UDC: 61 **ISSN 1409-9837**

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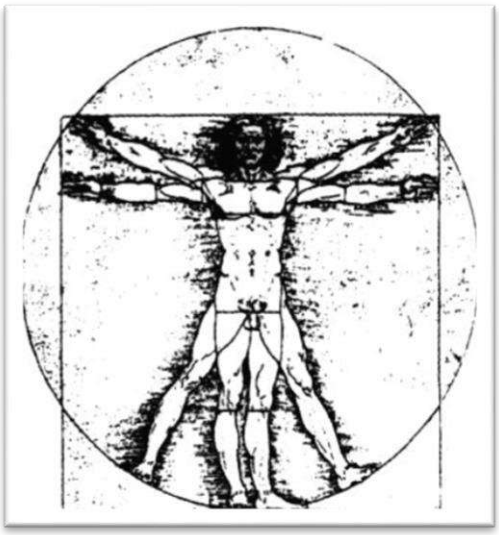

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ACTA MORPHOLOGICA

INTERNATIONAL JOURNAL OF THE MACEDONIAN ASSOCIATION
OF ANATOMISTS AND MORPHOLOGISTS

Vol. 18 (2) 2021

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

Published
Twice a year

CONTENT


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UDC: 616.72-007.77-097

ORIGINAL ARTICLE

DIAGNOSTIC UTILITY OF ANTI-CCP ASSAY IN PATIENTS WITH RHEUMATOID ARTHRITIS

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ABSTRACT

Introduction: The purpose of this research was to compare the diagnostic values of laboratory variables, to present quantitative evaluations of the diagnostic test with reference value for anti-cyclic citrullinated peptide (anti-CCP2) antibodies, rheumatoid factor (RF), C-reactive protein (CRP), and DAS 28 index in early diagnosis of untreated rheumatoid arthritis (RA).

Material and methods: Using the ELISA method of DIA-STAT™ Anti CCP (Axis-Shield Diagnostics), sera of 70 participants were examined (35 untreated patients with RA and 35 subjects from the healthy control group). RF and CRP were determined with the agglutination test (Latex test) in the same participants. At the same time, we determined the sensitivity, specificity, predictive value for positive and negative test and accuracy.

Results: Presence of anti-CCP 2 antibodies was 65.71%, 23/35 patients, while RF was present in 17/35 (48.57%). Twelve patients were anti-CCP2 and RF positive, 11 were anti-CCP2 positive, but RF negative. Five patients were anti-CCP2 negative and RF positive. In the healthy control group, one patient was anti-CCP2 positive, while 2 patients were RF positive.

Conclusion: Anti-CCP2 antibodies have higher sensitivity and specificity than RF in early RA.

Keywords: cyclic citrullinated protein (CCP), rheumatoid arthritis, rheumatoid factor

INTRODUCTION

Introducing every new diagnostic method brings about the necessity to evaluate its quality, i.e., to find the usefulness of information which should be compared to the risk for the patient and the price of the assay. Lately, due to technologic development, a lot of new diagnostic methods have been introduced. Although subjective estimation of the doctor responsible for the patient would be decisive in the choice of the offered diagnostic method, one objective quantitative evaluation of every method would help him for the most rational approach.

Rheumatoid arthritis (RA) is a systemic autoimmune disease, multifunctional in origin and is characterized with inflammation of the membrane of the peripheral joints, as well as extra-glandular manifestations, with major damages in the early phase [1]. The diagnosis of RA primarily is based on clinical, radiological and immune characteristics. The most often used serological test in the evaluation is rheumatoid factor (RF). The presence of RF is one of the criteria of the American College of Rheumatology (ACR) for classification of RA. IgG class is most commonly used and is found in 60-80% of patients with RA. RF is not specific for RA; it is often present in healthy individuals and in patients with other autoimmune diseases and chronic infections [2]. Contrasting their low specificity, positive RF is counted an important

predictor for evolution of RA. Antibodies like APF (antibodies to anti-perinuclear factor) and AKA (anti-keratin antibody) detected with indirect fluorescent using buccal epithelium or esophagus of rat [3,4] have high specificity for RA. Absence of donors of buccal cells is limiting the use of APF as a routine laboratory test. The antigen for these antibodies identified as epidermal filaggrin is intermediary filament, which is involved in the cornification of the epidermis [5,6].

The profilaggrin, present in kerato-hyaline granules of the buccal cells, is proteolytically liberated in filaggrin subunits during cell differentiation. In this phase, the protein is dephosphorylated and certain arginine residuals are converted in citrulline from the enzyme peptidyl-arginine deaminase (PAD) [7]. In 1998, Schllekers *et al*. reported auto-antibodies which react with linear synthetic peptides that contain unusual amino-acid citrulline. They are present in 76% in RA with specificity of 96%. The antibodies in patients with RA are predominantly of the IgG class and have high affinity [8]. The ELISA test, based on these cyclic citrullinated peptides (CCP), has superior characteristics in detection of RA [9], with different sensitivity and specificity [10]. The sensitivity of anti-CCP 2 test in different populations varies between 64% and 74%, while the specificity varies between 90% and 99% [11-16].

The aim of this study was to determine the diagnostic value of anti-cyclic citrullinated peptide (anti-CCP) antibodies in RA.

MATERIAL AND METHODS

Patients were included in this study based on the diagnosis of the disease according to the revised diagnostic criteria for classification of RA proposed in 1987 by the American Association for Rheumatism (ARA) [17]. For the aims of the classification, i.e. the patient to be included in the group of RA, he/she should satisfy 4 of the predicted 7 criteria.

The criteria from 1 to 4 are present at least 6 weeks. This study included 35 patients (28 women, 7 men) with RA, as well as 35 subjects (18 women, 17 men) as a healthy control group. Evaluation of patients was performed at the University Clinic for Rheumatology, Clinical Center "Mother Theresa", Skopje, Republic of North Macedonia

The mean age in the group with RA was 56.68 years (±6.79) (40-65 years), while in the control group 46.2 years (±12.49) (29-65). The mean duration of the disease was 43.97 months (±45.23) (6-168 months) (Table 1).

Table 1. Clinical characteristics of patients enrolled in this study

	RA Nº 35 mean ± SD (min-max)	**CONTROL HEALTHY GROUP Nº 35** mean ± SD (min-max)
Male / female relation	7/28	17/18
Middle average age (years)	56.68 ± 6.79 (40-65)	46.20 ± 12.49 (29-65)
Middle duration (time) of disease (month)	43.97 ± 45.23 (1.0-168)	-

Inclusion criteria

In the study were included patients with RA, age 18-65 years, newly diagnosed and previously untreated.

Exclusion criteria

From the study were excluded all patients with diseases or conditions that could directly or indirectly influence the results, such as:

1. Patients with previous history of disease of the spleen, thyroid gland, liver, kidney, hematological, cardiovascular, neurological, pulmonary disorders, autoimmune diseases, age < 18 years;

2. Patients with infective febrile condition and acute infections, malignant diseases;

3. Patients with uric arthritis, urine infections, lupus erythematosus, mixed connective tissue disorders**,** vasculitis;

4. Patients with history of previous blood transfusions, as well as overweight;

5. Patients with elevated glucose at 0 point, or elevated level of degradation products: serum creatinine, urine creatinine, serum urea, hypertension or disorder of the hematological or enzyme status.

Ethical criterion was fulfilled during our work, and all patients took part in this study voluntarily. Patients and control group subjects signed informed consents.

Clinical evaluation of activity of disease

The clinical evaluation is made by a subspecialist in the field. The activity of disease is evaluated using DAS index-Disease Activity Score (DAS 28) [18-21]. The index uses mathematical formula to obtain unique composite quantum score, comprising palpably painful joints (maximal number 28), swollen joints (maximal number 28), Westergen ESR and patient's entire evaluation for disease activity (0-100 mm Visual Analog Scale-VAS) and morning stiffness.

DAS index is ranged from 0 to 10 and score below 3.2 qualifies the disease as low active.

Laboratory evaluation

In order to make clinical evaluation of the disease, it was necessary to take into account the following variables: blood count and differential, reactants of the acute phase, anti-CCP2, C-reactive protein (CRP), rheumatoid factor (RF), erythrocyte sedimentation rate (ESR), alkaline phosphatase (AP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatine kinase (CK), lactic dehydrogenase (LDH), serum urea and creatinine. Examinations were made in time points - at 0 time, in the Institute of Public Health of the Republic of North Macedonia. Serum or plasma samples were stored for up to 24 hours at 2-8 °C prior to being tested. If testing was delayed for more than 24 hours, the sample was frozen at -20 °C or colder.

DIA-STAT™AntiCCP (Axis-Shield Diagnostics, Scotland) test is semi-quantitative/quantitative ELISA technique, based on detection of IgG antibodies in human serum or plasma, directed towards synthetic cyclic citrullinated peptides (CCP) that contain modified arginine residues. The test is an auxiliary tool in diagnosis of patients with RA. The test was performed according to the manufacturer's instructions.

Principles of work

The walls of micro-titer plate are laid with highly purified synthetic cyclic peptide that contains modified arginine residues. During the first incubation, specific auto-antibodies from diluted serum or plasma attach to the antigen layer surface on the wall. Then, it is washed in order to remove unattached components. During the second incubation, the conjugate, which is enzyme of monoclonal antibody for human IgG, binds to the surface of already attached auto-antibody. After the second wash, specific auto-antibodies are incubated with the substrate. Adding the stop-solution the reaction is stopped, resulting in colored end-product. The quantity of absorbed conjugate is expressed with absorption units. In the qualitative protocol, the quantity of the conjugate, bond with the sample is compared with the bond in the reference control. In the semi-quantitative protocol, the concentration of anti-CCP 2 auto-antibodies could be evaluated with interpolation of curve based on the standard.

C-reactive protein (CRP) was determined with the test for agglutination (Latex CRP test) (BioSystems S.A. Reagens&Instruments Costa Brava 30, Barcelona, Spain) [22-26]. The test result was considered negative for values below 6 mg/L in the serum.

Rheumatoid factor (RF) was determined with the test for agglutination (Latex CRP test) (BioSystems S.A. Reagens&Instruments Costa Brava 30, Barcelona (Spain) [26-30]. Values below 8 IU/ml were considered negative.

Statistical analysis

Data analysis was made with the statistical package Statistica 7.0.

For data processing we used statistical methods for measuring central tendency. For testing significance of the differences among more arithmetical means in the groups (independent samples) we used Freedman's analysis of variance. Testing significance of differences between two arithmetical means (dependent samples) was made with Wilcoxon Matched Pairs Test. P-value less than 0.05 was considered statistically significant.

RESULTS

Of the 35 patients with RA, 23 patients (65.71%) showed presence of anti-CCP2 antibodies. while RF was found in 17 patients (48.57%). Twelve patients were anti-CCP2 and RF positive (34.28%). Eleven patients (31.42%) were anti-CCP2 positive and RF negative, while 5 patients (14.28%) were anti-CCP2 negative and RF positive. Of 18 RF negative patients. 11 patients (61.11%) were anti-CCP2 positive. Of 12 anti-CCP2 negative RA patients, 5 patients (41.66%) were RF positive.

Of the 35 patients with RA, sensitivity to anti-CCP2 antibodies was 65.71%, while sensitivity to RF was 48.57%. In 17 RF positive RA patients, anti-CCP2 antibodies were found in 12 patients, so their sensitivity was 70.58% In 18 RF negative RA patients, anti-CCP2 antibodies were found in 11 patients, so their sensitivity was 61.11%.

In the healthy control group, 1 patient (2.85%) showed anti CCP2 positivity. while 2 patients (5.71%) were positive for RF (Table 2).

Table 2. Anti-CCP2 antibody and RF in RA and control healthy group

	RA Not treated N= 35 Positive (N) / Negative (N) **mean ± SD (min-max)**	**RA seronegative N=18** Positive (N) / Negative (N) **mean ± SD (min-max)**	**RA seropositive N=17** Positive (N) / Negative (N) **mean ± SD (min-max)**	**Control healthy group N=35** Positive (N) / Negative (N) **mean ± SD (min-max)**
Anti CCP 2 + ≥ 1.26	23/12 1.71 ± 0.69 (0.92-3.0)	11/7 1.56 ± 0.59 (0.93-2.6)	12/5 1.87 ± 0.77 (0.92-3.0)	1/34 0.95 ± 0.10 (0.90-1.38)
DAS 28 + ≥ 3.2	28/7 4.79 ± 1.56 (1.85-7.03)	13/5 4.56 ± 1.76 (1.85-7.03)	15/2 5.04 ± 1.33 (2.47-6.83)	0/35 0
MORNING RIGID + > 0 min	26/9 43.20 (± 65.13 (0-300)	14/4 57.50 ± 81.40 (0-300)	12/5 28.05 ± 38.72 (0-120)	0/35
RF + 30 ≥ IU/ml	17/18 346.15 ± 625.22 (0.00-1920)	0/18 0.00 ± 0.00 (0.00-0.00)	17/0 712.67 ± 743.72 (30-1920)	2/33 13.71 ± 38.73 (0.00-120)
CRP + 12 ≥ mg/L	14/21 46.86 ± 79.19 (0.00-384)	3/15 8.66 ± 24.62 (0.00-96)	13/4 87.31 (± 96.44 (0.00-384)	4/31 5.48 ± 12.80 (0.00-48)
SEDIMENTATION + ≥ 16	27/8 48.62 ± 39.81 (2.0-120)	13/5 43.94 ± 39.82 (2.0-120)	14/3 53.58 ± 40.39 (5.0-120)	4/31 9.42 ± 8.21 (2.0-44)

Diagnostic value of anti-CCP2 antibody in patients with rheumatoid arthritis (RA)

For anti-CCP2 antibody and RF in rheumatoid arthritis, sensitivity, specificity, predictive value of the positive and negative tests as well as their precision are shown in Table 3.

Anti-CCP2 antibodies had better diagnostic performances than RF sensitivity (65.71% *vs* 48.57%) and specificity (97.14% *vs* 94.28%) in the detection of RA.

Table 3. Diagnostic performance of anti-CCP2 antibody and RF in rheumatoid arthritis

	Anti CCP 2 RA N=35	Anti CCP 2 **RA^-** N=18	Anti CCP 2 **RA^+** N=17	**RF RA N=35**	RF **RA^-** N=18	RF **RA^+** N=17
SENSITIVITY %	65.71	61.11	70.58	48.57	0	100
SPECIFICATION %	97.14	97.14	97.14	94.28	94.28	94.28
PREDICTABLE VALUES FOR THE POSITIVE TEST %	95.83	91.66	92.30	89.47	0	89.47
PREDICTABLE VALUES FOR THE NEGATIVE TEST %	26.08	17.03	12.82	35.29	35.29	0
PRECISION %	81.42	84.90	88.46	71.42	62.26	96.15

Anti-CCP 2 antibodies and DAS 28 index of disease intensity

In the 35 patients with RA, DAS 28 > 3.2 was found in 28 patients (80%). In 17 seropositive RF patients, the presence of DAS 28 > 3.2 was found in 15 patients (88.23%). In these 15 patients DAS 28 > 3.2, anti-CCP2 positive were 10 patients (66.66%) and their M±SD (2.23±0.61), range (1.28-3.0).

In 18 RF negative patients, DAS 28 > 3.2 was found in 13 patients (72.22%). In these 13 patients DAS 28 > 3.2, anti-CCP2 positive were 9 (69.23%) and their M±SD (1.92±0.45), range (1.3-2.6).

Seropositive RF patients had higher titer of anti-CCP2 antibodies than seronegative [(1.87±0.77) (0.92-3.0) *vs* (1.56±0.59) (0.93-2.6)], but also higher DAS 28 index [(5.04±1.33) (2.47-6.83) *vs* (4.56±1.76) (1.85-7.03)]. There was no statistical significance (p=0.27) between these two groups regarding anti-CCP2 antibodies.

Although there was unequal representation of anti-CCP2 positive patients in DAS 28 > 3.2 seropositive and seronegative patients (10 *vs* 9 patients; 66.66% *vs* 69.23%), the range of titer of anti-CCP2 was higher in 10 patients RF seropositive with DAS 28 > 3.2 than in RF seronegative patients with DAS 28 > 3.2 (2.23±0.61 *vs* 1.92±0.45). There was no statistical significance (p=0.37) between these two group.

