal College of Physicians, 2008.
10. Funk G, Bauer P, Burghuber OC, Fazekas A, Hartl S, Hochrieser H, Schmutz R, Metnitz P, Prevalence and prognosis of COPD in critically ill patients between 1998 and 2008. Eur Respir J. 2013;41:792-9.
11. Anzueto A, Leimer I, Kesten S. Impact of frequency of COPD exacerbations on pulmonary function, health status and clinical outcomes. Int J Chron Obstruct Pulmon Dis. 2009;4:245-51.
12. Kramer B. Ventilator-associated pneumonia in critically ill patients. Ann Intern Med. 1999;130:1027–8.
13. Brochard L, Mancebo J, Wysocki M, et al. Noninvasive ventilation for acute exacerbations of chronic obstructive pulmonay disease. N Engl J Med 1995;333:817–22.
14. Bott J, Carroll MP, Conway JH, et al. Randomised controlled trial of nasal ventilation in acute ventilatory failure due to chronic obstructive airways disease. Lancet. 1993;341:1555–7.
15. Kramer N, Meyer TJ, Meharg J, et al. Randomized, prospective trial of noninvasive positive pressure ventilation in acute respiratory failure. Am J Respir Crit Care Med. 1995;151:1799–806.
16. Plant PK, Owen JL, Elliott MW. A multicentre randomised controlled trial of the early use of non-invasive ventilation for acute exacerbations of chronic obstructive pulmonary disease on general respiratory wards. Lancet. 2000;355:1931–5.
17. Nava S, Ceriana P. Causes of failure of noninvasive mechanical ventilation. Respir Care 2004; 49(3):295-303.
18. Rabe KF, Hurd S, Anzueto A, Barnes PJ, Buist SA, et al. Global strategy for the diagnosis, management and prevention of chronic obstructive pulmonary disease: GOLD executive summary. Am J Respir Crit Care. Med 2007;176: 532-55.
19. Meduri GU, Turner E, Abou-Shala N, et al. Non-invasive positive pressure ventilation via face mask: first-line intervention in patients with acute hypercapnic and hypoxemic respiratory failure. Chest 1996;109:179–93.
20. Anton A, Guell R, Gomez J, Serrano J, Castellano A, Carrasco JL et al. Predicting the result of noninvasive ventilation in severe acute exacerbations of patients with chronic airflow limitation. Chest 2000;117:828-33.
21. Confalonieri M, Garuti G, Cattaruzza MS, Osborn JF, Antonelli M, et al. A chart of failure risk for noninvasive ventilation in patients with COPD exacerbation. Eur Respir J. 2005;25:348-55.

22. Ambrosino N, Foglio K, Rubini F, et al. Non-invasive mechanical ventilation in acute respiratory disease due to chronic obstructive pulmonary disease: correlates for success. Thorax. 1995;50:755–7.
23. Celli BR, Cote CG, Marin JM, Casanova C, Montes de Oca M, Mendez RA, et al. The body-mass index, airflow obstruction, dyspnea, and exercise capacity index in chronic obstructive pulmonary disease. N Engl J Med. 2004;350(10):1005–12.
24. RaherisonG, Girodet PO. Epidemiology of COPD. Eur Respir Rev 2009;18:213-21.
25. Agusti A, Morla M, Sauleda J, et al. NF-kappaB activation and iNOS upregulation in skeletal muscle of patients with COPD and low body weight. Thorax. 2004;59:483–7.
26. Ezzell L, Jensen GL. Malnutrition in chronic obstructive pulmonary disease. Am J Clin Nutr. 2000;72:1415–6.
27. McKeever TM, Weston PJ, Hubbard R, et al. Lung function and glucose metabolism: An analysis of data from the third National Health and Nutrition Examination Survey. Am J Epidemiol. 2005;161:546–56.
28. Marquis K, Maltais F, Duguay V, et al. The metabolic syndrome in patients with chronic obstructive pulmonary disease. J Cardiopulm Rehabil. 2005;25(4):226–32.
29. Andreas S, Anker SD, Scanlon PD, et al. Neurohumoral activation as a link to systemic manifestations of chronic lung disease. Chest 2005;128:3618–24.
30. Chamberlain JS. Cachexia in Cancer-Zeroing in on myosin. N Engl J Med. 2004;351:2124–5.
31. Ottenheijm CAC, Heunks LMA, Sieck GC, et al. Diaphragm dysfunction in chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 2005;172:200–5.

UDC:616.717.46-002.4

AVASCULAR NECROSIS OF THE TRAPEZOID BONE

Samardziski Milan[1], Dew YT[2], Todorova T[3]
[1]University Clinic of Orthopaedic Surgery - Medical Faculty, "Ss. Cyril and Methodius" University, Skopje, R. Macedonia; [2]The Hand Center - Greenville, South Carolina, United States of America; [3] Medical Faculty, "Ss. Cyril and Methodius" University, Skopje, R. Macedonia

ABSTRACT

Avascular necrosis of the trapezoid bone is a rare condition, with very few cases described in the literature. There are numerous different causes for osteonecrosis, each with a different way in which the blood supply becomes obliterated. Immobilization, vascularized bone grafts, arthroplasty and osteotomy are possible treatment options.

Keywords: avascular necrosis, trapezoid bone

INTRODUCTION

Avascular necrosis of the carpal bones is a very rare condition [1, 2, 3, 4, 5]. The majority of cases reported concern about the avascular necrosis of the lunate bone, the scaphoid bone and the capitate bone. The usual symptoms are: weak grip, stiffness and wrist pain [2].

CASE REPORT

We report a case of 14-year-old patient, a child diagnosed with avascular necrosis of the trapezoid bone on the right limb as a consequence of previously experienced trauma. The patient underwent medical examination because of a possible fracture on his right hand. After through physical examination, we came to a conclusion that there were no restricted movements in the hand, nor changes in the skin or muscle atrophy. Hand examination showed light sensitivity to pain at the base of the thumb. The patient felt no pain to deeper touch or to flexion and extension movements of the second metacarpal bone. Motor function was preserved i.e. the patient was able to perform flexion movement of 60 degrees and extension movement of 60 to 80 degrees as well as complete prone and supine position. During the examination no instability was observed. X-ray revealed changes in the trapezoid bone and possible coronal fracture (Figure 1, 2), which was also confirmed with MRI scans (Figure 3, 4). There were no immobilization or restricted movements pursued.

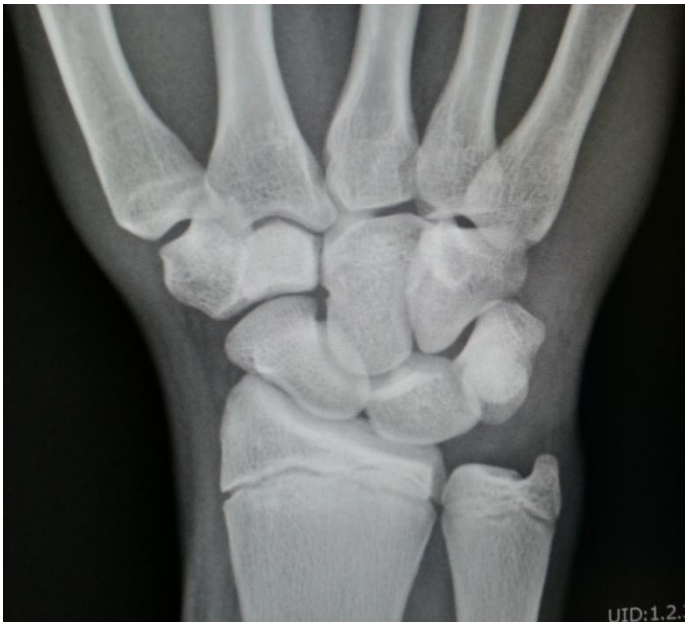


Fig. 1. X-ray of the hand (minimal sclerosis of the trapezoid is visible

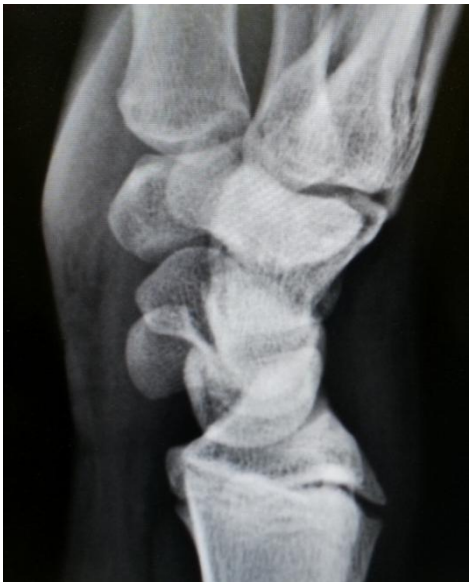


Fig. 2. X-ray -lateral view of the hand

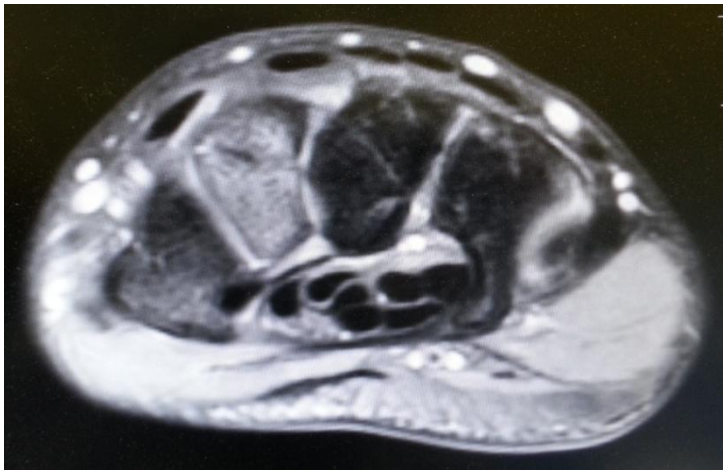


Fig. 3. MRI-transversal view of the hand (avascular necrosis of the trapezoid is detectable)

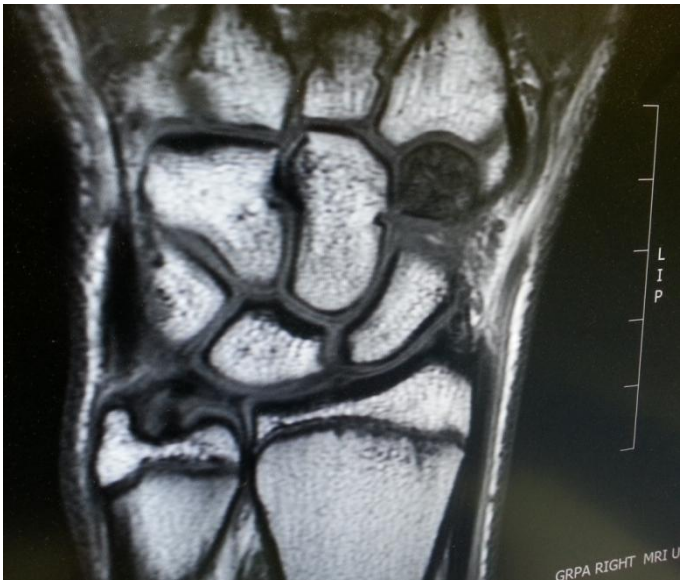


Fig. 4. On MRI-coronal view avascular necrosis of trapezoid is well defined

The medical examination after 3 months indicated that the patient's condition was significantly improved, the bone was still sclerotic but no collapse was observed. (Figure 5, 6, 7)

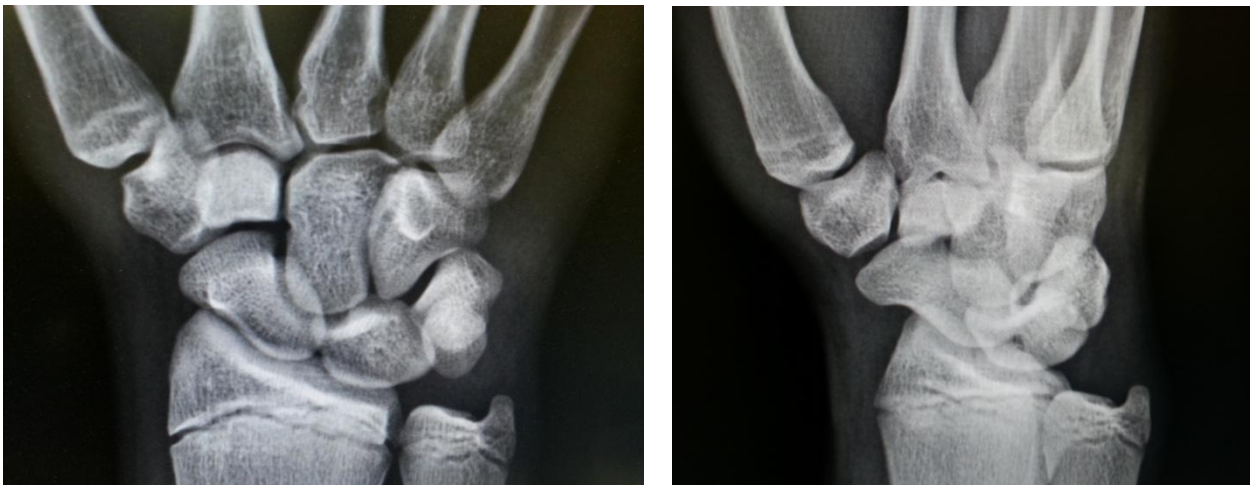


Fig. 5-6. Sclerosis of the bone necrosis is „late sign" on X-rays

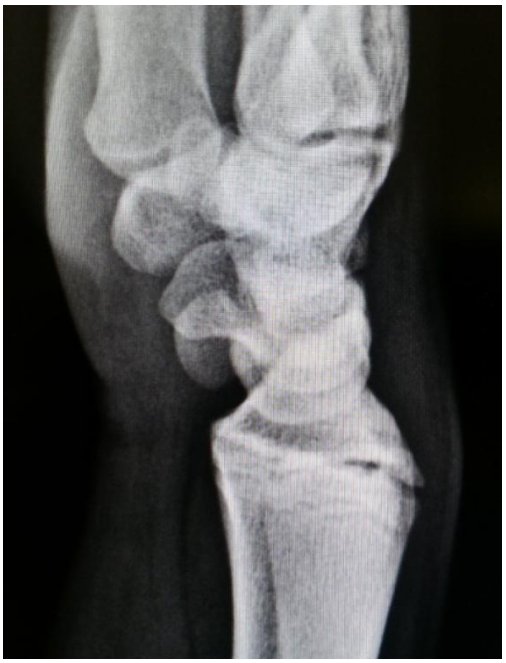


Fig. 7. Sclerosis of the necrotic trapezoid could easily be missed on lateral X-ray

DISCUSSION

Risk factors for osteonecrosis of the carpal bones are: trauma, alcohol consumption and corticosteroids [2]. In our case, the avascular necrosis of the trapezoid bone was a result of a trauma i.e. the patient was engaged in parkour and hiking. The necrosis of the bones may also be due to: arterial occlusion, microtrauma, intraosseous evaluated pressure and venous congestion. Once pathogenesis is completed, the bone begins with angiogenesis in order to reestablish vascularization, whereupon the resorption on the damaged bone tissue occurs [6, 7, 8, 9, 10]. Given that the articular surface uses its nutrients of the synovial fluid, the ability of the bone to regenerate the subchondral tissue will determine the extent of the collapse and the possible future fractures. The best way to confirm the diagnosis is MRI imaging due to greater sensitivity than the radiographic and bone scintigraphy techniques [4]. In certain cases, the osteoporosis, osteoarthritis and subchondral cysts can give a false picture for osteonecrosis on the very MRI imaging. The natural course of osteonecrosis is unpredictable. Revascularization of the bone may occur, but otherwise the condition can progress to bone sclerosis or fragmentation [2].

CONCLUSION

In most cases the treatment of avascular necrosis consists of immobilization, but the surgical interventions like arthodesis and osteotomy are not excluded. In the treatment of avascular necrosis, some surgeons suggest the use of vascularized bone grafts to stimulate the bone revascularization.

