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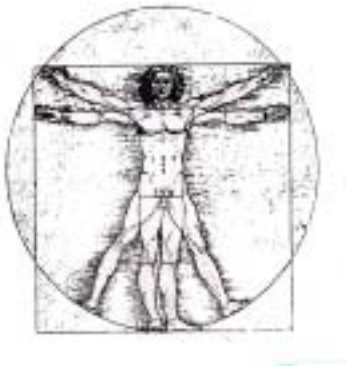

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CONTENT

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MORPHOLOGIC FEATURES IN TRANSITORY LUMBOSACRAL JOINTS

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Abstract

The aim of the study was to investigate the morphological features of transitory sacra and the morhostructural differences between these and sacra in normal lumbosacral junction. A total of 200 lumbar spine MR examinations from adults with low back pain over a period of six months were retrospectively selected and included in the study. The protocol consisted of sagittal, axial and coronal T1 and T2-weighted spin–echo sequences. The coronal and axial images were assessed for the presence of transitory lumbosacral vertebra. Of the total of 200 patients, 100 had normal and 100 had transitional lumbosacral junction. Measurements were made both on transitory and normal sacra using software based tools. The sacra with accessory articular facets were characterized with facets themselves being closer to each other, flatter and more coronally oriented. The partially lumbarized sacra were characterized with facets themselves being closer to each other, more sagitally oriented with facet joints on greater distance from the vertebral body. Transitory lumbosacral junction was characterized with increased lumbosacral angulation.

Key words: lumbar spine, MRI, transitional vertebra, Castellvi classification

Introduction

The sacrum is the final connection between the axial and the appendicular skeleton for transmission of load from the spine toward the hip bones at the sacroilac joints. Biomechanics of load bearing at the lumbosacral junction depends on its structural characteristics. Lumbosacral transitional vertebra (LSTV) is vertebra lying at the juncture of lumbar and sacral spinal segments that exhibit characteristics of the neighboring vertebral class. These characteristics usually regard to the shape and fusion patterns of their transverse processes. The incidence of this developmental spinal anomaly is between 4 and 30% in general population [1]. Controversial opinions exist regarding the clinical significance of this entity [2, 3]. Some authors have reported that a lumbosacral transitional vertebra is protective for disc degeneration at the transitional segment, but prone to greater disc degeneration at the level above [4, 5]. Other investigators have reported earlier occurrence and more severe degeneration in subjects with LSTV [4, 6]. It has been widely accepted that LSTV alter the biomechanics of the spine and contribute to low back pain [7-9].

The aim of the study was to investigate the morhological features of sacra bearing lumbosacral transitions and to examine the differences between transitional and normal sacra. These issues can help in understanding the structural and biomechanic characteristics of the transitional lumbosacral junctions.

Material and methods

Subjects

From MRI examinations of the lumbar spine performed in adults with low back pain over a period of six months (from September 2012 to February 2013) 200 subjects were retrospectively selected and included in the study. Patients with kyphoscoliosis, history of previous spine surgery, spinal fracture, other congenital spinal anomalies, tumor or infection were excluded. The study group included 100 patient with transitional lumbosacral vertebra (52 men, 48 women, mean age 52.75±9.42) and the control group consisted of 100 patients with normal lumbosacral junction (42 men, 58 women, mean age 45.87±10.5).

MR Imaging.

MR imaging examination of the lumbosacral spine was preformed with 1.5 T MR unit (Signa HDI) with a spinal surface coil. The imaging protocol consisted of a sagittal T1-weighted fast spin-echo sequence (FSE) (repetition time msec/echo time msec, 800/14; section thickness, 4 mm; field of view, 360x360 mm; matrix, 448 x 224), sagittal T2-weighted turbo spin-echo sequence (3520/102; section thickness, 4 mm; intersection gap, 10 mm; echo train length of 24), coronal T2-weighted fast spin-echo sequence (FSE) and a transverse T2-weighted fast recovery fast spin-echo (FRFSE) sequence at one or multiple levels (4,660/120; section thickness, 4 mm; intersection gap, 0.6 mm; echo train length of 27; field of view, 200x200 mm; matrix 320 x 256). Diagnostic imaging and measurements were performed by two diagnostic radiologists. Measurements were made on transitory or normal sacra using software based tools. Transitory sacra beared unilateral or bilateral accessory articulations, or owned lumbarized S1 segment partially or complete separated from the rest of the sacral mass. The interrater realibility of the MRI examinations and measurements was estimated using agreement percentage and Kappa

statistics between observers. Interobserver agreement ranged from substantial to excellent, with kappa values ranging from 0.62 to 0.81.

Transverse sacral interfacet distance, (TIF) - distance between the outer borders of sacral articular facets.

Perpendicular sacral body facet distance (PVFD) - perpendicular distance between the midpoint of the posterior border of the S1vertebral body and the coronal plane located on the dorsal margins of the sacral facet joints [Fig. 1].

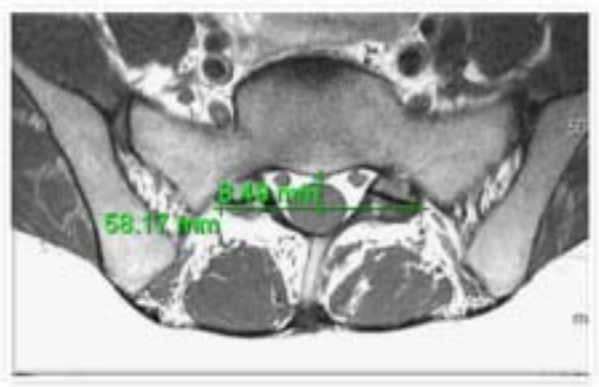

Fig.1. Transverse lumbosacral interfacet distance, (TIF) and perpendicular vertebral body facet distance (PVFD)

Right and left sacral facet angle to the sagittal plane (RSFA, LSFA) - on axial T2 images the orientation of the sacral facets was measured as an angle between the line in the midsagittal plane of sacrum and the line drawn tangential to the sacral articular facets [Fig.2].

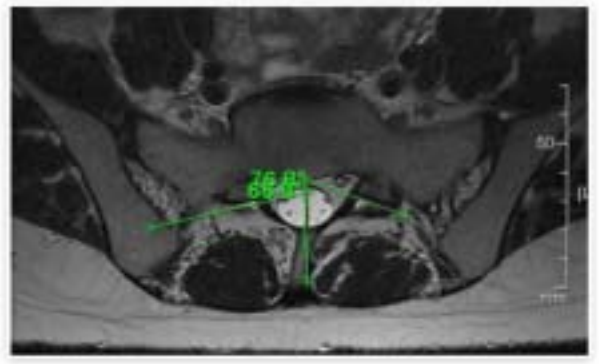

Fig.2. Right and left lumbosacral sagittal angle (RLSA, LLSA)

Lumbosacral angle (LSA) - on midsagittal T2 weighted images the angle was measured between the line parallel to the superior surface of sacrum and the horizontal plane [Fig.3].

Fig.3. Lumbosacral angle (LSA)

One way ANOVA was used for statistical comparison between different groups of lumbosacral transitory vertebra. A p value less than 0.05 was considered statistically significant.

Results

The study group consisted of 100 patients out of whom 94 were with sacralization and 6 with partial or complete lumbarization. The evaluated types of transitional lumbosacral vertebra according to Castellvi classification were: unilateral enlarged transverse process (TP) with articular fusion with sacral ala categorized as Castellvi type IIA found in 45(48%) subjects, bilateral enlarged TP with bilateral articular fusion with sacral ala categorized as IIB in 44 (47%) subjects, bilateral enlarged TP with complete osseus fusion with the sacral ala in 3 (3%) subjects. Mixed type with pseudoarthrosis on one side and complete osseus fusion on the other side was present in 2 (2%) subjects.

Analysis of the linear dimensions showed that the transverse lumbosacral interfacet distance was significantly smaller in transitory sacra than in normal ones. Perpendicular distance between sacral vertebral body and sacral articular surfaces was smaller in sacra with articulated or osseus fusion with L5, but larger in sacra with lumbarized S1 [Table 1].

Analysis of the orientation of the facet joints was measured as an angle between the line in the midsagittal plane of the first sacral vertebra and the line through each facet joint tangential to the sacral articular processes. The analysis showed that the facets in sacra with articulated or osseus fusion with L5 were more coronally oriented, while the facets in sacra with lumbarized S1 were more sagitally oriented compared with

Table 1. Comparison of the means of linear parameters in normal and transitory sacra

Linear dimensions	Normal sacra	Sacra with articulated or osseus fusion with L5	Sacra with lumbarized S1	Test of significance for difference in means
Transverse interfacet distance -TIFD	58.38 (±6.78)	55.29 (±6.78)	52.19 (±4.72)	P=0.03
Perpendicular vertebral body facet distance-PVFD	19.18 (±3,43)	18.74 (±3.39)	22.62 (±2.01)	P=0.006

Table 2. Comparison of the means of the right and left sacral facets angle to the sagittal plane in normal and transitory sacra

Angles	Normal sacra	Sacra with articulated or osseus fusion with L5	Sacra with lumbarized S1	Test of significance for difference in means
Right sacral facet sagittal angle-RSFA	46.86 (±11.64)	48.71 (±12.69)	41.89 (±6.63)	P=0.234
Left sacral facet sagittal angle-LFSA	47.81(±11.68)	49.32 (±10.85)	44.43 (±6.75)	P=0.4
Lumbosacral angle-LSA	32.46 (±6.99)	35.03 (±7.88)	35.39 (±7.79)	P=0.09

Table 3. Comparison of the means of the indices in normal and transitory sacra

Sum of anglesIndex	Normal sacra	Sacra with articulated or osseus fusion with L5	Sacra with lumbarized S1	Test of significance for difference in means
TIFD/PVBFD	3.16±0.79	3.06±0.75	2.34±0.40	P=0.009

the normal sacra, without significant differences. The angle measured between the line parallel to the superior surface of sacrum and the horizontal plane was identical to the lumbosacral angle measured on lateral radiographs of the spine. Increased lumbosacral angle was characteristic for the transitory lumbosacral junctions [Table 2].

The index that involve transverse sacral interfacet distance (TIFD) and perpendicular sacral body facet distance (PVFD) was smaller in transitory sacra than in normal ones because of the smaller absolute values of the parameter (transverse sacral interfacet distance) in transitory sacra than in normal ones [Table 3].

Discussion

This study was conducted to investigate speciûc morphostructural characteristics in transitory sacra that can be associated with transitional states at the lumbosacral junction that distinguish transitional from normal sacra. Sacral dimensions and position of articular surface areas can be detected with precise imaging techniques. Evaluation of dimensions in these transitional sacra may also help in understanding the biomechanics at these transitional L5–S1 junctions. Transitional sacra share some similar parameters, own flatter articular facets that lost their concavity and have their facet joints closer to the vertebral bodies, with the facets themselves being closer to each other.

The facet joints are highly loaded structures [10,11].Weight in transitory sacra is received through the sacral bodies, the facet joints, and the accessory facets toward the auricular surfaces. These joints at the top of the S1segment are subject to compressive force from the L5 above. A signiûcant proportion of the load transferred

to the facet joints is a shearing force. The facet joints resist the shearing forces, prevent forward translation of the L5 on the sacrum, and play a significant role (along with the anterior elements like the intervertebral discs) in preventing spondylolisthesis. Excessive translational or rotational stress at the facet joints may lead to structural failure and displacement of the facets [12,13].

The facet joints associated with the L5–S1 accessory articulations are possibly associated with diminished load bearing activity on account of their reduced articulating areas. Facet joints surfaces at normal lumbosacral junctions are typical in their orientation, angulation and direction [14]. Normal facet areas have been studied to defne the proportion of load bearing at these areas, their angulation with respect to the coronal and sagittal planes, and features of the curvatures on their articular surfaces [15]. The facet joints are signifcantly involved with the stability at the L5–S1 junction. Presence of rudimentary facets in transitional sacra substantiates on diminished quantum of load transferred through the facet joints. This situation might precipitate instability at the L5–S1 facet joints when a greater degree of stress affects the lumbosacral junction. Some facets in sacra with accessory articulations demonstrated loss of concavity and a more coronal orientation. The accessory articulations restrict lateral bending and rotation at this L5–S junction [16]. The value of the indices evaluated in transitory sacra exhibit transitional properties of these sacra. These indices defne the relative proportions of different dimensions of these sacra and possibly can be used to understand the biomechanics of load bearing in these transitional states. The altered biomechanics of weight transmission across L5/S1 articulations might precipitate a greater degree of stress and instability in this area. The relationship of transitional lumbosacral junction with back pain, disc disease, stenosis and other degenerative changes has been well documented and widely accepted [17].

Conclusions

Sacra with articulated or osseus fusion with L5 are characterized with facets themselves being closer to each other, flatter and more coronally oriented

Sacra with lumbarized S1 are characterized with facets themselves being closer to each other, more sagitally oriented settled on greater distance from the vertebral body.

Transitory lumbosacral junction is characterized with increased lumbosacral angulation

Transitional sacra are associated with altered morphologic characteristics and altered biomechanics of weight transmission across L5/S1 articulations.

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EVALUATION OF SEX-SPECIFIC DIFFERENCES OF ANTHROPOMETRIC PARAMETERS OF GROWTH IN 9 YEAR-OLD MACEDONIAN CHILDREN FROM URBAN AREA

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Abstract

Aim: Evaluation of sex-specific differences of anthropometric parameters that were used as indicators for growth in children at the age of 9 years.

Subjects and methods: The study included 225 healthy children aged 9 years from Macedonian nationality and urban area. Fourteen anthropometric parameters were measured that define longitudinal, circular and transversal parameters of the skeleton using standard equipment and measurement technique. The following indicators were calculated: weight-for-age [BW], height-for-age [BH] and BMI.

Results: The majority of anthropometric parameters showed no significant sex-specific differences, with exception of diameter of wrist, and circumferences of head, chest, and upper-arm, which were significant in favour of boys. Values of the 50th percentile in boys were as follows: 34.4kg for BW, 136.7 cm for BH and 18.25 kg/m^2 for BMI. The values of these parameters in girls were: 32.25 kg for BW, 133.05cm for BH and 18 kg/m^2 for BMI.

Conclusions: These results can be used as criteria for the assessment of the anthropometric characteristics and detection of deviations in growth and nutritional status in 9-year-old children.

Key words: anthopometry, growth, children

Introduction

One of the major characteristics in childhood is the continuing process of growth and development [1]. Nutrition is an important ecological phenomenon that has impact on all stages of growth and development. Therefore, its influence on growth has to be constantly monitored and assessed because growth is particularly sensitive on nutritional deficit or surpus [2]. Current knowledge points out to an increasing rate of risk factors for onset of non-communicable diseases such as obesity, its appearance and trend, and consequently a need has been imposed for evaluation and monitoring the growth and nutritional status [3]. Anthropometric examinations are non-invasive, simple, and easy adaptable to chidren's age [4]. They enable monitoring of the dynamics of child's growth and also indicate disorders in the growth and nutritional status during this childhood period [5]. Anthropometry can assess all these parameters by measuring certain dimensions that define longitudinal, transversal and circular dimensionality of the skeleton, mass and body volume.

Aim

Evaluation of sex-specific differences in anthropometric parameters that are used as indicators of growth in children at the age of 9 years.

Subjects and methods

The study included healthy 9-year-old children of both sexes and of Macedonian nationality, randomly selected from different urban regions in R. Macedonia. The total number of subjects (n=240) was divided into two groups based on the sex criterion (n=120 boys and n=120 girls).

Anthropometry

In line with the aim of the study 14 anthropometric variables were selected and measured according to the guidelines of the International Biological Programme [IBP]. The following anthropometric parameters were measured: for assessment of the longitudinal dimensionality of the skeleton - body height, length of the arm, length of the leg; for assessment of the transversal dimensionality of the skeleton - elbow diameter, wrist diameter, knee diameter, ankle diameter; for assessment of the mass and circular dimensionality, or volume of the body - body mass, chest circumference, head circumference, mid-upper arm circumference, forearm circumference, thigh circumference, calf circumference. Standard anthropometric instruments were used: anthropometer by Martin with reading precision of 1 mm; caliper square with reading precision of 1 mm, and elastic plastic tape with reading precision of 1 mm.

The following anthropometric indices were derived and calculated: BMI (weight divided by the square of height), weight-for-age, and height-for-age.

Definition

According to majority of authors defining the values of these anthropometric indicators is recommended by using the following percentile cut-off points [6-12]:

- Normal distribution (mean values) usually corresponds to percentile range from the 15th to the 85th percentile;
- Percentile range from the 5th to the 15th percentile denotes low values;

- Values under 5th percentile with greater probability points out to underweight as well as to delayed growth if the parameter height-for-age is analyzed;
- Values between 85th and 95th percentile for the indices weight-for-age, height-for-age and BMI are defined as high above the average, indicating children with growth above the average and children at risk of becoming overweight;
- Values above the 95th percentile point out to category of children with extremely high growth, that is, obese children.

Statistical analyses

The obtained data for the relevant variables were analyzed with descriptive statistics and presented with measures of central tendency and its deviation (arithmetic standard values ± standard deviation) as well as with ranges expressed as percentiles. Testing of the significance of the differences between two arithmetic series was performed by analysis of variance (ANOVA), and the statistical significance was defined as a p value <0.05.

Results

Mean values and standard deviations of the examined anthropometric parameters in 9-year-old children along with their sex-specific differences are presented in Tables 1 and 2.

Table 1 shows mean values and standard deviations for the following parameters: weight, height, BMI, length of the arms and legs and diameters of the elbow, wrist, knee and ankle.

Nine-year-old boys had body weight of 34.98 ± 7.5, height of 136.89 cm ± 6.29 and BMI of 18.5 kg/m^2 ± 3.08. Girls at the same age had the following values for the same parameters: weight 33.99 ± 6.62, height 135.5 cm ± 5.46 and BMI 18.42 kg/m^2 ± 2.79. In spite of the fact that mean values of these examined parameters were slightly higher in boys, sex-specific differences were not detected.

Sex-specific difference was found in one of the transversal parameters, wrist diameter, in favor of boys. Concerning the longitudinal parameters, length of arm and leg, sex-specific difference was not significant.

Table 2 presents mean values and standard deviations of the circumferences of the head, chest, mid-arm, forearm, thigh and calf.

Head circumference, chest circumference and mid-arm circumference showed sex-specific differences in favor of boys. Mean values of the other circular parameters [circumferences of forearm, thigh and calf] were slightly higher in boys, but the sex-specific difference was not significant.

Table1. Body weight, body height, BMI, lengths and diameters of the extremities in 9 year-old -children from R Macedonia [mean and standard deviation].

Sex	n	Body weight (kg)	Body height (cm)	BMI (kg/m^2)	Lengths (cm)		Diameters (cm)			
					Arm	Leg	Elbow	Wrist	Knee	Ankle
Male	120	34.98±7.5	136.89±6.29	18.5±3.08	59.92±3.28	78.032.19	6.2±0.87	4.47±0.42[b]	8.8±1.52	6.39±0.73
Female	120	33.9±6.62	135.5±5.46	18.4±2.79	59.49±3.71	77.87±2.03	5.97 ±0.93	4.3±0.47	8.51±1.89	6.22±1.89

[a]p<0.05 vs female children of the same age [ANOVA]

Table 2. Circumferences 9 year-old children from R. Macedonia (mean and standard deviation).

Sex	n	Circumferences (cm)					
		Head	Chest	Mid upper	Forearm	Thigh	Calf
Male	120	52.23±1.24[b]	65.11±5.94[b]	20.6.±2.92[b]	17.6±2.69	37.91±5.54	27.63±4.42
Female	120	51.66±1.4	63.44±6.45	19.6±2.82	16.94±2.23	37.47±5.19	27.21±4.51

[a]p<0.05 vs female children (ANOVA)

Table 3. Sex-specific percentiles of the indexes: weight-for-age, height-for-age and Body Mass Index in 9 year-old-children from R Macedonia

	PERCENTILES					
MALE	**5**	**15**	**50**	**85**	**90**	**95**
Weight-forage	25	27.3	34.4	39	43	45
Height-for-age	127	129.6	136.7	144.09	145	146.5
BMI	14	15.3	18.25	21.5	22.17	23.49
FEMALE						
Weight-for-age	25	27	32.25	38.1	41.8	44
Height-for-age	127	127.9	135.05	141.4	142	144
BMI	14	15.11	18	20.7	21.7	23.01

Sex-specific borderline percentiles of the indices weight-for-age, height-for-age and BMI in children at the age of 9 years are presented in Table 3.

Discussion

We examined several anthropometric parameters in our study, which define longitudinal, circular and transversal dimensionality of the skeleton, mass and body volume, and are used for assessment of growth and nutritional status in children. It was discovered that mean values of almost all examined anthropometric parameters were higher in boys. More significant sex-specific differences were observed in favor of boys. Some borderline percentile ranges of the anthropometric indices in 9-year-old children were also calculated.