The situation was almost equal in DAS 28 index in 9 RF seronegative, anti-CCP2 positive patients (5.69±1.37) range (3.31-7.03) in comparison with 10 RF seropositive anti-CCP2 positive patients (5.63±1.01) range (4.17-6.83).

There was no statistical significance between DAS 28 index in RF seropositive and seronegative patients (p=0.38), as well as between the two groups DAS 28 > 3.2. anti-CCP2 positive, but RF seropositive and seronegative patients (p=0.91) (Figure 1).

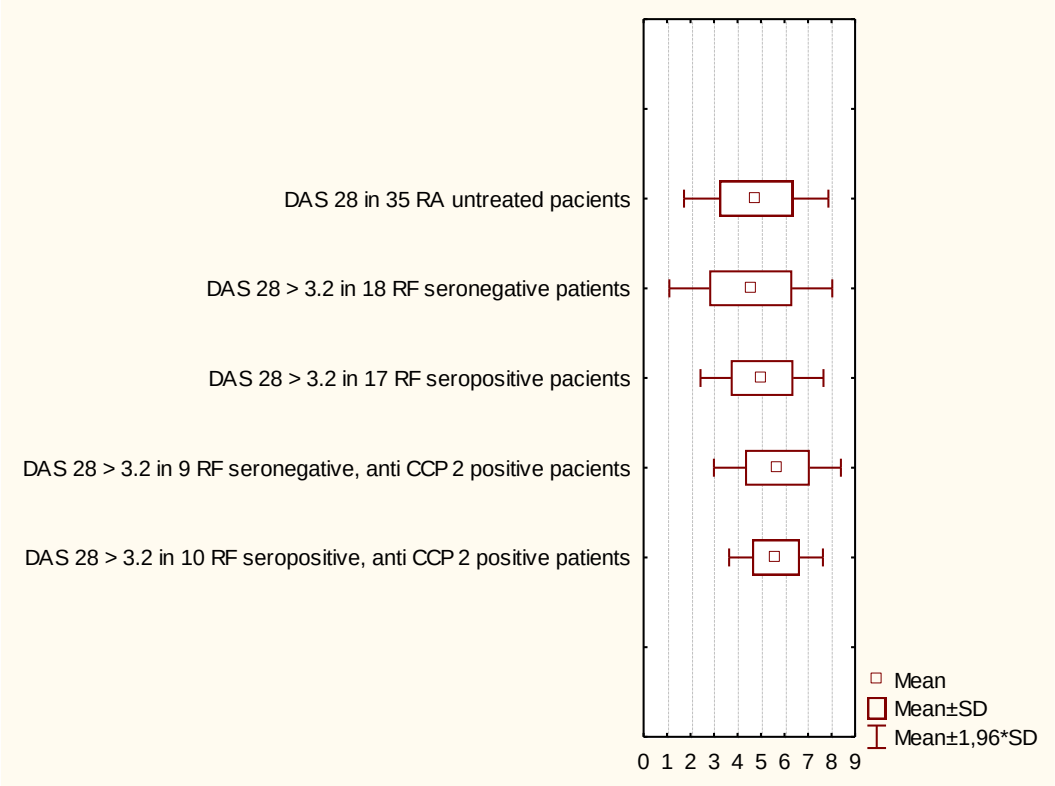

Fig. 1. Distribution of DAS 28 index

1. There was a statistical significance using Wilcoxon-matched test between anti-CCP2 in RA and healthy control group for $p < 0.05$ (p=0.0001).

2. There is a statistical significance using Wilcoxon-matched test between anti-CCP2 in RA and DAS 28, RF and CRP, ESR and morning stiffness in the same group for $p < 0.05$: (anti-CCP2 *vs* DAS 28 p=0,000; anti-CCP2 *vs* RF p=0.018; anti-CCP2 *vs* CRP p= 0.040; anti-CCP2 *vs* morning stiffness p=0.000; anti-CCP2 *vs* ESR p=0.000).

DISCUSSION

Numerous studies have shown the diagnostic application of anti-CCP2 generation, the involvement into the pathogenesis of disease, connection with clinical manifestation of RA, as well as their predictive and prognostic value. Up to date, researches regarding diagnostic performances of anti-CCP2 assay show the fact that this assay is highly specific for RA (90-98%), with sensitivity that varies from 40-80% in comparison to other inflammatory and systemic rheumatic diseases [10]. Beside patients with early RA, also patients with RA who are RF seronegative are our object of interest.

Reports for sensitivity to anti-CCP2 antibodies from first generation are approximately 68% (45-80%) sensitivity and 98% (96-100%) specificity [9]. Reports for sensitivity to anti-CCP2 antibodies from second generation are approximately 64-74%, with specificity 90-99% (11-16,31,32). The usefulness of anti-CCP2 assay is mostly visible in differentiation of early, undifferentiated arthritis [33]. Our findings for sensitivity of 65.71% and specificity of 97.14% correlate with these studies. As it could be noticed from the analyses of other laboratory variables from our study, anti-CCP2 antibody has comparable sensitivity and higher specificity than RF in optimal cut-off values. Relatively low sensitivity of anti-CCP2 assay could be due to the presence of heterogeneous group of antibodies directed towards different epitopes of citrulline molecule, to different sets of antibodies present in the serum of every patient, and to

the relatively small set of antigen determinants present on the molecule of the synthetic citrullinated peptide.

High specificity is especially useful in RF negative RA patients, showing specificity of 61.11%. Moderate sensitivity and high specificity allow anti-CCP2 antibody to be included as a classification criterion in RA.

High specificity is the most valuable characteristic of this assay, giving it high discriminatory power, with very little percentage of false positive results in comparison to the other systemic connective tissue disorders and advantage to other serological assays for RA. Anti-CCP2 antibodies are indicative for future development of RA in patients with early undifferentiated arthritis, and are better than RF for this purpose. Patients with positive anti-CCP2 antibodies have more difficult clinical features, increased joint discomfort and radiographic changes that need more intensive disease treatment [4]. Up to date researches show that in diagnostic procedure of RA it is necessary to use routinely at least 2 serologic markers (RF and anti-CCP2 assay) in order to substitute negative and emphasize positive values of each one of them. Their concomitant use would result in early and precise diagnosis of RA and timely and aggressive therapy. All patients with early and undifferentiated RA should be tested for RF. Its high titer is highly predictive for RA and erosive arthritis [12]. In patients with low titer of RF or negative for RF, anti-CCP2 antibodies should be determined. They have extra potential in detection of patients with RA and erosive arthritis. Also, anti-CCP2 antibodies are a useful marker in terms of predicting the course and prognosis of RA; higher titer of anti-CCP2 antibodies represents poorer prognosis [34]. Novel research in the field of serology markers for diagnosis of RA shows that combination of anti-CCP2 and anti-MCV (anti-mutated citrullinated vimentin) could be of value [35].

Although DAS 28 index, which unites not only laboratory variables, but also clinical markers for assessment of disease, has higher sensitivity (80%) and specificity (100%), however, anti-CCP2 antibody as an isolated variable dominates with its performances in diagnosis of early, undifferentiated RA.

It has to be mentioned that values obtained in this study were lower and deviated from the values given by the producer of DIA-STAT™ Anti CCP 2 (Axis-Shield Diagnostics) (sensitivity for anti-CCP2 79%, specificity 100%).

Data obtained in this study for anti-CCP2 antibodies were higher than previous tested kits from other researchers [12,31,36].

CONCLUSION

The anti-CCP2 assay is useful in everyday clinical practice in diagnosis of early, undifferentiated RA. We can conclude that anti-CCP2 antibodies are an excellent serologic marker in diagnosis and differential diagnosis of RA, clear predictor of the aggressive course of the disease, with the appearance of erosions and extra-articular manifestations. This contemporary diagnostic assay is implemented in routine clinical practice.

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UDC: 616.12-053.31-089.168-06:616.24-008.64

ORIGINAL ARTICLE

EVALUATION OF RESPIRATORY FAILURE FOLLOWING PEDIATRIC CARDIAC SURGERY

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ABSTRACT

Background: This study evaluated the respiratory failure (postoperative reintubation, respiratory acidosis, deterioration of gas blood, respiratory disfunctions, hypoxia) after congenital heart surgery.

Material and methods: To evaluate the impact of respiratory failure (within 48 hours postoperatively) in patients undergoing congenital heart surgery. This retrospective study included 45 operated patients (male and female aged 3 to 9 months) who had undergone cardiac surgery at the University Clinic for Pediatric Surgery in a period of two years.

Type of congenital heart diseases, perioperative and postoperative parameters (duration of cardiopulmonary bypass - CPB, cross-clamping of aorta, duration of stay in ICU and complications) were analyzed.

Results: Of a total of 45 operated patients, five required reintubation, and their average age was 7.5 months, and median body weight 7.8 kg. Perioperative procedures were prolonged (duration of CPB - 97 minutes, aortic cross-clamping time - 59 min. and duration stay in ICU -7.2 days), caused postoperative complications (chylothorax, respiratory infection and thoracic bleeding) and worsening of respiratory failure. We evaluated postoperative respiratory failure in five reintubated patients.

Conclusion: Prolongated perioperative and postoperative procedures were significantly associated with postoperative complications, worsening of the general condition and prolonged postoperative treatment.

Keywords: complications, cardiac surgery, pediatric population, perioperative and postoperative procedures

INTRODUCTION

In last decades the treatment of congenital heart diseases has become an operative routine in infant population [1,2]. Development and advances in surgical techniques and preoperative and postoperative management (preoperative diagnosis, anesthesia, cardiopulmonary bypass - CPB, and aortic cross-clamping, postintensive care and treatment) have altered the effects for mechanical ventilation following cardiac surgery [3,4,5].

However, reintubation in operated infants remains a significant concern and has been associated with long-term stays at intensive care unit (ICU), increased rates of complications and greater mortality. Early extubation allows earlier mobilization and verbal communication between the operated infant, parents and hospital staff involved. Prolonged mechanical ventilation in a child can be one of the most distressing experiences for the infants and their parents [6,7,8].

The incidence of congenital heart disease (CHD) in the infant population has been reported to be 3–12/1000 live births. In several studies discussing pediatric cardiac surgery, the percentile of reintubation was reported to be rare (3% to 7%).

In infant population, although technological and medical advances provided better devices (ventilators, machines for extracorporeal membrane oxygenation - ECMO, perfusors) and better postoperative care of intubated infants, the need of reintubation in the first 48 hours is still high (6% to 21%) [9,10].

Probably the main problem for the need of reintubation in the first days are infant's immature lungs (anatomic small-diameter airways, respiratory distress-hyaline membrane disease, followed with significant respiratory effort and reduced respiratory function, hypoxia, respiratory acidosis with hypercarbia, etc.). Prolonged perioperative procedures (duration of CPB and cross-clamping of aorta, ICU stay), postoperative complications and comorbidities also contribute to clinically manifested respiratory failure and need of reintubation [11,12,13].

The aim of our study was to present, analyze and evaluate respiratory failure and need of reintubation in the first 48 hours in the intensive care unit.

MATERIAL AND METHODS

This retrospective study included a total of 45 operated infants with congenital heart disease undergoing cardiac surgery at the University Clinic for Pediatric Surgery (The William Novick Global Cardiac Alliance) during a period of four years (2013-2016). From patients' data records we analyzed demographic parameters (such as age and gender), type of congenital heart diseases, comorbidities, duration of cardiopulmonary bypass (CPB), clamping of the aorta, duration of stay in ICU and complications.

Extubation criteria were fully awake operated patients with regular breathing, normal arterial blood gas (ABG) and normal oxygen saturation, hemodynamics stable, minimal chest tube drainage and normal postoperative echocardiogram. Reintubation was defined as reintubation at ICU within the first 48 hours with manifested respiratory acidosis, respiratory dysfunction, hypoxia, hemodynamic instability and consciousness.

The aim of this study was to present the evaluation of the respiratory failure in reintubated infants in the first 48 hours. Informed consent was obtained from the parents of the children.

RESULTS

During the study period, 45 infants with congenital heart diseases were operated and extubated in the first 48 hours. Five infants needed reintubation. The median age at surgery of these infants was 7.5 months (range 3-9) and the median body weight was 7.8 kg (range 4.5-9 kg). Three operated infants were male and two were female.

Table 1. Congenital heart diseases

Type of CHD	**N=5**
DORV	1 (20%)
Single ventricle	1 (20%)
TOF	1 (20%)
TGA	1 (20%)
VSD	1 (20%)

DORV – Double outlet right ventricle, TOF - Tetralogy of Fallot, TGA – Transposition of great arteries, VSD- Ventricular septal defect

A complex cardiac surgery with total correction of congenital heart diseases was made in all children (Table 1).

Table 2. Perioperative procedures and comorbidities

Characteristics	**N=5**
Duration of CPB, minutes	97 (58 - 150)
Duration of cross-clamping of aorta, minutes (min-max)	59 (30 -93)
Duration of stay in ICU in days (min-max)	7.2 (5 - 9)
K vitamin deficiency, N (%)	1 (20%)
Sy. Down N (%)	1 (20%)

Regarding perioperative procedures, the median duration of CPB was 97 minutes, duration of aortic cross-clamping was 59 minutes and median duration in ICU was prolonged (7.2 days) compared to the other operated children without reintubation. Two operated infants had comorbidities; the first one was with K vitamin deficiency and second was with Sy. Down (Table 2).

Extubation failure, with reintubation, occurred in five operated infants; there were three infants with postoperative complication chylothorax, one infant with respiratory infection and one infant with thoracic bleeding. All five infants were extubated in the first 12 hours, but due to the complications required reintubation (Table 3).

Table 3. Postoperative complications and time of reintubation after cardiac surgery in hours

Causes of reintubation	N= 5	Time of reintubation in hours
Chylothorax	3 (60%)	36
Thoracic bleeding	1 (20%)	16
Respiratory infection	1 (20%)	48

Reintubation was determined according to the following criteria: respiratory dysfunction (significant hypoxia with hyposaturation below 80%, hypercapnea, significant respiratory effort); hemodynamic instability (hipovolemia, hypotension and anemia) and deteriorated level of consciousness. The decisions for extubation and reintubation were made by an intensivist and the team caring for the patient (Table 4).

Table 4. Parameters for reintubation

Operated child with complication	sO2 %	pO2 mmHg	Respiratory effort	Hemodynamic instability	Consciousness
Chylothorax	80%	65	positive	positive	positive
Chylothorax	78%	55	positive	positive	positive
Chylothorax	75%	60	positive	negative	negative
Thoracic bleeding	85%	58	negative	positive	positive
Respiratory infection	79%	60	positive	negative	negative

DISCUSSION

This study aimed to evaluate respiratory failure and reintubation in operated infants with congenital heart diseases. Five of 45 operated infants undergoing cardiac surgery needed reintubation. All five reintubated infants were with comorbidities (two of them with Sy. Down and K- vitamin deficiency), prolonged perioperative procedures (duration of CPB, duration of

aortic cross-clamping, duration of stay in ICU) and complications (chylothorax, thoracic bleeding, respiratory infection).

In several previous studies many risk factors have been reported as possible that required reintubation (prematurity, genetic syndromes, diaphragmatic paralysis, cardiopulmonary bypass, procedure complexity, extracorporeal life support, delayed sternal closure, postoperative infections, and duration of mechanical ventilation) [14,15,16,17].

In our study the incidence of reintubation was 11.1% (45 operated infants /5 reintubated infants) and was relatively high when compared to the recently reported prevalence of 3% to 7% among infants after cardiac surgery. The median age of operated infants at surgery was 7.5 months and the median body weight was 7.8 kg.

In the studies of Gupta P *et al.* [18] the effects of prolonged perioperative procedures were confirmed to lead to reintubation. Similar results were obtained in our study, where the average duration of CPB was 97 (range 58 - 150) min., duration of aortic cross-clamping 59 (range 30 - 93) min. and consequently a prolonged stay in ICU 7.2 (5 - 9) days occurred. By comparing of the operated patients with patients who were not reintubated we confirmed a significantly higher percentage of prolonged ventilation and intensive medical treatment in the reintubated patients.