REFERENCES

1. Gelberman RH, Gross MS. The vascularity of the wrist. Identification of arterial patterns at risk. Clin Orthop. 1986;202:40-9.
2. Sturzenegger M, Mencarelli F. Avascular necosis of the trapezoid bone. J Hand Surg Br. 1998;23(4):550-1.
3. Bain GI, Durrant AW. Arthroscopic assessment of avascular necrosis. Hand Clin. 2011;27(3):323-9.
4. Cristiani G, Cerofolini E, Squarzina PB, Zanasi S, Leoni A, Rom-agnoli R, et al. Evaluation of ischaemic necrosis of carpal bones by magnetic resonance imaging. J Hand Surg.1990;15B:249–55.
5. D'Agostino P, Townley A William, Roulot E. Bilateral avascular necrosis of the trapezoid. J Hand Surg 2011;36A:1678–80.
6. Golimbu CN, Firooznia H, Rafii M. Avascular necrosis of carpal bones. Magn Reson Imaging Clin N Am 1995;3:281–303.
7. Cuenod P, Della Santa DR. Open dislocation of the trapezoid. J Hand Surg Br. 1995;20(2):185-8.
8. Panagis JS, Gelberman RH, Taleisnik J, Baumgaertner M. The arterial anatomy of the human carpus. part II: The intraosseous vascularity. J Hand Surg Am. 1983;8(4):375-82.
9. Gelberman RH, Panagis JS, Taleisnik J, Baumgaertner M. The arterial anatomy of the human carpus. part I: The extraosseous vascularity. J Hand Surg Am. 1983;8(4):367-75.
10. Freedman DM, Botte MJ, Gelberman RH. Vascularity of the carpus. Clin Orthop. 2001(383):47-59.

UDC:616.714.1-001.45-089.87-06-002.7

PERFORATING GUNSHOT INJURIES TREATED WITH DECOMPRESSIVE CRANIECTOMY AS A FOUNDATION FOR BOTH EARLY AND LATE SURGICAL COMPLICATIONS AND SECONDARY BACTERIAL SUPERINFECTIONS. A CASE REPORT.

Gavrilovska Dimovska Aleksandra[1], Milenkovik Z[2], Chaparoski A[1]

[1]University Clinic of Neurosurgery; [2]Clinic of Infectious Diseases and Febrile Conditions, Skopje, Macedonia

ABSTRACT

Perforating injuries to the head, though less prevalent among the general population compared to the closed head injuries, is a condition which represents a great challenge in management for every neurosurgical institution and is followed with poorer prognosis, numerous perioperative and postoperative surgical complications, and in certain cases by a severe disability. In this article we present a case of a patient with opened, perforating head injury inflicted with a firearm from a relative proximity that was treated in our neurosurgical institution. We discuss and present the latest surgical guidelines for management of patients with perforating gunshot wounds to the head, the perioperative preparation, planning and realization of an operative protocol, as well as the approach in treatment of the postoperative complications. In particular, we review the decompressive craniectomy and local debridement of the surrounding tissues, and the early complications and secondary bacterial infections which are directly inflicted to the postoperative outcome and incidence. The focus of interest of this article is also the choice of empiric antimicrobial therapy and prophylactic use of antibiotics.

Key words: intracranial hypertension, decompressive craniectomy, surgical infections

INTRODUCTION

Gunshot injuries to the head represent one of the main reasons for perforating trauma to the head and brain, and make up almost 35% of all the causes for death after brain injury for persons under the age of 45 [1]. These injuries to the head have the highest mortality rate - two thirds of the injured die at the place of the trauma, and are also the main cause of death in 90 % of the victims. For those who survive the initial trauma, there is still extensive discussion as regards the initial neurosurgical treatment [2, 3, 4, 5].

The decision for the continuing treatment including the best therapy from which the patient would have the most benefit is still full of controversies, even for the most up-to-date specialized neurotrauma centers. Patients with extensive brain disabilities, bilateral mydriasis, unstable and fluctuating vital parameters, or no truncal reflexes are the least likely to have any benefits from an operative treatment. In those cases supportive measures are indicated (namely the possibility of cadaveric explantations, or for the family to adjust to the new situation and the time frame for determining brain death). For those patients who can have an operative treatment, fast deterioration and signs of brain herniations warrant a rapid surgical intervention [1, 2, 3, 4].

Decompressive craniotomy could be performed in two different situations, such as prophylactic decompression or primary decompressive craniotomy, with or without debridement of the local tissue, in patients where the main point is evacuation of the intracranial lesion of some kind, all with the aim to avoid the postoperative rise of the intracranial pressure, not to control the resistance of the same one. The decision for a decompressive craniotomy is made without concerns for the value of the intracranial pressure and is usually based on the CT scans. Another option is the so called therapeutic decompressive craniotomy, or secondary decompressive craniotomy, which is indicated for control of the elevated intracranial pressure, which is resistant to all previously mentioned modalities .The same procedure could be performed after osteoplastic craniotomy and evacuation of the lesion in patients who develop massive unilateral or bilateral brain edema postoperatively, and the point of the intervention is surgical decompression and control of the elevated intracranial pressure [6, 7].

Very common complications are infections in patients with open trauma to the head and brain - these are associated with very high morbidity and mortality. The risk of local infections of the entry and exit wounds, of meningitis or of cerebral abscess is very high in patients with open trauma to the brain and head, especially because of the contamination with skin, hair, osseous fragments or alien bodies which are forcefully introduced into the brain with the trajectory of the projectile. Infective complications are more common with the presence of liquorea, communication of cranial cavity with paranasal sinuses and the nose cavity, and transventricular trajectory of the projectile. Higher incidence of intracranial infections are documented in military conditions (4 to 11%), compared with normal conditions (1 to 5%).

What is a rare theme for discussion is the importance of the right use of prophylactic antibiotic therapy against super infections with the victims with open gunshot trauma to the head and brain. The use of prophylactic and empiric therapeutic use of antibiotics is still big on controversies and is still the goal for more ongoing randomized trials [8, 9, 10].

CASE REPORT

The patient B.V., aged 63, was brought to the Urgent Surgical Center with a gunshot trauma to the head from a relative closeness. At first sight, the patient was conscious, agitated, when asked direct questions he gave confused answers; his GCS was with stable vital parameters - Ta 177/85 mmHg, pulse 73/min, SaO2 100%. His pupils were with the reflex of SAK, narrow and symmetric, without signs for focal neurologic deficits. When we made an inspection of the head, there were two wounds - one entry wound and one exit wound with bi-parietal projection. On the CT scan with bone and parenchyma window there was traumatic subdural hematoma on the right brain hemisphere and focal subarachnoid hemorrhagic on the right high parietal. There was impressive comminuted fracture high right parietal with abris fragment in the brain parenchyma from the same side, as well as an impressive fracture on the left side but without compression on the bone window. We did x-rays of the neck, thorax and abdomen, without any signs of trauma. The patient was admitted to the ICU unit on the Neurosurgery Department. After admittance we made absolute indication for operation, and after anesthetic preparation of the patient, we put the patient in the OR. Preoperatively, we introduced double broad-spectrum antibiotic prophylactic therapy with Ceftriaxone 2x1 grams and Amikacin from 500 mg 3x1, which was then continued postoperatively. The patient was put on the operation table in the supine position, with the head on the left, with exposed right frontotemporoparietal region on the head holder without any fixation. After isolation of the operating fieled, we did a concave incision on the full thickness of the skin including the periostheum in the projection of the right frontotemporoparietal region. After thorough haemosthasis on the skin and periostheum, we did trepanation round the depressive fracture with 4 holes with craniotomy, after which the bone flap was isolated with the fracture. After visualization of the durra, which was under pressure and elevated, with local defect of 1.5 to 1 cm, we did thorough haemosthasis and made a concave incision with excision of the defect of durra. Underneath there was an acute collection of subdural haematoma, which was evacuated immediately. Then we visualized the brain with the blood in the arachnoidal space. Brain giruses where wasted because of the global brain edema with laceration on the projection of lower parietal lobulus and supramarginal girus with detection of small bone fragment impetrated in the brain tissue. We did thorough haemosthasis and extracted the impetrated in the brain tissue. Local plastic of the durra was done using local periosteum, after which local defect of durra was closed. Bone flap was reintroduced on the pace of the craniotomy. Suture was done by the layers and epicranial drainage system was placed with passive drainage, which was extracted on the second postoperative day.

Postoperatively, the patient was with stable vital parameters, febrile, without signs of neurological lateralization. The wound was dry, clean, without signs of inflammation on the edges of the skin incision, without leakage from the same. In the course of the hospitalization, from the 8th day onward, he had sub febrile temperatures up to 39° from time to time, with headaches and leakage from the incision. We did a couple of lumbar punctures, after which the liquor was sent for cytological, biochemical and microbiological analyses. the liquor was bright, Pandy positive with minimal inflammatory elements, microbiologically sterile. During the hospitalization, the cytological findings from the liquor were that it was with albuminorachya, elevated lactates and elevated elements. Microbiologically, by way of an antibiogram, Enterobacter Cloacae, sensitive on Meronemum and Amikacinum, was isolated, after which he was placed on double antibiotic therapy. There were indications for lumbar drainage. During the antibiotic regiment there was an allergic reaction with generalized urticaria on the most of the sensitive antibiotics after which the treatment was continued with Meronemum and Vancomycinum. Those were administered simoultaneousy, 2 grams every 8 hours, with every risk for an allergic reaction for which was given a standard antiallergenic therapy. After subsequent cytological and microbiological analyses, Enterococcus sensitive to Vancomycinum, which was contraindicated because of an allergic reaction to it, was isolated.

On the control CT scans of the brain, there was suppurative subdural collection, with compression. It indicated a new operative treatment for the evacuation of the suppurative collection.

Over the course of the next couple of weeks, the treatment continued and there were alterations in the patient's condition ranging from consciousness to deep stupor. After a longer period of time, the patient slowly started to stabilize. On the control lumbar punctures there was tendency for betterment, which was supported by the clinical findings. After the patient was stable clinically and it was confirmed by the laboratory results, he was sent home in a good general and neurological condition.

DISCUSSION

In spite of its limitations, the Glasgow Coma Scale, which was introduced in 1974 by Teasdale and Jennett, is the most widely used system for grading head and brain traumas and is incorporated in numerous trauma classification systems. According to it, we can differentiate the easy (GCS 13-15), medium (GCS 9-12) and severe trauma to the head and brain (GCS 3-8), or those which deteriorate to the score of 8 or less for a shorter time interval. Although in the last couple of decades there was a significant understanding of the patophysiological mechanisms of brain injuries, what is in the core of the understanding is the notion that neurological damage does not start at the moment of trauma, but in the first couple of hours or days after the trauma. Today we understand the destroying effects of different cascades of complications which arise from these injuries, and which we can follow with contemporary monitoring machines on both clinical and biochemical levels [11, 12, 13].

The role of initial assessment of the extent of the injury in the clinical conditions of these injuries consists of using the standard measures of initial treatment and resuscitation in the patients with isolated severe trauma to the head and brain according to the guidelines of Advanced Trauma Life Support, which include maintaining airway, breeding and circulation, prevention of hypoxia, prevention of hypotension, short primary neurological examination (including assessment of the level of consciousness according to the GCS, examination of reactivity and equality of the pupils, and looking out for focal neurological deficits), inspection of face and cranium for entry and exit wounds, inspection of the spine for deformity or open fractures, full body examination and evaluation of other life threatening injuries [14].

Postoperative surgical infections have a significant role in morbidity and mortality in the patients with severe trauma to the head and brain, and have a significant influence on the length of the hospital stay, the outcome and the cost of the treatment. The risk from hospital infections, especially with this randomized group of patients, is highly elevated because of invasive monitoring and prolonged mechanical ventilation.

The incidence of preoperative and postoperative super infections is high because of contaminations introduced by foreign bodies, hair, skin, clothes and bone fragments which can be introduced in the brain from the trajectory of the projectile.

Tabel 1. Guidelines for surgical treatment of perforating brain injuries.

	Guidelines for surgical treatment of perforating brain injuries
1.	Debridement of devitalized tissue
2.	Removal of volume occupying lesion
3.	Removal of bone fragments and foreign bodies in the regions that are available
4.	Adequate haemostasis
5.	Reconstruction of durra
6.	Complete closure of skin on the place of the operative incision

According to Whitaker, over the course of the First World War, before the arrival of antibiotics, the rate of super infection was almost 60%. During the course of the Second World War, with the introduction of penicillin, there was a significant fall of incidence of surgical super infections (6-13%). Recent studies with high specter antibiotics show incidence 1-11%, with a trend for bigger incidence of super infections during war times [16, 17].

Most important risk factors for super infections are postoperative liquor fistulae and dehiscence of the operative wound.

Most common pathogen that is isolated from the liquor from the patient with super infection is Staphylococcus Aureus. Other common pathogens that are isolated are Streptococcus, Acinetobacter, Escherichia coli, Klebsiella and Enterobacter.

Inclusion of a broad spectrum antibiotic therapy is necessary in all the patients with open trauma to the head and it is also empirically based. According to scientific data, it is recommended to introduce broad spectrum antibiotic prophylaxis in the initial treatment of these patients. Though the dilemma as to which antibiotic regime is the most efficient is still unresolved. According to Kaufman and all 87% of neurosurgeons of the American Association of Neurological Surgery (AANS) use cephalosporins for treatment of surgical super infections, 24% use chloramphenicol, 16% use penicillin antibiotics, 12% use aminoglycoside, and 6% use vancomycin. The length of use of broad spectrum antibiotic therapy is also a part of the discussions, with very little scientific data to draw any conclusions yet. Both these problems are the goal of many prospective randomized studies [14, 15, 16, 17].

At our department, we routinely use two protocols for preoperative antibiotic prophylaxis in patients with open craniocerebral injuries. One of the protocols is with Ceftriaxone of 2 grams before operation, plus 2 grams every 24 hours for 5 days afterwards. The other protocol is Cefuroxime from 1.5 grams before operation, plus 750 mg every 12 hours for 5 days afterwards.

Most common complications from post operative super infections are secondary epilepsy and obstructive hydrocephalus. Epilepsy attacks occur during the hospital stay in 10% of the cases, though the rate for secondary epilepsy is from 35% to75% after the hospital treatment.

CONCLUSION

The goal of this paper is to present a study of a case of gunshot trauma patient to the head with perforating injury with bi-parietal trajectory who was treated at the University Clinic for Neurosurgery in Skopje. At the same time, from our point of view and with our expertise we give a short overview of guidelines for treatment of patients with open injury to the head, primary treatment, and the choice for primary decompressive craniotomy with debridement of devitalized tissue around the entry and exit wounds of the projectile and local plastic of the durra at the place of the injury with autologous transposition of local periosteum, the way of closure of the defect of durra with special accent on post operative treatment of surgical complications, especially of bacterial super infections.

Bacterial super infection in our case lead to suppurative meningitis, rise of subdural empyema at the place of decompressive craniotomy, limitation of use of antibiotic prophylaxis, and a very narrow window of treatment according to the antibiogram concerning the injured patient because of his multiple allergies to antibiotics.

Open gunshot injuries to the head considerably differentiate in mortality and morbidity from closed craniocerebral injuries, especially for the course of beginning, direct entry gate from the entry and exit wound of the projectile and destruction of the brain tissue and its structures along the trajectory of the projectile. Introducing the guidelines for conservative and surgical treatment of these patients it is very important notion for standardizing the treatment protocols for these patients which influence the rate of arising of post operative infections and the end of treatment. There is a trend for lesser aggressive debridement of deeply imbibed necrotic structures, bone fragments and foreign bodies along the trajectory of the projectile through the brain tissue and bigger inclusion of more aggressive antibiotic prophylaxis for the better outcome. Though there is still a need for bigger multi centric randomized control studies with the goal of continued studying and betterment of current guidelines.

REFERENCES:

1. Benzel EC, Day WT, Kesterson L, et al. Civilian craniocerebral gunshot wounds. Neurosurgery. 1991;29:67-72.
2. Haines SJ. Systemic antibiotic prophylaxis in neurological surgery. Neurosurgery. 1980;6:355-61.
3. Stone JL, Lichtor T, Fitzgerald LF. Gunshot wounds to the head in civilian practice. Neurosurgery. 1995;37:1110-2.
4. Levy ML, Masri LS, Lavine S, Apuzzo ML. Outcome prediction after penetrating craniocerebral injury in a civilian population: aggressive surgical management in patients with admission Glasgow Coma Scale scores of 3, 4, or 5. Neurosurgery. 1994;35:77-85.
5. Grahm TW, Williams FC Jr, Harrington T, Spetz ler RF. Civilian gunshot wounds to the head: a prospective study. Neurosurgery. 1990; 27:696-700.
6. Hagan RE. Early complications following penetrating wounds of the brain. J Neurosurg. 1971; 34:132-41.
7. Traumatic brain injury. A ESICM Multidisciplinary distance learning programmefor intensive care training (PACT), 2000.
8. Bullock MR, Povlishock JT, eds. Brain Trauma Foundation, AmericanAssociation of Neurological Surgeons, Congress of Neurological Surgeons,AANS/CNS Joint Section on Neurotrauma and Critical Care.Guidelines for the management of severe traumatic brain injury. 3rd ed J Neurotrauma. 2007;24(suppl 1):S1-S106.
9. Bullock MR, Chesnut R, Ghajar J, et al. Guidelines for the surgical managementof traumatic brain injury author group: acknowledgments. Neurosurgery. 2006;58(suppl 3):S2.
10. Aarabi B, Hesdorffer DC, Ahn ES, et al. Outcome following decompressive craniectomy for malignant swelling due to severe head injury. J Neurosurgery 2006;104:469-79.
11. Alderson P, Roberts I. Corticosteroids in acute traumatic brain injury: systemic review of randomized controlled trials. Br Med J.1997;314:1855-9.
12. Aarabi B, Hesdorffer DC, Simard JM, et al. Comparative study of decompressive craniectomy after mass lesion evacuation in severe headinjury. Neurosurgery. 2009;64:927-40.
13. Albanиse J, Leone M, Alliez JR, et al. Decompressive craniectomy for severe traumaticbrain injury: evaluation of the effects at one year. Crit Care Med. 2003;31(10):2535-8.
14. Bayston R, de Louvois J, Brown EM, Johnston RA, Lees P, Pople IK. Use of antibiotics in penetrating craniocerebral injuries. "Infection in Neurosurgery" Working Party of British Society for Antimicrobial Chemotherapy. Lancet. 2000;355:1813–7.
15. Cooper DJ, Rosenfeld JV, Murray L, et al . Decompressive craniectomy in diffuse traumatic brain injury. N Engl J Med. 2011;364(16):1493-502.
16. Kazim SF, Shamim MS, Tahir MZ, et al. Management of penetratingbrain injury. J Emerg Trauma Shock. 2011;4(3):395-402.
17. Ragel BT, Klimo Jr P, Martin JE, et al. Wartime decompressive craniectomy: technique and lessons learned. Neurosurg Focus. 2010;28(5):E2.