The obtained data were compared with similar anthropometric studies conducted in children from other countries and populations.

Longitudinal parameters are considered to be the most reliable indices of the physical growth in children [13]. On the other hand, transversal parameters are considered to be good indicators for bone maturity, and circular parameters along with body weight indicate the body volume, that is its mass. The knowledge about the importance of body mass and height as the basic somatic characteristics has been used in many diverse forms such as indices for precise interpretation of growth and nutritional status in children [14]. The height-for-age index shows the linear growth and the deviation in its value, which is being detected at the 5th percentile as a borderline value aimed to discover children with obstacles in the linear growth as a result of long-term cumulative misbalanced nutrition or health problems [5, 13]. Our values at the 50th percentile of the indices body weight and height were 34.4 kg and 136.7cm for boys and 32.25 kg and 133.05cm for girls at the age of 9 years. These values were moderately lower than those found in the NCHS reference population [11]. Values above the 95th percentile are useful in detecting children at risk of endocrine diseases, growth hormone producing tumors or other similar disorders. The index of body mass, widely known as BMI, together with the weight-for-age index, are parameters for monitoring the nutritional status in childhood [12, 14]. Fig. 1 shows the values of BMI above the 50th percentile in children from R. Macedonia (Mkd) and from other different countries and populations: Mexico (Mex), Zaragoza-Spain (E), America (USA and USA-1) and the reference NCHS population [11, 15-19].

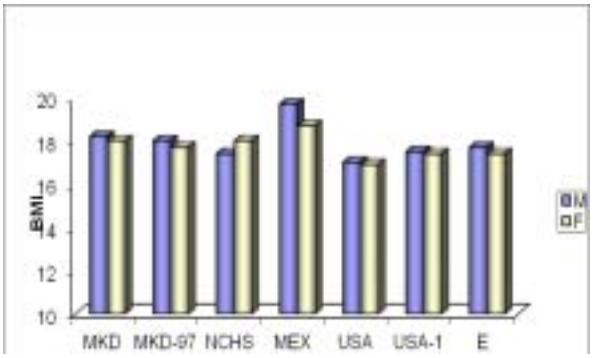

Fig.1. The values of the 50 th percentile of BMI in 9–years-old-children from Macedonian [Mkd] and different areas and population.

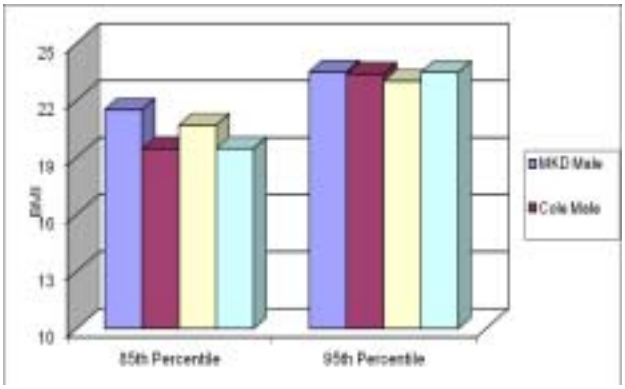

Fig. 2. The cut-off points for BMI of 85th for overweight and 95 th for obesity by sex for 9years old MKD children and Cole's cut off points from international survey

Fig. 2 presents the cut-off values for BMI at the 85th and 95th percentile in our male and female subjects and the relevant values obtained in the representative international reference sample of Cole et al [20].

This data that identify individuals at risk of overweight and obesity show that cut-off points obtained in our male subjects were moderately higher than in the international sample of Cole. On the contrary, the values of BMI at cut-off points in our female subjects were similar to those reported by Cole et al [20].

The detected differences between the children in our study and other studies and with reference to the standard values have shown the existence of population differences in anthropometric parameters that depend on many internal (genetic) and external exogenous factors [2,21-22]. It is necessary to comply with the WHO recommendations that stress the need for each country to prepare its own anthropometric standards. They are indispensable for classification and detection of growth and developmental disorders as well as nutritional status impairment in children at all age stages.

Conclusion

Based on the results obtained in this study, the following conclusions can be derived:

- Boys at the age of 9 years from Macedonian nationality and living in urban regions have shown higher values than girls for the parameters weight, height, that is longitudinal, transversal and circular parameters, except for mid-arm circumference.
- There were sex-statistically significant differences for the wrist diameter and for the circumference (head, chest and mid-arm) in favor of boys. There were no statistically significant differences for the other examined parameters (weight, height, BMI), length of arm and leg, 3 diameters (elbow, knee and ankle) as well as for circumference (forearm, thigh and calf).
- Sex-specific percentile ranges were also determined, that is, borderline percentiles or cut-off points for the anthropometric indices in 9-year-old children.

The results obtained are recommended to be applied in the everyday routine practice as anthropometric criteria for assessment and evaluation of growth and nutritional status. At the same time they might point out to certain imbalance as criteria in selection of individuals for further clinical examinations. Children who are in need of some kind of intervention might be identified by defining the cut-off values. This undoubtedly has a substantial importance in planning certain preventive measures and activities in the field of children's nutrition in one country.

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ATHROPOMETRIC PARAMETERS OF GROWTH IN CHILDREN AT THE AGE OF FOUR

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Abstract

Aim: Evaluation of sex-specific differences of anthropometric parameters as indicators of growth in children at the age of 4.

Subjects and methods: The study included a total of 200 healthy 4-year-old children of Macedonian nationality. Twelve anthropometric parameters were measured, defining longitudinal, circular and transversal dimensionality of the skeleton using standard technique and instruments for measurement. The following indices were selected and calculated: weight-for-age, height-for-age, weight-for-height and BMI. Skin-folds (triceps, scapula and abdomen) were also measured.

Results: Sex-specific differences for almost all anthropometric parameters were detected, but they were not significant. Girls showed higher values than boys regarding weight, height and BMI. Values at the 50th percentile in girls were 20 kg for BW, 108.1 cm for BH and 16.82 kg/m^2 for BMI. The values of these parameters in boys were 19.75 kg for BW, 108.25 for BH and 16.24 kg/m^2 for BMI.

Conclusion: The results obtained can be used as a criterion for assessment and detection of deviations in growth and nutritional status in children aged 4 years.

Key words: anthropometry, children, growth, nutritional status

Introduction

Continuing process of growth and development in preschool children is one of the major characteristics if this childhood period is considered to be very vulnerable to changes [1]. Nutrition is also an important factor that has impact on all stages of growth and development, and hence its influence has to be constantly monitored especially because growth depends on nutritional deficit or surpus. Current knowledge points out to an increasing rate of risk factors for onset of non-communicable diseases such as overweight and obesity [2]. Thus, overweight and its negative effects have imposed the need for evaluation of the growth and nutritional status during the preschool period. Anthropologic examinations, being non-invasive, simple, easy adaptable to children's age, enable monitoring of the dynamics of child's growth and also indicate disorders in the nutritional status during this preschool period [3]. Longitudinal, circular and transversal dimensionality of the skeleton might be defined by measuring the adequate anthropometric parameters.

Aim: Evaluation of sex-specific differences in anthropometric parameters as indicators of growth in children at the age of 4.

Subjects and methods

Healthy preschool 4-year-old children of both sexes and of Macedonian nationality living in urban areas were included in the study. The total number of subjects (n=200) was divided into two groups based on the sex criterion (boys n=110 and girls n=90). Twelve anthropometric parameters were measured in line with the guidelines of the International Biological Programme (IBP). They were: body mass; longitudinal dimensions: body height, length of the arm, length of the leg; transversal dimensions: elbow diameter, knee diameter; circular dimensions of the skeleton: head circumference, chest circumference, mid-upper arm circumference, forearm circumference, thigh circumference, and calf circumference, skin-folds of triceps, scapula and abdomen. Standard anthropometric instruments were used (anthrop -meter by Martin, caliper square, elastic plastic band and medical decimal scales). The following indices were calculated: weight-for-age, height-for-age, weight-for-height and BMI (weight divided by the square of height). Data obtained were analyzed with descriptive statistics presented with measures of central tendency and its deviations (arithmetic standard values and standard deviation) as well as with ranges (percentiles).

Results

Table 1 presents mean values and standard deviations of the following parameters: weight, height, BMI, length of the extremities (arms and legs) and diameters of the elbow and knee. Body weight of the 4-year-old boys was 19.58 ± 3.33, height 107.5 cm ± 5.40 and BMI 16.87 kg/m^2 ± 2.31. Girls at the same age had the following values for the same parameters: weight 19.81 ± 3.77, height 107.42 cm ± 7.03 and BMI 17.02 kg/m^2 ± 1.79. There was no significant difference between the examined parameters except for circular parameters (forearm circumference 15.09 cm ± 1.24 and thigh circumference 27.28 ± 3.70) where the significant difference was in favor of girls. There was also a difference in the skin-folds of triceps 13.2 cm ± 3.65; scapula 11.34 cm ± 4.12 and abdomen 9.58 cm ± 2.44; these values were higher in girls (Table 2). Sex-specific percentiles for the indices weight-for age, height-for-age and BMI in 4-year-old children are shown in Table 3. Borderline values (5th and 85th percentile) in 4-year-old girls were 95.3 cm (5th percentile) and 114.55 cm (85th percentile) for height-for-age, 14 and 23.1 kg for weight-for-age, 14.37 and 18.74 kg/m^2 for BMI. Boys had the following values for the same parameters: 99.97 cm (5th percentile) and 114

Table 1. Body weight, body height, BMI, lengths and diameters of the extremities in 5 year-old -children from R Macedonia (mean and standard deviation).

	n	Body weight (kg)	Body height (cm)	BMI (kg/m^2)	Lengths (cm)		Diameters (cm)	
					Arm	Leg	Elbow	Knee
Male	110	19.58±3.33	107.56±5.40	16.87±2.31	41.98±2.71	55.37±4.67	5.63±0.61	6.24±1.07
Female	90	19.81±3.14	107.42±7.03	17.02±1.79	42.56±3.64	57.77±5.32	5.66 ±0.67	6.40±0.72

Table 2. Values of skin-folds in boys and girls aged 4

	subscapula	*triceps*	*abdomen*
male			
X	9.76	13.37	8.36
SD	±3.28	±2.78	±1.81
female			
X	11.34	13.22	9.58
SD	±4.12	±3.65	±2.44

Table 3. Sex-specific percentiles of the indexes: weight-for-age, height-for-age and BMI in 4 year-old-children

	PERCENTILES								
MALE	**5**	**10**	**15**	**25**	**50**	**75**	**85**	**90**	**95**
Weight-for-age	14.95	15.9	16	17	19.75	22	23	23.1	24.1
Height-for-age	99.97	100.9	101.42	103	108.25	112.12	114	114	114.52
BMI	14.32	14.71	15.02	15.55	16.24	17.93	18.58	19.22	21.11
FEMALE									
Weight-for-age	14	15	15	18	20	21.37	23.1	25	25.35
Height-for-age	95.3	99	99.95	103.37	108.1	111.75	114.55	115.85	118.22
BMI	14.37	14.66	15.34	15.89	16.82	17.79	18.74	19.17	20.20

cm (85th percentile) for height-for-age, 14.95 and 23 kg for weight-for-age; 14.32 and 18.58 kg/m^2 for BMI.

Discussion

We examined several anthropometric parameters that define longitudinal, transversal and circular dimensionality of the skeleton and that are used for assessment of growth and nutritional status in preschool children. It was discovered that mean values of almost all examined anthropometric parameters were higher in girls [4]. More significant differences were observed in circumference of the extremities, particularly the forearm circumference and the thigh circumference that were higher in girls. Some percentile ranges of the anthropometric indices in 4-year-old children were also calculated. Data obtained were compared with similar anthropometric studies conducted in children from other countries and populations. Longitudinal parameters are considered to be the most reliable indices of the physical growth in children, and circular along with body weight indicate the body volume, that is its mass. The importance of the most stable anthropometric parameters, the basic being body weight and height, has been used in very diverse forms such as indices for presenting the growth and nutritional status in preschool children. The height-for-age index shows the linear growth and the deviation in its value, which is being detected at the 5th percentile as a borderline value aimed to discover children with obstacles in the linear growth as a result of misbalanced nutrition or health problems [5, 6, 7]. Our values at the 50th percentile of the indices body weight and height were 20 kg and 108.1 cm for girls at the age of 4 and 19.75 kg and 108.25 for boys at the same age. These values were slightly higher than those found in the NCHS reference population as well as higher than those reported by the WHO for this age. Values above the 90th and 95th percentile are useful in detecting children

at risk of endocrine diseases or other disorders in nutrition. The index of body mass, known as BMI, is a parameter that monitors the degree of nutrition in childhood. Values above the 50th percentile in our subjects were slightly lower than those found in subjects of Canada, USA, Germany, and slightly higher in countries such as India and Chile [8, 9]. The obtained differences between the children in our study and other studies and with reference to the standard values have shown the existence of population differences in anthropometric parameters that depend on many factors (internal and external). It is necessary to comply with the WHO recommendations that stress the necessity for each country to prepare its own anthropometric standards. They are indispensable for classification and detection of growth and development disorders as well as nutritional status impairment in children [10].

Conclusion

Girls aged 4 years have shown slightly higher values of the examined anthropometric parameters than boys, particularly in circular parameters such as circumference of the forearm and thigh. There were no other significant statistical differences. Percentile ranges from the 5th to the 95th percentile were also determined in children at the age of 4. The results obtained can be used as criteria for assessment and detection of deviations in the growth and nutritional status in 4-year-old children.

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PLANTAR DERMATOGLYPHIC FEATURES IN ROMA POPULATION

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Abstract

Dermatoglyphic population study has been conducted in healthy examinees of Roma nationality. The aim of the study was to establish and analyze individual dermatoglyphic features in healthy examinees of Roma nationality.

In the population study 15 dermatoglyphic parameters were read and classified on 400 right and left sole prints obtained from healthy individuals of Roma 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.

We have found that most fibular loops were on the first and the second digit, whorls were on the first and third digit but present in less than 20%; arches were more abundant on the fourth and fifth digit in both sexes. Double whorls on the third digit were present as complex patterns. Found triradii were d>b>c>a>pm>p>e>p'>p'' in males and d=c>b>a>pm>p>e>p' in females. Ridge count for the toe was 8 ridges both in males and females. In the halucal, thenar distal and first interdigital space ridge count was 13 in males and 14 in females. A-b ridge count was 12 ridges in males and 13 in females.

In conclusion, dermatoglyphic features on the sole in the Roma ethnic group are presented. We have made a comparison with other ethnic groups and we present differences between them. The results provide possibilities for further investigation of dermatoglyphics and their biological and genetic properties. Findings from this population studies can be compared with results of other authors.

Key words: dermatoglyphics, soles, Roma

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 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 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 purpose of this paper was an attempt to present a variety of true patterns occurring on the sole in Roma ethnic group. The group consisted of two hundred examinees, enough for a satisfactory description of the majority of pattern elements which may occur on the normal sole in the Roma population.

Material and method

In this paper we present a statistical analysis of plantar pattern frequencies and ridge count of the soles in 200 Roma people (100 males and 100 females). All prints were taken by Cummnis and Midlo's ink method. The prints were taken from healthy examinees of Roma nationality from Sutka 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. 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 that 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

Results

The results of the study are shown in Tables 1 and 2. The arches' frequency was the lowest on the first and most common on the fifth digit, the opposite was for the fibular loops for both sexes. Fibular loops were more abundant in males and arches were more frequently found in females on the first, second and third digit. Wd-double loop, which represents complex patterns, was present in small numbers.

The results of the basic triradii are shown in Fig.1 and 2 for males and Fig. 3 and 4 for females with the following formulas: d>b>c>a>pm>p>e>p'>p'' in males and c=d>b>a>pm>p>e>p'>p'' in females.

The most present patterns on the sole regions were distal loops-Ld as we can see in Fig.5 for males and the same finding was for females but in a lower percentage (Fig.6).

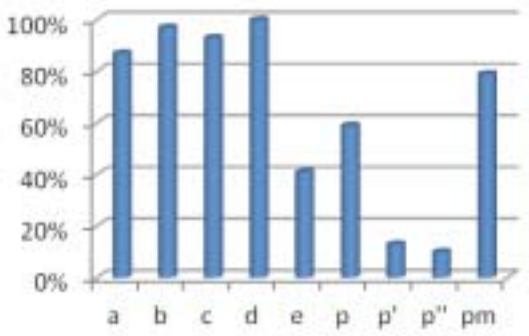

Fig. 1. Roma males right foot

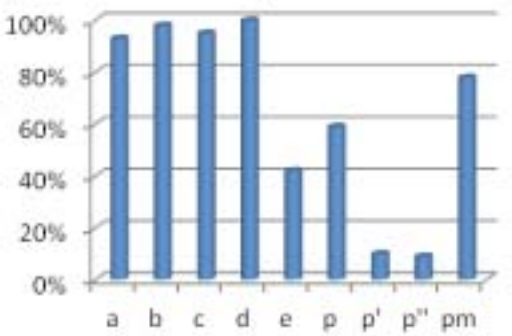

Fig. 2. Roma males left foot

Table 1. Distribution of patterns -Roma males

Patterns	First digit		Second digit		Third digit		Fourth digit		Fifth digit	
	right	left	right	left	right	left	right	left	right	left
W-c	12%	15%	4%	2%	9%	2%	3%	0%	0%	0%
L-f	63%	54%	66%	55%	38%	27%	22%	16%	0%	0%
A	19%	23%	22%	35%	29%	41%	73%	79%	100%	100%
A-t	3%	3%	0%	1%	0%	1%	0%	0%	0%	0%
Wd	3%	2%	8%	7%	24%	29%	2%	5%	0%	0%

Table 2. Distribution of patterns -Roma females

Patterns	First digit		Second digit		Third digit		Fourth digit		Fifth digit	
	right	left	right	left	right	left	right	left	right	left
W-c	9%	14%	6%	5%	12%	5%	2%	1%	1%	0%
L-f	53%	47%	50%	50%	17%	16%	8%	5%	0%	0%
A	35%	34%	43%	43%	52%	57%	87%	90%	99%	100%
A-t	2%	3%	0%	0%	0%	0%	0%	0%	0%	0%
Wd	1%	0%	1%	2%	19%	22%	3%	4%	0%	0%

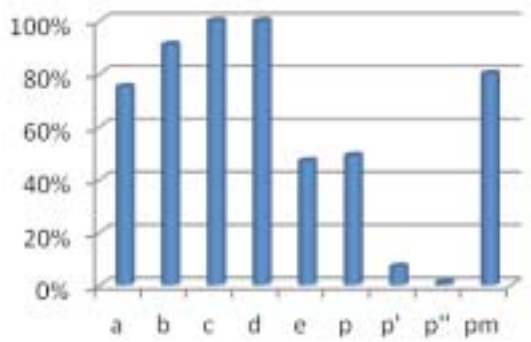

Fig. 3. Roma females right foot

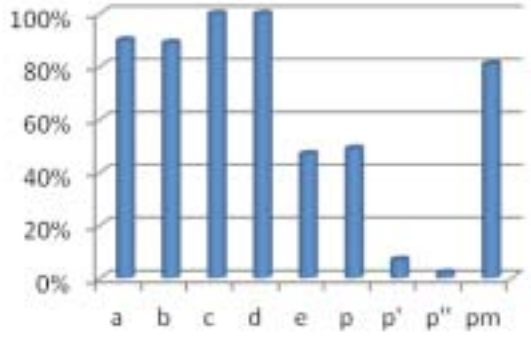

Fig. 4. Roma females left foot

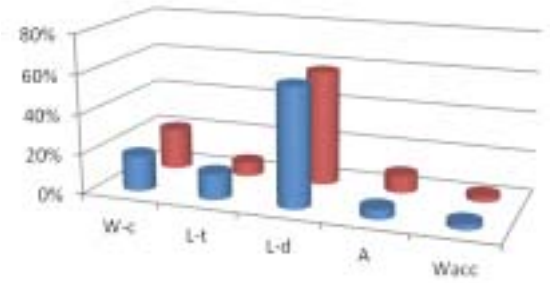

Fig. 5. Hal/Thd-Idp1-right and left, males

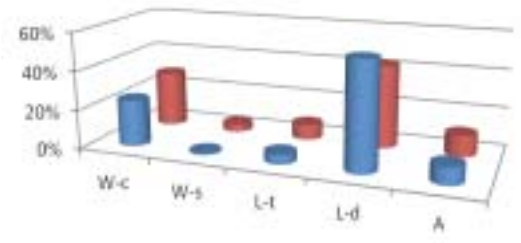

Fig. 6. Hal/Thd-Idp1-right and left, females

Total number of ridges was 8.66 in males and 8.00 ridges in females for the patterns on the big toe. Absolute ridge count for the patterns on the big toe was 13.73 in males and 13.03 in females. For the patterns on the sole the ridge count was 13.73 in males and 14.03 in females. Number of ridges between the a-b triradii was 12.46 in males and 13.30 in females.