Extubated patients in the first 24 hours were early discharged from ICU opposed to patients who needed reintubation. Heinle *et al*. found that infants who underwent early extubation after cardiac surgery had hospital stays 3 days less than those ventilated for longer periods [19]. In the study of Shinya Miura *et al.* respiratory failure and need of reintubation was found in 25 of 156 cases, 16.0% in the period of seven days [20].

Similar results were found in our study where the average stay in ICU was 7.2 days (range 5 – 9) days. All 5 operated patients were early extubated in the first 12 hours after operation, but the average period was 33.3 hours (range 16-48 hours). They were reintubated as a result of worsening of the general condition.

Several impaired parameters and worsening of the general condition were main reasons for reintubation. Hyposaturation below 85% caused decreased values of pO2 (hypoxia with respiratory acidosis) and hypercapnia (elevated values of pCO2). Further, respiratory efforts (tachypnea, apnea, impairing breathing) were presented in almost all cases; hemodynamic instability (hypotension, hypovolemia and anemia) manifested in one child and impaired consciousness in three operated infants.

The incidences of respiratory failure and infections were significantly lower in patients who were early extubated. The probable reason could be the associated ventilatory infections which causes acute systemic and pulmonary inflammatory response leading to potential alveolar collapse and atelectasis [21,22,23]. In our study we had one operated patient with respiratory failure and infection confirmed with microbiological tests and he required reintubation 48 hours after operation.

Chylothorax is a significant complication after pediatric cardiac surgery, with an incidence between 25 and 39% and is a significant cause of postoperative complication. It occurs due to a direct trauma to the thoracic duct and the lymphatic system [24,25,26].

In our study 3 patients had chylothorax, prolonged ICU stay and medical treatment. The incidence was 20%, which is a significant incidence and correlated with the previously reported one. The main reason was probably the complex access and management of cardiac surgeries.

One patient had hemodynamic instability, thoracic bleeding, followed by hypovolemia, hypotension and anemia. This postoperative condition can worsen breathing and can increase respiratory acidosis. Reintubation is an appropriate procedure for improvement of the condition.

CONCLUSION

In our study, the confirmed presence of postoperative complications such as chylothorax, respiratory infections and bleeding were additional factors for worsening of the condition and for needed reintubation. However, our outcomes are very poor and future work should focus on larger multicenter studies to confirm clinical results in early postoperative period in order to prevent possible complications that further worsen the general condition of operated children.

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UDC: 616.637-078

ORIGINAL ARTICLE

SUSCEPTIBILITY OF STRONG BIOFILM-PRODUCING UROISOLATES IN PLANKTONIC STATE VS. BIOFILM GROWTH MODE

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ABSTRACT

Introduction: Biofilms are defined as functional consortiums of microorganisms, attached to a surface, enclosed in self-produced hydrated polymeric matrix. Between microorganisms in biofilm and free floating microorganisms of the same species there are essential differences regarding: specific gene expression, ability for intercellular communication via biochemical signaling molecules, antimicrobial resistance. These biofilm features play a key role in the development of chronic and antibiotics tolerant infections. The ability of uropathogens to cause relapses of UTI directly correlates with their genetic capability for biofilm production on catheter surfaces or uroepithelium and with the innate recalcitrance to treatment of the biofilm itself.

Objective: To investigate the differences in pathogens resistance in relation to biofilm expression phenotype by comparing the antibiotic susceptibility of sessile cells and their planktonic counterpart, for biofilm forming bacteria isolated from patients with UTI.

Materials and methods: The study included 120 urine samples from outpatients, from both genders, over 18 years of age, with suspected UTI, referred for microbiological examination of urine at the Institute of microbiology and parasitology, Medical faculty, Skopje. A total number of 80 bacterial species, isolated in monobacterial culture were examined for biofilm production. For biofilm cultivation and biofilm biomass determination, adherence assay on 96-well microtitre plate and semi-quantitative spectrophotometric method were used. For 25 selected strong biofilm producers, antibiotic susceptibility to ciprofloxacin and sulfametoxasole trimetoprime of planktonic cells was tested by microdilution assay and compared with the antimicrobial sensitivity of bacterial biofilms (performed with the Calgary Biofilm Device)

Results: Distribution of susceptibilities, regarding the frequency of the sensitive, intermediate and resistant bacteria showed statistically significant difference in the antibiotic susceptibility of planktonic cells vs. their sessile counterparts ($p<0.01$) / Fisher´s Exact p. Results showed that bacteria are more resistant in biofilm communities as compared to planktonic form by comparison of MIC & MBEC assay.

Conclusion: Attached bacterial communities (biofilms) present with an innate deficit of antibiotic susceptibility not registered in the same bacteria grown at planktonic state.

Minimal biofilm eradication concentrations, determined applying the Calgary Biofilm Device, reveal that treatment of biofilms requires 20 to 1,200 times concentrations of a certain antibiotic to achieve the desired antimicrobial effect compared with planktonic cells of the same organisms which urges future changes in the conventional therapeutical approach.

Keywords: biofilm, urinary, infections, resistant

INTRODUCTION

Urinary tract infections (UTIs) are a group of diseases with infectious etiology resultign from microbial colonization of urine and infection of various parts of the urinary system involving the anatomical region from the renal cortex to the urethral meatus (including kidneys, ureter, renal pelvis, vesica urinaria and the urethra) and associated structures (perinephric fascia, prostate and epididymis)[1, 2, 3].

Recurrent urinary tract infections are defined as occurrence of 2 UTIs over a period of 6 months or 3 UTIs over a period of 12 months. Recurrent UTIs are mainly caused by reinfection with a different uropathogen, however they may occur in the form of relapse (recurrence caused by the same microorganism)[4].

Urinary tract infections are among the most common bacterial infections and represent significant global public health problem. Affecting approximately 150 million people annually worldwide UTIs (particularly complicated UTIs) impose substantial economic burden due to frequent hospitalization, losses of income, morbidity, and restricted activity.

Commonly used treatment for uncomplicated urinary tract infections includes combination of trimethoprim sulfamethoxazole (co-trimoxazole), trimethoprim, beta-lactam antibiotics, nitrofurantoin and fosfomycin tromethamine [5]. These antimicrobial agents primarily are used due to their good tolerance, antibacterial spectrum against suspected uropathogens, and favorable pharmacokinetic and pharmacodynamic profile [5, 6]. In the treatment of urinary infections, the effect on bacteriuria predominantly correlates with urinary rather than plasma concentrations of the antibiotic [5, 7].

Rising trend of antimicrobial resistance among the most common uropathogens, observed with antibiotics routinely used in UTI treatment is a cause of growing concern and the contributing risk factors remain in the focus of intensive screening and monitoring [8]. Based on current literature data, alongside the intrinsic resistance of some of the urinary pathogens, major threats for development multidrug resistant species is lack of introduced restrictions in antibiotic use and biofilm production [9, 10, 11, 12]. The effect of biofilms on susceptibility of the uropathogens is multifactorial hence it does not only represent a mechanistic optical, but may also confer additional resistance, diverse from other aspects of multidrug resistance (MDR).

Costerton & Donlan, 2002 stated perhaps the most appropriate definition of a biofilm: “A biofilm is a microbial sessile community of microorganismsl irreversibly attached to a substrate/surface/each other, embedded in a self-produced extracellular polymeric matrix (glycocalix) exhibiting altered pheno- and genotype“ [13].

Biofilms glycocalyx is one of the resistance factors of the encased communities of microorganisms. With its three-dimensional structure the glycocalyx is crucial for the high degree of heterogeneity of the biofilm hence the created chemical and nutritional gradient results with growth of multiple micro-environments in the biofilm structure. These unique growth conditions within the biofilm matrix are the initial step that directs the essential differences between sessile (attached) and planktonic (free-floating) microorganisms, primarily through specific gene expression [14, 15, 16] and ability for intercellular communication via chemical signal molecules known as auto-inducers.

To date there is no generally accepted stance on the exact mechanism of the vast resistance within the biofilm [17, 18, 19, and 20]. Biofilm mode of growth is proved to be associated with a significant increase in antimicrobial resistance, and fully formed; mature biofilms have shown resistance to antibiotic concentrations up to 10 -1000-fold higher compared to planktonic cultures [1]. Several mechanisms have been proposed to explain this phenomenon, including failure of the antimicrobial to penetrate the biofilm [23], slow growth rate and heterogeneity within the biofilm [13], induction of a resistant phenotype etc.

One of the key elements which despite numerous studies from this field remain unclear and undefined is the implacability of the results of conventional in vitro antibiotic susceptibility testing in the treatment of biofilm-related infections.

Aim

To investigate the differences in uropathogens resistance in relation to biofilm expression phenotype by comparing antibiotic susceptibility of sessile cells (minimal biofilm eradication concentration) and their planktonic counterpart (minimal inhibitory concentration) for biofilm forming bacteria isolated from patients with UTI.

MATERIALS AND METHODS

Bacterial strains

The study included urine samples obtained from 120 outpatients, males and females, over 18 years of age, with symptomatic UTI, referred for microbiological examination of urine at the Institute of microbiology and parasitology, Medical faculty, Skopje. A total number of 80 bacterial species, isolated in monobacterial culture were examined for biofilm production.

Isolation and identification of the strains

Midstream, clean-voided, urine samples were plated onto blood sheep agar or chromogenic (UTI) agar using calibrated loops (for the semiquantitative method). After 24 hour incubation the number of colony-forming units (CFU) in milliliter urine was determined. As part of evaluation of the urine culture Gram stain and microbial biochemistry tests were performed where necessary.

Biofilm assay

Biofilm forming ability and biofilm biomass determination were investigated by a semi-qualitative assay according to the method described by Christensen et al. with some modifications [24]. Suspension of one colony of each strain (loopful of test organism inoculated in 5 mL of trypticase soy broth) was incubated overnight at 37ºC. Then, 10 μl of stationary (18-24 h) TSB cultures were diluted 1:100 into 1000 μl of artificial urine to achieve 0.5 McFarland turbidity (~108 CFUs/ml) after which each bacterial suspension was inoculated into the wells of sterile, polystyrene, 96-well, flat-bottomed tissue culture plates. Following 24 hours incubation (without shaking) the residual broth was removed and the plates were washed with 200 μl 85% NaCl to remove the 'planktonic' bacteria before heat fixation at 60°C for 15 min. For evaluation of the biofilm producing capacity, 120 μl of 0.1% (wt/vol) crystal violet was added per well, left at room temperature for 15 min (in order to bind to any present attached microorganisms) and the plates were washed with running tap water.. At this point, biofilms were visible as purple rings on the walls of each well but the quantitative assessment of the biofilm mass requires elution of the bound dye so that the optical density (OD) can be determined. This was performed by adding 120 μl 75% ethanol per well. OD of the eluted solution was read in a ELISA microplate reader (Thermo Scientific, USA) at OD600 and the degree of absorbance was proportional to the concentration of dissolved dye, and thus to the amount of sessile microorganisms in the well.

Three wells inoculated with control strain in TSB served as positive control; wells containing sterile TSB served as a negative control; tests were done in triplicate, on three separate occasions, the results were averaged and interpretated according to Stepanovic et all [25].

Based on absorbance readings obtained from the blank (0.152) we calculated the ″cut-off″ OD value (ODc)(defined as three standard deviations (SD) above the mean OD of the negative control): In this study, the mean optical density value of the negative control wells measured at 600 nm was 0.152 and the optical density cut-off value was 1.61.

All isolates were classified as non- (OD≤ ODc), weak- (ODc<OD≤ (2xODc), moderate- (2 × ODc) < OD≤ (4 × ODc) and strong-biofilm producers (4 ×ODc) < OD:

From overall 80 bacteria examined for biofilm forming ability, 25 strong biofilm-producers were selected for the antimicrobial susceptibility testing.

Quality control

Reference strain *E.coli* ATCC 25922 was used as positive control for biofilm production as recommended by the National Committee for Clinical Laboratory Standards.

Microdilution method

Determination of the minimal inhibitory concentration (MIC) is considered a "gold standard" in antimicrobial susceptibility testing and is one of the most accurate and most commonly used methods in microbiological research. In this study we used microdilution method to investigate the effect (minimal inhibitory concentration-MIC and the minimal bactericidal concentration-MBC) of ciprofloxacin and sulfamethoxazole-trimethoprim (two commonly used uroantiseptics in clinical practice) on planktonic cells (cultivated on agar plates via conventional methods) of the selected 25 strong biofilm producers [26].

MICs were determined according to the EUCAST broth microdilution protocol using flat-bottomed 96-well microtitre plates. In each row of the plate a series of double dilutions with concentration range of 0.031-128 mg/L for sulfamethoxazole-trimethoprim and 0.004-128 mg/L for ciprofloxacin were done.

In each well 75 µl of diluted antibiotic was added with exception of the first column of each row (positive control of bacterial growth). Last column was left blank (medium only) and served as negative control. Frst seven rows (A-F) were inoculated with a suspension of test strains (in a cation-adjusted Mueller-Hinton broth) and the last row of the microtiter plate was inoculated with reference strain (E.coli ATCC 25922) for monitoring of the quality of the results.

The results were interpretated after 18 hours incubation at 35◦C according to EUCAST recommendations [27]. The lowest concentration of antibiotic with turbidity similar to the blank wells was registrated as the MIC. For determination of MBK (lowest concentration required to kill 99.9% of the inoculum) we inoculated 10 μl of the turbidity-free well on Mueller-Hinton blood agar. After 24 hours incubation at 35 ° C, MBK for each strain was defined by the agar plate with lowest concentration of antibiotic and no visible bacterial growth.

Breakpoints for interpretation of MIC of ciprofloxacin according to CLSI:

≤1.0 µg/mL (susceptible), 2.0 µg/mL (intermediate), and ≥4.0 µg/mL (resistant). Breakpoints for interpretation of MIC of sulfomethoxazole-trimethoprim according to CLSI: ≤2/38 µg/mL (susceptible) and >4/76µg/mL (resistant).

Minimal Biofilm Eradication Concentration

The minimal biofilm eradication concentration (MBEC) of the sessile microorganisms, i.e. biofilm producers with phenotypic expression of biofilm was determined by a modification of the Calgary Biofilm Device method which produces 96 equivalent biofilms for the assay of antibiotic susceptibilities by the standard 96-well technology [28].

Microtittre plates were inoculated with 200 µl of bacterial suspension in stationary phase of growth. Frst seven rows (A-F) were inoculated with a suspension of test strains and the last row of the microtiter plate was inoculated with reference strain (E.coli ATCC 25922).

Last column was left blank (medium only) and served as negative control.

- Step 1: for biofilm formation, a modified 96-peg polystyrene lid designed to fit into the wells of a standard microtiter plate without contact with the walls or the bottom of the well was used to close the microtiter plate containing stationary faze cultures of the bacteria and then incubated for 18-20 hours at 37°C.
- Step 2. For biofilm susceptibility test 200 of two-fold diluted volumes of antibiotic in a cation-adjusted Mueller-Hinton broth were added in a second microtiter plate. We used the same antibiotics as in the microdilution method (ciprofloxacin and sulfomethoxazole-trimethoprim) but the serial dilutions contained five-fold higher concentrations of the antimicrobials (range 2.5-640 μg/mL). Column one of each row was used as a positive control (with no antibiotic).
- Step 3: Biofilms were formed on the lids pegs of the first MTP, after which the lid was placed in a neutralizing recovery plate containing 200 μl sterile buffered saline (to remove residual planctonic cells) and along with the attached sessile bacteria was translocated onto the second 96-well plate in which dilutions of the specified antibiotics were prepared as described above.
- Step 4: After overnight of incubation at 37°C the 96-peg lid is removed from the antibiotic MTP, gently shaken to remove excess medium and rinsed three times in a MTP containing 200 μl sterile saline.
- Step 5: after rinsing, lid was placed on MTP with 200 μl TSB and biofilms formed on the pegs were disrupted in the recovery medium by u ultra-**sonication** for 5 minutes. After this procedure, the peg lid was replaced with a regular plate cover and the MTP was incubated for 20 hours at 37°C.