UDC:616.346.2-089.87-089.5

THE ANALGESIC EFFECT OF ULTRASOUND-GUIDED TRANSVERSUS ABDOMINIS PLANE (TAP) BLOCK FOR OPEN APPENDECTOMY

Toleska Marija[1], Kuzmanovska B[1], Kartalov A[1], Jankulovski N[2], Shosholcheva M[3], Zdravkovska M[4], Spirovska T[1]

[1]University Clinic for TOARILUC – KARIL, Medical Faculty Skopje; [2]University Clinic for Abdominal Surgery, Medical Faculty Skopje; [3]University Clinic St. "Naum Ohridski", Medical Faculty Skopje, University St. "Cyril and Methodius", Skopje; Faculty of Medical Sciences, University "Goce Delcev", Shtip, Republic of Macedonia

ABSTRACT

Background: Transversus abdominis plane (TAP) block is a (new) regional anesthetic technique that provides analgesia to the parietal peritoneum as well as the skin and muscles of the anterior abdominal wall, by introducing local anesthetic into the neuro-fascial plane between the internal oblique and the transversus abdominis muscles. We evaluated its analgesic efficacy in patients undergoing open appendectomy in a randomized controlled clinical trial.

Methods: Sixty adult patients undergoing open appendectomy were randomized to undergo standard care (n=30) or to undergo a right-sided TAP block with bupivacaine (n=30). All patients received standard anesthetic, and after induction of anesthesia, the TAP group received an ultrasound-guided TAP block. Each patient was assessed after operation by a blinded investigator at 2, 6, 12 and 24 hours after surgery.

Results: Ultrasound-guided TAP block significantly reduced postoperative visual analogue scale (VAS) pain scores at rest and on moving, reduced ketonal and tramadol postoperative consumption and reduced incidence of PONV in the TAP block group after surgery compared to standard care group. There were no complications attributable to the TAP block.

Conclusion: Ultrasound-guided TAP block provides effective postoperative analgesia during the 24 postoperative hours (as a part of a balanced postoperative analgesic regiment) after an open appendectomy.

Keywords: ultrasound, TAP block, open appendectomy

INTRODUCTION

Acute appendicitis is one of the causes for considerable abdominal pain and most common surgical emergency. Postoperative pain after an open appendectomy originates from somatosensory pain of the incision site and from viscera-peritoneum pain due to peritoneal inflammation and infection [1]. Postoperative pain from an appendectomy can be managed with intermittent systemic analgesics such as opioids, ketamine, nonsteroidal anti-inflammatory drugs (NSAIDs), paracetamol or continuous infusion of a mixture of drugs acting through different mechanisms [2]. Multimodal approaches to the provision of postoperative analgesia often incorporate blockade of the abdominal wall, such as ilioinguinal blockade or wound infiltration. The well documented pain relief with ilioinguinal nerve block and wound infiltration is reported to have had a limited duration of action of up to 6-8 hrs. [3].

A substantial component of the pain experienced by patients after abdominal surgery is derived from the anterior abdominal wall incision [4]. The anterior abdominal wall is innervated by nerve afferents that course through the transversus abdominis neuro-vascular fascia plane [5].

The abdominal wall has three muscle layers: external and internal oblique, and transversus abdominis. This muscular wall contains the T7-12 intercostal nerves, the ilioinguinal and iliohypogastric nerves and the lateral cutaneous branches of the dorsal rami of L1-3. The above nerves run in a neurovascular plane between the internal oblique and transversus abdominis muscles and represent the 'target' of local anesthetics [6]. Blocking these sensory nerves supply to the anterior abdominal wall has been reported to provide effective postoperative analgesia after open appendectomy [7].

Transversus abdominis plane (TAP) block is a (new) regional anesthetic technique that provides analgesia to the parietal peritoneum as well as the skin and muscles of the anterior abdominal wall. It was first described by Rafi in 2001 as an analgesic technique for abdominal incisions using a loss of resistance technique at the lumbar triangle of Petit. This technique is also known as anatomical landmark or "blind" technique [8]. Later it was described as technique by using the ultrasound guidance by Hebbard and al. in 2007 [9].

In recent years, transversus abdominis plane (TAP) block has been developed and considered as a peripheral nerve block for postoperative pain control after abdominal surgery including colon resection, caesarean section, prostatectomy, and laparoscopic cholecystectomy [10, 11, 12, 13, 14]. These studies suggested that TAP block used as a method of multimodal balanced analgesia reduces postoperative pain, opioid requirement during the 48 hours after surgery and adverse events from opioids such as sedation, nausea, vomiting, respiratory depression, and ileus.

MATERIAL AND METHODS

After obtaining approval from the Ethics Committee and written informed consent from the patient, there were examined 60 ASA 1-2 patients, aged 20-60 years, scheduled to undergo an open appendectomy, in randomized, prospective, interventional and controlled trial. Patients were excluded if there was a history of allergy to bupivacaine, ketonal and tramadol, coagulopathy, infection at the needle insertion site, pregnant women, women who are breastfeeding and ASA 3-5 patients.

Patients were divided into two groups: one group to undergo US-TAP block with 20 ml 0.5% bupivacaine (TAP block group) and the other group to receive standard care (control group). The primary outcome was time to first analgesia after surgery.

All patients received a standard general anesthetic with standard monitoring. Anesthesia was induced with midazolam 0.04 mg/kg, fentanyl 0.02 mg/kg, propofol 1-2 mg/kg, succinylcholine 1.5 mg/kg. Rocuronium was used for muscle relaxation 0.6 mg/kg. Anesthesia was maintained with oxygen, air and propofol 50-200 microgr/kg/min. Standard monitoring include continuous electrocardiography, noninvasive blood pressure every 5 minutes, heart rate, pulse oxymetry and capnometry. Additionally fentanyl 0.5-1 microgr/kg was injected to control blood pressure and heart rate within 20% of baseline. End-tidal carbon dioxide (CO2) was maintained at 30-35 mmHg. During the study prophylactic antiemetics were not given.

After induction of anesthesia, the patients from the first group had the block performed under ultrasound guidance on the right TAP, using Siemens Acusion X300 system (Siemens, Germany) ultrasound device and high-frequency (6-13 MHz) linear transducer. The skin was prepared with 10% betadine solution. After draping the needle insertion site, the probe was placed transversely on the anterolateral abdominal wall (on the level of the right anterior axillary line) between the iliac crest and the subcostal margin on the right side. The three muscles (external oblique, internal oblique and transversus abdominis) of the anterior abdominal wall were the identifier (Fig.1).

After identification of the neuro-fascial plane between the internal oblique and the transversus abdominis muscle, a 22G x 4” 50 mm needle (B. Braun, Stimuplex, Germany) was introduced anteromedial to the probe in the plane of the ultrasound beam and advanced in a posterolateral direction, by the “in-plane” technique. When tip of the needle reached the TAP between the internal oblique and transversus abdominis muscles, 1 ml of 0.5% bupivacaine were injected into the patients of the TAP block group, after negative aspiration, and the spread of the drug was confirmed. Then the remaining 19 ml was injected. The injectant was seen spreading in the TAP as a dark oval-shaped hypoechoic fluid pocket at TAP with real-time ultrasound imaging. The TAP block was performed by one investigator.

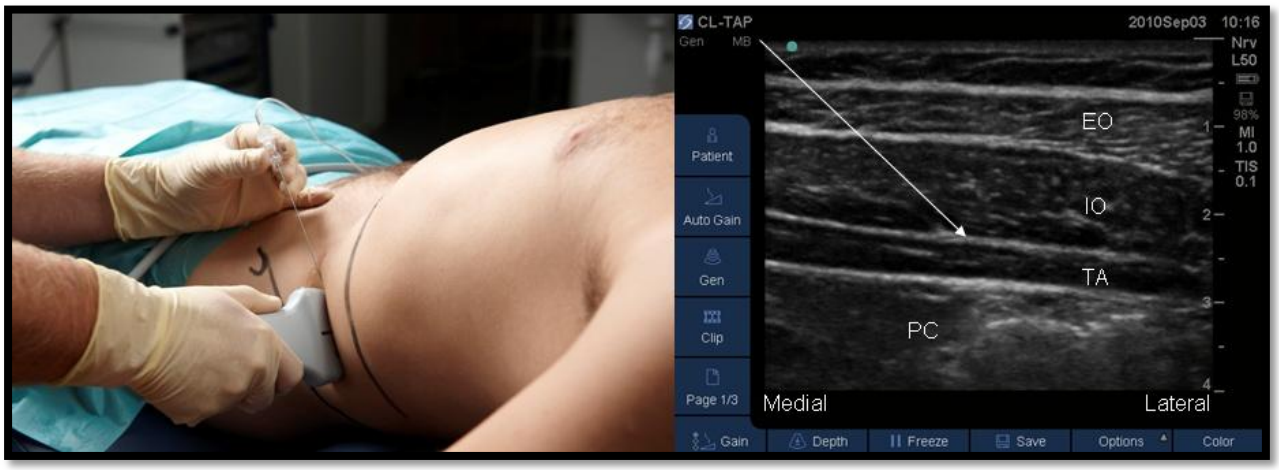


Fig. 1. TAP block as seen via ultrasound: probe-positioned transverse over the lateral wall of the abdominal wall, midway between the subcostal margin and iliac crest

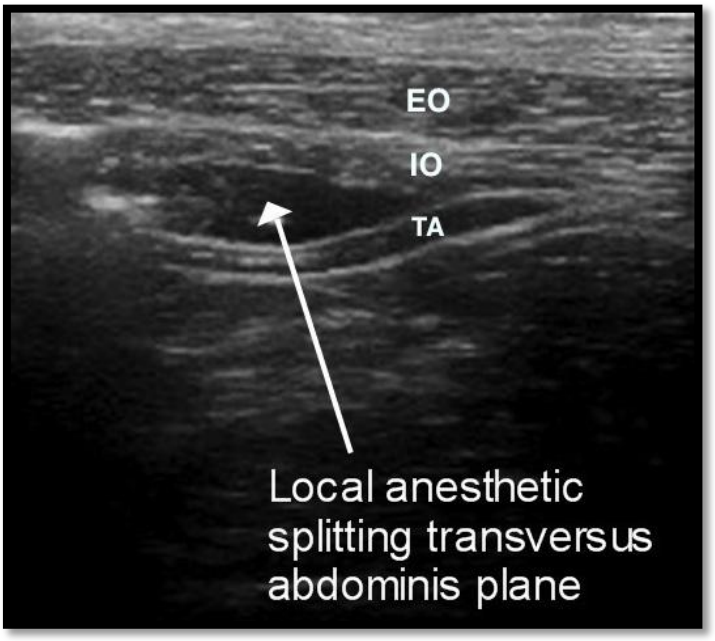


Fig. 2. Spreading of the local anesthetic into the TAP between m. obliquous internus and m. transversus abdominis.

After the completion of the surgical procedure, patients were transferred to the postanesthesia care unit (PACU) and stayed there for 2 hours. There, the patients were monitored. If the VAS score was over 3, then 100 mg ketonal was administered, whereas if the VAS score was over 6, then 100 mg tramadol was administered. The presence and severity of pain and nausea were assessed by an investigator blinded to group allocation. These assessments were performed in the recovery suite 2, 6, 12 and 24 hrs. after operation. All patients were asked to give scores for their pain at rest and on moving and for the degree of nausea at each time point. Pain severity was measured using a visual analogue scale (VAS). Nausea was measured using a categorical scoring system (none=0, mild=1, moderate=2, and severe=3). Rescue antiemetics were offered to any patient who had complained of nausea or vomiting.

The primary outcome measure in this study was the VAS pain scores at rest and on moving 2, 6, 12 and 24 hrs. after the operation. The secondary outcome measure included the postoperative opioid consumption. The third outcome measure included the incidence of postoperative nausea and vomiting (PONV). All these outcomes were systematically assessed by a member of the research team blinded to the group allocation.

STATISTIC ANALYSIS

Description of quantitative data was made with measures of central tendency (arithmetic average) and measures of dispersion (standard deviation). Description of qualitative data was made with relations and proportions. For determining the significance of differences between three and more arithmetic settings in dependent samples, Friedman ANOVA was being used. For determining the importance of differences between two arithmetic settings in dependent samples, Wilcoxon Matched Pairs Test was being used. For determining the relevance of differences between two arithmetic settings and two proportions in independent samples, Mann-Whitney U Test was being used. All results were to be noted as significant if their value was less than $p<0,05$.

RESULTS

Sixty patients entered this study. Thirty patients were randomized to receive standard care and the other thirty were randomized to undergo unilateral ultrasound-guided TAP block with bupivacaine. TAP was easily visualized and successfully done, without any complications or failure of the block, performed by one investigator.

In Table 1 and Diagram 1 average values of VAS score are shown in patients who were operated under general anesthesia and one-sided TAP block at rest and on movement after 2, 6, 12 and 24 hours from operation itself (N1). Analysis of variance showed that there were statistically important differences between average values of VAS score at rest (ANOVA Chi Sqr.=28,41 p=0,00001) as well as the VAS score on movement after 2, 6, 12 and 24 hours (ANOVA Chi Sqr.=41,46 p=0,000001) in this examined group. VAS score is smallest after 2 hours, and after that significantly raises, upon which it increases mostly after 24 hours.

Table 1. Mean values of VAS score in patients who received general anesthesia (GA) + one-sided TAP block at rest and on movement 2, 6, 12 and 24 hours after surgery (N1)

TIME	VAS SCORE AT REST				VAS SCORE ON MOVEMENT			
	AVERAGE	SD	MIN	MAX	AVERAGE	SD	MIN	MAX
After 2 h	1.6	0.76	1	3	1.8	0.80	1	3
After 6 h	2.0	1.14	1	6	2.6	1.67	1	7
After 12h	2.37	1.29	1	5	2.6	1.18	1	5
After 24h	2.6	1.59	1	7	3.3	1.59	1	6

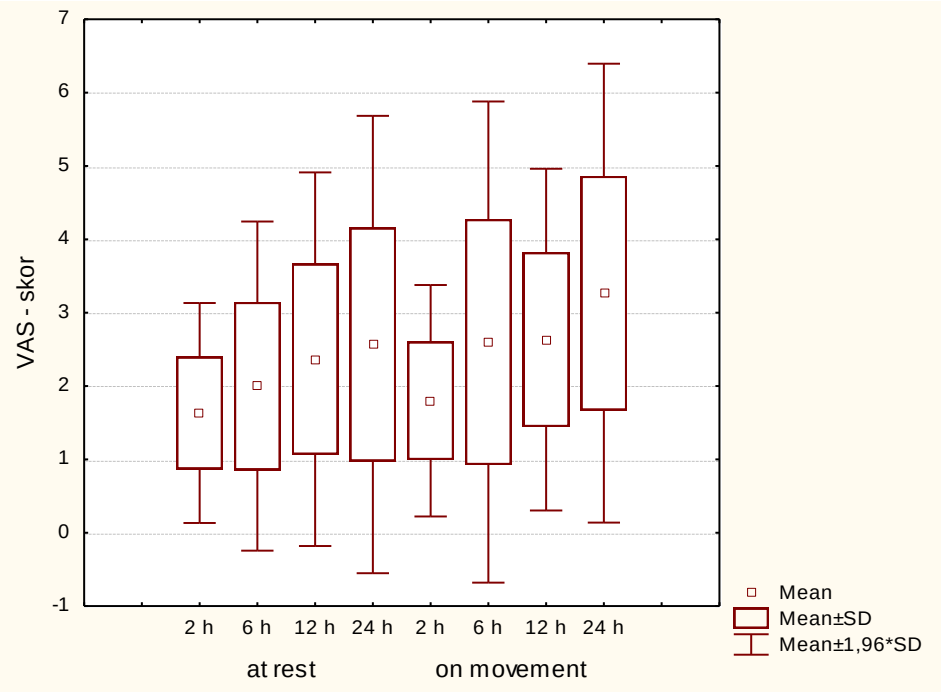


Fig. 3. Mean values of VAS score in patients who received general anesthesia (GA) + one-sided TAP block at rest and on movement 2, 6, 12 and 24 hours after surgery (N1)

In Table 2 and Diagram 2 mean values of VAS score are shown in patients operated in general anesthesia at rest and on movement after 2, 6, 12 and 24 hours from the operation itself (N2-CG). Analysis of variance showed that there were statistically important differences between mean values of VAS score at rest (ANOVA Chi Sqr.=43,75 p=0,000001) as well as the VAS score on movement 2, 6, 12 and 24 hours (ANOVA Chi Sqr.=47,15 p=0,000001) after surgery only under general anesthesia. VAS score is smallest after 2 hours, and after that significantly raises 6, 12 and 24 hours after surgery (Table 2, Diagram 2).