Discussion

The results show dermatoglyphic quantitative and qualitative plantar characteristics in the Roma population for the first time; therefore, they are of significance for the Roma 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 [4].

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; in Roma people 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 [5].

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 [6].

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 Roma people. In Jews' hallucal and hypothenar region there were whorls, tibial loops, and triradii were more abundant. Jews showed more ridges in transverse direction compared to the Roma and non-Jewish whites [7].

In conclusion, this paper has shown classification of the plantar dermatoglyphic traits present in the Roma population. They are different compared to the plantar dermatoglyphics in other nationalities and hence they can be interpreted as markings of the Roma 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.

It is hoped that further studies in dermatoglyphics will discover some new possible applications of skin ridge patterns.

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615.33.015.8:579.861.2]:616.12-089-053.31

STAPHYLOCOCCUS EPIDERMIDIS RESISTANT TO METHICILLIN AS A CAUSE OF SEPSIS IN CHILD AFTER CONGENITAL HEART SURGERY: A CASE REPORT

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Abstract

Staphylococcus epidermidis is a coagulase-negative staphylococcus primarily found as a part of the normal flora of the skin, mucous membranes and other body sites in humans. In the last two decades, the increase in the number of patients who have indwelling medical devices (intravenous catheters, prosthetic heart valves and other necessary implants), contributing to *S. epidermidis* become one of the important pathogens in hospital environment. We report a case of a patient with congenital heart disease who has developed sepsis twice in the postoperative period, by the same strain of *Staphylococcus epidermidis* resistant to methicillin (MRSE), but sensitive to vancomycin. At the beginning, the patient was treated with empiric therapy for septic conditions: cefotaxime and amikacin, but after receiving the microbiological findings, vancomycin was administered too. Rifampicin was additionally included. After several negative blood cultures and considerable improvement of the patient condition, he was discharged from the hospital. The second time, ceftriaxone was used for initial treatment of sepsis, but after obtaining additional findings (the same bacteria was isolated) vancomycin and amikacin were included. After 3 weeks of treatment, the patient was discharged from hospital afebrile in good health condition. However, the patient has developed motor changes.

***Key words*:** *Staphylococcus epidermidis*, MRSE, sepsis, blood culture, Bact/Alert

Introduction

The prevalence of Gram-positive pathogens isolated from blood culture specimens has increased over the past 20 years, especially in Intensive care units (ICUs) mainly because of the increased isolation of coagulase-negative staphylococci (CoNS), *Staphylococcus aureus and enterococci* [1, 2]. Coagulase negative staphylococci have great clinical significance, especially the following species: *Staphylococcus epidermidis*, *Staphylococcus haemolyticus* and *Staphylococcus hominis* and rarely the other species: *S. lugdunensis, S. capitis, S. sciuri, S. saprophyticus* [2, 3, 4].

Staphylococcus epidermidis is primarily found as a part of the normal flora of the skin, mucous membranes and other body sites in humans and animals. Although the true infections caused by *Staphylococcus epidermidis* are often community acquired, increasing the number of patients who have indwelling medical devices (intravenous catheters, prosthetic heart valves and other necessary implants), *Staphylococcus epidermidis* infections have become a greater threat to hospital patients. It is well-known that *Staphylococcus epidermidis* has the ability to produce biofilm on the plastic surfaces (most strains produce extracellular slime), which further complicates their eradication. Their presence and penetration in the circulation can lead to severe clinical conditions: sepsis, endocarditis, septic shock, which can be fatal. Such conditions often occur in immunocompromised patients, especially newborns and young children who have primarily indication for surgery because of the present congenital heart defect (atrial septal defect-ASD, ventricular septal defect-VSD, ect.) [5, 6].

S. epidermidis is most frequently isolated from clinical specimens and often there is a dilemma whether it is a causative agent of infection or simply a contaminant. This is very important in patients with compromised immune status [2, 7, 8].

Among isolates from nosocomial cases there is a higher rate of coagulase negative methicillin-resistant *Staphylococcus* (more often *Staphylococcus haemolyticus* and *Staphylococus epidermidis*), which infections are treated with vancomycin. According to clinical data, 70-80% of clinically important CoNS are resistant to methicillin [9, 10, 11].

Unfortunately, endocarditis and septicaemia caused by methicillin-resistant *Staphylococcus epidermidis* have been associated with significantly higher rates of persistent bacteraemia and in-hospital mortality than methicillin-susceptible isolates (production of glycocalyx "slime" that acts as glue promoting the adherence to biomaterials, causing resistance to phagocytosis and some antibiotics) [9, 12, 13, 14, 15]. Vancomycin and teicoplanin have become the first-choice antibiotics for these resistant pathogens [15, 16, 17, 18]. Resistance to vancomycin and teicoplanin (VRS strains) is rare. Of all isolates obtained from different specimens of hospitalized patients in the University Clinics in Skopje (*Staphylococcus aureus* and all CoNS), VRS strains have not been detected yet [19]. However, the use of both antibiotics has been gradually expanded; isolation of pathogens resistant to these antibiotics is to be expected.

The aim of this paper is to present a case of sepsis that repeated twice and was caused by the same strain of

Staphylococcus epidermidis with the same antimicrobial susceptibility.

Case report

A 3-week-old male infant was presented for a regular routine medical control to the primary health care provider, where the doctor on heart auscultation registered 3 - 4/6 systolic murmurs from behind the sternum (at the left sterna border). His mother also stated that the infant was getting tired quickly even after consuming a small amount of milk.

The child was sent for further examination at the University Clinic for Pediatric Diseases in Skopje. Following the actual clinical trials (electrocardiogram - EKG, chest x -ray, echocardiography-cardiac ECHO) doctors confirmed the existence of a congenital heart defect.

Diagnosis was established: large ventricular perimembranous septal defect, mild subinfundibular pulmonary stenosis and initial systemic pulmonary hypertension (Fig. 2). Middle heart enlargement and bronchopneumonia was seen on chest X-ray (Fig. 1).

Fig. 1. X-ray showed middle heart enlargement and signs of bronchopneumonia

Before surgery (at the age of five months), due to a high fever and difficulties in breathing the child was hospitalized at the University Clinic for Pediatric Diseases in Skopje suspected for bronchopneumonia. Microbiological examinations (throat swabs, tracheal aspirate) were negative, but despite this, the patient was treated with ceftriaxone. After only a few days of his admission to the hospital the child was discharged a febrile and in good general condition.

After two months (at the age of seven months), due to systemic pulmonary hypertension that the child developed, he was operated on at the Cardiac Surgery Center in Sofia, Bulgaria.

Three weeks after the operation, the patient was presented at the University Clinic for Pediatric Diseases in Skopje for regular medical control. Blood culture and tracheal aspirate were taken for microbiological analysis. The findings were negative.

Between 7 to 17 months of age, the child was hospitalized twice because of high fever and respiratory infections.

At the age of 17 months (on May 5, 2011) because of prolonged high fever (temperature of 39-40^{o} C), hyperaemic tonsils and pharyngeal mucosa observed on physical exam, the child was hospitalised again at the University Clinic for Pediatric Diseases in Skopje. On exam: the patient was anxious, with constant high temperature, had tachycardia of 140 bpm, tachypnea, leucocytes count was 13.7 X 10^9 with a predominance of neutrophiles (81%), CRP-125 U/L, sedimentation rate-29 and thrombocytosis of 734 X 10^9/L (an important factor associated with the infection; a normal platelet count ranges from 150–450 X 10^9/L).

There were negative urinary and pulmonary findings, and no visible skin changes.

Fig. 2. Echocardiographic image of a large ventricular perimembranous septal defect. (LV-left ventricle, DV-right ventricle, LA-left atrium, DV-right atrium, IVS-interventricular septum).

Immunoelectrophoresis (IEPH) showed low IgA = 0.08, and IgM and IgG were within the reference values: IgM = 0.39 and IgG = 6.18. The serological findings: BAB, HbSAg, antiHCV, antiCMV, anti EBV, antiHIV and antibodies for toxoplasmosis were negative. Cardiac ECHO was done several times and showed no endocardial bacterial vegetations and signs of infective endocarditis. Ultrasound of the liver was also normal.

Microbiological analyses were indicated: blood culture, tracheal aspirate, throat swabs and blood (serum) for rapid detection of atypical pathogens (for detection antibody): Influenza virus A and B, Adenovirus, Respiratory syncytial virus, Parainfluenza virus, *Chlamydia pneumoniae, Mycoplasma pneumoniae, Legionella pneumophila, Chamydophila pneumoniae*

and Coxiella burnetii, with commercial Pneumo-slide test (Vircell, Spain).

After 24 h, there was a positive signal from Bact/Alert apparatus. Gram-staining was performed and Gram-positive cocci in clusters were observed. The next day, after 24-h incubation of plates at 37°C, catalase-positive, DNA negative, white colonies without beta-haemolysis on sheep blood agar plate were observed. Gram staining was also done and the same Gram-positive cocci in clusters were seen. The isolate was identified as *Staphylococcus epidermidis* by VITEK-2 system (BioMerieux), resistant to methicillin. Antibiotic susceptibility was determined according to the CLSI standards (Laboratory Standard Institute) using a disc diffusion method and also with the automatic system VITEK-2. The MICs of 20 antimicrobial drugs were done. The bacterial isolate was resistant to all beta lactams, macrolides and quinolones. It was susceptible to glycopeptides (vancomycin and teicoplanine), linezolid, rifampicin, trimethoprime + sulphonamide.

The next day, three sets of blood cultures were sent for microbiological analysis: at zero time of collection, after 30 minutes and after 90 minutes from the first set. In two of them, the same bacteria with identical susceptibility to tested antimicrobial agents were isolated again: the same strain of MRSE.

High temperature and signs of septic condition continued. The third blood culture after incubation period of 5 days remained negative. The child was febrile (39ÚÑ) about 4 weeks and then subfebrile in a period of almost 1-2 weeks.

At the beginning, the child was treated with empiric therapy for septic conditions (cefotaxime and amikacin), but vancomycin was also administered as soon as the microbiological finding in the blood revealed *Staphylococcus epidermidis* methicillin resistant. In consultation with an infectologist, gentamicin and rifampicin were additionally included (instead of amikacin). After several negative blood cultures, the child was discharged from hospital in good health condition.

One month after this septic condition, the child was hospitalized again, due to fever (38ÚC), tongue covered with white plaques, aphthous ulceration in the mouth, tachycardia and tachypnea, and he refused to eat. Again, all laboratory and clinical findings were in favor of a septic condition.

Blood culture in the automatic system Bact/Alert (for FAN aerobic and anaerobic bottles) at the Institute of Microbiology and Parasitology in Skopje was analyzed, and the same bacteria (MRSE) with identical susceptibility were isolated again. Initial treatment began with ceftriaxone, but vancomycin and amikacin (topical nistatin solution was prescribed for the aphthae) were included as soon as the additional results were obtained.

After 3 weeks of treatment, the child was discharged from hospital afebrile in good health condition, and given advices for home treatment. Later, the child was again hospitalized due to pneumococcal bronchopneumonia. The patient developed motor changes (probably due to the cardiac defect, surgery and prolonged multiple hospitalizations, two of them with confirmed sepsis).

Discussion

It is generally known that surgical infections**,** either wound or systemic, are frequent causes of morbidity and mortality after cardiac surgery. Although the incidence of sternal wound infection remains lower than 2% to 3%, several patients have bloodstream and many serious systemic infections during, or immediately after a hospital stay for cardiac surgery.

Gordon et al. [20] have suggested that Staphylococcus aureus was the most common organism causing bloodstream infections after cardiac surgery. On the other side, Mossad et al. in their work [21] identified Staphylococus epidermidis as a significant agent causing sternal wound infection (the potential site of possibility to appear serious bloodstream infection later). They also showed that, not only was Staphylococcus epidermidis responsible for 23% of sternal wound infections in their study, but that 92% of them were caused by methicillin-resistant organisms.

Sherif B [22] has stressed out the risk factors for surgical site infections after open heart operation, diabetes mellitus, prolonged preoperative hospitalization, obesity, current cigarette smoking, large breast size, prolonged operative time, excessive operative or postoperative bleeding, usage of both internal mammary arteries (IMAs) for coronary artery bypass grafting (CABG), re-exploration for the control of hemorrhage, prolonged postoperative low cardiac output and prolonged postoperative artificial ventilation.

In the period of six months: 21.08.2006 to 22. 02. 2007 a total of 2211 blood cultures were analyzed in the Institute of Microbiology and Parasitology, Medical Faculty, in Skopje, of which 1181 (53.41%) were from adult patients and 1030 (46.59%) from children. Out of them, 202 (17.1%) blood cultures from adults and 82 (7.96%) from children were positive. CoNS were isolated from 33 blood cultures (16.3%) of adult patients and from 57 (69.51%) blood cultures of children. Only 11 strains (19.3%) of coagulasa negative staphylococci isolated from children were confirmed as true etiological agents of sepsis (with confirmed clinical signs and subsequent isolation of the same strain from two or three separate blood cultures) [19]. *Staphylococcus epidermidis* in six of those cases of sepsis with CoNS was confirmed to be an etiologic agent, which coincided with the findings from other healthcare institutions in Europe and in the world [23].

However, CoNS are undoubtedly important as potential participants in the etiology of intra-hospital infections because they can contaminate prosthetic devices (valves, shunts, joint replacements), during implantation or post-surgical manipulation [24, 25].

Having in mind all that has been said, one of the assumption is that, the bacteria being usually present on the skin were brought from outside during the operative process and surgical manipulations. Wilson [2] has found that coagulase-negative staphylococci are present on the skin overlying the sternum in 80% to 90% of the

population and are the most common cause of perioperative contamination in "clean" operations. Archer [3] in his study has shown that patients undergoing open heart operations are often colonized preoperatively with methicillin-susceptible strains of coagulase-negative staphylococci, but postoperatively with methicillin-resistant coagulasa negative strains. According to the literature data, sepsis occurring within 1 year after surgery is usually a result of intraoperative contamination by skin bacteria. Currently, infections from vascular catheters and surgical wounds are more frequent sources of infection [26, 27, 28].

The other assumption is that bacteria are usually present in hospital environment and during the surgical process and surgical manipulations can enter the body. The strains of coagulase negative staphylococci are present in hospital environment and they can first colonize the skin and then cause infection. An outbreak in hospitals, especially in ICU centers, oncology centers, trauma etc, is not excluded [29, 30]. There are registered strains that have the same clonal origin and are associated with smaller and larger outbreaks in hospitals in various parts of the world [29, 31].

Once colonized, bacteria can produce extracellular slime, biofilm that support and facilitate the adherence of bacteria on the surface of synthetic devices and protect the bacteria from phagocytosis and antimicrobial activity [32, 33, 34].

As mentioned previously, between 7 to 17 months of age our child was hospitalized twice because of high fever and respiratory infections. Intravenous therapy was applied, which might have caused bacteria enter the circulation through the skin and later be a cause for development of the septic condition.

On the other side, removing the central venous catheter caused rupture of the arterial wall and bleeding, and consequently the urgent ligation of the ruptured wall werethe key moments for occurrence of this infection. According to Boyce [35], who has analyzed the reasons for the development of the bacterial outbreak in the hospital, the common-source outbreak of infections among cardiac surgery patients was due to carriage of a S. epidermidis strain on the hands of a cardiac surgeon, which might have happened in our case, too.

Mylonakis et al. [25] evaluated 2,615 patients submitted to cardiac surgery and verified a low prevalence of sepsis (2%). Most of these patients were hospitalized in the intensive care unit; whereas nose and hand cultures taken from the personnel showed frequent carriage of multiply resistant *S. epidermidis*. In their study, the authors have explained that the characteristic features of infections due to CoNS are generally nosocomial. The epidemiology and pathogenesis of these infections are therefore associated with acquisition of nosocomial isolates by patients and hospital personnel. Exceptions are as follows: native valve endocarditis, many episodes of prosthetic valve endocarditis (PVE) occurring >1 year following valve implantation and some episodes of peritonitis in patients undergoing continuous ambulatory peritoneal dialysis (CAPD). From the literature data [12, 36], nosocomially acquired CoNS isolates are usually of the species S. epidermidis, while many CoNS infections acquired outside the hospital are due to species other than S. epidermidis [37, 38].

Finally, most nosocomially acquired isolates are often resistant to a number of antimicrobial agents, including methicillin and other beta-lactams: erythromycin, clindamycin, tetracyclins, trimethoprime sulfonamide, chlarythromicin, aminoglycosides.

Nosocomial infections caused by multiresistant staphylococci are a growing problem for many health care institutions [30, 39, 40], because such infections restrict the number of antibiotics available for treatment. This situation is particularly serious in the case of *S. epidermidis*, as an etiological agent of bacteremia, endocarditis, osteomyelitis, peritonitis, postoperative cardiac infections or urinary-tract infections, which are frequently associated with colonization of the catheters and implants [41, 42]. Within this context, the evaluation of antimicrobial agents' resistance in clinical and community isolates can contribute in understanding of the distribution and transmission of resistance and help in the selection of appropriate antimicrobial therapy [19, 20, 43, 44]. Resistance of coagulase-negative staphylococci to multiple antibiotics further complicates the therapy. Vancomycin was the most commonly chosen antibiotic for therapy. Our rates of methicillin resistance among coagulase-negative staphylococci are similar to the rates reported by others.

Staphylococcus epidermidis strain isolated twice in two separate sets of blood culture, in two separate episodes of sepsis in our case report showed resistance to many antimicrobial agents. After treatment with vancomycin, rifampicin and gentamicin, the infection was successfully cured and blood cultures were negative. Such a combination is recommended for successful treatment of septic conditions caused by MRCoNS and it can be used for endocarditis prevention; six weeks treatment with daptomicin can also give successful results. [10, 15, 39, 44].

Conclusion

Infections which occur during cardiac surgery must warn the clinicians to think and to keep an eye on them. Good prevention and maintaining a"clean" intervention during operative procedures will contribute to reduce the rate of morbidity and mortality in such patients.

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COMPARISON OF THE SUSCEPTIBILITY OF ESBL-POSITIVE AND ESBL-NEGATIVE ISOLATES OF *E. COLI* AND *KLEBSIELLA PNEUMONIAE* TO ANTIMICROBIAL AGENTS

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Abstract

The **aim** of this investigation was to compare the susceptibility between ESBL-positive and ESBL-negative isolates to different antimicrobial agents.

Material and methods: A total of 1207 consecutive non-repeat isolates of *E. coli* and *K. pneumoniae-Kp* (*E. coli*-804, *Kp*-403) were obtained from different clinical specimens from patients hospitalized at the University Clinics in Skopje over a one year period. Conventional microbiological procedures for the isolation and detection of the strains were used. Susceptibility to beta-lactams and non-beta-lactams was determined by standard disk diffusion method and by automated method using Vitek (for determination of the MIC-minimal inhibitory concentration). A total of 251 isolates were selected (*E. coli*-126 and *K. pneumoniae*-125) using tests for phenotypic detection of the ESBL-production. A total of 233 randomly selected strains which were ESBL-negative by phenotypic tests (*E.coli*-130 and *K.pneumoniae*-103) were used as a control strains for susceptibility testing.

Results: Difference of the susceptibility to imipenem between ESBL-positive and ESBL-negative strains of *E. coli* and *K. pneumoniae* was not detected. Both groups of strains were 100% susceptible to imipenem. Considering the rest of the tested beta-lactams, as well as non-beta lactams and uroantiseptics, there was a difference in the susceptibility comparing both groups of strains of *E. coli* and *K. pneumoniae*. This was due to the fact that ESBL-negative strains were highly susceptible to all tested antimicrobial agents, unlike the ESBL-positive ones (except in cases of cotrimoxazole and nitrofurantoin, where the difference in susceptibility between ESBL-positive and ESBL-negative strains of *E. coli* was not statistically significant, because both groups of strains were susceptible to those antimicrobial agents).These data are important for recommending adequate treatment of infections with ESBL-producing bacteria.

Key words: ESBL, *E. coli, Klebsiella pneumoniae*, susceptibility

Introduction

Since their first identification 25 years ago, ESBL-producing bacteria have become a serious clinical problem and cause of nosocomial infections. ESBL (extended spectrum beta lactamases) are enzymes which have the ability to hydrolyze penicillins, first- and second-generation cephalosporins, including oxyiminocephalosporins and monobactams (but not cephamycins and carbapenems) and which are inhibited by β-lactamase inhibitors such as clavulanic acid. ESBLs are encoded by transferable conjugative plasmids in bacteria of *Enterobacteriaceae* family (majority of ESBL-producing organisms are *E. coli* and *Klebsiella spp.)*. Most of the plasmids not only contain genes encoding ESBL enzymes, but also carry genes conferring resistance to several non-beta-lactam antibiotics. Hence, the most appropriate name for the ESBL-producing bacteria would be "multidrug resistant organisms". This resulted in limited treatment options and consequently, in clinical outcome (recommended therapy might not be effective, resulting in treatment failure and increased spread of these strains in the hospital environment). ESBL have spread globally and their prevalence varies among different medical institutions and geographic regions [1, 2].