We used new microtiter plate for each of the four steps. At the end of the procedure there should be visible growth in the first column (positive control) and absence of turbidity in the last column of each row (negative control).

Minimal biofilm eradication concentration (MBEC) was determined by measuring the turbidity of the wells at 495 nm and was defined as the minimal concentration of antibiotic required to eradicate the biofilm. OD495 reading (UV-Visible Spectrophotometer) of approximately 0.080, which is similar to the readings for the negative control was considered as negative for biofilm growth.

Statistical analyses

The statistical analyses were preformed in the statistical program Statistica 7.1 for Windows и SPSS Statistics 17.0

Descriptive statistics are presented as Mean; Std.Deviation; ±95, 00%CI; Minimum; Maximum); distribution of data was tested with: Kolmogorov-Smirnov test; Lilliefors test; Shapiro-Wilks test (p); Mann-Whitney U test (Z/p) was used to compare the absolute values of MIC (CIP) & MBEC (CIP); MIC (SXT) & MBEC (SXT); MIC (CIP) & MIC (SXT); MBC (CIP) & MBC (SXT); MBEC (CIP) & MBEC (SXT).

Significance was defined by $p<0,05$. Data were presented with tables and figures.

RESULTS

Susceptibility to ciprofloxacin (CIP)

Descriptive statistics of minimal inhibitory concentration (MIC), minimal bactericidal concentration (MBC) and minimal biofilm eradication concentration (MBEC) of ciprofloxacin for the isolates with strong biofilm production capacity from patients with UTI is presented in Table 1 and Figure 1.

MICs of the planktonic forms varied in range of 6,170±9,479 mg/L; ±95,00%CI: 2,257-10,082; lowest MIC value was 0,004 mg/L and peak MIC value was 32,000 mg/L.

MBCs of the planktonic forms varied from 11,857±19,431 mg/L; ±95%CI: 3,837-19,878; lowest MBC value was 0,008 mg/L; highest MBC value was 64,000 mg/L.

MBECs of the microorganisms with phenotypic expression of biofilm varied from 218,400±305,470 mg/L; ±95%CI: 92,308-344,492; with lowest and peak MBEC values of 5,000 mg/L and 1280,000 mg/L, accordingly.

Table 1. Descriptive statistics of MIC&MBC&MBEC of ciprofloxacin

Method	**N**	**mean±SD (min-max)**	**95% Confidence Interval (95%CI)**
MIC, (mg/L) (CIP)	25	6.17±9.48 (0.004-32)	(2.28-10.08)
MBC, (mg/L) (CIP)	25	11.86±19.43 (0.008-64)	(3.84-19.88)
MBEC, (mg/L) (CIP)	25	218.4±305.47 (5-1280)	(92.31-344.49)

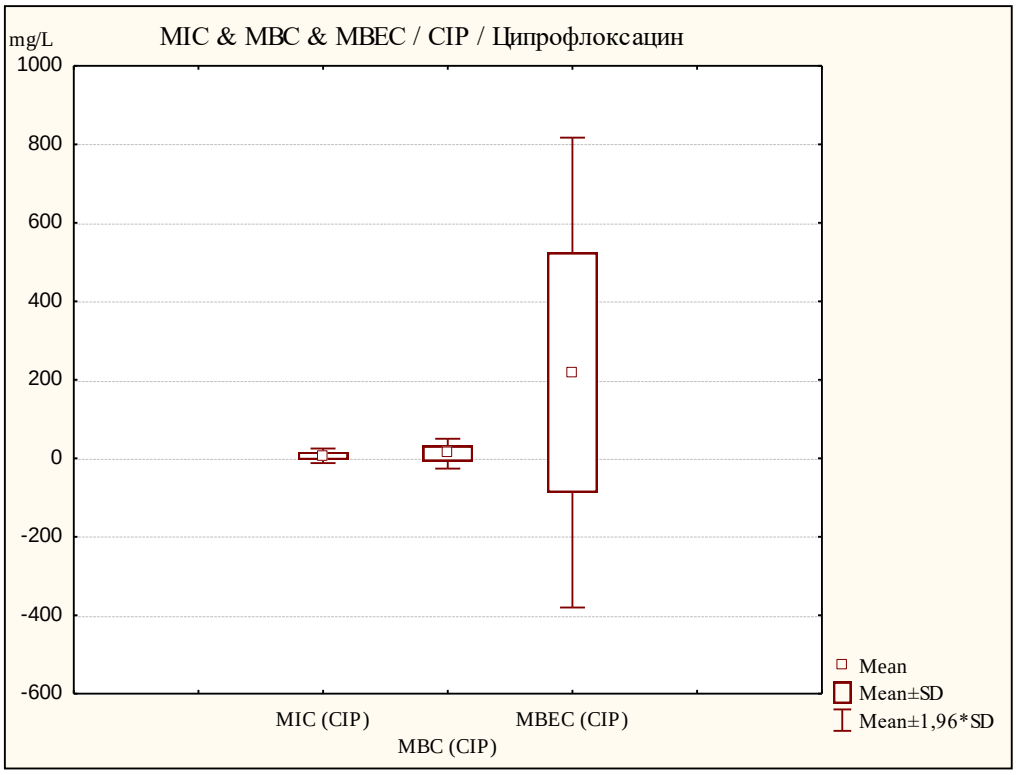

Fig. 1. MIC & MBC & MBEC of ciprofloxacin

Susceptibility to sulfomethoxazole-trimethoprim (SXT)

Descriptive statistics of minimal inhibitory concentration (MIC), minimal bactericidal concentration (MBC) and minimal biofilm eradication concentration (MBEC) of sulfomethoxazole-trimethoprim for the isolates with strong biofilm production capacity from patients with UTI is presented in Table 2 and Figure 2.

MICs of the planktonic forms varied in range of 17,481±25,413 mg/L; ±95% CI: 6,991-27,971; lowest MIC value was 0,031 mg/L and peak MIC value was 32,000 mg/L. MBCs of the planktonic forms varied from of 17,481±25,413 mg/L; ±95% CI: 6,991-27,971; lowest MBC value was 0,031 mg/L; highest MBC value was 64,000 mg/L. MBECs of the microorganisms with phenotypic expression of biofilm varied from 414,200±435,865 mg/L; ±95% CI: 234,284-594,116; with lowest and peak MBEC values of 5,000 mg/L and 1280,000 mg/L, accordingly.

Table 4. Descriptive statistics of MIC&MBC&MBEC of sulfamethoxazole-trimethoprim

Method	**N**	**mean±SD (min-max)**	**95% Confidence Interval (95%CI)**
MIC, (mg/L) (SXT)	25	8.24±11.85 (0.03-32)	(3.35-13.13)
MBC, (mg/L) (SXT)	25	17.48±25.41 (0.03-64)	(6.99-27.97)
MBEC, (mg/L) (SXT)	25	414.20±435.87 (5-1280)	(234.28-594.12)

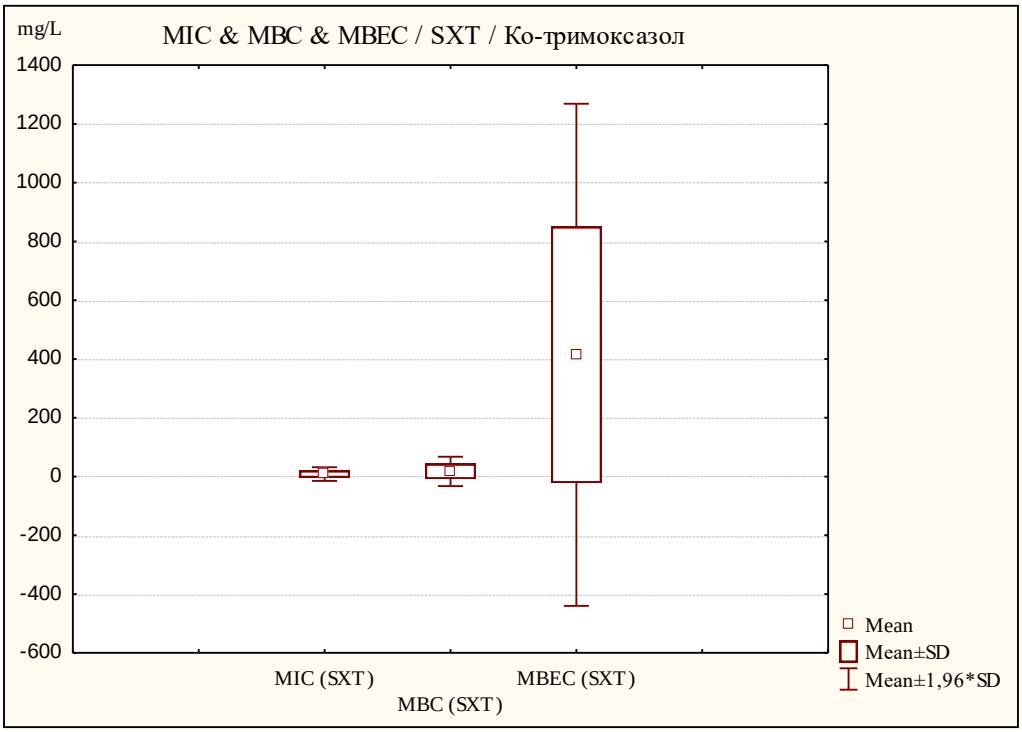

Fig. 2. MIC & MBC & MBEC of sulfomethoxazole-trimethoprim

Comparasion of susceptibility

For 25 selected strong biofilm producers, antibiotic susceptibility to ciprofloxacin and sulfomethoxazole-trimethoprim of planktonic cells (MIC) determined by microdilution assay was compared with the antimicrobial sensitivity of bacterial biofilms (MBEC) determined by Calgary Biofilm Device.

MICs, MBCs and MBECs values for ciprofloxacin and sulfomethoxazole-trimethoprim are presented in the table below.

Table 3. MICs, MBCs and MBECs values for ciprofloxacin and sulfomethoxazole-trimethoprim

Isolate	**MIC** (mg/L) **(CIP)**	**MBC** (mg/L) **(CIP)**	**MBEC** (mg/L) **(CIP)**	**MIC** (mg/L) **(SXT)**	**MBC** (mg/L) **(SXT)**	**MBEC** (mg/L) **(SXT)**
Klebsiella aerogenes	0,008	0,008	20	0,062	0,062	320
E. coli	2	4	320	2	4	1280
Klebsiella oxytoca	16	32	320	8	8	640
E. coli	0,004	0,008	5	16	64	1280
E. coli	8	8	640	2	4	320
Klebsiella aerogenes	16	32	80	0,062	0,125	320
Klebsiella pneumoniae	0,064	0.125	20	0,125	0,5	20
E. coli	0,016	0,031	10	0,031	0,031	640
E. coli	4	4	80	16	32	1280
E. coli	0,008	0,016	80	0,031	0,062	320
Enterobacter cloaceae	0,016	0,016	40	0,062	0,125	160
Klebsiella aerogenes	32	64	1280	8	16	160
E. coli	32	64	640	32	64	160
E. coli	8	32	640	32	64	640
E. coli	8	8	320	0,5	0,5	160
E. coli	0,031	0,064	10	16	64	640
E. coli	0,016	0,032	40	0,125	1	40
E. coli	0,008	0,016	20	0,062	0,5	320
E. coli	8	8	160	0,5	1	40
E. coli	4	8	320	32	64	1280
Klebsiella oxytoca	16	32	320	8	16	160
Morganella morganii	0,004	0,008	5	32	32	80
Proteus. mirabilis	0,016	0,016	40	0,062	0,125	80
Proteus. mirabilis	0,016	0,031	10	0,125	0,5	5
Klebsiella pneumoniae	0,031	0,062	40	0,25	0,5	10

Comparasion of susceptibility to ciprofloxacin - MIC (CIP) vs. MBEC (CIP)

Minimal biofilm eradication concentration of ciprofloxacin was for Z=-5, 31 and p<0,001(p=0,000) significantly higher than minimal inhibitory concentration of ciprofloxacin (Table 3).

Results showed that biofilm bacteria present a significantly increased antimicrobial resistance to ciprofloxacin compared with their planktonic counterparts.

Tested using MICs, 13(52%), 11 (44%) and 1 (4%) of the 25 strong biofilm producing uropathogens were susceptible, resistant and intermediate susceptible to ciprofloxacin, accordingly. Ciprofloxacin lost its efficacy towards all the isolates when the bacteria were grown in a biofilm with rate of resistance of 100% (25/25 isolates). The change in susceptibility was statistically significant to a level of $p < 0.001$ for all isolates tested.

Table 3. Comparasion of MIC (CIP) *vs.* MBEC (CIP)

Method	Rank Sum **MIC** (mg/L)	Rank Sum **MBEC** (mg/L)	**U**	**Z**	**p-level**
MIC (CIP**)** & MBEC (CIP)	364	911	39	-5.31	**0.000**

Comparasion of susceptibility to sulfomethoxazole-trimethoprim - MIC (SXT) vs. MBEC (SXT)

Minimal biofilm eradication concentration of SXT was for Z=-5, 66 and p<0,001(p=0,000) significantly higher than minimal inhibitory concentration of SXT (Table 4). Tested using MICs, 13(52%), 10 (40%) and 2 (8%) of the 25 strong biofilm producing uropathogens were susceptible, resistant and intermediate susceptible to SXT, accordingly.

Slime producing strains with phenotypic expression of biofilm tolerated significantly higher antimicrobial concentrations of sulfamethoxazole-trimethoprim compared with slime producers in planktonic state.

Table 4. Comparasion of MIC (SXT) vs. MBEC (SXT)

	Rank Sum **MIC** (ug/ml)	Rank Sum **MBEC** (ug/ml)	U	Z	p-value
MIC (SXT) & MBEC (SXT)	364	929	21	-5.66	**0.000**

DISCUSSION

In the past two decades, numerous studies have been performed on biofilm as a global problem and its involvement in the chronicity and recurrence of infections. These studies found strong correlation between biofilm production and recalcitrance to various antimicrobial agents. The biofilm protects the microorganism from the host's immune system, bacteriophages, dehydration, biocides, and emphasizes the virulence of the pathogen [29]. Considering the aforementioned this natural phenomenon poses a serious problem for public health. In addition to the impaired immune control of the infection, the most worrying feature of the biofilm-associated infections is the increased resistance to antibiotics of the biofilm communities [30]. The tolerance of biofilms to antibiotics is multifactorial but primarily results of genotypic and phenotypic adaptation of the sessile cells due to which they display multiple times higher resistance than the motile forms.

Gold standard for biofilm susceptibility testing is by determining the minimum biofilm inhibitory and eradication concentration (MBIC, MBEC) [31].

Studies of antibiotic efficacy against uropathogens conducted in this manner revealed that aminoglycosides and beta-lactam antibiotics can prevent the formation of "young" biofilms while fluoroquinolones, due to their highly penetrating capacity, exhibit their pharmacodynamic effect. in both "young" and mature biofilms. Published data indicate that this class of antibiotics is present in the biofilm for even up to two weeks after cessation of treatment [32, 33].

In the present study we compared the susceptibilitis of free-floating bacterial cells (represented by MIC) versus their biofilm counterparts (represented by MBEC) in 25 uroisolates confirmed as strong biofilm producers. The two antibiotics chosen for antimicrobial testing were sulfamethoxazole-trimethoprim (SXT) and ciprofloxacin (CIP), selected despite the raising rates of resistance, since they still represent drugs of choice for treatment of UTIs.

Results of the study showed that there was a significant difference ($p < 0.001$; $p = 0.000$) in the antibiotic susceptibility of plankton compared to sessile microorganisms to both tested antibiotics with MBEC (CIP) and MBEC (SXT) values for $Z = -5.31$ and $Z = -5.66$ higher than MIC (CIP) and MIC (SXT), respectively.