Table 2. Mean values of VAS score in patients who received GA at rest and on movement 2, 6, 12 and 24 hours after surgery (N2-CG).

TIME	VAS SCORE AT REST				VAS SCORE ON MOVEMENT			
	AVERAGE	SD	MIN	MAX	AVERAGE	SD	MIN	MAX
After 2 h	4.0	0.95	3	6	3.9	0.86	2	5
After 6 h	4.8	0.94	3	7	5.4	1.45	3	8
After 12h	4.6	0.89	3	6	5.6	0.89	4	7
After 24h	6.2	1.06	4	8	5.5	0.57	4	6

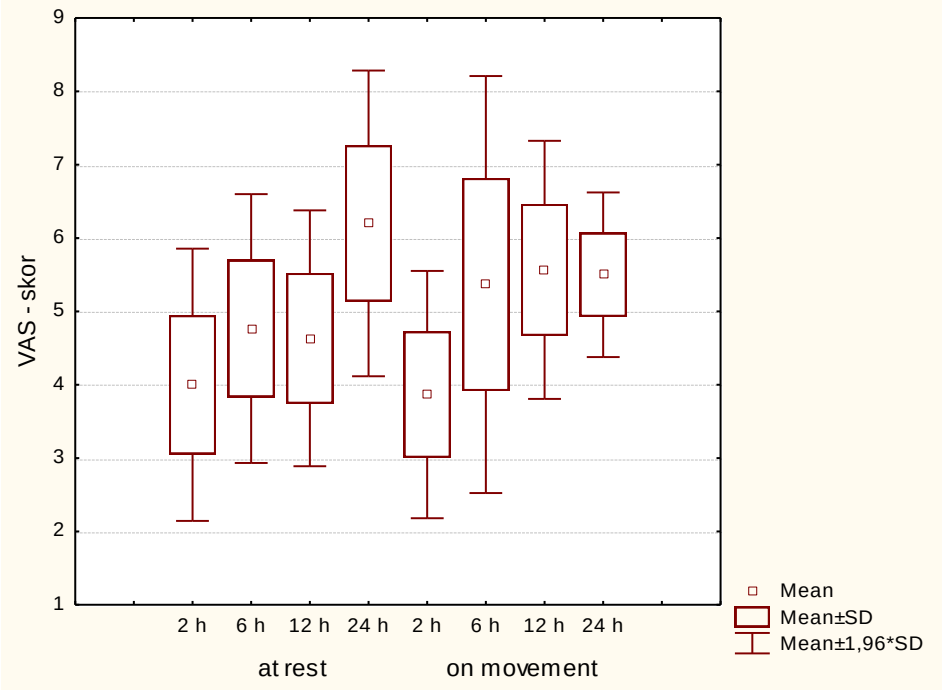


Fig. 4. Mean values of VAS score in patients who received GA at rest and on movement 2, 6, 12 and 24 hours after surgery (N2-CG).

In patients who received general anesthesia and patients who had one-sided TAP block, there is significant difference between VAS score at rest and on movement after 2, 6, 12 and 24 hours from the operation. In the patients which received only general anesthesia there was significant difference between VAS score at rest and on movement only 12 and 24 hours after surgery (Table 3).

Table 3. Significant difference in the mean values of VAS score in patients who received GA with or without one-sided TAP block at rest and on movement 2, 6, 12 and 24 hours after surgery

TIME	GA + ONE-SIDED TAP BLOCK (N1) AT REST / ON MOVEMENT	GENERAL ANESTHESIA (GA) (N2-CG) AT REST / ON MOVEMENT
After 2 h	p = 0,0431*	p = 0,5872
After 6 h	p = 0,0076*	p = 0,0913
After 12 h	p = 0,0249*	p = 0,0044*
After 24 h	p = 0,0012*	p = 0,0029*

*statistically significant differences (Wilcoxon Matched Pairs Test)

In Table 4 and Diagram 3 mean values of VAS score are given in patients who received general anesthesia with and without one-sided TAP block, at rest 2, 6, 12 and 24 hours after the operation. In Table 5 and Diagram 4 mean values of VAS score are given in two examined groups on movement after 2, 6, 12 and 24 hours. Analysis showed that VAS score is significantly higher in patients who received only general anesthesia at rest and on movement, as well as after 2, 6, 12 and 24 hours after operation, versus patients who received general anesthesia+one-sided TAP block (Table 6).

Table 4. Mean values of VAS score in patients who received GA + one-sided TAP block and only GA at rest 2, 6, 12 and 24 hours after surgery

TIME	GA + ONE-SIDED TAP BLOCK (N1)				GENERAL ANESTHESIA (GA) (N2-CG)			
	AVERAGE	SD	MIN	MAX	AVERAGE	SD	MIN	MAX
After 2 h	1.6	0.76	1	3	4.0	0.95	3	6
After 6 h	2.0	1.14	1	6	4.8	0.94	3	7
After 12h	2.37	1.29	1	5	4.6	0.89	3	6
After 24h	2.6	1.59	1	7	6.2	1.06	4	8

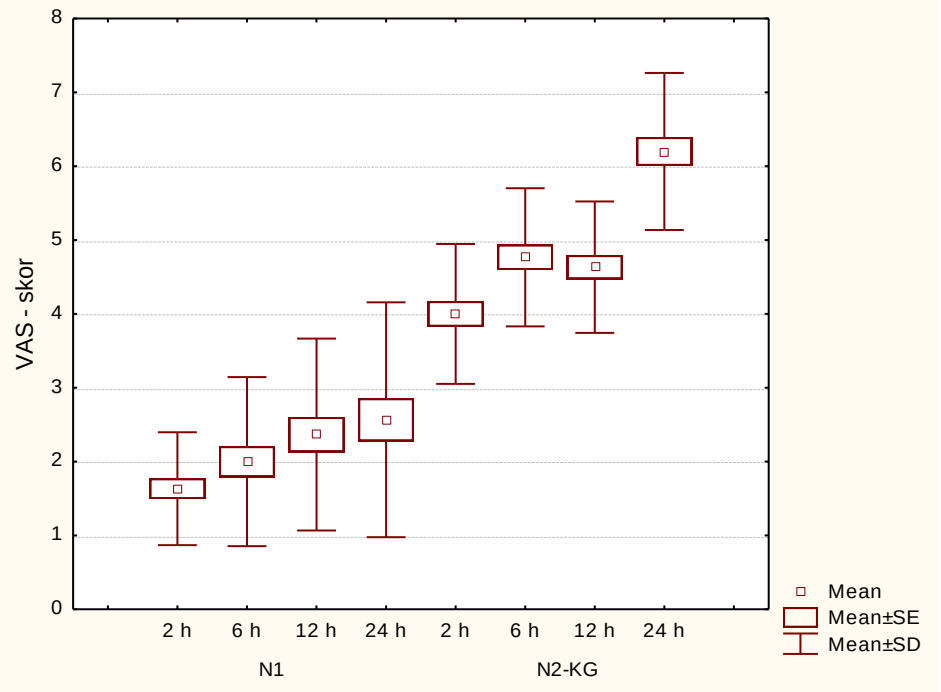


Fig. 5. Mean values of VAS score in patients who received GA + one-sided TAP block and only GA at rest 2, 6, 12 and 24 hours after surgery.

Table 5. Mean values of TAP block in patients who received GA + one-sided TAP block and only GA on movement 2, 6, 12 and 24 hours after surgery

TIME	GA + ONE-SIDED TAP BLOCK (N1)				GENERAL ANESTHESIA (GA) (N2-CG)			
	AVERAGE	SD	MIN	MAX	AVERAGE	SD	MIN	MAX
After 2 h	1.8	0.80	1	3	3.9	0.86	2	5
After 6 h	2.6	1.67	1	7	5.4	1.45	3	8
After 12h	2.6	1.18	1	5	5.6	0.89	4	7
After 24h	3.3	1.59	1	6	5.5	0.57	4	6

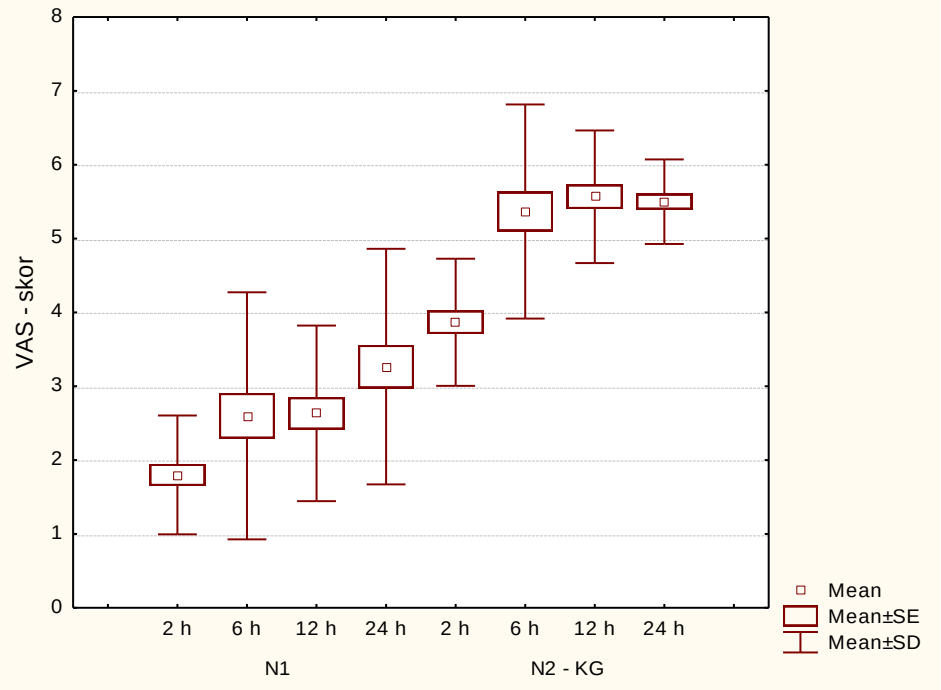


Fig. 6. Mean values of TAP block in patients who received GA + one-sided TAP block and only GA on movement 2, 6, 12 and 24 hours after surgery.

Table 6. Significant difference in the mean values of VAS score in patients who received GA + one-sided TAP block and only with GA at rest and on movement 2, 6, 12 and 24 hours after surgery

TIME	AT REST	ON MOVEMENT
	N1 versus N2 – CG	N1 versus N2 – CG
After 2 h	p = 0,000001*	p = 0,000001*
After 6 h	p = 0,000001*	p = 0,000001*
After 12 h	p = 0,000001*	p = 0,000001*
After 24 h	p = 0,000001*	p = 0,000002*

* statistically significant differences (Mann-Whitney U-test)

There is a significant difference between two examined groups (Mann-Whitney U Test : Z= -2,882 p=0,0039) in the need for opioids postoperatively. The patient who received general anesthesia had more need for opioids (70%) versus patients who received general anesthesia + one-sided TAP block (Table 7, Diagram 5).

Table 7. Distribution of examiners of two examined groups according to the need of opioids postoperatively

OPIOIDES POSTOPERATIVELY	GA + ONE-SIDED TAP BLOCK (N1)	GA (N2 – CG)	TOTAL
NO	22 (73.33%)	9(30%)	31
YES	8(26.67%)	21(70%)	29
TOTAL	30 (100%)	30(100%)	60

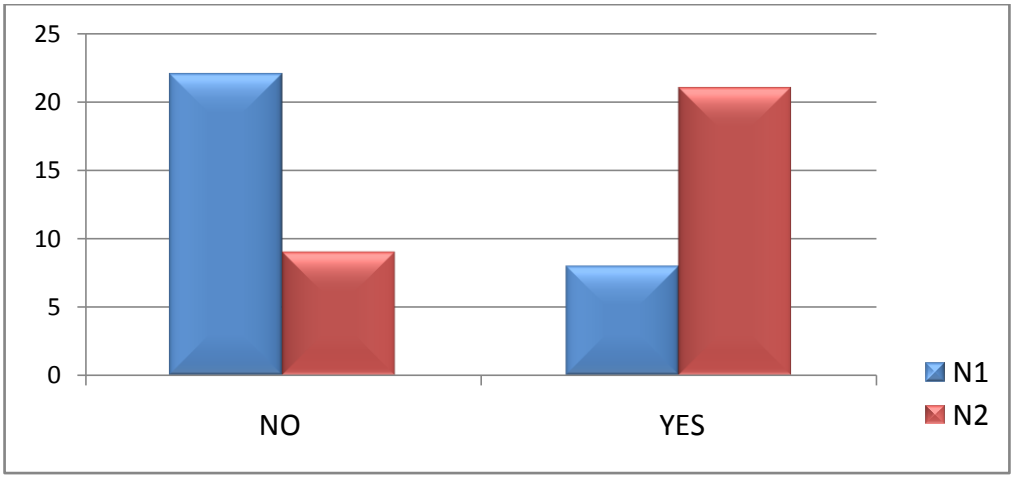


Fig. 7. Distribution of examiners of two examined groups according to the need of opioids postoperatively

There is a significant difference between two examined groups (Mann-Whitney U Test: Z= -3,245 p=0,0011) compared to the nausea postoperatively. The patients who received general anesthesia had significantly larger feeling of nausea versus patients who received general anesthesia + one-sided TAP block (Table 8, Diagram 6).

Table 8. Distribution of examiners of two examined groups according to the feeling of nausea postoperatively

FEELING OF NAUSEA	GA + ONE-SIDED TAP BLOCK (N1)	GENERAL ANESTHESIA GA (N2 – CG)	TOTAL
NONE	19 (63.33%)	6 (20%)	25
MILD	6 (20%)	8 (26.67%)	14
MODERATE	3 (10%)	11 (36.67%)	14
SEVERE	2 (6.67%)	5 (16.66%)	7
TOTAL	30 (100%)	30(100%)	60

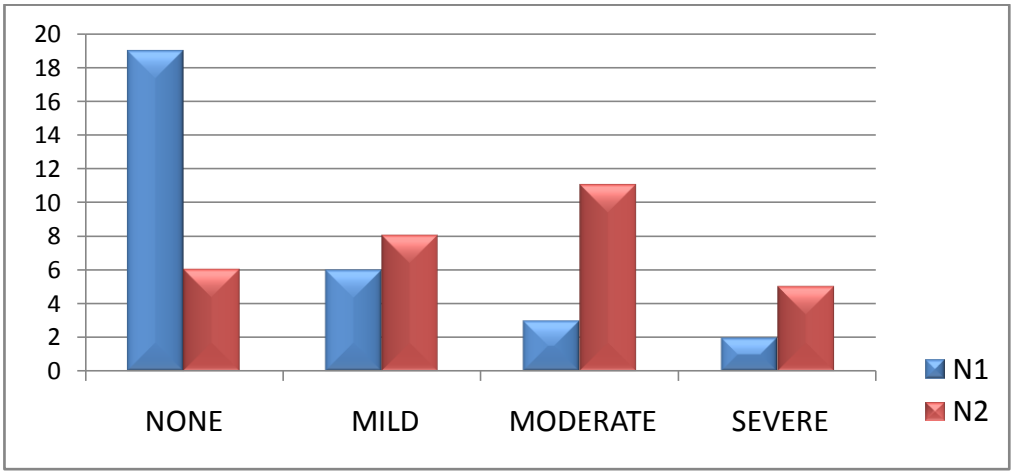


Fig. 8. Distribution of examiners of two examined groups according to the feeling of nausea postoperatively

VAS pain scores at rest and on moving were significantly lower in the TAP group. Postoperative ketonal and tramadol consumption was lower in patients who received unilateral TAP block. There was a significant difference in the incidence and severity of PONV in the two groups at 30 minutes after surgery but not at 24h after surgery.