Aim

The aim of this study was to compare the susceptibility between ESBL-positive and ESBL-negative isolates of *E. coli* and *Klebsiella pneumoniae* to different groups of antimicrobial agents (beta-lactams and non-beta-lactams).

Material and methods

A total of 1207 consecutive non-repeat isolates of *E. coli* and *K. pneumoniae-Kp* (*E. coli*-804, *Kp*-403) were obtained from different clinical specimens (such as urine, tracheal aspirate, blood, wound swab, punctuate) from patients hospitalized at the University Clinics (UC) in Skopje over a one year period. The isolates were identified on the basis of conventional microbiological procedures. Susceptibility was determined by standard disk diffusion method and by automated method using Vitek (for determination of the MIC-minimal inhibitory concentration).

Determination of the susceptibility

a) Standard disk diffusion method

Susceptibility of ESBL-positive and ESBL-negative isolates of *E. coli* and *K. pneumoniae* to the following beta-lactams was tested (concentrations in μg): piperacillin (100), imipenem (10), combinations of β-lactams/β-lactamase inhibitors (piperacillin-tazobactam-100/10, amoxicillin-clavulanic acid-20/10), cephalosporins (cefuroxime-30, ceftriaxone-30, ceftazidime-30, cefixime-5 and cefepime-30), as well as to non-beta lactams, such as aminoglycosides (gentamicin-10, amikacin-30), quinolones (ciprofloxacin-5) and antifolat (co-trimoxazole-1.25/23.75). Susceptibility of ESBL-positive and ESBL-negative urine isolates, in addition to all the above- mentioned

antimicrobial agents, the following uroantiseptics and quinolones were also tested: pipemidic acid-20, nitrofurantoin-300, ofloxacin-10 and norfloxacin-10.

Susceptibility of all isolates was determined by disk diffusion method on Mueller-Hinton agar (Oxoid, UK) following the zone size criteria as recommended by the Clinical and Laboratory Standards Institute (CLSI).

b) *Automated susceptibility testing using Vitek 2 compact system (bioMerieux, France)*

MIC of 17 antimicrobial agents: ampicillin, amoxicillin-clavulanic acid, piperacillin, piperacillin-tazobactam, cefazolin, cefoxitin, cefotaxime, ceftazidime, cefepime, imipenem, amikacin, gentamicin, ciprofloxacin, norfloxacin, tetracycline, nitrofurantoin and cotrimoxazole was determined. MIC values were interpreted as S, I and R by reference to CLSI breakpoints.

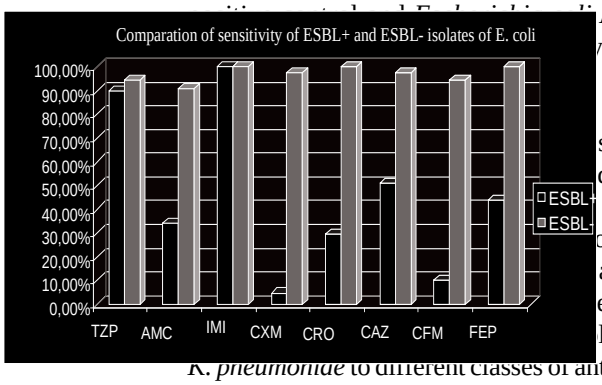
Screening and confirmatory tests for the phenotypic detection of ESBL-production

Modified double disk test and ESBL-agar were used as screening tests and ESBL-set, E-test and Vitek were used as confirmatory tests for phenotypic detection of the ESBL-production. Using these tests, 126 isolates of *E. coli* (15.7%) and 125 isolates of *K. pneumoniae* (31%) were selected as ESBL-producers.

A total of 233 randomly selected strains which were ESBL-negative by phenotypic tests (*E.coli*-130 and *K.pneumoniae*-103), were used as control strains for susceptibility testing.

Control strains

K. pneumoniae ATCC 700603 was used as a ATCC 25922 was ypic methods.



statistical program d. All results are

orrected and the applied to see the e susceptibility of lates of *E. coli* and *K. pneumoniae* to different classes of antimicrobial agents. Comparison of proportion was done by using chi square test with appropriate correction for antibiotic susceptibility among groups. A p value ≤ 0.05 was considered significant.

Results

1. Comparison of the susceptibility of ESBL-positive and ESBL-negative strains to beta-lactams (penicillins and cephalosporins)

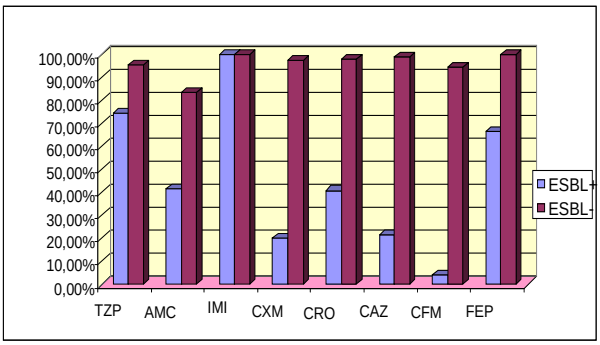
a) Comparison of the susceptibility of ESBL-positive and ESBL-negative strains of E. coli to beta-lactams (Fig. 1)

Difference of the susceptibility to imipenem between ESBL-positive and ESBL-negative strains of *E. coli* was not detected. Both groups of strains were 100% susceptible to imipenem. Considering piperacillin/tazobactam there was a difference in a susceptibility comparing both groups of strains, but it was not statistically significant (p=0,16). Both groups were susceptible to this combination (89.7% vs. 94.6%). Considering amoxicillin/clavulanic acid (34% vs. 90.8%), as well as all tested cephalosporins (cefuroxime – 4.8% vs. 97.7%; ceftriaxone – 29.4% vs. 100%; ceftazidime - 50.8 vs. 97.7%; cefixime - 10.3% vs. 94.6% and cefepime – 43.7% vs. 100%), the difference in susceptibility between ESBL-positive and ESBL-negative strains of *E. coli* was statistically significant (p=0.0000), due to the fact that ESBL-negative *E. coli* strains were highly susceptible to all tested beta-lactams.

TZP - piperacillin/tazobactam
AMC - amoxicillin-clavulanic acid
IMI - imipenem
CXM- cefuroxime
CRO- ceftriaxone
CAZ- ceftazidime
CFM- cefixime
FEP- cefepime

Fig. 1. Comparison of the susceptibility of ESBL-positive and ESBL-negative strains of *E. coli* to beta-lactams

b) Comparison of the susceptibility of ESBL-positive and ESBL-negative strains of K. pneumoniae to beta-lactams (Fig.2)



TZP - piperacillin/tazobactam
AMC – amoxicillin-clavulanic acid
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CXM- cefuroxime
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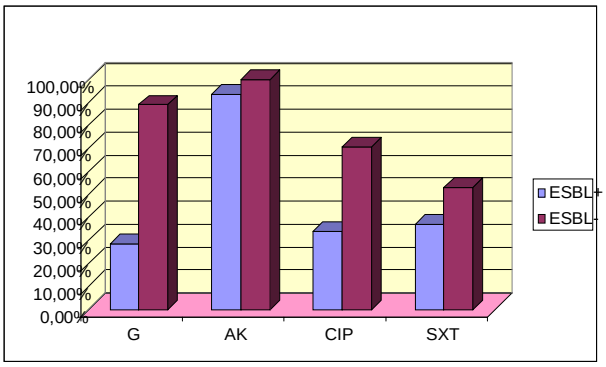
CAZ- ceftazidime
CFM- cefixime
FEP- cefepime

Fig. 2. Comparison of the susceptibility of ESBL-positive and ESBL-negative strains of *K. pneumoniae* to beta-lactams

Difference of the susceptibility to imipenem between ESBL-positive and ESBL-negative strains of *K. pneumoniae* was not detected. Both groups of strains were 100% susceptible to imipenem. Considering the rest of the tested beta-lactams, a difference in the susceptibility comparing both groups of strains was detected. This difference was statistically significant (p=0.0000), due to the fact that ESBL-negative strains of *K. pneumoniae* were highly susceptible to all tested beta-lactams.

2. Comparison of the susceptibility of ESBL-positive and ESBL-negative strains to non-beta-lactams (aminoglycosides, quinolones and antifolates)

a) Comparison of the susceptibility of ESBL-positive and ESBL-negative strains of E. coli to non-beta-lactams (Table 1, Figure 3)



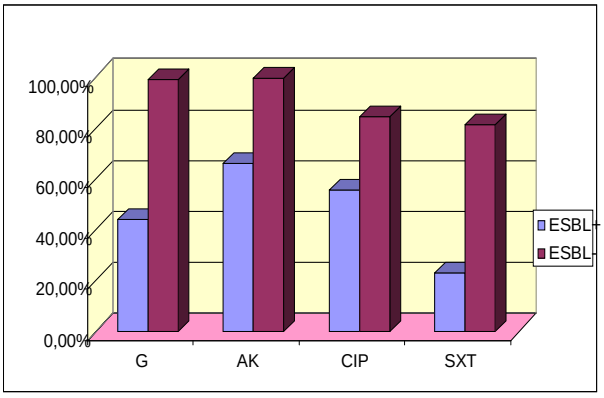
G- gentamicin
AK- amikacin
CIP-ciprofloxacin
SXT- co-trimoxazol

Fig. 3. Comparison of the susceptibility of ESBL-positive and ESBL-negative strains of E. coli

There was a difference in the susceptibility to gentamicin comparing both groups of strains (28.6% vs. 89.2%), which was statistically significant (p=0.0000). Statistically significant difference was also detected in the susceptibility to amikacin (p=0.044). This was due to the fact that ESBL-negative isolates of *E. coli* were highly susceptible to aminoglycosides, unlike ESBL-positive isolates.

Considering ciprofloxacin, the statistically significant difference between ESBL-positive and ESBL-negative strains of *E. coli* (34.1 vs. 70.8%) was detected (p=0.0001), while analysing cotrimoxazole, the difference in susceptibility between these two groups of strains was not statistically significant (37.3% vs. 53%) (p=0.097).

b) Comparison of the susceptibility of ESBL-positive and ESBL-negative strains of K. pneumoniae to non-beta-lactams (Table 1, Figure 4)



G- gentamicin
AK- amikacin
CIP-ciprofloxacin
SXT- co-trimoxazol

Fig. 4. Comparison of the susceptibility of ESBL-positive and ESBL-negative strains of K. pneumoniae

Difference of the susceptibility to all tested non-beta-lactams between ESBL-positive and ESBL-negative strains of *K. pneumoniae* was detected and it was statistically significant (p=0.0000), due to the fact that ESBL-negative strains of *K. pneumoniae* were highly susceptible to all tested non-beta-lactams.

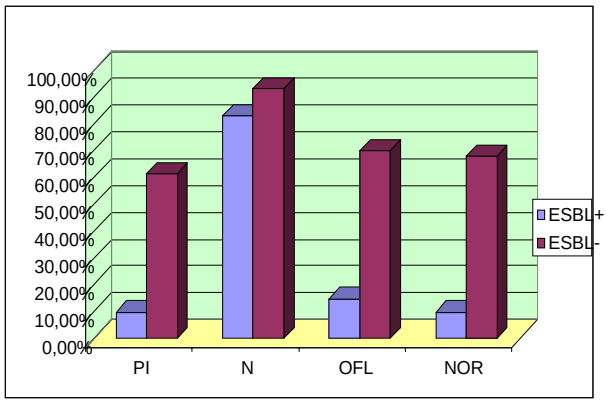
3. Comparison of the susceptibility of ESBL-positive and ESBL-negative urine strains of *E. coli* and *K. pneumoniae* to uroantiseptics (pipemidic acid and nitrofurantoin) and to quinolones (ofloxacin, norfloxacin)

a) Comparison of the susceptibility of ESBL-positive and ESBL-negative urine strains of E. coli to uroantiseptics and to quinolones (Table 2, Figure 5)

Considering uroantiseptics, the difference in the susceptibility between ESBL-positive and ESBL-negative strains of *E.coli* was detected only in case of pipemidic

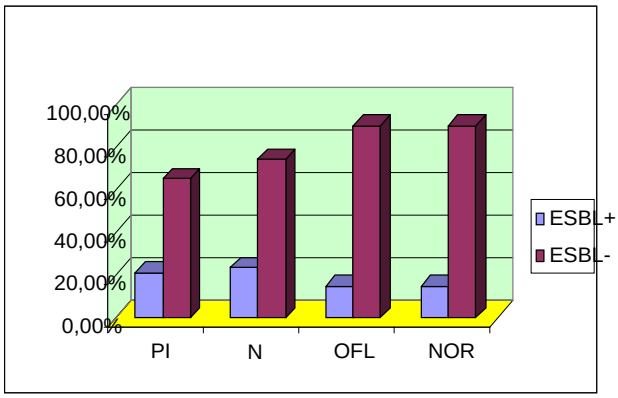
Table 1. Comparison of the susceptibility of ESBL-positive and ESBL-negative strains of *E. coli* and *K. pneumoniae* to non-beta-lactams

Bacteria	gentamicin		amikacin		ciprofloxacin		co-trimoxazol	
	S	%	S	%	S	%	S	%
***ESBL+E. coli* (126)**	36	28.6	118	93.7	43	34.1	47	37.3
***ESBL- E. coli* (130)**	116	89.2	130	100	92	70.8	69	53.1
***ESBL+K. pneumoniae* -125**	55	44	83	66.4	70	56	29	23.2
***ESBL-K. pneumoniae*- 103**	102	99	103	100	87	84.5	84	81.5



PI – pipemidic acid
N - nitrofurantoin
OFL - ofloxacin
NOR - norfloxacin

Fig. 5. Comparison of the susceptibility of ESBL-positive and ESBL-negative strains of *E. coli* from urine



PI – pipemidic acid
N - nitrofurantoin
OFL - ofloxacin
NOR - norfloxacin

Fig. 6. Comparison of the susceptibility of ESBL-positive and ESBL-negative strains of *K. pneumoniae* from urine

acid (10% vs. 61.7%) (p=0.023), while analysing nitrofurantoin, the difference in susceptibility between those two groups of strains was not statistically significant (83.3% vs. 93.3%). Both group of strains were highly susceptible to nitrofurantoin.

Considering quinolones, the difference in susceptibility between ESBL-positive and ESBL-negative strains of *E. coli* was statistically significant (p<0.05).

b) Comparison of the susceptibility of ESBL-positive and ESBL-negative urine strains of K. pneumoniae to uroantiseptics and quinolones (Table 2, Figure 6)

Difference of the susceptibility to both uroantiseptics and quinolones between ESBL-positive and ESBL-negative strains of *K. pneumoniae* was detected and it was statistically significant (p<0.05), due to the fact that ESBL-negative strains of *K. pneumoniae* were highly susceptible, unlike ESBL-positive strains.

Discussion

ESBLs are an increasingly important cause of transferable multidrug resistance in Gram-negative bacteria throughout the world. These bacteria have spread rapidly and have become a serious threat to human health worldwide [3, 4, 5]. The emergence of ESBL producing organisms seems to be the result of complex interactions between the type of ESBL, genetic background of the strain and selective pressures existing in ecologic niches. The heavy antibiotic usage (especially the third generation cephalosporins) is one of the selective pressures as well as the risk factor for acquisition of ESBL producing organisms. Therefore, clinicians should be familiar with the clinical importance of these enzymes and the potential strategies for dealing with them [6].

Considering all of the above-mentioned, determination of the susceptibility to various classes of antimicrobial agents is required in order to recommend the appropriate therapy for successful treatment of infections caused by ESBL-producing bacteria.

Beta-lactam antibiotics

In our study, the results of the susceptibility to combinations of beta-lactams/beta-lactamase inhibitors showed that ESBL-positive strains (89,7% of *E. coli* and 74,4% of *K. pneumoniae*) were more susceptible to piperacillin/tazobactam compared to amoxicillin-clavulanic acid (34% of *E. coli* and 42% of *K. pneumoniae* strains). These results are in accordance with the published data [7, 8]. Considering piperacillin-tazobactam, both ESBL-

Table 2. Comparison of the susceptibility of ESBL-positive and ESBL-negative urinary strains of *E. coli* and *K. pneumoniae* to uroantiseptics and quinolones

Bacteria	pipemidic acid		nitrofurantoin		ofloxacin		norfloxacin	
	S	%	S	%	S	%	S	%
ESBL+E. coli (60)	6	10	50	83.3*	9	15	6	10
ESBL- E. coli (60)	37	61.7	56	93.3*	42	70	41	68.3
ESBL+K. pneumoniae (46)	10	21. 7	11	24	7	15.2	7	15. 2
ESBL-K. pneumoniae (44)	29	65.9	33	75	40	91	40	91

***p= 0.11**

positive and negative *E. coli* strains were susceptible to this combination and there was no statistically significant difference between these groups of strains. There was a difference in susceptibility to piperacillin-tazobactam between ESBL-positive and negative *K. pneumoniae* strains. ESBL-negative strains were more susceptible to this combination. ESBL-negative strains of *E. coli* and *K. pneumoniae* were more susceptible to amoxicillin-clavulanic acid. Although these combinations were effective in vitro, the effect in vivo could be reduced due to overproduction of ESBL or porin changes. These combinations were inappropriate for treatment of infections caused by ESBL-positive bacteria, proved in animal models. It was concluded that the combination of beta lactam/beta-lactamase inhibitor could be effective, but inferior compared to the therapy with imipenem or piperacillin-tazobactam plus aminoglycoside [9, 10, 11]. In an other study (Rodriques-Bano et al.) the successful treatment of 43 cases of bacteremia caused by ESBL-positive strains and treated with piperacillin-tazobactam has been reported. This combination could be considered in treatment only after in vitro testing (if it shows in vitro activity). Mortality has decreased after the implementation of this antibiotic or carbapenem, compared to the implementation of cephalosporins or quinolones (9% vs. 35%, p=0.05). Many authors consider the use of the piperacillin-tazobactam combination as an initial empiric therapy by pointing out that it should be used instead of extended-spectrum cephalosporins in a strategy for decreasing the presence of ESBL-positive bacteria in hospital environment [12].

The EARSS (European Antibiotic Resistance Surveillance System) report for the period of 2001-2007 showed an increased resistance of *E. coli* and *K. pneumoniae* strains to the third-generation cephalosporins. Different ESBL enzymes vary in their ability of hydrolyzing the third-generation cephalosporins. Sometimes the MICs for ceftriaxone or ceftotaxime were ≤ 8 mg/L (which according to CLSI criteria determines susceptibility), but the clinical outcome after using these cephalosporins in treatment of infections caused by ESBL-positive strains was poor, besides apparent "susceptibility" [13]. The percentage of ceftazidime-resistant strains was higher in Europe, compared to that in the USA. Ceftazidime-resistant *K. pneumoniae* strains were 40% in ICU and 20% in other wards. Animal models showed that the inoculum effect is of clinical importance. If the third-generation cephalosporins were used in therapy, the effect would usually fail [14]. In our study the percentage of susceptible ESBL-positive strains varied according to tested cephalosporin. ESBL-positive strains of *E. coli* were more susceptible to ceftazidime, compared to other cephalosporins. ESBL-positive strains of *K. pneumoniae* were more susceptible to cefepime (66%), compared to cefuroxime, ceftazidime and cefixime. There was a statistically significant difference in susceptibility to all tested cephalosporins between ESBL-positive and ESBL-negative strains. ESBL-negative strains of *E. coli* and *K. pneumoniae* were more susceptible to all tested cephalosporins. Cefepime, as fourth generation cephalosporin is more stabile to the hydrolytic activity of ESBL. In many published data the susceptibility of the clinical isolates of *E. coli* and *Klebsiella spp*. to cefepime was between 52% and over 95%. According to these data, cefepime was recommended as drug of choice in treatment of infections caused by ESBL-positive strains. However, the clinical results were not very optimistic. If cefepime is used in treatment, it should be in higher doses, and it will be better to be combined with aminoglycosides, since in vitro cefepime shows a synergism with amikacin against ESBL-positive bacteria. In order to recommend this therapy, more clinical studies are needed [15, 16].

Carbapenems (imipenem, meropenem) show good in vitro and in vivo activity. All tested ESBL-positive strains of *E. coli* and *K. pneumoniae* were susceptible to these agents and they are recommended as drugs of choice for treatment of infections caused by ESBL-positive strains. In the case of non-life-threatening infections and in non outbreak situations, it is not necessary to administer carbapenems. This approach is intended to preserve the therapeutic value of these precious drugs. The heavy usage of carbapenems, in fact, may favor the selection of *Stenotrophomonas maltophilia* (a species naturally resistant to these drugs). It has been reported that *K. pneumoniae* isolates producing carbapenemases and different enterobacteria encoding metallo-β-lactamases have been recently detected in the Mediterranean area [17, 18, 19].