Attached bacteria in the biofilm were for approximately 20-1200 times more resistant, and at the upper limit of susceptibility range were MBECs of the strains highly susceptible to CIP and SXT (MICs ≤ 1 and $\leq 2/38$, respectively) according to the microdilution method. This data indicates an absence of positive correlation between the minimum inhibitory and biofilm eradication concentrations. The minimum biofilm eradication concentration of SXT was significantly higher than the minimum biofilm eradication concentration of CIP ($p < 0.05$; $p = 0.04$) which is probably due to the better penetration of fluoroquinolones through the biofilm layers. Similar results were reported by Sepandj et al. (2004) in the study of MICs and MBECs of ampicillin, cefazolin, cefotaxime, ciprofloxacin, gentamicin, and tobramycin in eight strains of E.coli and Pseudomonas spp. isolated from patients with peritonitis [34]. The same author in another study examined MICs and MBECs in gram-positive bacteria belonging to Enterococcus genus and discovered that in spite of the high susceptibility according to in vitro MICs, same isolates were either resistant or intermediate susceptible when determining their MBECs [35].

Aforementioned findings were supported by the results obtained by Naves et al. and Abdallah et al. during two recent studies on biofilm forming isolates from patients with UTI, relation to catheterization and differences in the in vitro susceptibility of planktonic and biofilm strains [35, 36]. Consistent with this Passerini de Rossi et al. reported that results for susceptibility to levofloxacin and ciprofloxacin of 32 strains of Stenotrophomonas maltophilia differ in relation to the method applied; namely, isolates which were sensible by the broth dilution test showed resistance when using the Calgary device [37].

CONCLUSION

Recommendations for antibiotic treatment are usually based on conventional methods for susceptibility testing performed on bacteria traditionally grown on a enriched media, rather than stationary phase organisms growing in biofilms and are not applicable in the treatment of biofilm-associated infections.

Results of present and similar studies show that current antibiotics used as empiric first line antimicrobial agents in treatment of urinary tract infections, often show ineffectiveness in vitro susceptibility tests when performed on sessile microorganism in biofilm mode of growth.

Significantly higher antibiotic resistance observed in bacteria with phenotypic biofilm expression makes the clinical predictive value of conventional susceptibility tests limited. Our data provide an insight in the origin of the recurrence of infections and pathogens recalcitrance to antibiotics routinely tested in the laboratory by the disk diffusion method on planktonic forms of bacteria.

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UDC: 616.711-007.55-053.6

ORIGINAL ARTICLE

RESULTS OF OUR EXPERIENCE IN CORRECTION OF ADOLESCENT IDIOPATHIC SCOLIOSIS WITH POSTERIOR SEGMENTAL INSTRUMENTATION AND FUSION WITH ALL-POLYAXIAL PEDICLE SCREW CONSTRUCT

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ABSTRACT

Introduction: This retrospective study of 30 adolescent idiopathic scoliosis patients was undertaken to analyze the effectiveness of high density thoracic and lumbar all-polyaxial pedicle screws and rods construct in patients undergoing surgical correction of adolescent idiopathic scoliosis (AIS).

Material and methods: The studied group of patients included 27 female and 3 male patients, average age 16.8 years (12-25 at the age of surgery). The used implants were polyaxial pedicle screws and titanium-alloy rods. A combination of scoliosis correction maneuvers (rod translation, rod derotation, segmental direct vertebral derotation, compression-distraction and in-situ bending) were used depending on the individual case. Standard preoperative and postoperative scoliosis x-ray films with PA and lateral views were used for assessment of radiographic parameters. The follow-up period was average 22.5 months (6-48 months).

Results: We analyzed preoperative and postoperative Cobb angle, coronal balance, thoracic trunk shift, apical vertebral translation, clavicle angle, shoulder height, thoracic kyphosis, lumbar lordosis and overall sagittal balance.

Conclusion: The statistical analysis showed a statistically significant correction of the scoliotic curve reflected through improvement of the analyzed radiographic parameters.

Keywords: idiopathic scoliosis, polyaxial pedicle screw, high-density construction

INTRODUCTION

Idiopathic scoliosis is the most common type of spinal deformity in children and adolescents. This type of spinal deformity accounts for about 65% of cases. In contrast, about 15% are congenital and about 10% are secondary to neuromuscular disease [1]. The rest are rare cases of spinal deformities as part of some syndromes.

Idiopathic scoliosis is a complex three-dimensional spinal deformity, with curvatureon parts of the spine, in the shape of the letter "S" or "C", greater than 10 degrees (measured radiographically according to Cobb's method), with simultaneous rotation of the vertebrae around the vertical axis [2]. This type of spinal deformity lacks congenital anomalies in the formation and segmentation of the vertebrae.

The present rotation of the vertebrae makes the difference between the so-called structural curves and compensatory curves of the other parts of the spine.

Although the exact cause of this deformity can not be confirmed (idiopathic scoliosis), in recent years, in the framework of basic research on the possible causes of this deformity, a genetic basis is being evaluated [3,4]. About 30% of patients have a family history of spinal deformity.

Multiple gene loci have been proven to recur in patients with idiopathic scoliosis. Therefore, genetic tests are already underway to help in the early diagnosis and prognosis of the course of spinal deformity (Scoliscore test) [5].

It occurs most often in the period of 10-14 years of age, in the pre-pubertal and early pubertal period, therefore marked as adolescent idiopathic scoliosis. During this period, due to the active hormonal status of developing children, rapid progression of the deformity occurs. In girls, this is associated with the first menarche and there is usually a gradual progression of the deformity to the period of stunting. In boys, progression is usually faster and occurs over a shorter period of time, usually before growth stops [6].

Juvenile idiopathic scoliosis occurs between the ages of 4 and 10 years and accounts for about 10-15% of cases of idiopathic scoliosis. Early onset is usually associated with greater curve magnitude in adolescence [6].

Infantile idiopathic scoliosis occurs before 4 years of age. Over 90% of this type of scoliosis is spontaneously corrected. For the remaining 10%, regular monitoring of the condition and assessment of possible neuroaxial anomalies is required [6].

The clinical examination shows [1]:

- Asymmetry of the shoulder or pelvic girdle, depending on the type of curvature
- Positive Adams test - if vertebral rotation is present
- Ligament laxity – in case of collagenopathy
- Superficial sensory deficit is being exacerbated in case of neurological disorders associated with neuroaxial anomalies (Figure 1).

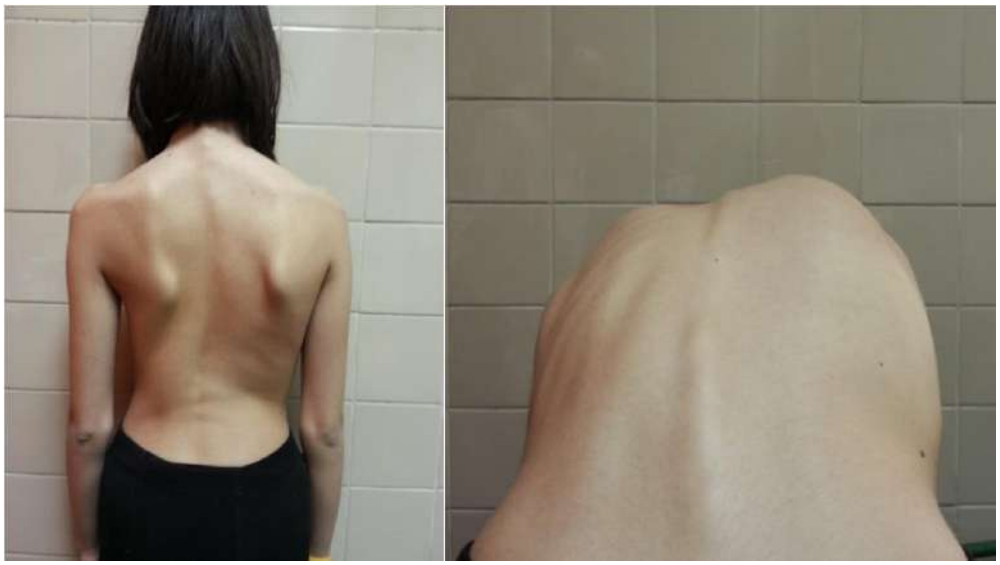

Fig. 1. Characteristics present scoliosis in a patient on clinical examination

For radiographic assessment of the type of deformity, morphology, magnitude, and growth potential, an X-ray of the spine in PA and lateral projection is performed. The flexibility of the spine is assessed by lateral bending radiographs.

Magnetic resonance imaging is not routinely performed in these patients. It is reserved for patients with present neurological signs or atypical curves.

This retrospective cohort study of 30 patients with adolescent idiopathic scoliosis is performed to analyze the efficacy of a high-density all polyaxial pedicle screw construct in the surgical correction of adolescent idiopathic scoliosis (AIS).

MATERIAL AND METHODS

The study analyzed the cases of 30 patients, 27 female, 3 male, with a mean age of 16.8 years (12-25 years in the period of surgery). The median follow-up period was 22.5 months (6 to 48 months). According to the Lenke Classification [7] patients' curves were as follows:

Lenke classification	No. of patients	Lenke classification	No. of patients
1A	4	3B	3
1B	7	3C	4
1C	5	5C	2
2C	4	6C	1

Preoperative and postoperative full spine panorama radiographs were analyzed by a surgeon according to the recommendations of the Spinal Deformity Study Group Guidelines [8].

The following parameters were analyzed:

- Cobb angle
- Coronal balance
- Thoracic trunk shift
- Apical vertebral translation
- Clavicle angle
- Shoulder height
- Thoracic kyphosis
- Lumbar lordosis
- Sagittal balance

All patients underwent posterior segmental instrumentation and fusion with a high-density construct (two implants at each instrumented level), entirely constructed of titanium polyaxial pedicle screws and titanium alloy rods.A combination of maneuvers was used to correct the deformity (rod translation, rod derotation, direct segmental derotation, compression-distraction and in-situ banding) depending on the individual case.

The surgeries were performed at the University Clinic for Orthopedic Surgery, Skopje.

Our study was approved by the Ethics Committee of the Faculty of Medicine at Ss. Cyril and Methodius University in Skopje, R. N. Macedonia.

RESULTS

The analysis with Z Wilcoxon Matched Pairs Test (p <0.0001sig) showed Cobb angle correction of 59.31% on proximal thoracic curves (28.85 ± 12.9SD preoperatively, at 11.74 ± 10.1SD postoperatively), 74.24% correction of major thoracic curves (28.85 ± 12.9SD, 11.74 ± 10.1SD), 76.5% of thoraco-lumbar curves (two cases), and 80.73% correction of lumbosacral curves (38.72 ± 17.1 SD, 7.46 ± 7.5SD postoperatively).

For the parameters expressed in centimeters it is arbitrarily accepted that the values ± 2.5 cm are considered as an interval of normal values.

The other parameters are the following:

Table 1. Overview of preoperative and postoperative values of the correction of coronal balance, thoracic trunk shift, apical vertebral translation, clavicle angle, and shoulder height

Type of correction	Prepoperative	Postoperative	p-value	%
	mean±SD	mean±SD		
Coronal Balance (cm)	2.01 ± 1.9	1.34 ± 1.1	p=0.16 ns	33.33 %
Thoracic Trunk Shift (cm)	2.45 ± 1.9	1.79 ± 1.4	p=0.102 ns	26.94 %
Apical Vertebral Translation (cm)	7.01 ± 2.2	1.67 ± 1.3	p=0.000002	76.18 %
Clavicle Angle (cm)	5.35 ± 3.4	1.50 ± 1.4	p=0.00003	71.96 %
Shoulder Height (cm)	3.07 ± 2.2	0.91 ± 1.1	p=0.00026	70.36 %

Patients were assigned to groups in order to make a more detailed analysis of the variables. The groups were as follows: a group where parameter values were preoperatively and postoperatively the same (unchanged), preoperatively above normal limits (elevated), which remained elevated postoperatively, then preoperatively elevated, postoperatively normal, preoperatively normal, postoperatively normal values, but decreased, preoperatively normal, postoperatively normal values, but increased, preoperatively normal, and postoperatively increased (Table 2, 3).

Table 2. Overview of the subgroups regarding changes of the analyzed parameters - coronal balance, thoracic trunk shift and apical vertebral translation

Variable	Coronal Balance	Thoracic Trunk Shift	Apical Vertebral Translation
	n (%)	n (%)	n (%)
Pre-op (same)–Post-op (same)	3 (10)	3 (10)	
Pre-op (elevated) –Post-op (elevated)	1 (3.33)	2 (6.67)	7 (23.33)
Pre-op (elevated)–Post-op (Normal)	8 (26.67)	10 (33.33)	18 (60)
Pre-op (normal)– Post-op (normal, lower)	7 (23.33)	3 (10)	4 (13.33)
Pre-op (normal)– Post-op (normal, elevated)	9 (30)	9 (30)	
Pre-op (normal)–Post-op (elevated)	2 (6.67)	3 (10)	1 (3.33)

The analysis showed that in 90% of patients coronal balance values postoperatively were within normal limits of, in 83.33% thoracic trunk shift values postoperatively were within normal limits and 73.33% of patients had normal values for apical vertebral translation postoperatively.

Regarding the correction of thoracic kyphosis, the values were 45.79 ± 13.1SD preoperatively, 42.82 ± 8.9 SD postoperatively, i.e. there was a reduction of 6.49%. The values of lumbar lordosis were 59.44 ± 14.9 SD preoperatively, 64.16 ± 6.9SD postoperatively, i.e. there was an increase of 7.94%. Despite these seemingly small corrections of the sagittal parameters, a correction of the sagittal balance of 33.33% was observed (3.69 ± 2.9SD preoperatively, 2.46 ± 2.2SD postoperatively).

Table 3. Overview of the subgroups regarding changes of the analyzed parameters - thoracic kyphosis, lumbar lordosis and sagittal balance

Variable	Thoracic Kyphosis	Lumbar Lordosis	Sagittal Balance
	n (%)	n (%)	n (%)
Pre-op (same)–Post-op (same)	-	1 (3.33)	-
Pre-op (elevated) –Post-op (elevated)	3 (10)	-	10 (33.33)
Pre-op (elevated)–Post-op (Normal)	7 (23.33)	1 (3.33)	3 (10)
Pre-op (normal)– Post-op (normal, lower)	9 (30)	7 (23.33)	11 (36.67)
Pre-op (normal)– Post-op (normal, elevated)	11 (36.67)	21 (70)	5 (16.67)
Pre-op (normal)–Post-op (elevated)	-	-	1 (3.33)

The analysis showed that in 90% of patients the values of thoracic kyphosis were within normal limits postoperatively, in 96.66% of patients the values of lumbar lordosis were within normal limits. Sagittal balance values were within normal limits in 63.34% of patients.
Table 4 shows the changes in the values of the clavicle angle and shoulder height. Changes in the angle of the clavicle follow the change and the rising side of the corresponding shoulder.

Table 4. Overview of the subgroups regarding changes of the analyzed parameters - clavicle angle and shoulder height

Variable	Clavicle Angle	Shoulder Height
	n (%)	**n (%)**
Leveled shoulders	10 (33.33)	10 (33.33)
Pre-op right shoulder higher – Post-op left higher (in normal interval)	7 (23.33)	9 (30)
Pre-op left shoulder higher – Post-op right shoulder higher (in normal interval)	4 (13.33)	2 (6.67)
Left and right shoulder improvement	6 (20)	6 (20)
From leveled shoulders pre-op to worsening post-op	3 (10)	3 (10)

The analysis showed that in 90% of patients the postoperative radiographic parameters showed that the shoulder height was within normal limits.