DISCUSSION

The use of ultrasound-guided sensory block of the anterior abdominal wall with local anesthesia for postoperative pain reliefs is an attractive method because of its simplicity and safety. Effective analgesia has shown to reduce postoperative stress response and accelerate recovery from surgery [15]. TAP block is a promising technique with a potential for wide application in providing analgesia after surgery involving the anterior abdominal wall [16].

The studies reported so far have utilized a bilateral TAP block for midline lower abdominal surgery and for Caesarean section using a landmark technique [11, 13, 17]. Hebbard et al. in his study subsequently described an ultrasound-guided technique for the TAP block [9]. In 2008, Hebbard described another ultrasound-guided TAP block technique designed for upper abdominal surgery referred to as the oblique (anterior) subcostal approach [18, 19]. This approach is mainly used for upper abdominal incisions. Borglum et al. described an ultrasound-guided, four-point, single-shot technique that combines the posterior and oblique subcostal techniques in an effort to provide wider bilateral analgesic coverage [20]. Carney et al. described mid-axillary approach, mainly used for analgesia for incisions below the umbilicus [21]. Another study described the posterior approach that gives analgesia from Th5-L1 and is spreading in the paravertebral space [22]. In order to create a prolonged analgesia, a catheter can be placed under the ultrasound, thus adding a continuous local anesthetic infusion [23].

We studied the efficacy of unilateral, ultrasound-guided TAP block in patients with an anterolateral abdominal wall incision for open appendectomy.

The results of our study show that the patients who received TAP block had significantly reduced VAS pain scores at rest and on moving, ketonal and tramadol postoperative consumption is significantly reduced and also the incidence of PONV after the operation in the first 30 min is reduced.

Pain after surgery of acute appendicitis has two sources. Somatosensory pain originates from the surgical wound on the anterior abdominal wall and the visceroperitonitic pain due to the inflammation and infection of the appendix [1]. TAP block ameliorates the pain from the surgical wound. In our study, the patients who received the TAP block had significantly reduced postoperative ketonal and tramadol consumption. This reduction is on the lesser scale when compared with the previous clinical studies on TAP block in patients undergoing elective laparotomy and Caesarean section [11, 17]. This could be explained by the probable absence of the visceroperitonitic inflammatory pain component in elective surgical patients. Ten patients in the study had perforated appendicitis. Ketonal and tramadol postoperative consumption was significantly greater in the standard group patients with perforated appendicitis when compared to the TAP group patients with perforated appendicitis.

Local anesthetic infiltration to the surgical wound can be an alternative to TAP block in patients undergoing open appendectomy. The duration of action is short-termed, 2-6 hrs and a systematic review has shown that there was no evidence for improved pain relief after appendectomy in adult patient [3].

Niraj et al. in his study has used ultrasound-guided TAP block with 0.5% bupivacaine in open appendectomy and morphine consumption and VAS pain scores were much less in the first 24 hrs [24].

Cho et al. has used 0.5% levobupivacaine in ultrasound-guided TAP block in open appendectomy and has reduced VAS scores on pain significantly up to 12 hours postoperatively [25].

TAP block caused a 25% reduction in the amount of used intra-operative fentanyl compared to the control group.

In one study, ultrasound-guided TAP block with (0.4 ml/kg) 0.25% bupivacaine provides prolonged postoperative analgesia and reduces analgesic use without any clinical side-effects after appendectomy in children [26].

In our study the success rate of TAP block was 96.6%. The one TAP block that did not work was probably due to operator error of injecting the local anesthetic into the TAP. Although ultrasound guidance improves accurate needle placement into the neuro-fascial plane, visibility can be impaired in the obese patients.

This study has the following limitations: the study assessments were limited to 24 hrs. McDonnell et al. had shown the efficacy of TAP block in reducing postoperative morphine consumption to last for 36 hrs. after a single-shot injection. We limited our study to 24 hrs. because the majority of patients no longer require systemic opioid therapy after 24 hrs. upon the open appendectomy. The frequency of the study assessments was limited to the immediate postoperative period and within 24 hrs. after surgery. Surgery for acute appendicitis is usually performed on an urgent basis and the majority of cases are not done during the daily working hours, but during the early hours of the night.

The pathophysiology of postoperative pain is complex and the pain after open appendicectomy is often undertreated. Opioids, which have been the mainstay of postoperative analgesia in appendicitis, inhibit only one component of the pain pathway. A multifaceted approach achieves synergy in drug actions offering better analgesia using smaller doses of individual drugs [27]. We conclude that ultrasound-guided TAP block holds considerable promise as a part of balanced postoperative analgesic regiment for patients undergoing open appendectomy.

REFERENCES

1. Aida S, Baba H, Yamakura T, Taga K, Fukuda S, Shimoji K. The effectiveness of preemptive analgesia varies according to the type of surgery: a randomized, double-blind study. Anesth Analg 1999; 89:711-6.
2. Buvanendran A, Kroin JS. Useful adjuvants for postoperative pain management. Best Pract Res Clin Anaesthesiol 2007;21:31-49.
3. Moiniche S, Mikkelsen S, Wetterslev J, Dahl JB. A qualitative systematic review of incisional local anaesthesia for postoperative pain relief after abdominal operations. Br J Anaesth 1998;81:377-83.
4. Wall PD, Melzack R. Pain measurements in persons in pain. In: WallPD, Melzack R, eds. Textbook of Pain, 4-th Edn. Edinburgh, UK: Churchill Livingstone, 1999;409-26.
5. Netter FH. Back and spinal cord. In: Netter FH, ed. Atlas of Human Anatomy. Summit, NJ, USA: The Ciba-Geigy Corporation, 1989;145-55.
6. Newman K, Ponsky T, Kittle K, Dyk L, Throop C, Gieseker K, Sills M, Gilbert J. Appendicitis 2000: variability in practice, outcomes, and resource utilization at thirty pediatric hospitals. J Pediatr Surg 2003; 38:372-9.
7. Carney J, Finnerty O, Rauf J, Curley G, McDonnell JG, Laffey JG. Ipsilateral transversus abdominis plane block provides effective analgesia after appendectomy in children: a randomizes controlled trial. Anesth Analg 2010;111:998-1003.
8. Rafi AN. Abdominal field block: a new approach via the lumbar triangle. Anaesthesia 2001;56:1024-6.
9. Hebbard P, Fujiwara Y, Shibata Y, Royse C. Ultrasound-guided transversus abdominis plane (TAP) block. Anaesth Intensive Care 2007;35:616-7.
10. Bharti N, Kumar P, Bala I, Gupta V. The efficacy of a novel approach to transversus abdominis plane block for postoperative analgesia after colorectal surgery. Anesth Analg 2011;112:1504-8.
11. McDonnell JG, Curley G, Carney J, Benton A, Costello J, Maharaj CH, et al. The analgesic efficacy of transversus abdominis plane block after cesarean delivery: a randomized controlled trial. Anesth Analg 2008;106:186-91.
12. Carney J, McDonnell JG, Ochana A, Bhinder R, Laffey JG. The transversus abdominis plane block provides effective postoperative analgesia in patients undergoing total abdominal hysterectomy. Anesth Analg 2008;107:2056-60.
13. O'Donnell BD, McDonnell JG, McShane AJ. The transversus abdominis plane (TAP) block in open retropubic prostatectomy. Reg Anesth Pain Med 2006;31:91.
14. Ra YS, Kim CH, Lee GY, Han JI. The analgesic effect of the ultrasound-guided transverse abdominis plane block after laparoscopic cholecystectomy. Korean J Anesthesiol 2010; 58: 362-8.
15. Kehlet H. Surgical stress: the role of pain and analgesia. Br J Anaesth 1989;63:189-95.

16. Niraj G, Kelkar A, Fox A. Application of the transversus abdominis plane (TAP) block in the intensive care unit. Anaesth Intensive Care 2009; 37 (in press).
17. McDonnell JG, O'Donnell BD, Curley GCJ, et al. The analgesic efficacy of transversus abdominis plane block after abdominal surgery. Anesth Analg 2007;104:193-7.
18. Hebbard P. Subcostal transversus abdominis plane block under ultrasound guidance. Anesth Analg 2008; 106(2):674-5.
19. Hebbard P, Barrington MJ, Vasey C. Ultrasound-guided continuous oblique subcostal transversus abdominis plane blockade. Reg Anesth Pain Med 2010;35:436-41.
20. Borglum J, Maschmann C, Belhage B, Jensen K. Ultrasound-guided bilateral dual transversus abdominis plane block: a new four-point approach. Acta Anaesth Scand 2011;36:568-71.
21. Carney J, Finnerty O, Rauf J, Bergin D, Laffey JG, McDonnell JG. Studies on the spread of local anaesthetic solution in transversus abdominis plane blocks. Anaesth 2011;66:1023-30.
22. Carney J, McDonnell JG, Bhinder R, Maharaj CH, Laffey JG. Ultrasound guided continuous transversus abdominis plane block for post-operative pain relief in abdominal surgery. Reg Anesth Pain Med 2007;32: 410.
23. Maeda A, Shibata SC, Kamibayashi T, Fujino Y. Continuous subcostal oblique transversus abdominis plane block provides more effective analgesia than single-shot block after gynaecological laparotomy. Eur J Anaesth 2015;32:514-5.
24. Niraj G, Searle A, Mathews M, Misra V, Baban M, Kiani S, Wong M. Analgesic efficacy of ultrasound-guided transversus abdominis plane block in patients undergoing open appendicectomy. Br J Anaesth 2009;103:601-5.
25. Cho S, Kim YJ, Kim DY, Chung SS. Postoperative analgesic effects of ultrasound-guided transversus abdominis plane block for open appendectomy. J Korean Surg Soc 2013;85:128-33.
26. Shaaban AR. Ultrasound guided transversus abdominis plane block versus local wound infiltration in children undergoing appendectomy: A randomized controlled trial. Egyptian J Anaesth 2014;30:377-82.
27. Horvath G, Joo G, Dobos I, Klimscah W, Toth G, Benedek G. The synergistic antinociceptive interactions of endorphin-1, with dexmedetomidine and/or S(+) ketamine in rats. Anesth Analg 2001;93:1018-24.

UDC:616.831.94-005.1

EARLY VERSUS DELAYED SURGICAL TREATMENT IN PATIENTS WITH ANEURYSMAL SUBARACHNOID HEMORRHAGE DUE TO RUPTURED INTRACRANIAL ANEURYSM ADMITTED WITH HUNT AND HESS GRADE 2

Filipce Venko, Caparoski A, Sumkovski R, Elezi F, Kamiloski T
University Department of Neurosurgery Skopje

ABSTRACT

Purpose: To evaluate the effect of the timing of early versus late surgery in patients with subarachnoid hemorrhage due to ruptured intracranial aneurysm admitted in Hunt and Hess grade 2, on the outcome of the treatment.

Materials and methods: In the period of 2011 until 2015, 224 patients were treated and operated due to subarachnoid hemorrhage caused by a ruptured intracranial aneurysm at the Clinic of Neurosurgery in Skopje. At admission, the patients were evaluated with the Hunt and Hess scale for their neurological condition. The diagnostics was done using the unenhanced Computerized Tomography (CT) scan, CT angiography or catheter angiography. Based on the scan findings the patients were randomized in seven groups according to the localization of the aneurysm: anterior communicating artery, posterior communicating artery, middle cerebral artery, pericallosal artery**,** aneurysms of the vertebrobasilar circulation, multiple aneurysms and aneurisms on the ophthalmic artery. The existence of intarcerebral hematoma or intraventricular hemorrhage was also assessed on admission. During the treatment part of the patients were operated on in an acute stage, i.e. in the first 72 hours after admission or in a delayed stage after 14 days of admission.

Results: Out of total 76 patients admitted as "conscious, alert, oriented with severe headache and nuchal rigidity" in 65 (85.50%) early surgical treatment was applied and in comparison with only 11 (14.50%) delayed surgical interventions. 53 (69.70%) of the patients operated in the early stage had good outcome, 7 (9.20%) of the patients had minor complications (doing every day chores by his/her self), 4 (5.30%) of the patients were with severe complications (needed other person's help constantly) and 1 (1.30%) patient had lethal outcome. From the patients operated in a delayed stage 10 (13.20%) had good outcome and 1 (0.30%) patient had severe complication (needed other person's help constantly) and 1 (1.30%) patient had lethal outcome.

Conclusion: In our study the patients admitted with H&H grade 2, i.e. patients who were conscious, alert, oriented with highly expressed meningeal signs and who were operated on in the early stage (in the first 3 days of admission) had insignificantly grater probability of a good outcome Glasgow Outcome Scale.

Key words: intracranial aneurysm, subarachnoid hemorrhage, early surgery, delayed surgery

INTRODUCTION

The incidence of cerebral aneurysm is not known in full, having in mind the high rate of non-ruptured aneurysms in the general population. Subarachnoid hemorrhage represents a small part of the cerebrovascular diseases, and at the same time a significant cause of morbidity and mortality. The annual rate of not traumatic subarachnoid hemorrhage is 10-15 in 100000. The acute mortality is between 25 and 50%. In most cases the subarachnoid hemorrhage is caused by cerebrovascular malformations, while in 10-20% there aren't any vascular lesions in case of subarachnoid hemorrhage [1, 2, 3]. The form for evauluation that has been most often used to evaluate neurological condition of the patients is the Glasgow Coma Scale and the Hunt and Hess scale. Although there are some disadvantages in regards to the inability to identify the focal neurologic deficit, the Hunt and Hess scale is the perfect form for evaluation of the patient's level of consciousness [2].

The aim of the study is to evaluate the outcome, using the Glasgow Outcome Scale in comparison with the timing for surgical treatment (early-in the first 72 hours versus delayed-after two weeks surgical treatment) in patients with aneurysmal subarachnoid hemorrhage admitted in Hunt and Hess grade 2.

MATERIAL AND METHODS

In the period of 2011 until 2015, 224 patients were treated and operated due to subarachnoid hemorrhage caused by a ruptured intracranial aneurysm at the Clinic of Neurosurgery in Skopje. At admission, the patients were evaluated with the Hunt and Hess scale. The diagnostics was done using the unenhanced Computerized Tomography (CT) scan, CT angiography or catheter angiography. Based on the scan findings the patients were randomized in seven groups according to the localization of the aneurysm: Acom – anterior communicating artery, Pcom – posterior communicating artery, MCA - middle cerebral artery, Peric A – pericallosal artery**,** VB – aneurysms of the vertebrobasilar circulation, multiple aneurysms and Ophtalmica – aneurisms on the ophthalmic artery. The existence of intarcerebral hematoma or intraventricular hemorrhage was also assessed on admission.

During the treatment part of the patients were operated on in an acute stage, i.e. in the first 72 hours after admission or in a delayed stage after 14 days of admission. Of the total number of patients (224), 76 were admitted in H&H grade 2, i.e. they were conscious, alert, oriented with severe headache and nuchal rigidity. Sixty five of them were operated in the early stage and 11 in a delayed stage. During the treatment events of re-bleeding, hydrocephalus or development of vasospasm were notified. The outcome was evaluated with Glasgow Outcome Scale on the day of hospital discharge.

RESULTS

In our study 224 patients with subarachnoid hemorrhage were included; 102 (45.50%) were men and 122 were women. In table 1 and Graph 1 the shown data refer to the localization of ruptured intracranial aneurysms in comparison with the gender of the patients.

In men, 41 (18.30%) of the aneurysms were located on the art. comunicans anterior, 26 (11.60%) of the aneurysms were located on the art. comunicans posterior, 27 (12.10%) of the aneurysms were located on the art. cerebri media, 2 (0.90%) of the aneurysms were located on the art. pericallosa, 1 (0.40%) of the aneurysms was located on vertebro-basilar circulation n, 4 (1.80%) were multiple aneurisms and 1 (0.40%) aneurysm was located on art. ophtalmica.

In women, 29 (12.90%) of the aneurysms were located on the art. comunicans anterior, 33 (14.70%) of the aneurysms were located on the art. comunicans posterior, 43 (19.20%) of the aneurysms were located on the art. cerebri media, 2 (0.90%) of the aneurysms were located on the art. pericallosa, 4 (1.80%) of the aneurysms was located on vertebro-basilar circulation, 10 (4.50%) were multiple aneurisms and 1 (0.40%) aneurysm was located on art. ophtalmica.

Evaluation of the correlation of the localization of the ruptured intracranial aneurysms with the gender of the patients with the Fisher's Exact Test=9,30 and $p>0,05$($p=0,128/0,120-0,137$) showed that there was no statistically significant preference for the localization in comparison with the gender of the patents.