Aminoglycosides

According to EARSS data, there is a trend of increasing resistance of *E. coli* and *K. pneumoniae* strains to aminoglycosides in many countries. For *E. coli*, it varies from 5-10% in Northern European countries to 20-38% in Eastern European countries similar data for *K. pneumoniae* strains have been published. The highest resistance (over 40%) is detected in Bulgaria, Greece and Israel [20]. Aminoglycosides should be used in treatment of infections caused by ESBL-positive strains if they showed susceptibility in vitro. Oteo et al have presented data of the susceptibility of 1962 strains of *E. coli* collected from 27 hospitals in Spain. The resistance to aminoglycosides was higher in ESBL-positive strains compared to ESBL-negative ones [21]. Similar results were obtained in our study. ESBL-positive strains of *E. coli* (29%) and *K. pneumoniae* (44%) were susceptible to gentamicin, while 94% of *E. coli* strains and 66.4% of *K. pneumoniae* strains were susceptible to amikacin. Both groups of strains were more susceptible to amikacin than to gentamicin. There was a statistically significant difference between ESBL-positive and ESBL-negative strains to aminoglycosides. ESBL-negative strains were more susceptible to both gentamicin and amikacin.

Fluoroquinolones

Although the resistance to fluoroquinolones is chromosomally encoded, the fact that the resistance could be transferred via plasmids is worrisome as well. This might be the way of spreading the resistant phenotype in different bacterial strains. Paterson et al in a multicentric

study in 12 hospitals from 7 countries confirmed that among *K. pneumoniae* isolates, 18% were resistant to ciprofloxacin [22]. According to EARSS data, the percentage of resistant *E. coli* strains in Europe is rapidly increasing (except in Estonia where the resistance of 7% has not changed since 2001). Countries with the resistance over 35% are: Malta (35%), Cyprus (40%) and Turkey (53%). Similar were data for the percentage of quinolone resistant *K. pneumoniae* strains. The higher percentage of resistance was found in Greece (55%), Israel (42%) and in Bulgaria (41%) (20). In our study, the percentage of ESBL-positive strains susceptible to ciprofloxacin was 34% for *E. coli* and 56% for *K. pneumoniae* strains. For determination of the susceptibility of urine isolates, two quinolones were included: ofloxacin and norfloxacin. The percentage of susceptible *E. coli* strains was 15 and 10 for ofloxacin and norfloxacin, respectively. The percentage of susceptible *K. pneumoniae* strains was 15.2% for both quinolones.

Uroantiseptics

Susceptibility of all urine isolates to pipemidic acid and nitrofurantoin was tested. 10% of ESBL-positive strains of *E. coli* and 22% of *K. pneumoniae* were susceptible to pipemidic acid.

Nitrofurantoin is a synthetic antimicrobial agent, which has been in use for more than 50 years. Its mechanism of action is still not well-known. The value of MIC ≤ 32 mg/L is considered as susceptible. According to these criteria, > 90% of clinical isolates of *E. coli* and 45% of *K. pneumoniae* were susceptible to nitrofurantoin [23]. The low percentage of the resistance was described in another study. Out of 1142 urine isolates of *E. coli* isolated from outpatients in Canada and USA, 1.1% was resistant to nitrofurantoin [24]. In a study conducted by Puerto et al, the percentage of susceptible *E. coli* clinical isolates was 71%. In hospitals in Latin America the percentage of resistance was 13% and in Italy 20%. A statistically significant difference in susceptibility to nitrofurantoin was detected between ESBL-positive and ESBL-negative strains of *K. pneumoniae* [25]. In our study, 83% of ESBL-positive strains of *E. coli* and 24% of *K. pneumoniae* were susceptible to nitrofurantoin. There was no difference in susceptibility between ESBL-positive strains of *K. pneumoniae* between the two uroantiseptics, while in case of *E. coli* more ESBL-positive strains were susceptible to nitrofurantoin compared to pipemidic acid.

Conclusion

There was no difference of the susceptibility to imipenem between ESBL-positive and ESBL-negative strains of *E. coli* and *K. pneumoniae*. Both groups of strains were 100% susceptible to imipenem. Considering the rest of the tested beta-lactams, there was a difference in the susceptibility comparing both groups of strains of *E. coli* and *K. pneumoniae*, which was statistically significant. It was due to the fact that ESBL-negative *E. coli* and *K. pneumoniae* strains were highly susceptible to all tested beta-lactams, unlike the ESBL-positive ones.

Difference of the susceptibility to all tested non-beta-lactams between ESBL-positive and ESBL-negative strains of *E. coli* and *K. pneumoniae* was detected and it was statistically significant, due to the fact that ESBL-negative strains of *E. coli* and *K. pneumoniae* were highly susceptible to all tested non-beta-lactams (except cotrimoxazole, where the difference in susceptibility between ESBL-positive and ESBL-negative strains of *E. coli* was not statistically significant).

Difference of the susceptibility to both uroantiseptics and quinolones between ESBL-positive and ESBL-negative strains of *E. coli* and *K. pneumoniae* was detected and it was statistically significant, due to the fact that ESBL-negative strains of *E. coli* and *K. pneumoniae* were highly susceptible, unlike ESBL-positive strains (except for nitrofurantoin, where the difference in susceptibility between ESBL-positive and ESBL-negative strains of *E. coli* was not statistically significant, because both groups of strains were highly susceptible to nitrofurantoin).

These data are important for recommending adequate treatment of infections with ESBL-producing bacteria. The first-line therapy for ESBL-producing bacteria is imipenem, as well as piperacillin-tazobactam. Cefepime in high concentration and in combination with an aminoglycoside should be recommended as the second-line therapy. Drug of choice for the urinary tract infections is nitrofurantoin, but only for ESBL-positive *E. coli* isolates. Judicious usage of extended-spectrum cephalosporins, periodic surveillance of antibiotic resistance patterns and infection control measures for the surveillance are crucial to overcome the problems associated with ESBLs.

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SYNOVIAL SARCOMA: FREQUENCY OF MR PARAMETERS IN 10 CASES

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Abstract

Purpose: To present frequency of magnetic resonance (MR) imaging parameters of histological proven synovial sarcomas.

Material and methods: MR imaging findings of 10 cases, 6 males and 4 females, 13-60 years old (median age 34 years) of synovial sarcoma were retrospectively analyzed. Twenty three MR imaging parameters were evaluated: size, margins, depth, compartmental localization, T1 and T2 weighted signal intensities and homogeneity, presence of hemorrhage, fluid-fluid levels, multilocularity, calcifications, "triple sign", septations and involvement of neurovascular structures, bone and joints. For presence of calcifications additionally plain film was analyzed in all cases. Descriptive statistical analysis was done.

Results: Most of the cases (80%) were smaller than 5 cm. Deep localization had 60% of the cases and 80% were extracompartmental. Ill defined margins had 70% of the cases. On T2 weighted images all cases were with inhomogeneous high signal intensity. On T1 weighted images predominantly were with isosignal intensity (90%) and inhomogenous 70% of the cases. In 90% inhomogeneous peripheral enhancement was present after contrast material application. Lobularity, multilocularity, cystic areas and calcifications were present in 20-30%. Only one case had fluid-fluid levels and one "triple sign". Infiltration of neurovascular structures, bone and joints, was present in 10-30% of the cases.

Conclusion: Imaging features of synovial sarcoma are not pathognomonic. Most of the cases were with inhomogeneous high signal intensity mass on T2 and with inhomogeneous peripheral enhancement after contrast material application. Although the lesions are small, their inhomogeneity can help to differentiate small size malignant lesion like synovial sarcoma from benign one.

Key words: synovial sarcoma, MR imaging, MR parameters

Introduction

Soft tissue sarcomas constitute about 1% of all solid tumors [1]. Synovial sarcoma is the fourth most common malignant primary soft-tissue neoplasm, occurring primarily in young adults [2], and constituting 7-10% of all soft-tissue sarcoma [3, 4]. It is an aggressive sarcoma that is pathologically defined as a mesenchymal spindle cell tumor that displays variable epithelial differentiation [5].

High percentage (80-95%) of the synovial sarcomas is reported in the extremities, with two-thirds primary occurrence in the lower limbs and often close to the joints. Rare sites of origin include head and neck, paravertebral region, chest and abdominal wall [2].

Magnetic resonance (MR) imaging is the most valuable imaging technique for the preoperative local staging of soft-tissue tumors in musculoskeletal system. At MR imaging, most synovial sarcomas are poorly defined and infiltrative [6]. Imaging presentation is often similar to other soft tissue sarcomas. When synovial sarcoma is large might have areas of high signal intensity on T1-weighted MR images, which represent hemorrhage. One-third of the tumors appears with high, iso- and hypointense signal on T2-weighted images, representing solid, cystic and fibrous part of the tumor with areas of hemorrhage, the so-called "triple signal intensity" feature [7]. Some MR imaging features of synovial sarcoma have been described previously in different studies [6, 7].

The aim of this study is to present magnetic resonance imaging features of 10 cases of histologically proven synovial sarcoma. The idea was to make consecutive evaluation of 23 MR parameters and to present frequency of each MR parameter.

Material and methods

We have performed a retrospective evaluation of MR images of 10 cases of histologically confirmed synovial sarcoma over a period from 2006 to 2013. There were 6 males and 4 females, ranging in age 13 - 60 years (median age 34 years).

MR images were obtained with 1 T and 1.5 T systems (Philips and General Electric). Images were obtained by using T1-weighted, T2-weighted sequences, and T1 fat sat before and after CM application, in all planes. Plain films were done in order to assess presence of calcifications as an additional examination to MR imaging.

Twenty three MR images parameters were evaluated: size, depth (deep/superficial), compartmental localization (intra-/extracompartmental), signal intensity and homogeneity on T1-weighted images, and on T2-weighted images, enhancement after contrast material application (diffuse or peripheral; homogeneity), margin demarcation, lobularity, multilocularity, presence of hemorrhage, fluid-fluid levels, calcifications, septations,

"triple sign", and involvement of neurovascular structures, bone and joints.

Size was estimated by measuring the greatest diameter of the lesion. Location of the tumor was classified as upper or lower extremity, and trunk location. Superficial or deep localization was assessed; superficial tumor was located above the superficial fascia without invasion of the fascia. The lesion is considered to be intracompartmental if it is localized within one anatomical compartment [8]. If two or more compartments are affected, then the lesion is extracompartmental. Margin definition was described in terms of how distinct was from surrounding tissues (well / ill defined). The presence of septations and lobulations was analyzed. The signal intensity relative to muscle (high, low and iso signal intensity) as well as homogeneity (homogeneous, inhomogeneous) of the lesion was analyzed in all sequences. Enhancement pattern (peripheral or diffuse) and homogeneity of the lesion after intra venous contrast material application was also analyzed. Presence of "triple signal intensity" feature was assessed, which means differentiation of solid (intermediate signal intensity), cystic (high signal intensity) and fibrous part (low signal intensity) of the tumor. Presence of calcifications was analyzed on MR imaging and plain film as well. Involvement of the joint represents projections of the soft tissue mass into the expected confines of a joint capsule or when an intraarticular ligament or tendon is involved. The relationship to adjacent bone (periosteal reaction, cortical erosion, medullary invasion) and neurovascular structures (displacement, infiltration) were evaluated. Descriptive statistical analysis was done on all evaluated parameters.

Results

Most of the cases (80%) were localized at lower extremity, four cases (40%) around knee and 3 cases (30%) around ankle and one on the thigh. One case was localized on the hand and one on the neck (Table.1).

Table 1. Distribution of synovial sarcoma

Location	Total
Lower extremity	
thigh	1 (10%)
knee	4 (40%)
ankle	3 (30%)
Neck	1 (10%)
Upper extremity	
hand	1 (10%)
Total	10 (100%)

Table 2. Frequency of MR parameters in synovial sarcoma.

MR parameters	Number (%)
Size (cm)	
<5	8 (80%)
>5	2 (20%)
Localization	
deep	6 (60%)
superficial	4 (40%)
Compartmental localization	
intracompartmental	2 (20%)
extracompartmental	8 (80%)
Margins	
well defined	3 (30%)
Signal intensity T1	
low	1 (10%)
iso	9 (90%)
inhomogeneous	7 (70%)
Signal intensity T2	
high	10 (100%)
inhomogeneous	10 (100%)
KM-enhancement	
diffuse	1 (10%)
peripheral	9 (90%)
inhomogeneous	10 (100%)
Edema	3 (30%)
Lobularity	2 (20%)
Cystic areas	2 (20%)
Calcifications	3 (30%)
Necrosis	4 (40%)
Hemorrhage	0
Fluid-fluid levels	1 (10%)
"triple sign"	1 (10%)
septatons	1 (10%)
multilocularity	2 (20%)
Involvment of:	
neurovascular structures	1 (10%)
bone	1 (10%)
joint	3 (30%)

Most of the synovial sarcomas (80%) were smaller than 5 cm at the time of diagnosis. Deep localization had 60% of the cases and 80% were extracompartmental. Ill definition of the margins was present in 70% of the cases. On T1 weighted images, synovial sarcomas were predominantly with isosignal intensity compared with the muscle in 90% of the cases, with inhomogeneity in 70% of the cases. On T2 weighted images, all cases of synovial sarcoma showed high inhomogeneous signal intensity (Fig.1). After contrast material application all cases were inhomogeneous and 90% were with peripheral enhancement (Fig.2). Only one case had diffuse enhancement of the lesion. Lobularity, multilocularity,

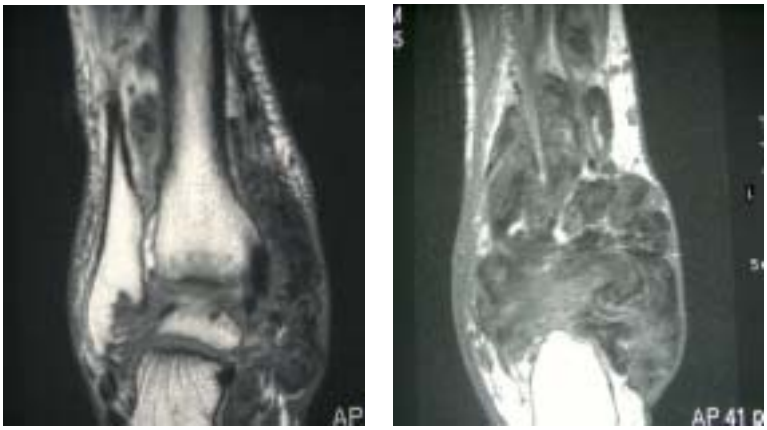

Fig. 1. Intraarticular synovial sarcoma. Large inhomogeneous lobulated lesion with bone erosion. a) coronal T1 weighted image – low to iso signal intensity lesion b) coronal and sagital T2 weighted image –inhomogeneous lesion.

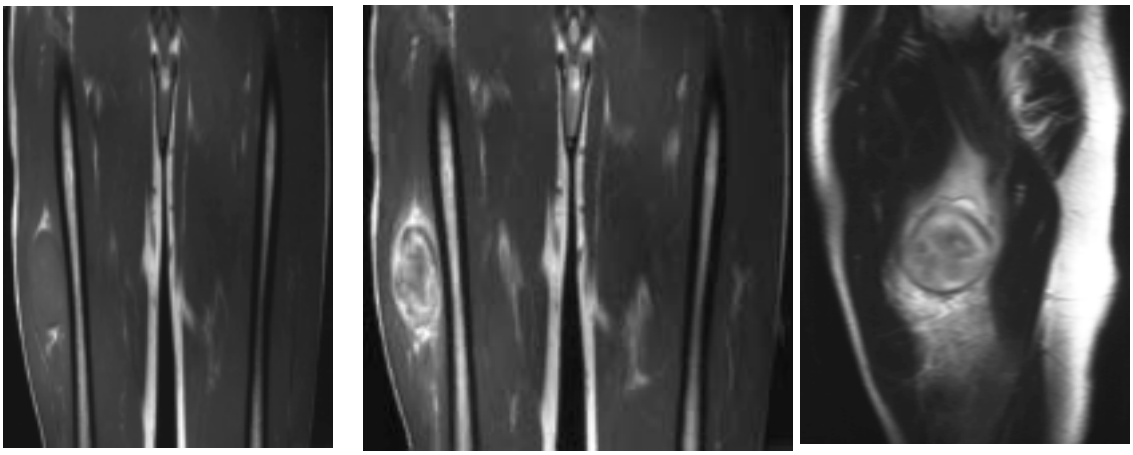

Fig. 2. Intramuscular synovial sarcoma. a) coronal T1 weighted image- isosignal intensity, homogenous lesion.; b) coronal T2 weighted image – high signal intensity, inhomogeneous lesion with small peritumoral edema; c) coronal T1 weighted image after contrast material application- inhomogeneous enhancement.

cystic areas and calcifications were present in 20-30%. Only one case had fluid-fluid levels and one "triple sign". Involvement of neurovascular structures, bone and joints, was present in 10-30% of the cases (Table 2).

Discussion

Synovial sarcoma is a rare, malignant mesenchymal neoplasm that occurs most often in the extremities of the young adults. Because of its high contrast resolution MR imaging is a method of choice for local staging of soft tissue tumors. Combination of MR findings, with clinical and pathologic data in a large series of patients for a given type of mass, can determine potentially characteristic features and MR parameters that suggest specific diagnoses.

Most of the synovial sarcomas are located in the extremities, in 80% to 95% of the cases. Lower extremity is most often affected, accounting for 60-71% of cases, whereas 16%-25% occur in the upper limb [3, 9, 10]. Less commonly affected sites include the head and neck region with 5% of the cases [11]. In our study larger percentage (80%) of synovial sarcomas were localized in the lower extremity in comparison to the literature data. There was one case in the neck region and one in the upper extremity.

Most patients with synovial sarcoma range in age from 15 to 35 years [2, 9]. Tateishi et al. in a study of 30 patients demonstrated median age at diagnosis of 27, with a range from 10-61years [12]. Our patients were with median age of 34 years with age range 13-60 years, which is similar to the literature data.

Superficial lesions may be smaller, but when deep they can reach larger dimension. Lesions ranging from 2 cm to >14 cm have been described [12]. Most of the synovial sarcomas tend to be large >5 cm in over 85%. However, in one study mean size of the lesion was reported to be 4.2 cm [13]. In our analyzed cases 80% of the tumors were smaller than 5 cm.

Radiographs appear normal in approximately 50% of the cases of synovial sarcoma, particularly those with small lesions [2]. Synovial sarcomas detected at radiography typically appear as nonspecific, round to oval juxta-articular soft-tissue masses. Calcifications are identified up to 30% of synovial sarcomas at radiography [2]. The most characteristic finding is the presence of multiple small focal calcifications and this feature is seen in about 20–30% of cases, typically in the periphery of the lesion [2, 14, 15]. Peripheral calcification with or without osseous invasion may be seen on radiographs and CT [16]. In our study, calcifications were present in 30% of the cases at radiography. MR often fails to demonstrate small calcifications seen on plain films.

Kransdorf et al. described presence of clear margin, in more than 50% of the cases [2]. Well-defined margins have been seen in some cases of synovial sarcoma, which have been described as a probable sign of benignity. In our study 70% of the cases had ill defined margins. Morton et al. also reported that most synovial sarcomas were poorly defined and infiltrative [6]. Relative clarity of tumor margins can be the reason for misdiagnosing the synovial sarcoma as benign lesion. In the study of Blacksin et al. one-third of the cases had homogeneous, well circumscribed lesion, but they noted that those cases were among the smallest ones with mean length of 4.8 cm [17]. Small lesions may be homogeneous and thus simulate benign pathology [17]. However, in our study we found that although the lesions were small the slight irregularity of the margins was present in 70% of the cases, which made the diagnosis for malignancy easier.

The majority (66%) of synovial sarcomas can display mildly inhomogeneous to complex signal characteristics [17]. On MR imaging synovial sarcomas are usually non-specific heterogeneous mass, although certain features do help to differentiate them from the other sarcomas [18]. Lesions are usually multilocular with internal septa [7], and presented as heterogeneous multilocular mass with internal septa in 67%-75% of the cases [6, 12, 17]. That is well seen on T2-weighted images of larger lesions. In our study only 20% of the synovial sarcomas were presented as multilocular mass and internal septa were found in 10% of the cases.

On MR imaging most synovial sarcomas (>90%) are hypointense relative to fat, and nearly isointense relative to muscle on T1-weighted MR images [14]. On T2-weighted images marked inhomogeneity is the rule, and internal septation may be seen [6]. This is more frequently found in lesions more than 5 cm in diameter that have heterogeneous signal pattern in more than 85% of cases vs 60% for smaller lesions [7]. Our reported cases in 80% were smaller than 5 cm, but they showed inhomogeniety of the lesion in T1-wi and T2-wi in 70% to 100% of the cases, respectively.