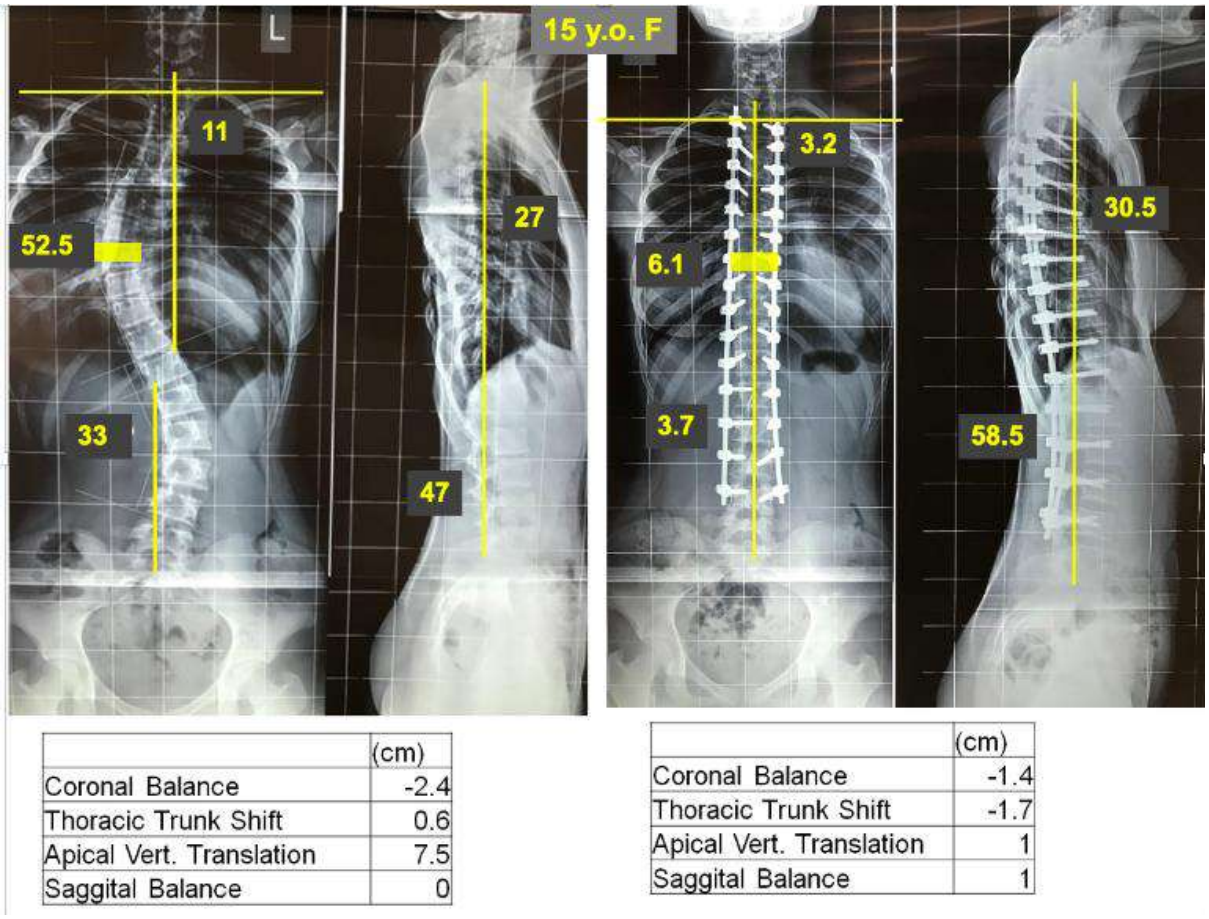

Fig. 2. Example of radiographic analysis of the investigated parameters

DISCUSSION

Unlike in the past, today we should not focus only on the correction of the coronary deformity. The surgical strategy should be aimed at effective correction of the sagittal and coronary alignment and balance of the spine because the closer we bring them to the normal parameters, the greater the patients' satisfaction of the surgical intervention. Ilharebrod [9] in his study from 2018 indicated that the literature regarding the correction of spinal deformities did not support the preservation of mobile segments (selective fusion) and that no association was found between distal fusion level and low back pain as a consequence of the fusion.

The same study showed that sagittal positioning of the spine was more important than the distal instrumental level to avoid disc degeneration at adjacent non-instrumental levels.

Numerous studies on the quality of life of patients with spinal deformities have shown that scoliosis causes mental dysfunction and psychosocial problems of the patient and family that are not commensurate with the severity of the deformity [10,11]. Gandehari*et al*. in their extensive study of 135 patients, who were surgically treated, followed for two years postoperatively and evaluated with the SRS-30 questionnaire, showed that aesthetic experience and appearance was the most important factor determining patient satisfaction and quality of life related with the health condition [12]. Therefore, special attention must be paid to maximize the gibbus correction and shoulder asymmetry.

In our study, in 90% of patients postoperative radiographs showed shoulder height values to be within normal range (± 2.5 cm).

The initial analysis of the parameters in our series as well as the "behavior" of the curves of the parts of the spine, showed consistency with the change of the parameters, their correction, in the published large series of patients treated with the same method.

In a study by Lehman *et al*., who analyzed 114 cases of operated patients with adolescent idiopathic scoliosis, an average correction of 68% for major thoracic curve, 50% for proximal thoracic curve, and 66% for thoraco-lumbar / lumbar curve [13] was obtained. Our study showed Cobb angle correction of 59.31% on proximal thoracic curves (28.85 ± 12.9SD preoperatively, 11.74 ± 10.1SD postoperatively), 74.24% correction of major thoracic curves (28.85 ± 12.9SD, 11.74 ± 10.1SD), 76.5% of thoraco-lumbar curves (two cases), and 80.73% correction of lumbosacral curves (38.72 ± 17.1 SD, 7.46 ± 7.5SD postoperatively).

The design of pedicle screws, whether they are polyaxial, monoaxial or uniplanar, as well as their use for the correction of spinal deformities is a topic of constant debate. Kuklo *et al*. [14] in their study comparing correction performed with monoaxial versus polyaxial pedicle screws in 35 operated patients with the most common Lenke Type 1 scoliosis showed that the constructs with both monoaxial and polyaxial pedicle screws showed excellent coronal correction, but the monoaxial screws achieved better derotation and restoration of thoracic symmetry.

In the analysis of our surgical method with the use of high-density polyaxial pedicular screws construct, we obtained a significant coronl correction of the deformities where in 90% of patients coronal balance values were within normal limits postoperatively, in 83.33% were within normal limitsfor the thoracic trunk shift postoperatively and 73.33% of patients had normal values for apical vertebral translation postoperatively.

A study by Blondel, Lafage *et al*. [15] showed that polyaxialpedicle screws achieved a significantly greater correction of thoracic kyphosis. This is an important fact because one of the most important features of idiopathic scoliosis is the applanation of thoracic kyphosis (hypokyphosis), which is important to correct within the sagittal positioning of the spine postoperatively.

In our study, in 90% of patients the thoracic kyphosis values were within the normal range postoperatively (normal limits according to the Scoliosis Research Society- SRS are 20-50 degrees), in 96.66% of patients the lumbar lordosis values were within the normal limits (normal limits according to SRS are 40-85 degrees). Sagittal balance values were within normal limits (± 2.5 cm) in 63.34% of patients.

Uniplanar pedicle screws have been present in recent years. Although they have been shown to achieve greater apical vertebral derotation compared to polyaxialin Lenke Type 5 curves according to a study by Tao Lin *et al.* [16], no studies are yet available to confirm their efficacy in a larger series of patients with other types of curves and compared to polyaxial implants.

There is still debate about whether to focus on low- or high-density structures. High-density structures provide greater power of correction, but are associated with longer duration of surgery and greater blood loss.

A very important element in the correction of spinal deformities is its retention. The study by Hwang, Samdani *et al.* [17] analyzed 800 patients regarding the reduction of the correction postoperatively. Decreased correction postoperatively is usually associated with the presence of pseudoarthrosis, loosening of the instrumentation, or an "adding-on" phenomenon. In their study, these factors were excluded as a reason for postoperative reduction of correction. They associated the reduced correction to a positive correlation with a greater magnitude of the thoracic curves, as well as the use of hybrid constructs (laminar hooks in combination with pedicle screws or sublaminar wires which are essentially low-density structures). On the other hand, they have proven that the use of pedicle screws reduces the incidence of loss of correction.

In lower-density construct, the duration of the surgery is shorter and blood loss is lower as shown in the study by Shen *et al*. [18]. An important issue with low-density constructs is that they can be associated with implant loosening, as well as with the possibility of "Crankshaft" phenomenon in skeletally immature patients [19].

CONCLUSION

The analysis of our series of operated patients with adolescent idiopathic scoliosis, as well as a review of the literature on this topic shows that the high-density construction made entirely of polyaxial pedicle screws and rods is a powerful tool for three-dimensional correction of adolescent idiopathic scoliosis.

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UDC: 616.24-005.6/.7

ORIGINAL ARTICLE

EVALUATION OF THE RELATIONSHIP BETWEEN PULMONARY ARTERIAL OBSTRUCTION INDEX AND SEVERAL CT MARKERS OF RIGHT VENTRICULAR DYSFUNCTION IN PATIENTS WITH ACUTE PULMONARY EMBOLISM

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ABSTRACT

Introduction: Pulmonary thromboembolism (PTE) ranks third in mortality among the most common cardiovascular diseases. Usual cause of death in acute pulmonary embolism (APE) patients is development of right ventricular dysfunction (RVD).

Aim: The study aimed to evaluate the relationship between pulmonary arterial obstruction index (PAOI) and several computed tomographic (CT) markers of right ventricular dysfunction (RVD), using computed tomographic pulmonary angiography (CTPA) in patients with acute pulmonary embolism (APE).

Material and methods: We evaluated the initial and follow-up CTPAs of 22 patients with acute pulmonary embolism (APE) from January 2017 to January 2018, who had previous echocardiographic examination. We calculated the pulmonary arterial obstruction index (PAOI) and several CT markers of right ventricular dysfunction (RVD), then made a comparison with the echocardiographic findings in order to detect patients with acute pulmonary hypertension (PAH) and right ventricular dysfunction (RVD). All patients underwent CTPA according to appropriate diagnostic protocol for acute pulmonary embolism (APE) on a 64-slice Somatom Definition AS + computed tomography (Siemens Healthineers, USA).

Results: There was a strong and statistically significant positive correlation between the pulmonary arterial obstruction index and the right ventricular diameter as well as the PAOI and the right and left ventricular diameter ratio, on the first and follow-up measurements (r=0 .5306, p=0.011 and r=0.5359, p=0.010; r=0.5568, p=0.007 and r=0.6077, p=0.003).

Conclusion: The semi-quantitative measurements of pulmonary arterial obstruction index (PAOI) acquired on CTPA not only enable thrombi quantification, but also risk stratification for undesirable outcomes. Such and similar studies can aid the selection of appropriate CT protocols for acute pulmonary embolism (APE) diagnosis and appropriate therapy selection, as well as the avoidance of additional and unnecessary diagnostic examinations.

Keywords: pulmonary arterial obstruction index (PAOI), right ventricular dysfunction (RVD), computed tomography pulmonary angiography (CTPA), acute pulmonary embolism (APE), pulmonary arterial hypertension (PAH)

INTRODUCTION

Pulmonary thromboembolism (PTE) is a cardiovascular disease with a high rate of mortality. The most common cause of death in patients with acute pulmonary embolism (APE) is right ventricular dysfunction (RVD) [1, 2, 3]. Pathophysiologically speaking, RVD is the

result of a sudden increase of the right ventricular filling due to mechanical pulmonary artery obstruction and pulmonary vasoconstriction, all driven by neurohumoral factors.

Although the first diagnostic line of RVD is presented by echocardiography [3], computed tomographic pulmonary angiography (CTPA) is the preferred imaging modality for the diagnosis of PTE in stable patients, due to its accuracy and 24-hour availability in many institutions [4].

Several studies have evaluated various CT signs in order to determine the risk of developing RVD and predicting the patient's outcome, such as right atrial diameter, right ventricular diameter, right-to-left ventricular diameter ratio, pulmonary artery diameter, pulmonary artery and aortic diameter ratio, the superior vena cava diameter, the azygos vein diameter, contrast reflux into the inferior vena cava and hepatic veins, and the right to left interventricular septal shift [6, 7, 8, 9].

The APE diagnostic CT criteria are direct visualization of a non-occlusive or occlusive endoluminal thrombus in a blood vessel of normal or enlarged diameter. In addition to the signs of RV overload and dilatation, the APE outcome is determined by semi-quantitative CT measurement of the pulmonary arterial obstruction index (PAOI) which is a good predictor of RVD development. PAOI can distinguish between patients with or without RVD.

Given the fact that RVD indicates a high recurrence rate and a potentially fatal outcome of PTE, even after anticoagulation, it is of utter importance to analyze the relationship between pulmonary obstruction index (PAOI) and CT parameters of RVD. At the same time, PAOI is an important prognostic factor of right ventricular response to the APE.

OBJECTIVES

The aim of this study was to evaluate the relationship between the pulmonary artery obstruction index (PAOI) and several CT markers of RVD, using computed tomographic pulmonary angiography (CTPA) in patients with APE, as well as to determine the thrombus resolution rate and the variations that CT markers of RVD undergo on the follow-up CTPAs after therapy.

MATERIAL AND METHODS

Study population - This study included the initial and follow-up CTPAs of 22 patients with APE during January 2017 - January 2018. It had a retrospective approach and was performed in a single diagnostic center. All patients had previously undergone echocardiography. Data was retracted from the computer data archiving system of the Institute of Radiology and analyzed by a subspecialist in thoracic and cardiovascular radiology. All patients were aged 18 years and older. PAOIs and several CT markers of right ventricular failure were measured and compared with echocardiographic findings in order to detect patients with acute pulmonary hypertension and RVD. All patients had a follow-up CTPAs performed after appropriate anticoagulant therapy, and thrombus resolution rate. RVD regression was also evaluated.

CTPA Scan Protocol - CTPA was performed according to an appropriate diagnostic protocol for APE on a 64-slice Somatom Definition AS + computed tomography (Siemens Healthineers, USA). A bolus of 70-100 ml iodine contrast was injected with a concentration of 300 mg / ml in the left antecubital vein, with a flow rate of 3-5 ml per second, using an automated injection system. The CT scan was performed after the start of the contrast medium injection with a bolus-tracking technique. All examinations were performed with a single breath technique, in a supine position and cranio-caudal direction. The following parameters were used: cross-sectional thickness of 1 mm, cross-sectional interval of 0.5 mm, scan time of 2.76 ms with a delay of 3 seconds, average voltage of 120 kV and exposure of 108 ms. Axial CT scans were transferred to a workstation that enables post-processing with multiplanar reconstruction, and all were analyzed on a mediastinal and parenchymal window.

Calculating PAOI and signs of RVD - The CT examinations mentioned in the introduction analyzed a number of parameters for RVD including the RV/ LV diameters ratio > 1 that was limited in hemodynamically stable PTE patients. The right / left ventricle ratio was calculated through the short axes of the RV and LV in the axial plane, from the endocardial edge of the free wall to the interventricular septum (Figure 1A). The diameters of the pulmonary trunk were measured on the axial scans at the level of continuation with the right pulmonary artery, where we also calculated the diameter of the ascending aorta. The superior vena cava diameter was obtained from the axial scans at the inflow point of the azygos vein (Figure 1B). A significant contrast reflux in the inferior vena cava was considered in those exams where the contrast reached the intrahepatic segment of the vein (Figure 1C). A significant interventricular septum shift was considered in findings where we observed a straightened or convex D-septum on the left (Figure 1D).