Table 1. Distribution of the patients according to the gender in comparison with the localization of the aneurysm

Sex		Localization							Total
		Art. Comunic. Anterior	Art. Comunic. posterior	Art. Cereb. media	Art. Pericall.	Verteb.-basilar	Multipli anevrizmi	Art. Ophtal.	
Men	Number	41	26	27	2	1	4	1	102
	%	18.3%	11.6%	12.1%	0.9%	0.4%	1.8%	0.4%	45.5%
Women	Number	29	33	43	2	4	10	1	122
	%	12.9%	14.7%	19.2%	0.9%	1.8%	4.5%	0.4%	54.5%
Total	Number	70	59	70	4	5	14	2	224
	%	31.3%	26.3%	31.3%	1.8%	2.2%	6.3%	0.9%	100 %

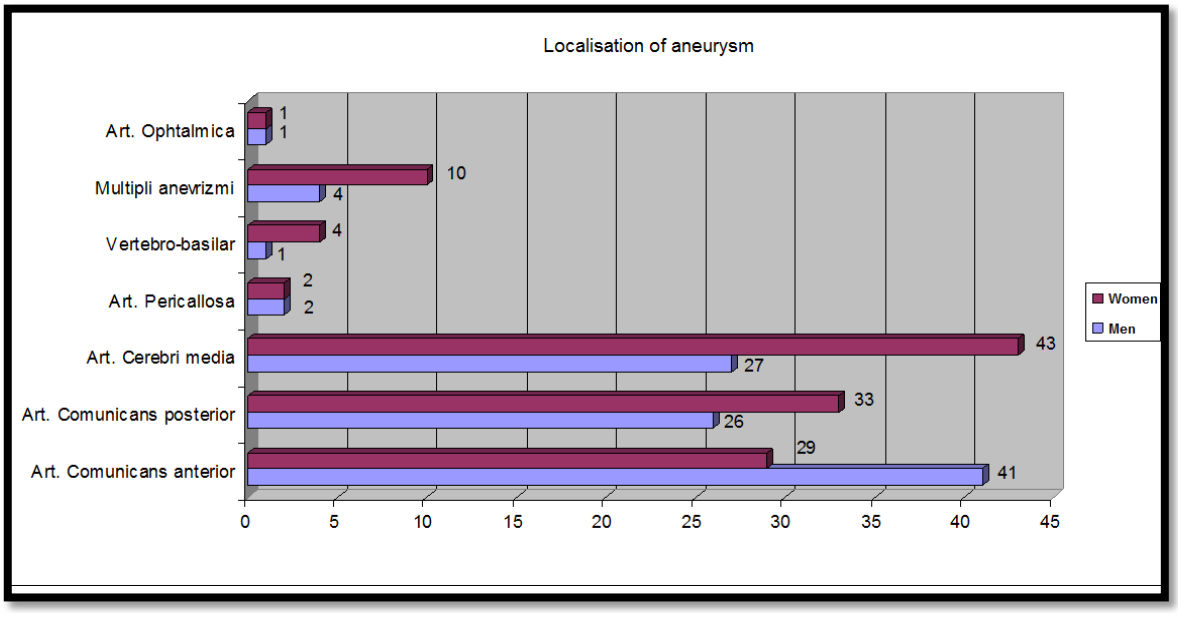


Fig. 1. Distribution of the patients according to the gender & Localization of the aneurysm

The data referring to the correlation of the applied operational treatment with to the status of the patient on admission according to Hunt & Hess are presented at Table 2.

Out of total of 42 (18.80%) patients admitted in H&H grade 1 as "conscious, alert, oriented with slight nuchal rigidity", in 37 (16.50%) subjects early surgical treatment was applied (in the first 72 hours of the moment of bleeding). In 5 (2.20%) of the patients delayed surgical treatment was applied (after the fourteenth day of the bleeding).

Out of total of 76 (33.90%) patients admitted in H&H grade 2 as "conscious, alert, oriented with severe headache and nuchal rigidity" in 65 (29%) subjects early surgical treatment was applied (in the first 72 hours of the moment of bleeding), and in 11 (4.90%) of the patients delayed surgical treatment was applied (after the fourteenth day of the bleeding).

Out of total of 66 (29.50%) patients admitted in H&H grade 3 as " with drowsiness/ confusion, with or without neurologic deficit" in 55 (24.60%) subjects early surgical treatment was applied (in the first 72 hours of the moment of bleeding), and in 11 (4.90%) of the patients delayed surgical treatment was applied (after the fourteenth day of the bleeding).

Out of total of 23 (10.30%) patients admitted in H&H grade 4 as "stuporous with or without neurologic deficit" in 18 (8.0%) subjects early surgical treatment was applied (in the first 72 hours of the moment of bleeding), and in 5 (2.20%) of the patients delayed surgical treatment was applied (after the fourteenth day of the bleeding).

Out of total of 17 (7.60%) patients admitted in H&H grade 5 as "in comatose status" in 16 (7.10%) subjects early surgical treatment was applied (in the first 72 hours of the moment of bleeding), and in 1(0.40%) of the patients delayed surgical treatment was applied (after the fourteenth day of the bleeding).

Evaluation of the correlation of the applied surgical treatment with the patients status at admission according to Hunt & Hess with the Fisher's Exact Test=2,25 and p>0,05(p=0,695/0,683-0,707) showed that there was no statistically significant difference in the timing for intervention in correlation with the Hunt & Hess grades.

Table 2. Status at admission according to Hunt & Hess in correlated with timing for the surgical treatment

			Timing or surgical treatment		Total
			In early stage	In delayed stage	
Status	conscious, alert, oriented with slight nuchal rigidity	Count	37	5	42
		% of Total	16.5%	2.2%	18.8%
	conscious, alert, oriented with severe headache and nuchal rigidity	Count	65	11	76
		% of Total	29.0%	4.9%	33.9%
	drowsiness/ confusion, with or without neurologic deficit	Count	55	11	66
		% of Total	24.6%	4.9%	29.5%
	stuporous with or without neurological deficit	Count	18	5	23
		% of Total	8.0%	2.2%	10.3%
	comatose status	Count	16	1	17
		% of Total	7.1%	0.4%	7.6%
Total		Count	191	33	224
		% of Total	85.3%	14.7%	100%

Patients admitted in H&H grade 2 as "conscious, alert, oriented with severe headache and nuchal rigidity"

Out of total 76 patients admitted as "conscious, alert, oriented with severe headache and nuchal rigidity" in 65 (85. 50%) early surgical treatment was applied and in comparison with only 11 (14.50%) delayed surgical interventions.

From the patients operated in early stage, 53 (69.70%) had good outcome, 7 (9.20%) of the patients had minor complications (doing every day chores by his/her self), 4 (5.30%) of the patients were with severe complications (needed other person's help constantly) and 1 (1.30%) patient had lethal outcome.

From the patients operated in a delayed stage 10 (13.20%) had good outcome and 1 (0.30%) patient had severe complication (needed other person's help constantly) and 1(1.30%) patient had lethal outcome.

Statistical evaluation of the patients outcome in correlation with the timing for operative treatment with Fisher's Exact Test=1,77 and p>0,05(p=0,699/0,687-0,710) showed that there were no statistically significant difference in the outcome in comparison with the operative timing (Table 3).

Table 3. Distribution of the data referring to outcome in patients who underwent surgery taking into consideration the timing in which the surgery took place

		Outcome				Total
		good	minor complications (doing every day chores by his/her self)	complications (needed other person's help constantly)	lethal outcome	
In early stage	Count	53	7	4	1	65
	% of Total	69.7%	9.2%	5.3%	1.3%	85.5%
In delayed stage	Count	10	0	1	0	11
	% of Total	13.2%	0.0%	1.3%	0.0%	14.5%
Total	Count	63	7	5	1	76
	% of Total	82.9%	9.2%	6.6%	1.3%	100%

The results referring to the predicted values of the timing of the surgical treatment of the patients for "Good & Minor complications" are presented in Table 4.

The patients operated on in early stage (1) have 1,20 times (Exp(B)=1,20)(95%CI:0,13-11,37) grater possibility of "Good & Minor complications" outcome than the patients operated in a delayed stage, but the evidence is not significant for p>0,05(p=0,87).

Table 4. Predicted values of the timing of the surgical treatment of the patients for "Good & Minor complications"are presented in Table 4.

Table 4. Operated & Outcome / Prediction

Operate								95% C.I.for EXP(B)	
Step 1[a]		B	S.E.	Wald	Df	Sig.	Exp(B)	Lower	Upper
	In early stage (1)	18	1,15	03	1	87	1.20	13	11.37
	Constant	2.30	1.05	4.82	1	03	10.00		

a.Variable(s) entered on step 1: Operated.

DISCUSSION

The quandary for early versus delayed surgical treatment is present in all the patients with aneurysmal subarachnoid hemorrhage, and especially in patients with grade 2 and 3 according to Hunt & Hess. The early surgery eliminates the possibility of re-bleeding and is associated with good outcome [4, 5, 6]. According to the results from the International Cooperative Study on the Timing of Aneurysm Surgery, which is a prospective non randomized study in which 722 patients in 27 sites across USA were enrolled, 70.9% of the patients who were operated on in the early stage i.e. in the first 3 days after admission had good recovery and 62.9% of the patients who were operated on in the delayed stage i.e. two weeks after admission had good recovery. A lot of factors influence the decision for the timing of the treatment. They are connected to the patient – the most important are the neurological stage and the age, i.e. the worse the grade the greater the risk for re-bleeding and vasospasm [6, 7, 8]. Furthermore, the cerebral edema which doesn't affect the outcome of the early and the delayed operations of the patients with bad neurological status at admission, should be taken in consideration. In that sense the patients with signs of cerebral edema and older patients are good candidates for endovascular treatment. Good candidates for an early surgery are the patients with high risk of vasospasm, i.e. those with thick layer subarachnoid hemorrhage shown on CT scan [4, 9]. The delayed surgery can be taken into consideration where vascular lesions as gigantic aneurysms exist, when a longer time period of temporary clipping is expected [6, 10].

CONCLUSION

In our study the patients admitted with H&H grade 2, i.e. patients who were conscious, alert, oriented with highly expressed meningeal signs and who were operated on in the early stage (in the first 3 days of admission) had insignificantly grater probability of a good outcome Glasgow Outcome Scale 1-2 versus the patients operated on in the delayed stage, i.e. two weeks after the admission at the clinic.

REFERENCES

1. Inagawa T. Seasonal variation in the incidence of aneurysmal subarachnoid hemorrhage in hospital- and community-based studies. J Neurosurg. 2002;96(3):497-509.
2. Lantigua H, Ortega-Gutierrez S, Schmidt JM, et al. Subarachnoid hemorrhage: who dies, and why? Crit Care. 2015;19:309. doi: 10.1186/s13054-015-1036-0.
3. Nakagawa T, Hashi K, Kurokawa Y, Yamamura A. Family history of subarachnoid hemorrhage and the incidence of asymptomatic, unruptured cerebral aneurysms. J Neurosurg. 1999;91(3):391-5.
4. Lanzino G, Kassell NF. Double-blind, randomized, vehicle-controlled study of high-dose tirilazad mesylate in women with aneurysmal subarachnoid hemorrhage. Part II. A cooperative study in North America. J Neurosurg. 1999;90(6):1018-24.
5. Lanzino G, Kassell NF, Dorsch NW, Pasqualin A, Brandt L, Schmiedek P, Truskowski LL, Alves WM. Double-blind, randomized, vehicle-controlled study of high-dose tirilazad mesylate in women with aneurysmal subarachnoid hemorrhage. Part I. A cooperative study in Europe, Australia, New Zealand, and South Africa. J Neurosurg. 1999;90(6):1011-7.
6. Le Roux PD, Winn HR. Management of cerebral aneurysms. How can current management be improved? Neurosurg Clin N Am. 1998;9(3):421-33.
7. Le Roux PD, Elliott JP, Downey L, et al. Improved outcome after rupture of anterior circulation aneurysms: a retrospective 10-year review of 224 good-grade patients. J Neurosurg. 1995;83(3):394-402.
8. Mackey J, Brown RD Jr, Moomaw CJ, Sauerbeck L, et al. Unruptured intracranial aneurysms in the familial intracranial aneurysm and international study of unruptured intracranial aneurysms cohorts: differences in multiplicity and location. J Neurosurg. 2012;117(1):60-4.
9. Lanzino G, Couture D, Kassell NF. New developments in cerebrovascular surgery. Curr Opin Anaesthesiol. 2000;13(5):497-502.
10. Winn HR, Almaani WS, Berga SL, Jane JA, Richardson AE. The long-term outcome in patients with multiple aneurysms. Incidence of late hemorrhage and implications for treatment of incidental aneurysms. J Neurosurg. 1983;59(4):642-51.

UDC:616.66-007.26-089.84

RETROSPECTIVE COMPARATIVE STUDY OF PRIMARY ANTERIOR HYPOSPADIAS REPAR USING SNODGRASS AND MATHIEU METHODS

Chadikovski Vladimir[1], Dimov A[1], Todorovic L[1], Simeonov R[1], Efremov Lj[2], Petrovski M[1]
[1]University Clinic for Pediatric Surgery, [2]GFK Skopje, University " Ss. Cyril and Methodius",
Skopje, R. Macedonia

ABSTRACT

Introduction: Hypospadia a congenital anomaly in which the urethral meatus is ectopically positioned on the ventral side of the penis. There are several classifications depending on the abnormal position of the urinary meatus.Three types of hypospadia are most frequent: anterior, penile and posterior.Hypospadia is result of incomplete tubularization or fusion of the urethral plate leading to an abnormal location of the meatus. The etiological cause for development of hypospadias lies in the disordered embryology of the penis due to disorders of hormone receptors, genetic disorders, enzyme disorders, influence of external environment and endocrine disorders. The incidence of hypospadias is reported to be approximately 1 in 200 live-born male babies.

Material and methods: A total of 300 patients operated on for anterior hypospadia at the University Clinic of Pediatric Surgery in Skopje were included in the study. Patients were divided in two groups, each one containing 150 children, who underwent surgery using the surgical techniques by Mathieu (Meatal-based flap technique) and Snodgrass (Tubularized incised plate urethroplasty).

Results: The following parameters were assessed: duration of surgery, days of hospitalization, complications (urethral meatal stenosis, urethral diverticulum, urethral fistula, urethroplasty disruption). The results obtained were analyzed with numeric and qualitative statistical methods.

Conclusion: The study has shown that Snodgrass surgical procedure known as Tubularized incised plate urethroplasty is superior and more acceptable in formation of anterior hypospadias than the Mathieu procedure . Cosmetic outcome and functionality of the penis meet all the criteria for anterior hypospadias repair using the Snodgrass procedure and it is a method of choice. However, in cases where there is no healthy urethral plate the surgical technique by Mathieu is recommended.

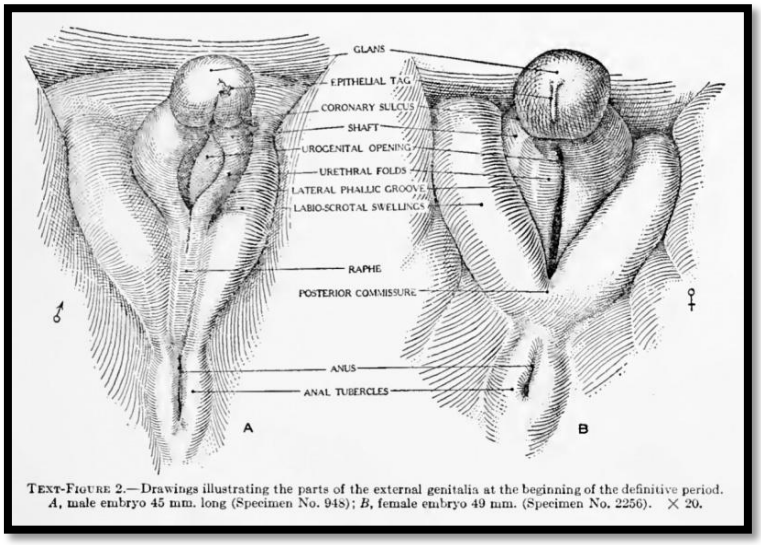
***Keywords*:** hypospadia, child, surgery, Mathieu, Snodgrass

INTRODUCTION

Hypospadia is a congenital anomaly in which the urethral meatus is ectopically positioned on the ventral side of the penis; there is absence of prepuce on the ventral side and misplaced median raphe of the penis [1]. There are several classifications depending on the abnormal position of the urethral meatus. Most often hypospadiasis is divided into anterior, penile and posterior [2]. Hypospadias is a result of incomplete tubularization or fusion of the urethral plate leading to an abnormal location of the meatus [2]. The incidence of this anomaly is approximately 1 per 200 live-born male babies [3]. The etiological cause for development of hypospadias is due to disorders of hormone receptors, genetic disorders, enzyme disorders, influence of the external environment and endocrine disorders [3, 4]. Observations related to racial and familial incidence suggest that genetic factor in inheritance of multifactor disorders speak in favour of 5α-reduction type 2 deficiency that results in conversion of testosterone in dihydrotestosterone. Associated anomalies with hypospadias are cryptorchidism, prostate utricle and intersexuality. Hypospadiac meatus can be stenotic. Also, ventral curvature of the penis can be observed during ejection. Some children with hypospadias can present with obvious endocrinopathies. Intersexual evaluations are mainly limited to those with scrotal and perineal urethral openings as well as in patients with bilateral cryptorchidism.