Kransdorf et al. described synovial sarcomas with heterogeneous intensity on T2 weighted images in 35% of the cases [2]. In rare cases the tumor may be mostly hyperintense due to extensive intratumoral hemorrhage. Small areas of high signal on T1-weighted images are more often encountered (45%) that correspond with small foci of hemorrhage [7, 14]. The incidence of intratumoral hemorrhage was found to be 73% in one study which represent very high percentage, and is generally uncommon in most of soft-tissue sarcomas other than synovial sarcoma [19, 20]. Kransdorf et al. reported presence of cystic components and hemorrhage from 25% to 40% in synovial sarcomas [2]. High signal on T1 and T2- weighted images presenting hemorrhage was found in 40% of the cases. In our study on T1 weighted images, isosignal intensity was predominant, in 90% of the cases, with inhomogeniety in 70% of the cases. On T2-weighted images, all cases of synovial sarcoma showed high inhomogeneous signal intensity. As in the study of Rangheard et al. in our study MR signs for hemorrhage were absent [13]. This may be due to small size of the lesions.

In 35% of the cases areas of hyperintensity, isointensity and hypointensity relative to fat, the so-called “triple signal intensity” can be seen [9, 19]. A triple signal pattern on T2-weighted images is described in one third of all synovial sarcomas [7, 15]. It consists of a high signal similar to fluid, intermediate signal intensity equal to or slightly hyperintense relative to fat, and low signal intensity closer to that of fibrous tissue. This triple signal pattern on T2-weighted images together with the small high signal foci on T1-weighted images is suggestive for synovial sarcoma. Jones et al. described 82% of their lesions as inhomogeneous that included “triple signal intensity” [7]. We found “triple signal intensity” in 10% of the cases.

In general fluid-fluid levels do not help to differentiate benign from malignant neoplasm [21]. It remains a non-specific finding and can occur in a wide range of bone and soft tissue tumors [22]. However, they have been present in almost one-fifth of cases with synovial sarcoma [9, 19]. Extensive loculation with multiple fluid-fluid levels can be present in 13-25% of the cases [6, 17] that are nonspecific finding for exact type of soft tissue

neoplasm. In our study, only 10% of the cases had a fluid-fluid level and it can be due to the smaller size of the lesions.

Because of its extensive vascular supply, synovial sarcoma enhances markedly after injection of contrast medium. Heterogeneous enhancement after injection of contrast material is generally seen in 83%-100% of cases [2, 6, 7]. Hypervascular areas are strongly enhancing, whereas necrotic and cystic areas remain hypointense. Jones et al. did MR analysis of synovial sarcomas on unenhanced standard spin-echo images [7]. We analyzed enhancement of the lesions after contrast material application and we found peripheral inhomogeneous enhancement in 90% of the cases.

Neurovascular involvement may be suspected in cases where the tumor margin abuts and displaces the neurovascular bundle. In one study this was confirmed at operation in two out of five cases [6]. It is not unusual and has been reported in 17%-24% of cases of synovial sarcoma [6, 7]. We found neurovascular involvement in 10% of the cases, which is much less than previously described.

More than half of all synovial sarcoma are intimately related to bone. Bone destruction was previously seen on radiography in 11% of the cases [3]. In contrary, Jones et al. reported 21% of the cases with bone destruction, which is the highest reported rate of synovial sarcomas that have contact with the bone [7]. In 5–30% of cases there is periosteal reaction, bone erosion (related to pressure from the adjacent tumor) or even bones invasion. In our study bone destruction was present only in one case (10%).

Conclusion

Although imaging features of synovial sarcoma are not pathognomonic, the findings of a soft-tissue mass, particularly if calcified, near but not in the joint in a young patient are very suggestive for this diagnosis. Radiography gives additional information to MR images for intratumoral calcifications. A relatively well-defined inhomogeneous high signal intensity mass on T2 with internal septation and “triple signal” can be suggestive for synovial sarcoma.

In our study although the lesions were small in size, their inhomogeneity on MR images makes easier to differentiate small size malignant lesion like synovial sarcoma from benign one. It is important to make sequences after contrast material application which improved the diagnosis of synovial sarcoma.

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VALIDITY OF RADIOLOGICAL METHODS IN DIAGNOSING AND TNM STAGING OF MALIGNANT TUMORS OF PANCREAS

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Abstract

The aim of this study is to compare two imaging methods, multislice CT and MRI in diagnostics and staging of pancreatic carcinomas. With this study, superiority of abdominal magnetic resonance over CT in diagnosing of pancreatic tumors is confirmed. With the realization of MRCP- magnetic resonance cholangiopancreatography, it is been allowed imaging of biliary ducts and the pancreatic duct which are of significant importance in evaluation of tumors of pancreatic head. The stated radiological diagnosis is confirmed with ERCP and histopathological findings.

Total of 120 examinees participated in this study, who were patients headed for Radiological department in GCH ''8 September" for diagnostic purposes, whereupon in 23(19.17%) of them, tumor of pancreas was diagnosed.

Conclusion With multislice CT, presence of tumor formation is diagnosed and TNM staging is being performed. Its advantage is the short period of examination, although with disability of localization of suspected lesions with dimensions less than 2 cm and with difficulties in differentiation from benign cases in which the head of pancreas is increased in volume from the malignant tumors.

With CT, it is not clear and not possible precise presentation of billiary ducts, on the other hand, with MRCP, with which biliary ducts and pancreatic ducts are well presented, with the quality of gained sequences identical to quality of images made with ERCP, which is known as the gold standard for the detection of diseases of pancreatobiliary system for the last 30 years.

This study confirms the validity of abdominal MRI and MRCP like the most valuable radiological tool in diagnosing malignant tumors of pancreas.

Key words: MRI, CT scan of abdomen, tumors of pancreas.

Introduction

Magnetic resonance imaging (MRI), although a radiological technique, is not based on usage of X-rays, but in a strong magnetic field. In addition to high magnetic field, radio frequency waves, and a computer for production of detailed picture of organs, soft tissues, blood vessels and other structures are also used. The gained images are documented on films or CD-s [1].

Magnetic resonance is used since 1980 and until now there is no information of possible side effects of magnetic field. Advantages of MR of abdomen are: non-invasiveness, lack of X-rays usage [2]. Application of contrast medium is not always necessary. The only exclusive criterion is presence of a metal component in patient's body. The high sensitivity of this method in diagnostics of tumors of pancreas, comes as result of combination of more projections with standard slices, reconstructions in all plane levels and using more pulse sequences.

With magnetic resonance, MR cholangiopancreatography (MRCP) can be possibly accomplished, which allows imaging of biliary ducts and the pancreatic duct. Imaging of the blood vessels is also possible with the usage of MR angiography. All this enables more precise determination of the diagnosis, TNM staging [3]. Nevertheless also a more accurate further follow up of patients with tumor of pancreas [4].

When we compare the validity of MRI in correlation with CT scan in diagnostics of carcinomas of pancreas, it is concluded that MRI as a diagnostic technique enables correct differentiation between tumors and lesions who may be confused with wrong CT scan diagnosis [5]. For instance, chronically hypertrophied pancreas may be misdiagnosed like tumor [6].

Aim of study The aim of this study is to compare two radiologic methods, multislice CT and MRI in diagnostics and staging of pancreatic carcinomas. With this study, superiority of abdominal MRI in diagnosing of pancreatic tumors is confirmed.

With the realization of MRCP- magnetic resonance cholangiopancreatography, it is been allowed imaging of biliary ducts and the pancreatic duct which are of significant importance in evaluation of tumors of head of pancreas [7]. The stated radiological diagnosis is confirmed with ERCP and histopathological results.

Material and methods

The research launches prospective clinical study, which was implemented in City General Hospital(CGH) '' 8 September " in Skopje, Macedonia. In this study, 120 patients participated, who were send in Radiological Department for realization of CT and MR diagnostic procedures. All participants signed an informed consent. On the other hand, the study was also approved from the ethical commision in General City Hospital ''8th of september''.

Patients, who had clinical presentation of suspected pancreatic tumor or suspicion for from the previously made Ultrasound examination, were suggested and undergone imaging studies like Computed Tomography and Magnetic Resonance.

Computed tomography is a radiological method based on utilization of Roentgen (X-rays) with an

advantage of shorter examination time. MRI of abdomen is a technique with which there is no X- ray ionization. With different pulse sequences and DWI +/- contrast enhanced studies, diagnosis of lesions less than 2 cm can be precisely evaluated. In addition, with MRCP implementation, biliary ducts and pancreatic duct are well visualized, which is very important for further treatment of pancretic tumors.

Statistical analysis

For processing and analysis of statistical data, SPSS 13.0 for Windows-statistical program was used. Categorical variability is presented with N (%), and the differences between them were being testes with Chi-square test, Yates corrected and Fisher exact test. Quantitative variables are represented with mean ±SD, and the differences between them are tested with Student t-test. The values of $p< 0,05$ were taken as statistically significant.

Results

Total of 120 examinees participated in this study, who were patients headed for Radiological Department in CGH ''8th of September" for diagnostic purposes, whereupon in 23(19,17%) of them, tumor of pancreas was diagnosed. Considering the localization of tumor, in most patients, in 20(16,67%) of them were localized in head of pancreas, in 2(1,67%) patients carcinoma was localized in body of pancreas, whereas in 10(8,33%) patients it was localized in pancreatic tail. In 2 patients the tumor lesion has been found both in body and tail of pancreas.

The gender predisposition indicated that, tumor of head of pancreas is insignificantly more often found in male patents, whereas the frequency of appearance of tumors of both head and tail of pancreas is equal in both ganders.

The average age of the examinees is 64±8,2 years in group with tumor in the head of the pancreas,57,0±12,7 for examinees with tumor in body of pancreas, however the group of examinees with tumor of tail of the pancreas is with average age of 61,2 ±8,9.

Patients with tumor in head of pancreas are with insignificantly older average age from patients with diagnosed tumor of tail of the pancreas (p=0,2).

The average age of examinees with tumor in head, body and tail of the pancreas insignificantly depends of the gender.

In the graphic below it is symptomantology shown the reasons why patients where headed for further examinations.

Pain is dominant symptom in all patients, very high percentage of examinees with tumor in the head of pancreas have jaundice and loss of weight.

Patients with tumor in the head of pancreas manifest more variety and abundant of symptoms in relation with patients having tumor in body and tail of pancreas.

In table 3, TNM staging is presented, in which patients with tumor in head; body and tail of pancreas are diagnosed.

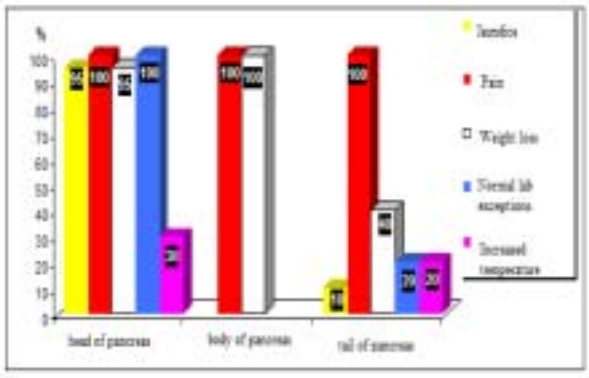

Fig. 1. Reasons why patients where headed for further examinations

In all 30 patients with pancreatic tumors, Computed Tomography scan and Magnetic Resonance imaging of abdomen were performed. With implementation of accordingly selected pulse sequences, diagnosis of pacreatic tumor is confirmed with significant percentage with MRI of abdomen.

In 15 patients of them, CT scan findings also detected presence of tumor of pancreas, respectively the percentage of correspondence of CT scan with MRI findings expresses 50%.On the other hand, the percentage correspondence of MRCP findings with ERCP results expresses 66,67%(20/30).

After the diagnosed tumor of pancreas, 17(56,67%) of patients were subjected to surgical intervention, however in 20(66,67%) of the patients ERCP was performed.From the results of the table 5 we can see that in 90%(9/10) of the operated patients ERCP was not undertaken, and 40%(8/20) of patients in whom previously ERCP was done were operated. Statistical analysis implied that patients with tumor of pancreas are significantly more often followed by an surgical intervention, without previously ERCP method (p=,017)

Discussion

The incidence of pancreatic carcinoma has an increasing trend in the last five years. It is mostly localized in the head of pancreas. The appearing symptoms are: abdominal pain, jaundice and weight loss. With the appearance of the symptoms, tumor is been diagnosed in a short period of time [8]. The first step of diagnosing is: laboratory and Ultrasound examinations of abdomen. When the previous stated symptoms persist, computed tomography scan and magnetic resonance imaging are indicated, and the abnormal laboratorial parameters are detected [9, 10].

Ultrasound findings can confirm presence of suspected hypoechoic mass localization but in the most cases ultrasound is false negative and is also very operator dependent method [11, 12].

With multislice CT, presence of tumor formation is diagnosed and TNM staging is being performed [13]. Its advantage is the short examination time, although with

Table 1. Gender presentation and localization of tumors of pancreas

Gender	Tumor of pancreas		
	In head of pancreas n=20	In body of pancreas n=2	In tail of pancreas n=1
female n(%)	8(40,0%)	1(50,0%)	10 (50,0%)
male n(%)	12(60,0%)	1(50,0%)	10 (50,5%)
Tested differences	Chi-square=0,24df=1 p=0,6	Yates corrected==0,35 p=0,5	Chi-square=0,1df=1 p=0,7

Table 2. Age presentation of tumors of pancreas

Age (mean ± SD) years		
Tumor in head of pancreas	Tumor in body of pancreas	Tumor in tail of pancreas
64±8,2 min=38 max=70	57,0±12,7 min=48 max=66	61,2 ±8,9
Male 63,3±9, 4Female 65,25±6,4	Male 66Female 48	Male 60,8±10,6Female 61,6±8,2
t = 0,5 p=0,6		t = 0,13 p=0,9

Table 3. TNM staging of tumors of pancreas

TNM STAGING	Tumor in head of pancreas	Tumor in body of pancreas	Tumor in tail of pancreas
TX – no primar Tumor is confirmed	/	/	1(10%)
TO – no signs for primar Tumor	/	/	1(10%)
Tis - Ca in situ	/	/	1(10%)
T1 - Tumor < 2 cm	/	/	1 (10%)
T2 - Tumor > 2 cm	1 (5%)	1	5 (50%)
T3 – Tumor spreads in surrounding structures	2 (10%)	1	3 (30%)
T4 – Tumor spreads in surrounding structures infiltrating blood vessels	17 (85%)		1 (10%)
NX – no regional LN	/	/	/
NO – metastasis in regional LN absent	7 (35%)	1	6 (60%)
N1 –metastasis in regional present	13 (65%)	1	4 (40%)
Increased LN in hilus of hepar	6 (30%)	/	
MX - metastasis are not diagnosed	/	/	/
MO –metastasis absent	12 (60%)	1	7 (70%)
M1 –metastasis present	8 (40%)	/	2 (20%)
Metastasis in hepar	8 (40%)	/	2 (20%)

Table 4. Correlation between performed CT/MRI and localization of tumors of pancreas

CT/MRI findings' correspondence	**N**	**(%)**
Tumor of pancreas	15/30	(50%)
Tumor of head of pancreas	9/20	(45%)
Tumor of body of pancreas	1/2	(50%)
Tumor of tail of pancreas	6/10	(60%)

Table 5. Correlation between operational interventions and performed ERCP in patients with tumor in pancreas

ERCP	Operational intervention		total
	No	Yes	
No	1 (10%)	9 (90%)	10
Yes	12 (60%)	8 (40%)	20
Total	13	17	30

disability of localization of suspected lesions with dimensions less than 2 cm and with difficulties in differentiation from benign cases in which the head of pancreas is increased in volume from the malignant tumors.

With CT, it is not a clear and possible precise presentation of billiary ducts, on the other hand, with MRCP method, with which biliary ducts and pancreatic duct are well presented, with the quality of gained scans identical to quality of images made with ERCP, which is known as the gold standard for the detection of diseases of pancreatobiliary system for the last 30 years [14].

This study confirms the validity of MRI of abdomen combined with MRCP like the most precise radiological method in diagnosing pancreatic tumors. All patients diagnosed with the neo infiltrative lesion of pancreas were selected in accordance to the estimated further treatment.In the group of patients who were advanced stadium, ERCP with therapeutic aim was indicated- stent implantation. Patients, who according to TNM staging were indicated to undergo surgical intervention, were taken into operation, and the stated radiological diagnosis was confirmed with postoperative pathohistological diagnosis.

In small percentage of patients, percutaneus biopsy was indicated and pathohistological confirmation was made before surgical intervention [15]. All patients were continuously followed up, for detection of possible mistakes in preoperative staging.

Conclusion

With multislice CT, presence of tumor formation is diagnosed and TNM staging is being performed [14]. Its advantage is the short period of examination, although with disability of localization of suspected lesions with dimensions less than 2 cm and with difficulties in differentiation from benign cases in which the head of pancreas is increased in volume from the malign tumors.

With CT, it is not clear and gives no possible precise presentation of billiary ducts, on the other side, with MRCP method, biliary ducts and pancreatic ducts are well presented, with the quality of gained scans identical to quality of images made with ERCP, which is known as the golden standard for the detection of diseases of pancreatobiliary system for the last 30 years [15].

This study confirms the validity of MRI of abdomen and MRCP to be the most precise radiological method in diagnosing the tumors of pancreas.

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LONG-TERM FOLLOW-UP AFTER NEO-ADJUVANT CHEMOPTHERAPY AND SURGERY IN 29 PATIENTS WITH HIGH-GRADE NON-METASTATIC OSTEOSARCOMA OF EXTREMITIES

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Abstract

Objectives. Currently, 80-85% of patients with osteosarcoma on the extremities can be safely treated with wide resection and limb preservation.

Background. Neo-adjuvant chemotherapy and a number of options for reconstruction after osteosarcoma resection (especially in chemotherapy-sensitive tumors) have increased long-term survival rates.

Methods. From the group of 47 patients with high-grade osteosarcoma, 8/47 patients were excluded, owing to lung metastases at first presentation or pelvic localization. Another 10/39 patients were excluded from the study due to primary indication for ablative surgery. Seventy-five percents of the patients (29/39) were treated with limb-sparing surgery. The mean age was 23.4 ± 14.5 years (range 8-63). Mean follow-up was 49.9 ± 23.1 months (range 23-108). All patients received neo-adjuvant chemotherapy protocol according to the Scandinavian Sarcoma Group XIV. After neo-adjuvant chemotherapy a clinical and radiological response of the tumor has been observed.

Results. Response to neo-adjuvant chemotherapy was good in 16/29 patients (55.2%). Local recurrence appeared in 17/29 patients (58.6%). Lung metastases appeared in 18/29 patients or 62.1%. Mean survival time of the patients was 53 months, and 10% of the examinees survived longer than 105 months. Up-to-date 10/29 patients (34.5%) are disease or event-free.

Conclusion. There was significant different overall survival time in our study between the groups of patients with a good response to neo-adjuvant chemotherapy compared to the group of patients with a bad response (p=0.0002). Furthermore, overall survival time in our group of patients was shorter than the time reported in the literature.

Key words: neo-adjuvant chemotherapy, limb-sparing surgery, osteosarcoma

Introduction

Osteosarcoma is a very rare malignant bone tumor with an incidence of 4-6 cases in 1,000,000 inhabitants and appears mostly in the young and active population aged 10- 30 years [1]. Before 1970, amputation was the primary treatment for high-grade osteosarcoma and 80% of patients died of lung metastatic disease. Despite aggressive and radical surgery, 5-year survival was low (10-20%) [2].