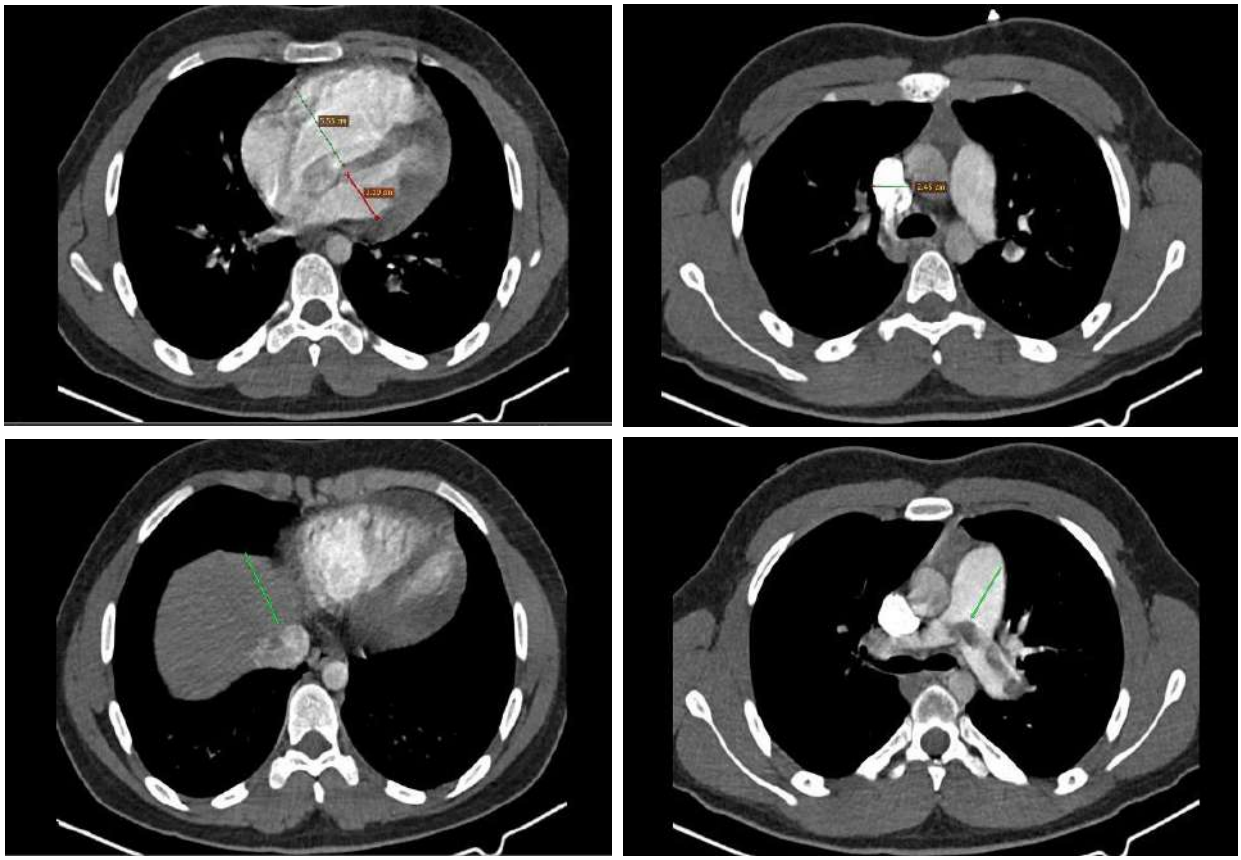


Fig. 1. A: Measurements of the right and left ventricular diameters on axial scans. B: Measuring the superior vena cava diameter at the inflow level of v. azygous. C: Assessment of contrast reflux into the inferior vena cava. D: Detection and quantification of thrombi in the pulmonary arterial system using PAOI.

In the study of Qanadli *et al*. [10], which was the foundation for defining the pulmonary obstruction index, the arterial trunk of each lung contained 10 segmental branches (3 for the upper lobes, 2 for the middle lobe and lingula, and 5 for the lower lobes). The presence of a thrombus in a segmental artery was given a value of 1 point. If a proximal arterial trunk was affected, the value given was the total number of non-visualized segmental arteries arising from the trunk. Regarding whether there was a complete or partial occlusion of the blood vessel lumen, values from 0-2 were given (0 - no, 1 - partial occlusion, 2 - complete occlusion). Therefore, the maximum CT obstruction index was 40. We calculated the percentage value according to the formula {(n x d) / 40} x 100, where n is the number of segmental arteries

affected (minimum 1, maximum 20), and d is the degree of obstruction of the affected blood vessel (minimum 1, maximum 2) (Figure 1G). Obstruction index of 40-60% in patients without hypotension is associated with PTE with intermediate, i. e. high risk [11, 12].

Echocardiography findings - We collected and analyzed the echocardiography findings of all patients in the first 48 hours of admission. RVD on echocardiography was defined as right ventricular hypokinesia and one or more of the following findings present: right ventricular dilatation (end-diastolic diameter> 30 mm); right and left ventricular end-diastolic diameters ratio > 1; paradoxical septal movement and pulmonary hypertension.

RESULTS

A total of 22 patients with radiologically confirmed APE on initial CTPAs, as well as a control group of the same patients who underwent follow-up CTPA after appropriate therapy, in order to exclude possible residues or disease complications were included in the study. According to a previously performed echocardiogram, patients were divided into two groups - those with and those without pulmonary arterial hypertension. The first group consisted of 9 patients (5 men and 4 women), while the second group of 13 patients (7 men and 6 women) (Figure 2).

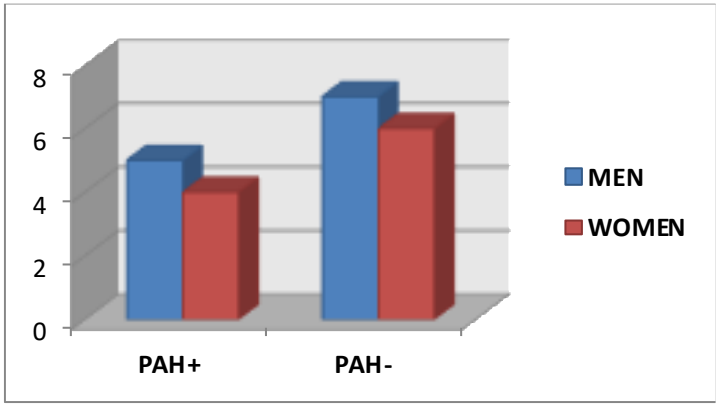


Fig. 2. Gender distribution in both groups of patients

Of the 22 patients, 10 were women (45.45%) and 12 men (54.55%). The patients age varied from 18 to 89 years, with an average of 55.18 ± 20.08. The average body weight was 78.82 ± 12.03 kg, with variations from 47 to 95 kg. Of the 22 patients, 7 (31.82%) were smokers, the rest were non-smokers. Systolic blood pressure average was 136.59 ± 20,84, diastolic 83.18 ± 10.18, and the pulse average was 95 ± 15.94. Six were diagnosed with deep vein thrombosis (DVT) (27.27%), while the remaining 16 did not have DVT. Table 1 depicts the mean values and standard deviations of PAOI and cardiological CT markers of RVD after the first measurement, as well as their minimum and maximum values. The average value of PAOI after the first measurement was 48.86 ± 28.42 with a minimum value of 15%, a maximum of 100% and the average value of the RV / LV diameters ratio was 1.17 ± 0.27 after the first measurement, with a minimum value of 0.9 and a maximum of 2.0. The remaining values are shown in Table 1.

Table 1. The average values of PAOI and CT markers of RVD on the initial CTPAs

CT markers	mean value	median±SD (min-max)
$PAOI_1$ (%)	48.86	38.75±28.42 (15-100)
RV_1 (mm)	46.86	46±6.94 (36-64)
LV_1 (mm)	40.45	40±4.16 (32-49)
RV/LV_1	1.17	1.1±0.27 (0.9-2)
PA_1 (mm)	29.59	29±4.22 (20-38)
AO_1 (mm)	32.23	33±4.49 (24-40)
PA/AO_1	0.92	0.9±0.14 (0.7-1.3)
SVC_1 (mm)	20.82	20.5±3.45 (12-27)

(PAOI-pulmonary obstruction index, RV-right ventricle, LV-left ventricle, PA-pulmonary artery, AO-aorta, SCV- superior vena cava)

The other measurements as part of the radiological evaluation of RVD exhibited a significant contrast reflux in the inferior vena cava to the middle segment of the hepatic veins in 9 patients (40.91%) and a significant interventricular right to left septal shift in 4 patients (18.18%). Peripheral triangular zones of consolidation, i. e. pulmonary infarction were detected in 14 patients (63.64%), atelectasis in 7 (31.82%), and pleural effusion in 8 patients (36.36%). We can conclude that there was a statistically significant difference for the initial PAOI measurements in both groups for $p < .05$ (Table 2). A statistically significant difference of $p < .05$ was also seen for the RV diameter and RV/ LV diameter ratio in both groups. The other parameters in both groups showed a statistically insignificant difference.

Table 2. Overview of the initial CTPA average parameter values in relation to positive or negative PAH and t-test

CT markers	PAH Positive (N=9) median±SD	PAH Negative (N=13) median±SD	p-value
$PAOI_1$ (%)	63.89± 29.58	38.46± 23.31	0.02
RV_1 (mm)	50.22± 8.54	44.54± 4.61	0.03
LV_1 (mm)	39.44± 6.00	41.15± 2.23	0.18
RV/ LV_1	1.3± 0.36	1.08± 0.14	0.03
PA_1 (mm)	31± 3.54	28.62± 4.50	0.10
$AO1_1$ (mm)	34± 4.58	31± 4.16	0.06
PA/ AO_1 (mm)	0.9± 0.19	0.9±0.11	0.43
SVC_1 (mm)	21.44± 2.65	20.38± 3.95	0.25

(PAOI-pulmonary obstruction index, RV-right ventricle, LV-left ventricle, PA-pulmonary artery, AO-aorta, SCV- superior vena cava)

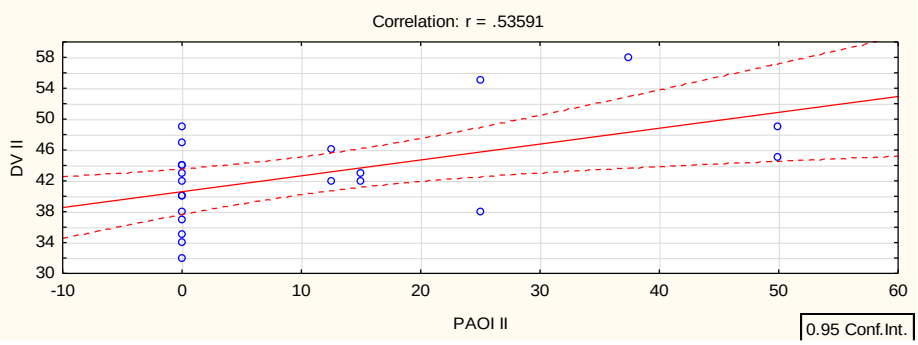
A correlation was made between PAOIs from the initial measurements with the other markers of RVD, using the Pearson's correlation. After the first measurements, there was a medium strong statistically significant positive correlation between PAOIs and RV diameter ($r=0.531$, $p=0.011069$), as well as a moderately strong statistically significant positive correlation between PAOI and RV / LV diameters ratio ($r=0.557$, $p =0.007112$). The remaining parameters showed a weak and statistically insignificant positive and negative correlation with PAOI.

The difference between average values of PAOI, RV, RV / LV, PA and PA / AO in the first and second measurements was statistically significant for p <0.05. The difference between average values of SVC diameter in the first and second measurement was statistically insignificant for p> 0.05. The correlation of PAOI and RV diameter and the RV/ LV diameters ratio in the first and second measurements depicted a strong and statistically significant positive correlation (r=0.5306, p=0.011 and r=0.5359, p=0.010; r=0.5568, p=0.007 and r=0.6077, p=0.003). The correlation of PAOI and the pulmonary artery and PA/ AO diameters ratio in the first and second measurements exhibited a weak, statistically insignificant correlation (r=0.0079, p=0.972 and r=0.0566, p=0.1844, p=0.411 and r=0.0726, p =0.748) (Table 3, Figure 2).

Table 3. Overview of the correlation of PAOI and other examined parameters during first and second measurements

FIRST MEASUREMENT	CORRELATION	SECOND MEASUREMENT	CORRELATION
PAOI	RV 0. 5306 p= 0. 011	PAOI	RV 0. 5359 p=0. 010
PAOI	RV/ LV 0. 5568 p= 0. 007	PAOI	RV/ LV 0. 6077 p=0. 003
PAOI	PA 0. 0079 P= 0. 972	PAOI	PA 0. 0566 p=0. 803
PAOI	PA/ AO -1. 844 p= 0. 411	PAOI	PA/ AO 0. 0726 p=0. 748
PAOI	SVC 0. 3808 p=0. 080	PAOI	SVC 0. 4685 p=0. 028

A



B

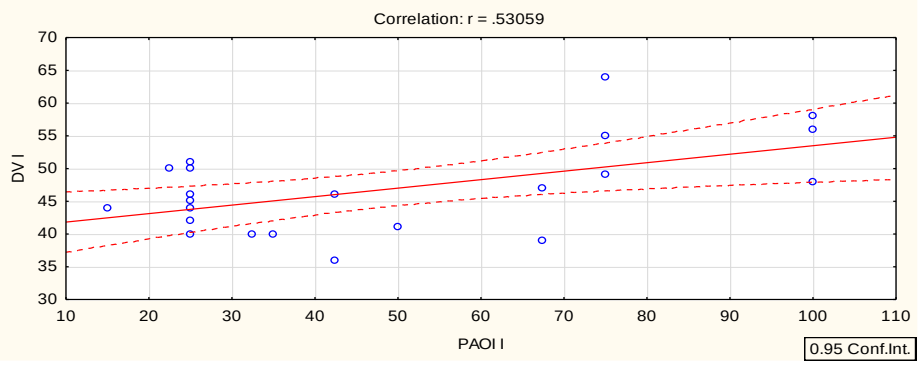


Fig. 3. Correlation of PAOI and RV diameter in the initial and follow- up measurements, A and B

The ROC analysis indicated that PAOI contributed to the diagnosis of right heart failure with 72.73% (p = 0.000) (good predictor), closer to the ideal value of 1.0 and above the worst value of 0.5 (Figure 3). Cut-off indicated the value of the variable, the range that predicted a positive state and according to the coordinates on the ROC curve, sensitivity was 85% whereas the specificity was 60.5%, which corresponded to the value - 41% (cut-off) (Figure 2).

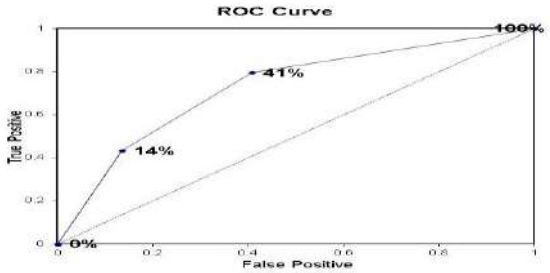


Fig. 4. ROC curve for PAOI

The ROC analysis indicated that the right and left ventricular diameters ratio contributed to the diagnosis of right heart failure with 90.91% (p = 0.000) (excellent predictor), closer to the ideal value of 1.0 and above the worst value of 0.5 (Figure 5). According to the coordinates on the ROC curve the sensitivity was 100%, whereas the specificity was 81.8%, which corresponded to the value - 0.9 (cut-off) (Figure 4).

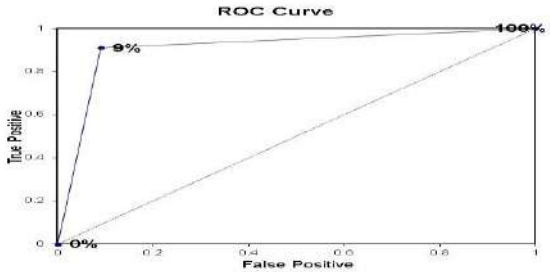


Fig. 5. ROC curve for RV/ LV diameter ratio

The number of patients without pulmonary arterial hypertension (PAH) in the follow-up measurements increased from 13 to 17. According to the dynamic index, an increase rate of 30.8% and a decrease rate of 44.4% were registered (Table 4).

Table 4. Decrease rate of PAH in follow-up measurements.

PAH/ MEASURE	I		II	
	N.	%	N.	%
POSITIVE	13	59.1	17	77.3
NEGATIVE	9	40.9	5	22.7
TOTAL	22	100.0	22	100.0

DISCUSSION

Semi-quantitative measurements of PAOI made on CTPA enable APE severity quantification, as well as risk stratification of undesirable outcomes. In our study, PAOI exhibited a positive and statistically significant correlation with echocardiographic findings of RVD and with certain CT markers of RVD, such as the RV diameter and the RV/ LV diameter ratio. PAOI manifested a close relationship with the RV diameter in patients with APE and thus it can be used as a prognostic marker for its outcome. In as many as 55% of patients, there was a complete resolution of thrombi during the follow-up CTPA (30 to 180 days).