EMBRYOLOGY

Up to the seventh week, the external genitalia are similar in both sexes (Fig. 1A and B). Distinguishing sexual characteristics begin to appear during the 9th week, but the external genitalia are not fully differentiated until the 12th week. Early in the fourth week, proliferating mesenshyme produces a genital tubercle in both sexes at the cranial end of the cloacal membrane. Labioscrotal swellings and urogenital folds soon develop on each side of the cloacal membrane. The genital tubercle elongates to form a primordial phallus. At the end of the 6th week the urorectal septum descends towards the cloacal membrane, fuses with it and separates it into dorsal (anal) and ventral urethral membrane. The urogenital membrane lies in the floor of a median cleft, the urethral groove, which is bounded by the urethral folds. One week later, the anal and the urethral (urogenital) membrane perforate and form the anal and the urogenital orifice. Masculinization of the indifferent external genitalia is induced by testosterone produced by the interstitial cells of the fetal testes. As the phallus enlarges and elongates to form the penis, the left and right urethral folds form the lateral walls of the urethral groove on the ventral surface of the penis [5].



TEXT-FIGURE 2.—Drawings illustrating the parts of the external genitalia at the beginning of the definitive period. *A*, male embryo 45 mm. long (Specimen No. 948); *B*, female embryo 49 mm. (Specimen No. 2256). × 20.

Fig. 1. Embriological development of genital organs

This groove is lined by a proliferation of endodermal cells, the urethral plate, which extends from the phallic portion of the urogenital sinus. The urethral folds fuse with each other along the ventral (lower) surface of the penis to form corpus spongiosum around the urethra. The surface ectoderm fuses in the median plane of the penis, forming the penile raphe and enclosing the corpus spongiosum and the urethra within the penis. At the tip of the glans penis, an ectodermal ingrowth forms a cellular ectodermal cord, which grows toward the root to meet the urethra [5]. As this cord canalizes, its lumen joins the previously formed urethra. This completes the terminal part of the urethra and moves the external urethral orifice to the tip of the glans penis.

During the 12th week, a circular ingrowth of ectoderm occurs at the periphery of the glans penis. When this ingrowth breaks down, it forms the prepuce (a covering fold of a skin) (Fig. 2.). The corpus cavernosus (cavernous body) and corpus spongiosus (spongious body) develop from mesenchyme in the phallus. The labioscrotal swellings grow toward each other and fuse to form the scrotum. The line of fusion of these folds is clearly visible as the scrotal raphe (raphe scroti) [5, 6, 7].

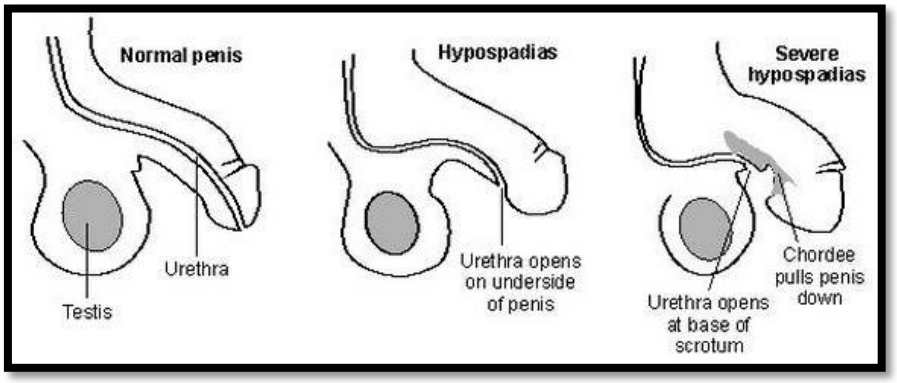


Fig. 2. Hypospadias

Hypospadias results from inadequate production of androgens by t he fetal testes or inadequate receptor sites for the hormones. These defects result in failure of canalization of the ectodermal cord in the glans of the penis or failure of fusion of the urethral folds. As a consequence, there is incomplete formation of the spongy urethra. Different forms of hypospadias are due to the different time of their occurrence, the degree of hormonal sensitivity or underdevelopment of the receptors [5, 6, 7] (Fig 2).

MATERIAL AND METHODS

This study analyzed data of performed surgeries at the University Clinic of Pediatric Surgery in Skopje during the period from 1999 to 2013. The main goal of hypospadias repair is to get a normal functionality and appearance of the penis, without curvature and a wide meatus. Since this anomaly has a wide spectrum and range of forms (from glandular, penile, penoscrotal to perineal) there are more than 200 techniques for its management.

Our investigation included a total of 300 patients with anterior hypospadias, of whom 150 underwent surgery using Snodgrass procedure (group I) and 150 patients underwent surgery using Mathieu procedure (group II). The patients' age ranged from 1.5 to 10 years. Routine examinations, such as blood count, urinary status and echotomography of urinary pathways were done in all patients. The operation was carried out under general anesthesia. Foley's catheter was used a derivation procedure, and in some cases percutaneous cystostoma

RESULTS

The obtained results are presented in two sections. The first section gives basic analysis which relates to primary surgery (children with first surgery), while the additional analysis is given in the second section were post-surgeries (e.g. the second surgery on the same children) are presented.

Description of patients for basic analysis

The cohort consisted of 150 randomly chosen patients who underwent both techniques (primary surgery). There were a total of 242 patients operated on with Mathieu technique in the period from 1999 to 2013, and a total of 197 patients with Snodgrass technique. Regarding religion and age of children, the results are presented below.

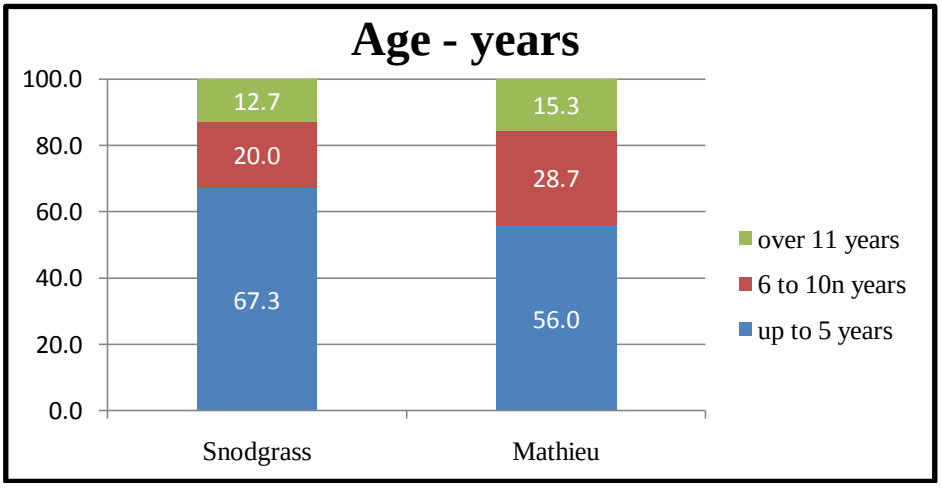


Fig. 3. Age of children

RESULTS FOR BASIC ANALYSIS

The analysis started with the year of performed surgery and continued throughout the entire period (from 1999 to 2013).

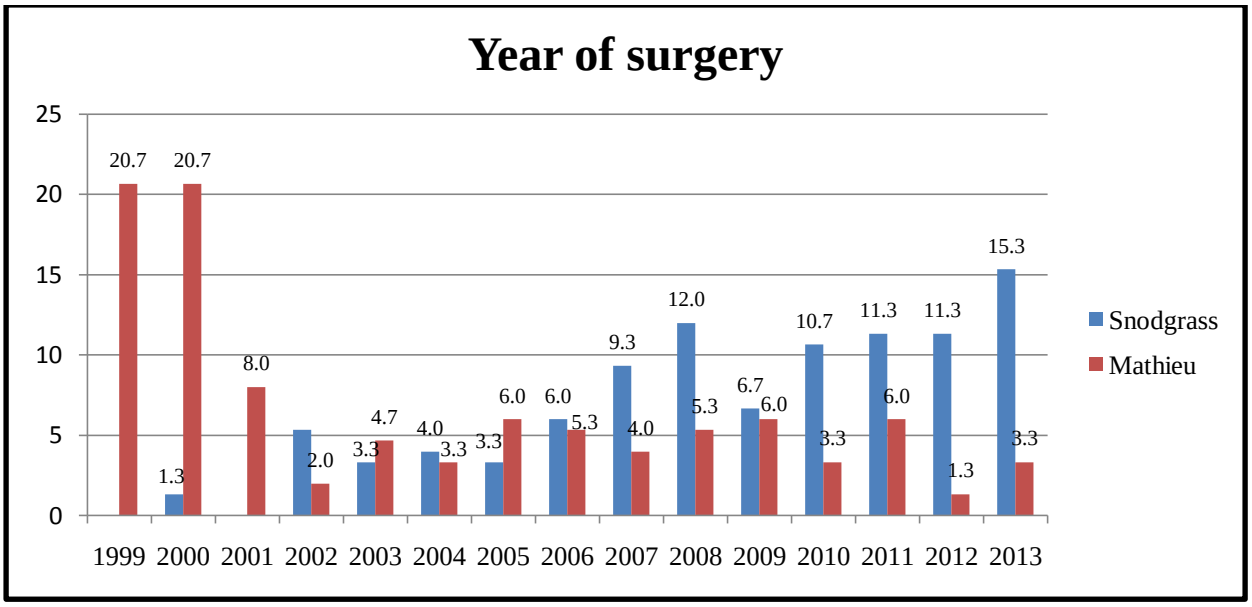


Fig. 4. Presentation of surgeries by year

The Figure 4 shows that the tendency of using Snodgrass technique is increasing with years (ascending trend), while the situation is quite opposite of using Mathieu technique (descending trend), e.g. the number is decreasing with years.

The table 1 show the statistical results for key measures.

Table 1. Presentation of basic statistical parameters for key measures

Measures	Type of surgery	N	Mean	Std. deviation	Std. error mean
Age years	Snodgrass	150	5.32	3.58	0.29
	Mathieu	150	6.17	3.77	0.31
Duration of surgery minutes	Snodgrass	139	98.67	29.77	2.52
	Mathieu	146	95.05	31.21	2.58
Duration of therapy - days	Snodgrass	150	8.04	3.16	0.26
	Mathieu	150	10.57	4.22	0.34
Days of hospitalization	Snodgrass	150	9.85	3.33	0.27
	Mathieu	150	13.33	5.45	0.45
Days of catheter	Snodgrass	149	7.79	3.33	0.27
	Mathieu	150	10.71	4.98	0.41
Days with temperature	Snodgrass	50	1.66	0.96	0.14
	Mathieu	72	2.11	1.53	0.18

The table 2 shows the statistical test used to detect whether there was a significant difference among the measures.

Table 2. Presentation of tests

Measures		Levene's Test for Equality of Variances		t-test for Equality of Means		
		F	Sig.	t	df	Sig. (2-tailed)
Age years	Equal variances assumed	0.80	0.37	-1.99	298.00	0.05
	Equal variances not assumed			-1.99	297.26	0.05
Duration of surgery minutes	Equal variances assumed	0.49	0.49	1.00	283.00	0.32
	Equal variances not assumed			1.00	283.00	0.32
Duration of therapy - days	Equal variances assumed	13.27	0.00	-5.89	298.00	**0.00**
	Equal variances not assumed			-5.89	276.05	**0.00**
Days of hospitalization	Equal variances assumed	30.18	0.00	-6.67	298.00	**0.00**
	Equal variances not assumed			-6.67	246.57	**0.00**
Days of catheter	Equal variances assumed	24.35	0.00	-5.96	297.00	**0.00**
	Equal variances not assumed			-5.97	260.20	**0.00**
Days with temperature	Equal variances assumed	4.45	0.04	-1.84	120.00	0.07
	Equal variances not assumed			-1.99	118.89	0.05

DISCUSSION

The largest number of Snodgrass surgeries lasted from 61 to 120 minutes, while the largest number of Mathieu surgeries lasted from 61 to 90 minutes. The highest percentage of patients undergoing Snodgrass surgery received therapy up to 7 days, while the majority of patients undergoing Mathieu surgery received therapy for 8-14 days; hence, patients undergoing Snodgrass surgery received therapy for shorter period [8, 9, 10, 11].

In general, patients who had undergone Snodgrass surgery were hospitalized for a shorter period compared to those who had undergone Mathieu surgery; thus, the results speak in favor of Snodgrass technique.

In a higher percentage of surgeries with Mathieu procedure cystostoma was used, while in a higher percentage of surgery with Snodgrass procedure Foley's catheter was used. The results have shown similar percentage of given local antibiotic in cases treated by both techniques [12, 13].

The analysis of results has shown that there were a lower percentage of patients with elevated body temperature among cases treated with Snodgrass technique, which means that they speak in favor of Snodgrass compared to Mathieu technique [14, 15].

Almost all mean measures of Snodgrass cases had lower values compared to Mathieu, except for duration of surgery in minutes Mathieu technique showed better results than Snodgrass.

The mean measures in Snodgrass cases were lower than in Mathieu cases ($p< 0.01$) regarding the following measures: duration of therapy, days of hospitalization and days of catheter presence (values are bolded). The given results show the superiority of Snodgrass technique compared to Mathieu for the given measures [17, 18, 19].

CONCLUSION

Our study has shown that Snodgrass surgical technique (Tubularized incised plate urethroplasty) is superior and most acceptable in formation of the neourethra in cases of anterior hypospadias than the Mathieu technique (Meatal-based flat technique). It is also important to emphasize that duration of surgery is shorter and the percentage of complications is lower when using Snodgrass surgery. The cosmetic outcome and normal functioning of the penis meet all the criteria of anterior hypospadias repair using Snodgrass procedure and it is a method of choice, but in cases where there is no healthy urethral plate the Mathieu surgical technique is recommended.

REFERENCES

1. Snodgrass W, Koyle M, Manzioni G, Hurwitz R, Caldomone A, Ehrlich R. Tubularized incised plate hypospadias repair: results of multicenter experience.J Urol. 1996; 839-41.
2. Snodgrass W. Tubularized incised plate urethroplasticy for distal hypospadias. J Urol 1994;151:464-5.
3. Hesham ME.Primary distal hypospadias repair: Tubularized incised-plate urethroplasty (Snodgrass) versus the perimeatal-based flap (Mathieu).J Plast Reconstr Surg 2004;28:55-61.
4. Aminisharifi A, Taddayun A, Assdolahpoor A, Khezri A. Combined use of Mathieu procedure with plate incision for hypospadias repair: a randomized clinical trial. Urol.2008;72:305-8.
5. Keith LM, Persaud TV.The developing human. Clinically oriented embryology; 2008:271-3.
6. McElreavey K, Fellous M. Sex determination and Y chromosome.Am J Med Genet 1999;89:176.
7. Moore KL, Dalley AF. Clinically Oriented Anatomy, 6thed. Baltimore, Williams&Wilkins, 2006.
8. Keith LM, Arthur F, Dalley I, Anne MR. Clinically oriented anatomy; 2010:419-23.
9. Anger MT, Murphy LI. The history of urology. Thomas Springfield, Illinois 1972: 1874b; p 454.
10. Anger MT. Hypospadias peno-scrotal complique de coudure de la verge: redressment du penis et urethra-plastie par inclusion cutaneezguerison. Bull Soc Chir Paris. 1875;179.
11. Bevan AD. A new operation for hypospadias. JAMA.1917; 68:1032.
12. Davis DM. The pedicle tube graft in the surgical treatment of hypospadias in the male. Surg Gynecol Obstet. 1940;71:790.
13. Davis DM. The surgical treatment of hypospadias especially scrotal and perineal. Plast Reconstr Surg. 1950; 5:373.
14. Duckett W. MAGPI (meatal advancement and glanuloplas-ty):a procedure for subcoronal hypospadias. Urol Clin North Am 19811); 5211-8513.
15. ElGanainy EO, Abdelsalam YM, Gadelmoula MM, Shalaby MM. Combined Mathieu and Snodgrass urethroplasty for hypospadias repair: a prospective randomized study. Int J of Urol.2010; 17:661-5.
16. Oswald J, Korner I, Riccabona M. Comparation of the perimetal- based flap (Mathieu) and the tabularized incised-plate urethroplasty (Snodgrass) in primary distal hypospadias. BJU Int. 2000; 85 (6):725-7.
17. Imamoglu MA, Bakirtas H. Comparation of two methods-Mathieu and Snodgrass- in hypospadias repair. Urol Int. 2003;71(3):251-4.
18. Morandi M, Morandi A, Ghaderpanah F. Copmparation of Snodgrass and Mathieu surgical techniques in anterior distal shaft hypospadias repair. Urol J.2005;2:28-31.
19. Hasoon M.Comparative study in anterior distal hypospadias reconstruction utilizing different techniques (Mathieu and Snodgrass): Outcome, complications and failure. J Pharmacy. 2013;3:53-9.