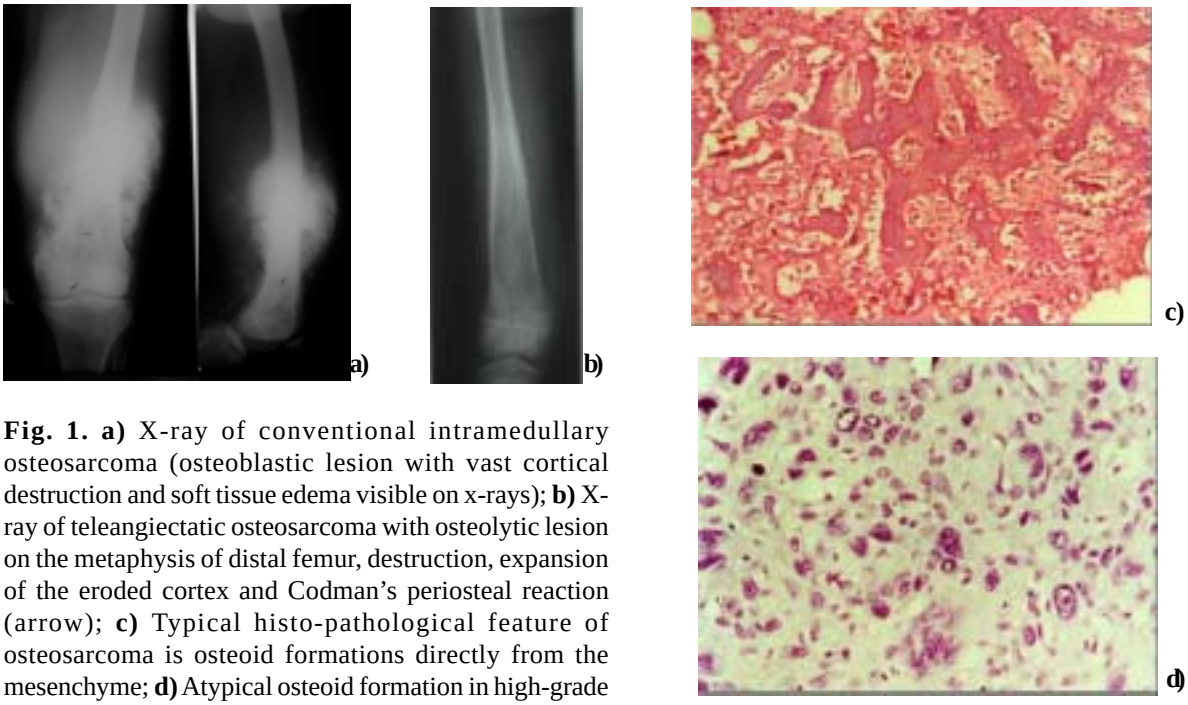

Fig. 1. a) X-ray of conventional intramedullary osteosarcoma (osteoblastic lesion with vast cortical destruction and soft tissue edema visible on x-rays); **b)** X-ray of teleangiectatic osteosarcoma with osteolytic lesion on the metaphysis of distal femur, destruction, expansion of the eroded cortex and Codman's periosteal reaction (arrow); **c)** Typical histo-pathological feature of osteosarcoma is osteoid formations directly from the mesenchyme; **d)** Atypical osteoid formation in high-grade anaplastic osteosarcoma typifies the diagnosis.

After 1980, improvement of chemotherapeutic protocols with neo-adjuvant chemotherapy, better preoperative planning and modern reconstructive options after resection of osteosarcoma led to better survival rates of patients with limb-sparing procedures [3, 4, 5].

A multidisciplinary approach to diagnosis and treatment, combination chemotherapy and a number of options for reconstruction after osteosarcoma resection (especially in chemotherapy-sensitive tumors) have increased long-term survival rates from 60 to 80%. Amputations, once a dominant treatment for malignant bone tumors, now are rarely and very selectively used. Most patients with extremity-localized osteosarcoma are candidates for limb-sparing procedures because of the effective chemotherapeutic agents and regimens, the improved imaging modalities, and advances in reconstructive surgery. Application of neo-adjuvant chemotherapy improves survival rates and functional outcome in patients with non-metastatic, high-grade osteosarcoma of the extremities [6].

Before consideration of limb preservation, the patient needs to be appropriately staged and assessed through a multidisciplinary approach. Some elements of the disease may warrant concern, including relative contraindications to such procedures. The main risk of limb-salvage procedures is that complications sometimes may cause a delay of chemotherapy [7].

Depending on cytological or histopathological features of the tumor matrix or tumor cells, osteosarcomas are divided into two groups. In the first group there are patients with *low-grade osteosarcoma* and surgery alone has the primary role of treatment. In the second group there are patients with *high-grade osteosarcoma* (Fig. 1). In this group of patients "sandwich therapy" is strongly preferred (neo-adjuvant chemotherapy - surgery - adjuvant chemotherapy) [3, 8, 9].

Considering the localization of the osteosarcoma in the diameter of the bone, it can be central (or conventional) or peripheral (periostal, juxtracortical or parosteal). The conventional type of osteosarcoma is most often seen in every day practice. There are rare variants of osteosarcoma such as: small-cell, giant cell-rich and teleangiectatic osteosarcoma, which can be differential diagnostic and treatment problem [10, 11].

Surface osteosarcomas (periostal, parosteal or juxtracortical) arise on the outer surface of the long bone metaphysis, sparing the medullar canal (Fig. 2a). The peak incidence is in the second and third decade, affecting more females than males. These osteosarcomas are considered to have their low-grade and high-grade variants [12, 13].

Material and methods

Following the "wave of modern" poly-chemotherapy, in the period of 2005-2013, a prospective study was done. In this period, 47 patients with high-grade osteosarcoma were treated [13].

According to the exclusion criteria, 8/47 patients were excluded, owing to lung metastases at first presentation or pelvic localization. Another 10/39 patients were excluded from the study due to primary indication for ablative surgery (amputation or disarticulation). Seventy-five percent of the patients (29/39) were treated with limb-sparing surgery (Table 1). Fourteen (48%) patients were male and 15 (52%) were female. The mean age was 23.4 ± 14.5 years (range 8-63). Mean follow-up was 49.9 ± 23.1 months (range 23-108).

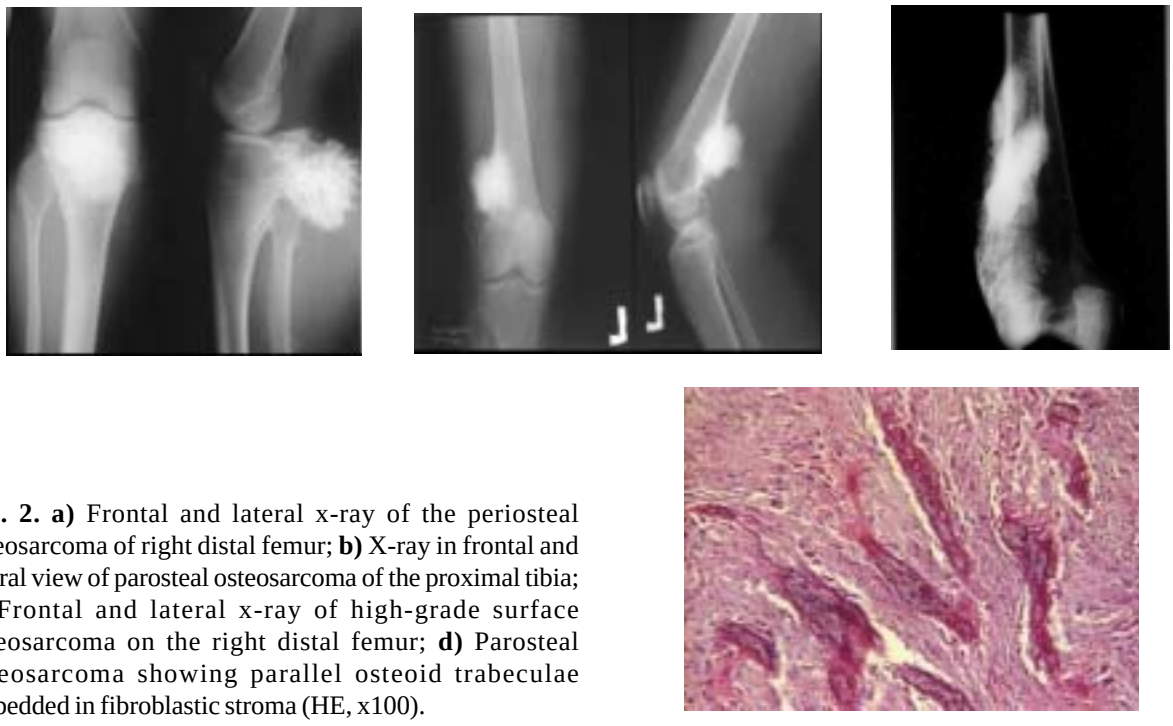

Fig. 2. a) Frontal and lateral x-ray of the periosteal osteosarcoma of right distal femur; **b)** X-ray in frontal and lateral view of parosteal osteosarcoma of the proximal tibia; **c)** Frontal and lateral x-ray of high-grade surface osteosarcoma on the right distal femur; **d)** Parosteal osteosarcoma showing parallel osteoid trabeculae embedded in fibroblastic stroma (HE, x100).

All patients received the Scandinavian Sarcoma Group XIV neo-adjuvant chemotherapy protocol (SSG XIV). Patients received 2 cycles of preoperative chemotherapy (high dose methotrexate of 1200 mg/m^2, cisplatin 45 mg/m^2/day ×2 days, and doxorubicin 75 mg/m^2), (Fig. 3).

After resection, a detailed histopathological assessment of the specimen was done to determine the extent of necrosis of the tumor tissue. Histopathological assessment of the specimen did not only identify the extent of tumor necrosis, but gave information on tumor-free margins, too. Considering the percentage of necrotic tumor tissue, patients were classified into two groups. The first group experienced a good response to chemotherapy (>90% necrosis of the tumor). The second group had a poor response to chemotherapy (>10% viable tumor).

Regarding a good or poor response of the tumor to chemotherapy, patients were assigned to different branches of the protocol (Fig. 3). All 29 patients received 3 courses of postoperative chemotherapy (the same as preoperative). Patients with a poor response received 3 more cycles of chemotherapy with high dose of ifosfamide (2000 mg/m^2/ day ×5 days plus Mesna) every 3 weeks (Fig. 3).

We have analyzed the following parameters (Table 1) of the clinical and radiological data after neo-adjuvant chemotherapy:
-age,
-gender,
-time of follow-up,

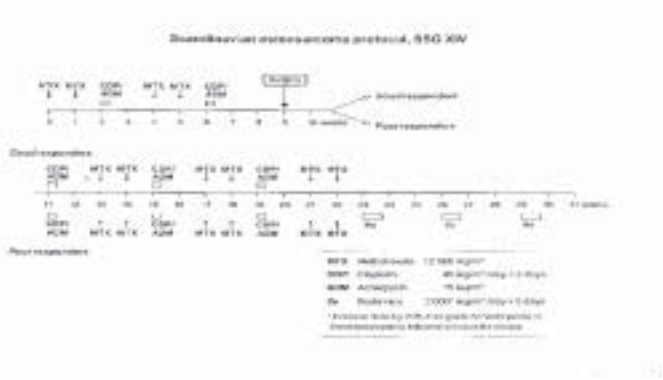

Fig. 3. Scandinavian Sarcoma Group Protocol XIV

-necrosis of the resected tumor after neo-adjuvant chemotherapy (poor or good response),
-decrease of pain,
-reduction of tumor diameter,
-tumor pseudo-capsule seen on MRI,
-sclerosis seen on radiographs or CT,
-local recurrence and metastases.

Most commonly used imaging techniques were: plain-film radiographs (as “gold” standard), Tc-99m bone scintigraphy, CT of the affected site or of the lungs and CT or conventional angiography [9]. Positron emission tomography (PET-scan) and Thallium scintigraphy have not been used.

Plain-film radiographs in two orthogonal plains showed mixed osteosclerotic and osteolytic tumor, affecting the metaphysis of the bone (Fig. 4). The destruction in some cases was so advanced that

Table 1. Clinical data of patients with high-grade osteosarcoma of the extremities, treated with neo-adjuvant chemotherapy.

Patie. No.	*Age (y.)*	*Gender (m.)*	*Follow-up*	*Response to neoad. chemoth.*	*Decrease of pain*	*Decrease in diameter*	*Pseudo-capsule*	*Sclerosis*	*Recurrence (m.)*	*Metastases (m.)*	*Deceased after (m.)*
1	25	M	30	P	0	1	0	0	0	22	30
2	13	M	32	P	1	1	1	0	0	27	32
3	23	M	50	G	1	1	U	1	0	29	-
4	16	F	44	P	1	1	1	1	0	38	44
5	15	F	68	G	1	1	1	1	50	57	68
6	14	M	51	G	1	1	1	1	0	0	-
7	8	F	50	G	1	1	1	1	29	43	50
8	13	M	45	G	1	1	1	1	0	0	-
9	16	F	54	P	1	1	1	1	0	36	54
10	17	F	23	P	0	0	0	0	6	12	23
11	54	F	38	P	1	1	U	1	0	0	38
12	14	F	107	G	1	1	1	1	0	0	-
13	63	M	106	G	1	1	1	1	96	100	106
14	17	M	67	P	1	0	1	1	54	60	67
15	16	M	59	G	1	1	1	0	0	0	-
16	20	F	54	P	1	1	1	0	0	46	54
17	20	F	47	G	1	1	1	1	0	0	-
18	16	M	10	P	0	0	0	0	2	4	10
19	39	F	61	P	1	0	1	1	53	57	61
20	14	M	26	P	0	0	0	0	19	19	26
21	8	M	40	G	1	1	1	0	0	0	-
22	44	F	59	G	1	1	U	1	0	0	-
23	14	M	40	G	1	1	1	1	0	30	40
24	44	F	35	P	0	1	0	0	21	28	35
25	15	F	108	G	1	1	1	0	0	0	-
26	15	M	27	P	0	0	0	0	2	11	27
27	48	F	43	G	1	1	1	1	0	0	-
28	24	F	33	G	1	1	1	1	18	0	-
29	34	M	51	G	1	1	0	1	35	45	51

M*: male;* **F***: female;* **G***: good response after neo-adjuvant chemotherapy (necrosis >90% of the tumor);* **P**: *poor response after neo-adjuvant chemotherapy (>10% viable tumor);* **U** *-unknown or missing data;* **1***-yes;* **0***-no or none.*

pathological fractures or complete bone erosion were present (Fig. 4b). There was a typical periostal reaction due to aggressive expansion of the tumor, forming hairy, sun-ray or velvet-like specula of neoplastic bone. In some cases "Codman's triangles" (arrows on Fig. 4a) were present**.** Plain-film radiographs were used in correlation with bone scintigraphy and CT to detect local recurrence or bone and lung metastases. Additional data for diagnosis and decision-making process was obtained using a "computer assisted diagnosis" in analysis of the x-rays [14, 15].

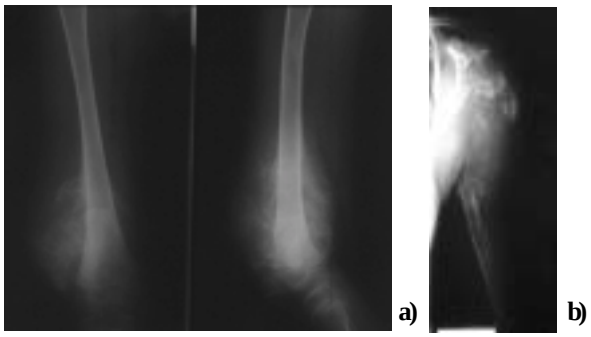

Fig. 4. a) X-ray in two orthogonal planes of typical mixed sclerotic and lytic osteosarcoma of the distal femur. Tumor has penetrated bone and formed a soft tissue mass with Codman's triangles. **b)** Frontal plane X-ray of osteosarcoma causing pathological fracture on proximal humerus with small, confluent cloud-like densities, destroying the bone completely.

Computer tomography (CT) scan of the affected extremity was useful in visualization of the intra and extra-osseous extent of the tumor, especially when extensive necrosis and surrounding edema were present. High-definition CT scans obtained a three dimensional view of the tumor in relation to adjacent neurovascular structures, especially when contrast medium was used (Fig. 4). All patients with osteosarcoma underwent CT scanning of the chest and lungs for detection of pulmonary metastases. After surgery, in patients with non-metastatic osteosarcoma, CT scans of the lung were repeated every three to six months in the following two years [6].

Obtaining an MRI prior to surgical resection permitted accurate planning of the osteotomy and gross tumor excision for achieving a "wide" surgical margin. Skip metastases on MRI were easily detectable in the same bone or in the adjacent joint. MRI studies (which are inferior to high-definition CT scans) for lung metastases detection were not regularly done [5].

Biopsy and staging. Biopsy was the key step in the diagnosis and treatment of osteosarcoma. Improperly performed biopsy could compromise the treatment plan. It was mandatory to place the biopsy in the line of definite surgical approach for osteosarcoma resection. A large needle biopsy (core biopsy) sometimes was preferable, because it was less invasive, with lower risk for skin necrosis, infection and pathological fracture. If no representative osteosarcoma tissue was obtained, an open biopsy was done.

One must state that obtaining an accurate histopathological diagnosis of the tumor (especially of osteosarcoma) may be a very delicate task. Osteosarcoma can be divided into high-grade or low-grade variants, depending on cellularity, pleomorphism, anaplasia and number of mitoses [10, 16, 17].

1This fact and the data for presence or absence of osteosarcoma metastases were enough to do the Enneking's surgical staging [18].

Treatment. A multidisciplinary approach was obligatory in the diagnosis and treatment of osteosarcoma. To achieve high standards in treatment specialized radiologists, pathologists, orthopedic, vascular and other surgeons (specialized in oncologic surgery), pediatric oncologists, specialized physical therapist and often social workers were needed [6]. High-grade osteosarcoma patients without clinically detectable lung metastases **were presumed to have micrometastases**. In these patients the treatment consisted of preoperative (neo-adjuvant) chemotherapy, wide or radical surgical resection and postoperative (adjuvant) chemotherapy i.e. "sandwich therapy". Parosteal osteosarcoma or low-grade intra-medullar osteosarcoma patients were treated with wide or radical surgical resection alone. Chemotherapy was reserved only for cases with high-grade malignant transformation. These cases were treated with preoperative (neo-adjuvant) chemotherapy similar to that used for conventional osteosarcomas [4, 19, 20, 21].

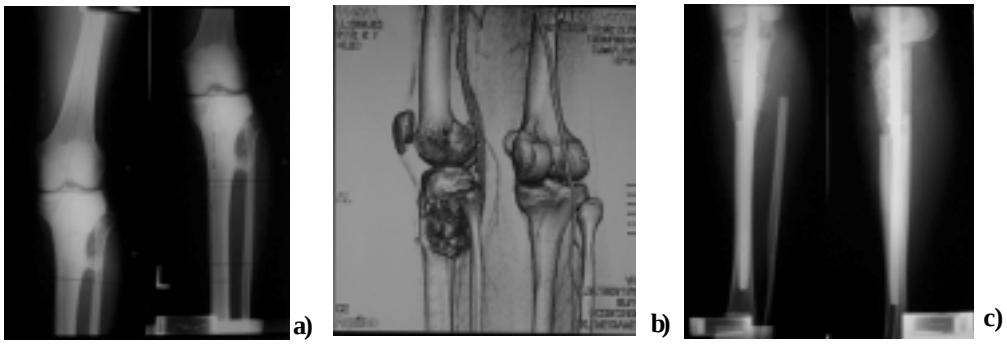

Fig. 5. a) X-ray of the proximal tibia osteosarcoma; **b)** CT angiography of the osteosarcoma, with visualization of the arteries; **c)** X-ray after modified Campanacci resection arthrodesis of the knee.

Surgery. The two primary surgical options were tumor resection with limb-salvage, and amputation. Surgical margins in excision included resection of tumor, pseudo capsule, and a cuff of normal tissue en block. Meticulous preoperative planning before biopsy and definitive surgery ensured better results. The limb-salvage surgery for osteosarcoma patients was possible due to the use of preoperative (neo-adjuvant) chemotherapy and to advancement in musculoskeletal imaging, prosthetic implant design and surgical technique (Fig. 5). During surgical treatment basic principles of limb-salvage procedures were kept in mind [22, 23, 24].

When "negative" tumor margins were obtained, a large skeletal defect was often present, requiring reconstruction of the bone, muscles, other soft tissues, and the skin. Patient age, tumor location and extent of resection determined the appropriate surgical alternatives.

Several options for limb-sparing were available: resection arthrodesis and other similar techniques with special indications (Fig. 7c), modular or special expanding endoprostheses (Fig. 5), cortico-spongious or bulk auto graft. For ablative surgery patients, disarticulation of the hip or shoulder griddle, rotationplasty, femoral or below knee, humeral or other amputations were far more appropriate [25].