UDC:616.718.5-001.5-089.8

MINIMALLY INVASIVE TREATMENT USING CONTRA LATERAL ELEVATION OF TIBIAL PLATEAU FRACTURES- RESULTS AND PROTOCOL OF TREATMENT

Gavrilovski Andreja[1], Dimovska – Gavrilovska A[2], Nikolov Lj[1], Kaftandziev I[1], Poposka A[3]
[1]University Clinic of Traumatology – TOARILUC; [2] University Clinic of Neurosurgery; [3]University Clinic of Orthopedics – TOARILUC, Skopje, Macedonia

ABSTRACT

Tibial plateau fractures, also known as proximal tibial articular fractures are most commonly described by the term complex knee trauma because these kinds of injuries are associated with asignificant damage to two or more of the following compartments: the soft-tissue envelope, ligamentous stabilizers and the bony structures of the knee. Therefore the preoperative care, the surgical method of choice and postoperative care must be elected carefully and planned in advance due to further possible various early and late complications.

This study elaborates tibial plateau fracture patterns according to the Schatcker classification (type I, II, III and IV) treated with a minimally invasive surgical method using contra lateral elevation of tibial plateau. Our goal was to present that this type of treatment proved to be superior above others in terms of the amount of blood loss, length of the surgery, complications and functional results observed 6 months after the procedure.

Methods: In the period between April 2011 to June 2015, at the Clinic of Traumatology in Skopje, 16 patients (8 males and 8 females) were treated with minimally invasive surgical method using a contra lateral elevation of tibial plateau. The patients were evaluated 6 months after surgery, Tegner and Lysholm functional scoring was done.

Results: Seventeen patients (8 males and 9 females) were treated at the Clinic of Traumatology in Skopje, with minimally invasive surgical method using contra lateral elevation of tibial plateau. Postoperative examination of the knee took place on the 6th day after surgery. In one patient physical examination has shown positive anterior drawer findings, a result of an anterior cruciate ligament injury. Another one had positive finding in the valgus test, a result of medial collateral ligament injury. The first patient can walk only with walking stick and the second one has slight valgus deformity. All patients were evaluated by Tegner and Lysholm scoring system. The average score was 87.76 (minimal score of 44 to maximal score of 100). The standard deviation in the statistical analysis and calculations was 16.51.

Conclusion: Minimally invasive surgical method using a contra lateral elevation of tibial plateau used for treatment of tibial plateau fracture patterns according to the Schatcker classification (type I, II, III and IV) is a surgical method with no major complications and shorter recovery period. Also the hospital stay was shorter, the verticalisation was quicker and faster return of the patients in the social and working environment was noted.

Key words: platotibial fractures, minimal invasive, Tegner and Lysholm scoring system

INTRODUCTION

Tibial plateau fractures are defined as complex fractures because they are placed in the group of articular fractures. The tibial plateau as upper surface and the medial and lateral condyle of the femur as lower surface are the bone structures of the knee. Their surfaces are coated with articular cartilage and medial and lateral meniscuses are placed beneath. Medial collateral ligament, lateral collateral ligament, anterior cruciate ligament and posterior cruciate ligament, as soft tissue structures are a part of the knee. All these elements of the knee give proper function and stability of the knee joint [1].

The ligaments and meniscus are attached to the tibial bone, so when trauma occurs, they can be injured. According to Bennett and Browner in 96% of the cases complex knee trauma is accompanied by injury of cruciate ligaments, in 67% of the cases there is also trauma on the menisci and in 85% of the cases the fractures are followed by injuries of the collateral ligaments [1].

Anatomically on the proximal region of the tibia 3 parts or 3 plateaus- lateral, central and medial plateau can be recognized. On the lateral plateau (lateral third of proximal tibia) the lateral meniscus lies where lateral collateral ligament is attached. On the posterior-lateral part of the lateral tibial plateau the head of the fibula is connected. Peroneal nerve is possitionated a round fibula`s head from lateral to anterior side. Cruciaate ligaments are attached on the central plateau which is elevated uneven part i.e. intercondylar eminence. In front and behind the eminence the menisci are attached. Behind the intercondylar eminence vascular vessels of the knee joint are located. On the medial plateau (medial third of proximal tibia) medial meniscus lies and medial collateral ligament is attached [2, 3, 4].

Accurate determination of fracture pattern and soft-tissue injury is necessary when developing a treatment plan, everything should be precisely and cautiously considered, because of the possible complications. According the newest principles of a minimally invasive treatment the best option is to do indirect reposition of joint structures without indirect visualization under fluoroscope. Minimally invasive treatment using contra lateral elevation of tibial plateau fractures is in agreement with the latest updates in these types of surgeries.

This kind of treatment is superior above the others in terms of the amount of: blood loss; because the joint`s space is not opened and the incision is smaller; risk of postoperative infection is lower; the hospital stay is shorter; shorter recovery period, resulting in sooner rehabilitation processs, which leads to full recuperation from the injury.

It was our aim to show that tibial plateau fracture patterns according to the Schatcker classification (type I, II, III and IV) treated with minimally invasive surgical method using a contra lateral elevation of tibial plateau is superior above the others due to decreased amount of blood loss, length of the surgery, complications and functional results observed 6 months after the procedure.

MATERIALS AND METHODS

In the period between April 2011 to June 2015 in the Clinic of Traumatology-Skopje 16 patients (8 of them males, and 8 females) were treated with minimally invasive treatment using contra lateral elevation of tibial plateau.

Patients had to meet the following basic inclusion criteria:

1. Tibial plateau fractures type I, II, III or IV according to the Schatcker classification.
2. Fractures only of the tibial plateau bone.
3. Fractures of the tibial plateau bone, accompanied by one or more fractures of other bones.
4. The general health condition of the injured should be good enough to allow surgery in the next 3 to 5 days after the trauma occurred.

Exclusion criteria were:

1. Patients with tibial plateau fractures type I, II, III or IV according to the Schatcker classification, but with high ASA score, a result of prior health disorders such as: inadequately treated diabetes mellitus, cardiomyopathia chronic, elderly patients etc.
2. Tibial plateau fractures type V or VI according to the Schatcker classification
3. Fractures only of the tibial plateau bone, or accompanied by one or more fractures of other bones. However the general health condition of the injured don`t allow patient to be operated in the next 3 to 5 days after the trauma event. Proper reposition of fracture fragments cannot be achieved after the fifth day with the minimally invasive method.

The mean age at the time of surgery of male patients was 45 years (from 33 to 64 years), and the mean age of female patients was 56 years (from 27 to 70 years).

At hospital admission radiographs and CT scans were made. Laboratory investigations and ECG test were done. Clinical and radiological evaluation showed that 2 patients have had Schatcker type I fracture, five had Schatcker type II fracture, 8 patients had Schatcker type III fracture and one patient have had Schatcker type IVfracture. The time between trauma and surgery was 3 to 5 days. During that period the edema was reduced. Patients were laid in supine position, placed on surgical table for extension, conducted into spinal anesthesia. Traction was applied to injured shanks in neutral position. Reposition was guided under fluoroscope. After isolation of the operating field, in parallel with tuberositas tibiae and incision was made (1.5 cm long) and another one (also 1.5 cm long) behind and to the contra lateral side of the fracture. After reaching the bone, with 4 consecutive drills (2.8 mm to 6 mm) 1 hole was made into the bone. The fracture fragments were again appropriately repositioned, controlled under fluoroscope, and pointed reduction forceps was placed to hold the preferred position. Then a periosteal elevator was inserted beneath the depressed articular fragments, and by slow and meticulous pressure the articular fragments were elevated. This produces a large cavity in the metaphysis that it must be filled with bone, but in our cases hydroxyapatite bone graft substitute. At the end were cancellous screws for definitive fixation was used. The knee was immobilized placed in knee brace.

Postoperatively, patients received antibiotics, analgesic drugs, drugs for prophylaxis of blood clots. The first day after surgery, control lab test was made. The seventh day after surgery sutures were taken out. The patients were evaluated 6 months after surgery, Tegner and Lysholm functional scoring was done [2, 3]. The maximal score was one hundred points, equivalent to non objective or subjective problems with knee function. Tegner and Lysholm scoring system evaluate 8 sections: 1. Limp; 2.Support; 3. Pain; 4.Instabillity; 5.Locking; 6.Swelling; 7.Stair-climbing; 8.Sqautting.

RESULTS

Seventeen patients (8 males and 9 females) were treated at the Clinic of Traumatology in Skopje, with minimally invasive surgical method using contra lateral elevation of tibial plateau. The length of the surgery was approximately 30 minutes. Because of the small incision no significant blood lost was noticed (confirmed by the control blood count test) and during the surgery no blood derivates were given. The third day after the surgery all patients were discharged to homecare. Crutch walking was begun, but no weight bearing was permitted until the 15th post operative day. After that patients were referred to physical therapy, where gradual weight bearing was started and specific exercise given. After the first post operative month full weight bearing was accomplished.

Postoperative examination of the knee took place on the 6th day after surgery. In one patient physical examination has shown positive anterior drawer findings, a result of an anterior cruciate ligament injury. Another one had positive finding in the valgus test, a result of medial collateral ligament injury. The first patient can walk only with walking stick and the second one has slight valgus deformity.

All patients were evaluated by Tegner and Lysholm scoring system. The average score was 87.76 (minimal score of 44 to maximal score of 100). The standard deviation in the statistical analysis and calculations was 16.51.

DISCUSSION

Because of the complex nature of the tibial plateau fractures, the right treatment for different type of these fractures was subject of discussion for a long time.

Initially tibial plateau fractures type I, II, III or IV according to the Schatcker classification were treated conservatively, regardless of the soft tissue injuries, depression of plateau, residual knee instability, varus and valgus angulations [4, 5, 6].

With the medical progress of medicine, the point of view for treatment of these fractures has started to change. In the past they were treated by open arthrotomy due to depression of tibial platea, reposition and fixation of the fragments was done with plates and screws. However the size of depression of fragments was not considered [7, 8, 9].

According to the latest worldwide studies and case reports, the most important indications for treatment of tibial plateau fractures are residual knee instability, varus and valgus angulations [10].

Treatment methods proposed for these fractures include: arthrotomy, reposition and fixation of the fragments with plates and screws; arthroscopy with percutaneal fixation; minimal invasive treatments with small incisions and fixation with contoured plates and minimal invasive treatments with traction with percutaneous fixation [11].

Open arthrotomy, reposition and fixation of the fragments with plates and screws is adequate in complex, comminuted fractures. But this method is less used because of residual knee instability, limited flexion, and high risk of infection.

Unlike this method, during the last of years, the minimally invasive surgery is more appreciated and shows better results. Different surgeons propagate different type of minimally invasive surgery. But it has been shown that no matter which minimal invasive surgery is used for treatment, the functional result are almost the same [12, 13, 14]. Our results are in accordance with previously published studies.

The golden surgical technique is the arthroscopical reposition with elevation of the depressed plateau, cement application into bone defect to prevent further depression [14] and percutaneous fixation.

CONCLUSION

Minimally invasive surgical method using the contra lateral elevation of tibial plateau used for treatment of tibial plateau fracture patterns according to the Schatcker classification (type I, II, III and IV) is a surgical method with no major complications and shorter recovery period. Also the hospital stay was shorter (2 days), there was a negligible amount of blood loss, and positive functional results were observed 6 months after the trauma and procedure.

REFERENCES

1. Bennett WF, Browner B. Tibial plateau fractures: a study of associated soft tissue injury. J Orthop Trauma. 1994; 8(3):183-8.
2. Kraus TM, Martetschlager F, Muller D, et al. Return to sports activity after tibial plateau fractures: 89 cases with minimum 24-month follow-up. Am J Sports Med. 2012;40(12):2845-52.
3. Muller D, Sandmann GH, Martetschlager F, Stockle U, Kraus TM. Tibial plateau fractures in alpine skiing--return to the slopes or career end? Sportverletz Sportschaden. 2014;28(1):24-30.
4. Apley AG. Fractures of the lateral tibial condyle treated by skeletal traction and early mobilisation: a review of sixty cases with special reference to the long-term results. J Bone Joint Surg Br. 1956; 38B(3):699-708.
5. Apley AG. Fractures of the tibial plateau. Orthop Clin North Am. 1979;10(1):61-74.
6. Bakalim G, Wilppula E. Fractures of the tibial condyles. Acta Orthop Scand. 1973; 44(3):311-22.
7. Brown TD, Anderson DD, Nepola JV, et al. Contact stress aberrations following imprecise reduction of simple tibial plateau fractures. J Orthop Res 1988; 6(6):851-62.
8. Tscherne H, Lobenhoffer P. Tibial plateau fractures: management and expected results. Clin Orthop Relat Res. 1993; 292:87-100.
9. Hohl M. Treatment methods in tibial condylar fractures. South Med J 1975; 68(8):985-91.

10. Rasmussen PS. Tibial condylar fractures as a cause of degenerative arthritis. Acta Orthop Scand. 1972; 43(6):566-75.
11. Moore TM, Patzakis MJ, Harvey JP. Tibial plateau fractures: definition, demographics, treatment rationale, and long-term results of closed traction management or operative reduction. J Orthop Trauma 1987; 1(2):97-119.
12. Ballmer FT, Hertel R, Notzli HP. Treatment of tibial plateau fractures with small fragment internal fixation: a preliminary report. J Orthop Trauma. 2000; 14(7):467-74.
13. Caspari RB, Hutton PM, Whipple TL. The role of arthroscopy in the management of tibial plateau fractures. Arthroscopy 1985; 1(2):76-82.
14. Vendeuvre T, Babusiaux D, Breque C, et al. Tuberoplasty: Minimally invasive ostesynthesis technique for tibial plateau fractures. Orthop Traumatol Surg Res. 2013; 99(4 Suppl):S267-72.

ERRATUM CORRIGENDUM

The article “Our preliminary experience with spect ^{99m}Tc-HMPAO brain perfusion scans in diagnosis of dementia” from the authors Makazlieva T, Vaskova O, Crcareva B, Zdraveska-Kocova M, Krsteska R, Stoeva M, Mukaetova-Ladinska E was published in Acta Morphol. 2015;12(1):22-28. Due to an unintentional technical error in the printing process the abstract will be published in the journal Acta Morphol. 2015;12(2).

We sincerely apologize to the authors for the unintentional technical errors in the printing process.

UDC:616.892.3-073.916

OUR PRELIMINARY EXPERIENCE WITH SPECT ^{99M}TC-HMPAO BRAIN PERFUSION SCANS IN DIAGNOSIS OF DEMENTIA

Makazlieva Tanja[1], Vaskova O[1], Crcareva B[1], Zdraveska-Kocova M[1],
Krsteska R[2], Stoeva M[2], Mukaetova-Ladinska E[3]
[1]Institute of Pathophysiology and Nuclear Medicine, Medical Faculty, Skopje
[2]Psychiatric Hospital, Psychogeriatric Center - Skopje
[3]Institute for Ageing and Health, Newcastle University

ABSTRACT

Introduction: Dementia is a clinical syndrome caused by a spectrum of different etiopathogenetic disorders, such as neurodegenerative, infective, expansive or other morphological processes in the brain tissue. Several studies indicate the possibility of differential diagnosis of dementia according to the findings of brain SPECT study.

Aim: The objective of the paper is to present our preliminary experiences with SPECT 99mTc-HMPAO brain perfusion scintigraphy in the diagnosis of dementia.

Materials and methods: ^{99m}Tc-HMPAO SPECT brain perfusion was performed on 9 patients selected after detailed neuropsychiatric investigations conducted by specialist psychiatrist. Obtained images were reconstructed with commercial software Mediso Brain SP128 and the qualitative and semiquantitative analysis of the data was carried out.

Results: We found hypoperfusion defects in all evaluated patients, in most cases unilateral hypoperfusion in the left hemisphere, respectively in 56% of evaluated patients and in six out of nine, respectively in 67%, hypoperfusion of the left temporal lobe. Most common finding was parietal hypoperfusion, found in 8 patients (89%) and left mesial temporal lobe hypoperfusion, detected in 6 patients (67%).

Conclusion: Findings of all the evaluated patients showed indisputable defects in perfusion which in correlation with clinical criteria give useful guidance in diagnosis of dementia. We concluded that it is necessary to continue with the prospective study of larger number of patients and with multidisciplinary approach in order to obtain relevant conclusions with statistical significance.

Key words: ^{99m}Tc-HMPAO SPECT, dementia, brain perfusion

INFORMATION FOR AUTHORS

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

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