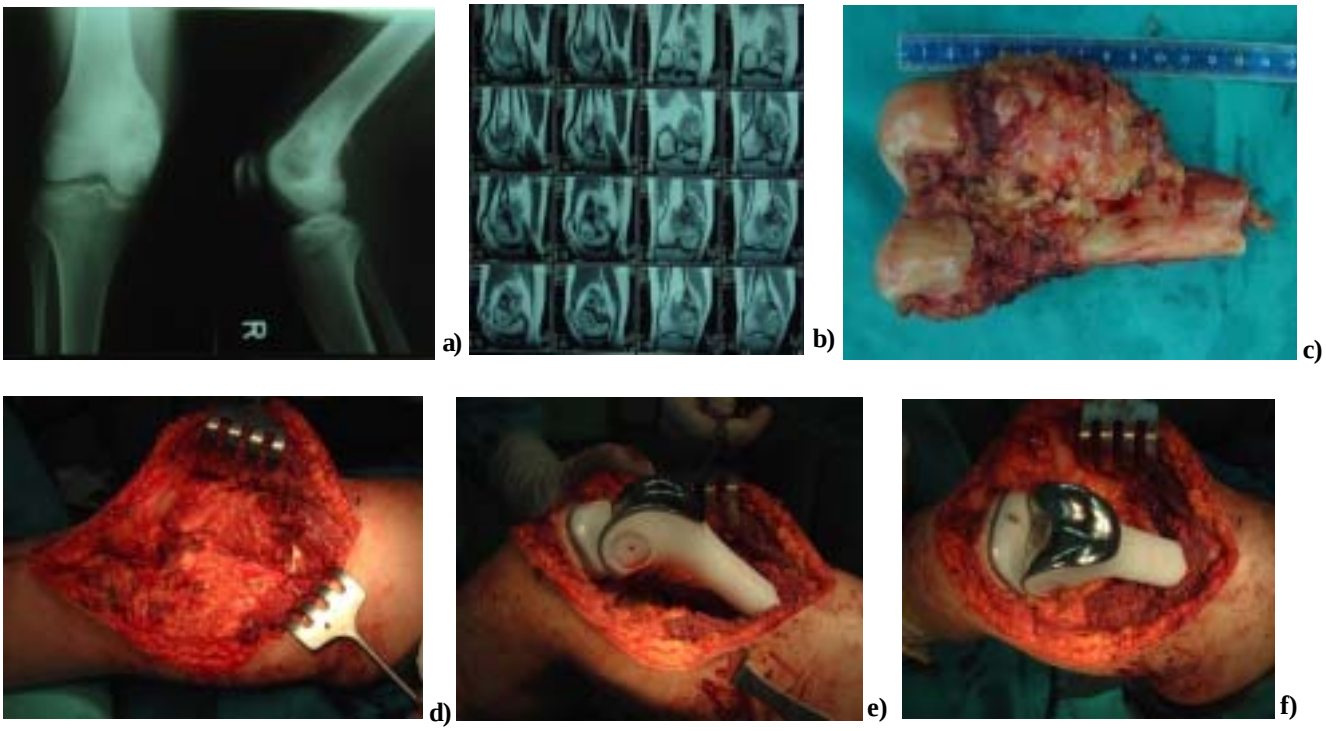

Fig. 6. a) x-ray of high-grade chondroblastic osteosarcoma of right distal femur in a girl aged 17; **b)** anterior and lateral MRI view of the lesion; **c)** photo of the resected tumor; **d)** tumor site ready for reconstruction; **e, f)** reconstructed right femur and knee (Link modular endoprosthesis).

There were a few relative contraindications taken into consideration for limb-salvage surgery: wrong site or ill-planed biopsy; massive encasement of neurovascular bundles; extensive tumour involvement in soft tissue, muscles or skin; complex or complicated (i.e. with infection) pathological fractures; expected inequalities of the extremities more than 8 cm; and exceptionally poor effect of the neo-adjuvant chemotherapy.

In the process of decision making for limb-salvage surgery versus amputation the "rule of three" was very helpful. For extremity survival the bone (1), nerves (2), blood vessels (3), and muscle and skin (4) were necessary to be preserved. If osteosarcoma involved one or two of the former structures, limb preserving was possible. If any three of the former were involved, amputation was taken into consideration [5, 24].

Postoperative follow-up. After chemotherapy, patients were closely followed by the orthopedic surgeon and the oncologist. The patients were monitored for local recurrence, distant or systemic metastases and complications related to reconstruction of the extremity. CT scanning of the chest, plain film radiographs of the reconstructed extremity and meticulous physical examinations were recommended every three months for the first two years and at least every six months from the second year through the fifth year, and subsequently on a yearly basis. Also, annual bone scintigraphy is mandatory for the first two years after completion of the chemotherapy [5, 9].

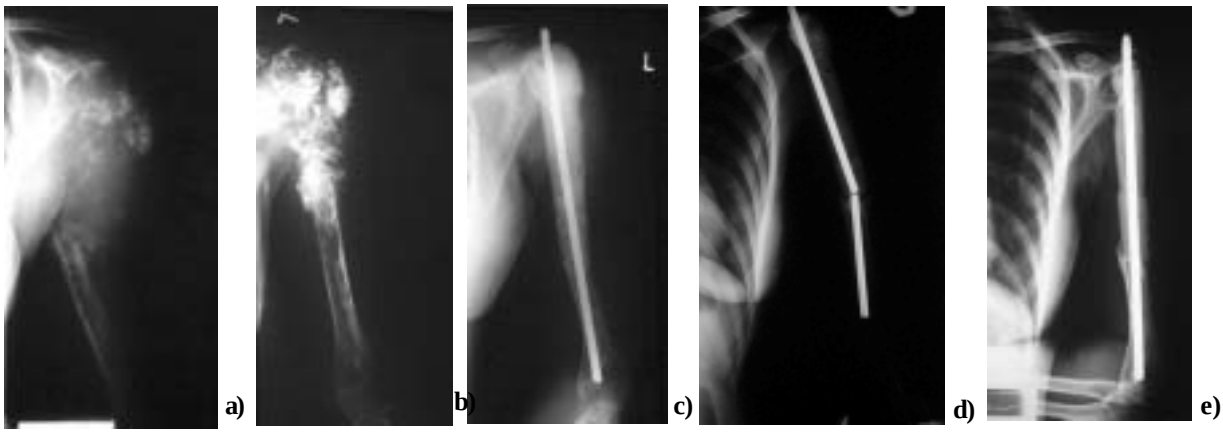

Fig. 7. a) Fourteen-yearold female osteosarcoma patient with pathological fracture of the left proximal humerus at the first presentation. The patient had preoperative (neo-adjuvant) chemotherapy with Swedish Sarcoma Protocol XIV. **b)** Excellent response (>90% tumor necrosis) with sclerosation after neo-adjuvant chemotherapy (arrow shows the site of pseudo-healing of the pathological fracture). **c)** Radiograph of the humerus after wide resection of the osteosarcoma, and first stage reconstruction of the bone with intramedullary rod and bone cement. **d)** "Stress-fracture" of the intramedular rod 6 years after the treatment. **e)** Considering the financial possibilities, reconstruction with new intramedular rod was sufficient for the patient's function.

Results

Various effects of neo-adjuvant therapy, such as: remission of pain, reduction of the size of the tumor, sclerosation, pseudo capsule formation, decreasing of neo-vascularisation, tumor necrosis and decrease of the elevated alkaline phosphathase and lactate dehydrogenase levels were recorded. After neo-adjuvant chemotherapy, a clinical and radiological response of the tumor has been observed. There was reduction, or more often complete remission of pain.

This was usually followed by decrease or normalization of serum alkaline phosphathase and lactate dehydrogenase levels (if elevated). An increased density on plain radiographs (Fig. 7b) with decreased vascularity on the angiograms was associated/was noticed.

Clinical and radiographic reduction in tumor size was observed in more than half of the patients. This was more due to a decrease of the surrounding inflammatory and reactive tissue than to an actual reduction in tumor size. Neo-adjuvant chemotherapy in some patients decreased the size of the primary tumor (Fig. 7) by reducing its neo-vascularity and promoting tumor demarcation from surrounding tissue with pseudo-capsule (Fig 7b).

Response to neo-adjuvant chemotherapy was good (more than 90% necrosis of the tumor) in 16/29 patients (55.2%). The examinees with a good response to neo-adjuvant therapy had significantly longer overall survival time than the patients with a poor response (Fig. 8).

Ten percents of the patients with poor response survived for more than 65 months (Fig. 9), while 58% of the patients with a good response survived for more than 100 months (Log-Rank test=3.74 p=0.0002).

Local recurrence appeared in 17/29 patients (58.6%). The examineås without local relapse had significantly longer overall survival time than the examined

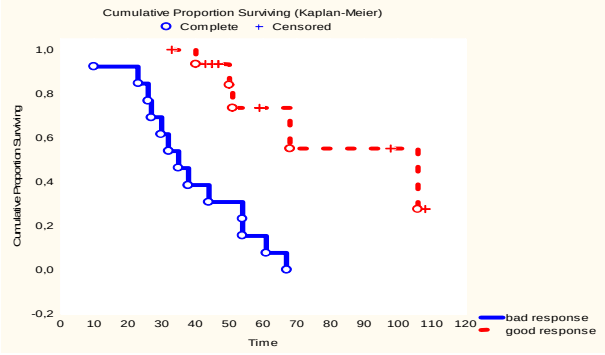

Fig. 8. Neo-adjuvant chemotherapy response in correlation with survival in patients treated with SSG XIV chemotherapy protocol.

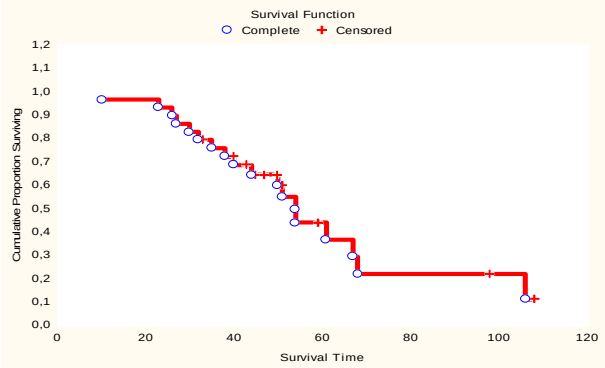

Fig. 9. Disease and event free survival time of the patients with extremity localized high-grade osteosarcoma treated with SSG XIV neo-adjuvant chemotherapy protocol and surgery (10% of the examinees survived longer than 105 months).

patients with relapse. Ten percents of the patients with relapse survived more than 100 months, while 48% of the examined with no local relapse were alive even after 100 months (Log-Rank test p=0.0002). Most of the tumor relapses were seen in the patients by 22 months after surgery. The 3 patients with early local recurrences had secondary extirpation of the relapsed tumor and one of them had to be amputated.

Lung metastases appeared in 18/29 patients or 62.1%. The examinees with metastases had significantly shorter overall survival time than the metastasis-free patients. Four percents of the examined patients with metastases survived longer than 100 months, while 90% of the examined with no metastases were alive even after 100 months (Log-Rank test p=0.0002).

Plain radiograph or CT-scan sclerosis of the tumor after neo-adjuvant chemotherapy was seen in 18/29 patients (62.1%). Pseudo-capsule was seen in 19/29 patients (65.5%), but in 3/29 (10.3%) MRI imaging showed inconclusive data. Cystic necrosis on MRI scans after neo-adjuvant chemotherapy was seen in 14/29 patients (48.3%). Inconclusive results for cystic necrosis were found in 3 and data was missing for 1 patient.

Up-to-date mean survival time of the patients was 53 months (4.4 years), and 10% of the examinees survived longer than 105 months (8.7 years) as presented in Figure 9. Disease and event-free are 10/29 (34.5%) of the patients.

Discussion

Prior to the introduction of chemotherapy, when amputation was the primary treatment for patients with osteosarcoma, the predicted long-term survival was 15-20%. Dismal survival rates were presumably attributable to pulmonary metastatic disease, whether clinically obvious or occult [8]. Survival rates dramatically increased during 1970's and 1980's with the pioneer work of Rosen and Jaffe. Currently, long-term survival rates are 60% to 70% for patients with localized osteosarcoma and for extremity localized up to 80% [26]. Despite the use of modern neo-adjuvant chemotherapy the 10-year survival rates decline significantly to 20% in patients with clinically detectable metastases [27]. Most of the patients ultimately die because of respiratory failure caused by the metastatic burden [9, 13, 25]. Excluding high-grade surface osteosarcoma, which has similar prognosis to that of conventional osteosarcoma, the surface (parosteal and periostal) osteosarcoma variants have the best prognosis of all. The 10-year survival rates for this group of patients are up to 85% [5, 12, 25]. The site of the lesion has prognostic importance. Best survival rates are expected in patients with appendicular localization of the osteosarcoma. Central localization (as pelvis, ribs and vertebrae) are less common sites of osteosarcoma, but with the poorest prognosis. Osteosarcoma of the jaws is associated with an especially good prognosis, whereas some osteosarcoma involving the scull has a very poor prognosis [11, 28]. Badly planned and ill preformed biopsy can complicate the final surgery and may decrease survival rates due to local spreading or risk for early metastatic disease [16, 17].

The overall treatment results in high-grade osteosarcoma are less impressive than widely presumed. Whereas classical osteosarcoma survival has indeed increased, in other subgroups, comprising more than 40% of the entire osteosarcoma population , the prognosis has been modestly improved. Today still more than half of an unselected osteosarcoma population eventualy succumbs to the disease, despite the current multimodal primary tretatment as well as second-line chemotherapy and surgical metastatectomies [4, 25, 27].

Neo-adjuvant chemotherapy enables limb-sparing in majority of patients with extremity localised osteosarcoma. During the past 20 years dramatic advances have been made in the treatment of non-metastatic osteosarcoma in terms of cure rate and quality of life for survivors. These advances are mainly due to the development of effective adjuvant and neo-adjuvant chemotherapeutic regimens. Reports on the progress and controversis in the treatment of osteosarcoma occurred with respect to the construct, expirimental design and interpretation of the many important studies which led to these remarkable results [22].

The survival rate of patients can be as high as 60%-75% when both the primary tumor and the solitary lung metastasis are adequately resected [3, 27]. The rate of surgical site recurrence is 4% to 16% for both limb-salvage and amputations. However, surgical treatment associated with a limb-sparing operation is also associated with a significant number of complications and requires extensive rehabilitation. Complications following limb-salvage reconstructions include wound complications, infections, mechanical failure, and nonunion. The main risk of limb-salvage procedures is that complications sometimes may cause a delay of chemotherapy. The reported incidence of complications with limb-salvage surgical techniques is 4% to 38% [5, 20, 29, 30].

The evaluation variables influencing systemic and local recurrence and final outcome are extremely important in defining risk-adapted treatments for patients with nonmetastatic osteosarcoma of the extremity. Upon multivariate analysis, age d" 14 years, high serum levels of alkaline phosphatase, tumor volume >200 mL, a dual-drug regimen chemotherapy, inadequate surgical margins, and poor histologic response to treatment maintained independent prognostic values on the outcome of nonmetastatic osteosarcoma of the extremities. These factors must be considered when deciding risk-adapted treatments for osteosarcoma patients. [22, 28, 30] Amputation remains the indicated treatment when these factors are taken into concideration or tumor resection to disease-free margins leaves a nonfunctional limb [5, 8].

Conclusion

With advances in neo-adjuvant chemotherapy, radiographic imaging, and reconstructive surgery, most patients with osteosarcoma now can be offered limb-sparing treatment. As reported in literature, if treatment and management principles of high-grade osteosarcoma

with neo-adjuvant therapy are followed, long-term 60-80% overall survival rates could be easily achieved.

Our results are slightly different from those published in the literature. In our study, there was significantly different overall survival time between the groups of patients with a good response to neo-adjuvant chemotherapy compared to the group of patients with a bad response (p=0.0002). Furthermore, overall survival time in our group of patients was shorter than the time reported in the literature. Up-to-date mean survival time of the patients was 53 months (4.4 years), and 10% of the examinees survived longer than 105 months (8.7 years). Up-to-date 10/29 patients (34.5%) are disease and event-free.

The key factor for increased survival rates was introduction of modern protocol with neo-adjuvant chemotherapy. In spite of the recorded differences in the results reported in the literature, the treatment regimen with neo-adjuvant chemotherapy is promising and encouraging.

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BILATERAL INGUINAL HERNIA, LAPAROSCOPIC REPAIR WITH SINGLE MESH (CASE SERIES)

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Abstract

Simultaneous repair of bilateral inguinal hernia is a persistent problem in hernia surgery. Introduction of laparoscopic hernia repair attenuated perioperative stress and the need for pain control; it simplified postoperative course and shortened the postoperative inability for work. Bilateral hernia repair become reality with the introduction of the tension-free technique, especially for laparoscopic hernia repair. We present a case series of patients with bilateral inguinal hernia. They were treated with transabdominal preperitoneal (TAPP) laparoscopic technique with a placement of one large mesh. The main advantage of laparoscopic hernia repair for bilateral inguinal hernia is reduced postoperative pain and early return to daily activities.

Key words: bilateral inguinal hernia, laparoscopy, laparoscopic hernia repair, TAPP

Introduction

The most common surgical intervention in general and digestive surgery is hernioplasty for inguinal hernia. Approximately 2 million hernioplasties are performed in the world annually of which 800 000 in the USA [1]. The anterior open approach technique with prosthesis known as Lichtenstein technique is accepted as a "golden standard" [2]. Minimally invasive, laparoscopic techniques are growing in popularity and in the USA 1 in 5 hernioplasty is laparoscopic, and in Europe 16% of all hernioplasties is laparoscopic. At the moment two minimal invasive techniques are used, TAPP (TransAbdominal PrePeritoneal) and TEP (Totally ExtraPeritoneal) [3]. There are no significant differences between the two techniques [4, 5] yet compared to open techniques there are significant differences with regard to postoperative pain, shorter hospital stay and shorter convalescence [6, 7, 8]. Simultaneous repair of bilateral inguinal hernia is a persistent problem in hernia surgery. Introducing tension free techniques and especially laparoscopic repair techniques have resulted in reducing perioperative stress and better pain control; it also simplified postoperative course and shortened the postoperative inability to work [9, 10, 11]. Bilateral hernia repair become reality with the introduction of tension-free technique, especially for laparoscopic hernia repair. Another advantage of TAPP technique is the ability to discover occult hernias [12]. In some patients there is an occult contralateral or ipsilateral inguinal, femoral or obturator hernia, which can be discovered and treated with laparoscopic TAPP technique, thus reducing the morbidity and symptoms of undiagnosed and untreated hernia.

The aim of this paper was to present a case series of patients with bilateral inguinal hernia treated with laparoscopic technique. These are preliminary results of a larger study that can lead to better planning and changing the design of the study.

Material and methods

We present 5 patients treated in the period 15.01-15.04.2013 at the University Clinic for Digestive Surgery in Skopje. All included patients were male, 18-70 years of age with bilateral inguinal hernia not descending under the lower edge of pubic symphysis during straining and ASA (American Society of Anesthesiologists) graduation 1 or 2. Preoperatively a single dose of the 3-rd generation cefalosporine was given. Standard laparoscopic TAPP procedure was performed on each patient in general anaesthesia in 15^{o} Trendelenburg position without urinary catheter (3, 13, 14).The direct and indirect hernia sac were reduced and a preperitoneal space was freed, the transversalis fascia in larger direct hernias was attached to Cooper's ligament with one or two tackers. Large polypropylene mesh (masse > 40gr/m^2) in a form of "bikini" was used, 25-27 cm wide and 10-12 cm high in the medial part and 8 cm in lateral part. Mesh fixation was performed with spiral titanium "tackers", 1-3 on the Cooper ligament bilateral, 1-2 on each rectus muscle and one laterally on the level of anterior superior iliac spine on both sides. Peritoneum was also fixed over the mesh with tackers.

Evaluation included operative time, time of ambulation of the patient, use of analgesia after hospital discharge (number of tablets of Metamizol NA or Ibuprofene used daily), postoperative morbidity: hematoma, seroma, wound infection and convalescence on the 7-th postoperative day. The evaluation was performed on the day of hospital discharge and on the 7-th postoperative day.

Results

All patients were operated according to the presented technique and materials. The results were: median operative time 76 min (from 60 to 90 min), hospital discharge median POD 1,6 (from 1 to 3). Two patients after discharge used analgesia and three did not. All patients on the 7-th POD did not use analgesia and were able to perform moderate physical activity.

One patient had urinary retention and catheterization was performed, the discharge from hospital was on POD 3. During surgery occult large femoral hernia was detected along with a large direct inguinal hernia on the right side. We did not fix the femoral hernia sac and seroma occurred.

Table 1 – Preliminary results

Operative time	76 min (from 60 to 90 min)
Discharge from hospital	
POD 1	3
POD 2	1
POD 3	1
analgesia	
Ø	3
2 <TD	1
2 >TD	1
seroma	1
Urinary retention	1
POD 7	5

- **POD** (Postoperative Day), 1 –counting 24 hours from the surgical intervention, 2 -48 h ....
- **TD** (Tablets Daily), three groups of patients: I. without analgesia, II. Patients using 2 or less tablets, III. Using more than 2 tablets (we measured analgesia after hospital discharge).

- **POD 7** – evaluation on the 7-th day for performing moderate physical activity.

Discussion

The results in the case series are preliminary and include a small number of patients; yet we can conclude that the advantage of laparoscopic TAPP technique is early mobilization and ambulation of the patient, reduced postoperative analgesia and early return to daily activities.

By using TAPP technique occult hernia can also be discovered, which might be overlooked if conventional techniques are used.

It is necessary to conduct a larger study in order to confirm the importance of the results.

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\ |	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
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