ROC analysis of selected parameters demonstrated that the RV/ LV diameter ratio had the greatest influence in predicting right heart failure and contributed to the diagnosis with 90.91%, with a cut-off value of 0.9, whereas the PAOI participated in the diagnosis with 72.73% and the cut-off value was 41%.

Our goal was to show that values of PAOI and CT markers of RVD decreased on the follow-up CTPAs. We found a statistically significant correlation between the resolution degree of PAOI and the reduction in RV diameter, as well as reduction in RV / LV diameter ratio on the follow-up CTPAs.

CONCLUSION

The disadvantages of this study include the retrospective design, as well as the small population size, echocardiographic findings based on a written result, CTPA examinations performed without ECG-gating and different time interval of the follow-up CTPAs.

The semi-quantitative measurements of PAOI acquired on CTPA not only enable thrombi quantification, but also risk stratification for undesirable outcomes. Such and similar studies can aid the selection of appropriate CT protocols for APE diagnosis and appropriate therapy selection, as well as the avoidance of additional and unnecessary diagnostic examinations.

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UDC: 616.33/.342-002.44-007.251-08

ORIGINAL ARTICLE

CURRENT STRATEGY IN THE TREATMENT OF PERFORATED PEPTIC ULCER

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ABSTRACT

Background: Perforated peptic ulcer (PPU) is one of the most common emergencies in gastrointestinal pathology.

The purpose of this study is to understand the importance of this entity in modern medical practice and to assess and define the current strategy in the treatment of this disease.

Material and methods: This is a prospective, randomized study of 75 subjects with perforated peptic ulcer who underwent inpatient and post-hospital treatment based on a previously established protocol. The hospital protocol consisted of the application of a serological test for *Helicobacter pylori*, a questionnaire, and a clearly defined surgical procedure (covering the perforation site with a vascularized loop of the omentum with or without prior suturing of the perforation opening). The posthospital protocol consisted of the application of first and second line eradication treatment for *H. pylori* and acid suppression therapy to treat the underlying ulcer disease and the application of non-invasive diagnostic tests and upper gastrointestinal endoscopy to determine the success of the eradication treatment and healing the ulcus.

Results: The presence of *H. pylori*-specific IgG antibodies was confirmed in 66 (88.0%) patients, 25 or 33.3% of the participants used NSAIDs and 4 or 5.3% of subjects had a negative serological test for *H. pylori* and did not use NSAIDs. Eradication of *H. pylori* after first and second line treatment was successful in 63 i.e. 95.5% of *H. pylori* positive subjects, and in 3 i.e. 4.5% was unsuccessful. In accordance with the results of the upper GI endoscopy after 16 weeks follow-up, out of 66 *H. pylori* positive subjects the ulcer was cured in 63 i.e. 95.5%, while 3 i.e. 4.5% of the respondents still had the ulcer. Of the 46 *H. pylori*-positive participants who did not use NSAIDs after 16 weeks follow-up, the ulcer was cured in 44 i.e. 95.7%, and in 2 i.e. 4.3% of the participants still had an ulcer. In accordance with the results of upper GI endoscopy after 16 weeks of follow-up, the ulcer was cured at 70 i.e. 93.3% of all 75 participants included in the study, while the ulcer was still present in 5 i.e. 6.7% of participants.

Conclusion: Depending on the etiological factor, after simple closure of the perforation with an omental patch, outpatient medical treatment is necessary.

It is obligatory to confirm the effectiveness of the treatment, i.e. ulcer healing and eradication of *H. pylori*.

Keywords: perforated peptic ulcer, simple closure, omental patch, eradication therapy for *H. pylori*, ulcer healing

INTRODUCTION

Peptic ulcer perforation (PPU) is a frequent cause of hospitalization, which affects 2-10% of patients with peptic ulcer. It presents with an overall mortality of 10% although various authors had reported incidence between 1.3% and 20% [1].

To Cheng in 1984 verified duodenal perforation in a preserved body from 167 BC in China [2].

Henriette-Anne, daughter of King Charles I, died at the age of 26 in 1670 after a day of severe abdominal pain. The autopsy showed a small opening in the anterior wall of the stomach and peritonitis [3].

Reporting 50 cases of perforated peptic ulcer in 1843, Edward Crisp stated that the symptoms of this disease were so typical that hardly anyone could make a mistake in the diagnosis.

In the past, the high mortality rate following surgery for a perforated duodenal ulcer led to the emergence of nonoperative management.

The basic idea for conservative treatment came from Crisp who noted that perforations of the stomach were filled by adhesions to the surrounding viscera which prevented leakage from the stomach into the peritoneal cavity.

Wangesteen recognized the process of self-closure of the perforation opening and in 1935 reported 7 cases of non-surgical treatment of a perforated ulcer [4].

In 1946, Herman Taylor reported 28 cases of non-operatively treated perforated peptic ulcers. His method was based on effective gastric decompression, parenteral nutrition, and analgesia. This method is also used in the current practice of selected cases [5,6].

The selection is made on the basis of clinical findings (painless patient with soft and insensitive abdomen) and water-soluble gastroduodenography (spontaneously closed perforation opening, presence of ulcer and no leakage of contrast in the abdominal cavity of the gastroduodenogram) [7].

Johan Mikulicz-Radecki is the first surgeon who surgically resolved a perforated peptic ulcer by simply closing the perforation opening [5].

In 1929, Cellan-Jones published a rapid method of treating a perforated duodenal ulcer, which consisted of placing a long vascularized omental loop at the perforation site and fixing it with 4 to 6 sutures without primary closure of the opening to avoid narrowing of the duodenum.

In 1937, Roscoe Reid Graham of Toronto reported 51 successfully treated cases of a perforated duodenal ulcer with a free, unvascularized piece of omentum that he fixed with 3 sutures above the perforation site without closing the opening (omentoplasty), and concluded that routine gastroenterostomy is not required.

By Graham's modified technique (omentopexy) the surgeon first closes the perforation opening and then places a vascularized omental loop over the perforation site, which fixes it to the ends of the sutures that close the opening [4].

Today, surgeons usually close the perforation opening first and then place a vascularized loop of omentum over the suture line. In surgical practice, surgeons often say they used a Graham's patch, but they actually use a vascularized patch described by Cellan-Jones.

Mouret et al first described laparoscopic intervention for perforated duodenal ulcers in 1990 [8].

In 1910, Dragutin (Carl) Schwartz, a Croatian surgeon born in Varaždin, uttered the famous saying that without the presence of acid, there is no development of an ulcer (no acid no ulcer). This axiom became the norm in medicine in the following decades and the treatment of ulcers was based on this principle [9].

Respecting Schwartz's axiom, surgeons researched surgical techniques that would lead to acid reduction. This rule has been surgically accomplished in the past by removing the ulcer

by simultaneously reducing the acid-secreting oxytocin mucosa or by vagal denervation of the stomach or by a combination of gastrectomy and vagotomy.

The medical management of peptic disease was revolutionized by the advent of H2 receptor antagonists with subsequent discovery of PPI.

Barry J. Marshall and Robin Warren who identified *Helicobacter pylori* (*H. Pylori*) revolutionized the management of peptic disease.

The Maastricht III, The Maastricht/Florence IV and The Maastricht/Florence V Consensus Report lay down guidelines for therapy of *H. pylori* by using triple drug regime [10].

H. pylori infection and the use of nonsteroidal anti-inflammatory drugs (NSAIDs) are the most common etiological factors for peptic ulcer disease (PUD). Ulcers can also occur in people who do not have *H. pylori* infection and who have not used NSAIDs (idiopathic ulcers).

MATERIAL AND METHODS

It is a prospective, randomized study of 75 subjects with perforated peptic ulcer primarily treated with surgical life-saving procedure (covering the perforation site with a vascularized loop of the omentum with or without prior suturing of the perforation hole). After that on these 75 subjects an algorithm of further therapeutic treatment and diagnostic procedures was applied, in order to cure the disease and verify that cure.

The sample of participants in the group was made by respecting the set inclusion and exclusion criteria.

Inclusion criteria were patients diagnosed with perforated peptic ulcer who underwent life-saving surgery during hospitalization, and were discharged from hospital in good general condition.

Exclusion criteria were patients diagnosed with perforated gastroduodenal ulcer who underwent definitive surgery, persons under 14 years of age and psychiatric patients.

Serological testing of IgG antibodies to *H. pylori* was performed during hospital treatment.

The researchers used a specially designed questionnaire for this purpose, with guaranteed confidentiality of the obtained data.

After discharge from hospital, outpatient therapy started immediately (first and second line therapy for eradication of *H. pylori* or acid suppression therapy alone).

As the first-line treatment for *H. pylori*, triple therapy was used for 14 days with amoxicillin 1000 mg, clarithromycin 500 mg and pantoprazole 40 mg all twice daily, and after that pantoprazole 40 mg daily for the next 6 weeks.

If the first-line *H. pylori* eradication therapy failed, a second-line *H. pylori* treatment was started immediately thereafter.

Levofloxacin 500 mg with metronidazole 400 mg both twice daily and pantoprazole 40 mg daily was used as a second-line *H. pylori* eradication treatment for 10 days.

For NSAID users without the presence of *H. pylori*, therapy with pantoprazole 40 mg twice daily during the first 2 weeks and pantoprazole 40 mg daily for the next 6 weeks was used.

For patients with non-Helico non-NSAID perforated peptic ulcers, therapy with pantoprazole 40 mg twice daily was used for the first 2 weeks and for the next 10 weeks pantoprazole 40 mg once daily.

Urea breath test (UBT) and feces antigen test (FAT) were used to verify the success of the first and second line eradication treatment (4 weeks after the end of first and second line treatment). If one of these tests was positive the eradication treatment was considered unsuccessful.

Follow-up endoscopy was performed 16 weeks after hospital discharge for surveillance of ulcer healing. Successfully healed ulcer lesion was defined as the absence of an ulcer lesion on upper gastrointestinal endoscopy.

The endpoints were initial ulcer healing and eradication of *H. pylori*.

Databases were created using specific computer programs for that purpose. Their processing was performed using standard descriptive methods. The attributive statistical series were analyzed by determining the coefficient of relations and proportions, and by determining the statistical significance between the detected differences - Difference test.

The Shapiro-Wilk`s test examines the normal distribution of variables. For CI (confidence interval ± 95% CI) the statistical significance for an error level less than 0.05 (p) is defined. The results are presented in tabular form.

RESULTS

The prospective study registered 75 patients with perforated peptic ulcer, out of which 63 patients (84.0%) were male and 12 (16.0%) were female (Table 1). The percentage difference between the two sexes is statistically significant for $p < 0.05$ (Difference test, $p = 0.0000$). The ratio between men and women is 5.3:1.

The most common age group is the one from 61 to 80 years (30 i.e. 40.0%) and the group from 41 to 60 years (26 i.e. 34.7%) i.e. 74.7% of patients were between 41 and 80 years old.

The age group of 15 to 20 years is represented by 4 patients, i.e. 5.3%, the age group of 21 to 40 years is represented by 13 patients, i.e. 17.3%, and in the group over 80 there are 2 patients, i.e. 2.7% (Table 1).

The percentage difference between the age groups 61 to 80 years and 41 to 60 years versus the other age groups is statistically significant for $p < 0.05$ (Difference test, $p = 0.0000$).

Table 1. Demographic data of patients with perforated peptic ulcer

Gender	number	%
male	63	84.0
female	12	16.0
Age		
<=20	4	5.3
21-40	13	17.3
41-60	26	34.7
61-80	30	40.0
>80	2	2.7

The presence of *H. pylori*-specific IgG antibodies was confirmed in 66 (88.0%) patients, and in 9 (12.0%) patients *H. pylori*-specific IgG antibodies were not confirmed.

The percentage difference between the two groups (*H. pylori* positive and *H. pylori* negative) is statistically significant for $p < 0.05$ ($p = 0.0000$). The ratio between *H. pylori* positive and *H. pylori* negative subjects was 7.3:1.

Of 66 *H. pylori* positive patients, 20 i.e. (30.3%) used NSAIDs, while 46 i.e. (69.7%) did not use NSAIDs.

25 or 33.3% of all the participants used NSAIDs, while 50 or 66.7% of the participants denied the use of NSAIDs. Out of 25 NSAID users, 20 i.e. (80.0%) were *H. pylori* positive, while in 5 i.e. (20.0%) no presence of *H. pylori* IgG antibodies was detected.

4 or 5.3% subjects had a negative serological test for *H. pylori* and did not use NSAIDs (Table 2).

Table 2. Etiological factors in patients with perforated peptic ulcer

H. pylori	number	%	*H. pylori* positive NSAID	number	%
positive	66	88.0	did not use	46	69.7
negative	9	12.0	used	20	30.3
NSAID users			Non Helico Non NSAID		
yes	25	33.3	yes	4	5.3
no	50	66.7	no	71	94.7

After the application of first-line eradication treatment, urea breath test was negative in 60 i.e. 90.9% of participants, and 6 participants, i.e. 9.1% were positive.

The presence of *H. pylori* antigen in stool after application of first-line treatment was not verified in 64 i.e. 97.0% of participants, and 2 i.e. 3.0% of subjects demonstrated the presence of *H. pylori* antigen in stool.

Of the 66 subjects confirmed to have *H. pylori*-specific IgG antibodies, according to the results of urea breath test (UBT) and feces antigen test (FAT) after first-line treatment, and according to the established methodology, the eradication of *H. pylori* was successful in 59 i.e. 89.4% of the participants, and 7 participants, i.e. 10.6% were unsuccessful. The percentage difference was statistically significant for $p < 0.05$ ($p = 0.0000$, Difference test).

Out of seven participants who received second-line treatment, due to unsuccessful first-line treatment, urea breath test (UBT) was negative in 4, i.e. 57.1%, and positive in 3, i.e. 42.9% of the participants, while the *H. pylori* antigen test in stool (FAT) was negative in 5 subjects, i.e. 71.4%, and 2 participants, i.e. 28.6% were positive.

According to these results and the established methodology, second-line treatment was successful in 4 i.e. 57.1% of the participants, and in 3 participants, i.e. 42.9 was unsuccessful (Table 3).

Table 3. Efficacy of 1 and 2 line treatment for eradication of *H. pylori* in 66 *H. pylori* IgG positive PPU patients

First line treatment			Second line treatment		
Urea breath test (UBT)	number	%	Urea breath test (UBT)	number	%
negative	60	90.9	negative	4	57.1
positive	6	9.1	positive	3	42.9
Feces antigen test (FAT)			Feces antigen test (FAT)		
negative	64	97.0	negative	5	71.4
positive	2	3.0	positive	2	28.6
Efficacy/eradication of *H. pylori*			Efficacy/eradication of *H. pylori*		
successful	59	89.4	successful	4	57.1
unsuccessful	7	10.6	unsuccessful	3	42.9

Of the 46 *H. pylori* positive subjects who did not use NSAIDs (Helico Group), in relation to the set goal after 16 weeks of follow-up and according to the results of the upper GI endoscopy, the ulcer was cured in 44 i.e. 95.7% of the subjects, while in 2 subjects i.e. 4.3% of subjects the ulcer was still present. The percentage difference was statistically significant for $p < 0.05$ ($p = 0.0000$, Difference test).

Of the 66 *H. pylori* positive subjects, 20 of whom used NSAIDs (Helico + NSAID group) after 16 weeks of follow-up, the ulcer was cured in 63 i.e. 95.5% of the respondents, while in 3 i.e. 4.5% of the subjects the ulcer was still present. The percentage difference was statistically significant for $p < 0.05$ ($p = 0.0000$, Difference test).

Of the 5 NSAID users subjects who did not show the presence of *H. pylori*-specific IgG, and who underwent acid-suppressive therapy for 8 weeks, healing of the ulcer after 16 weeks of follow up was confirmed by upper gastrointestinal endoscopy in 4 i.e. 80.0% of subjects, while in 1 subject (20%) the ulcer still existed.

In 4 Non Helico Non NSAID subjects after 16 weeks of follow up the ulcer was cured in 3 i.e. 75.0 % of the subjects and the ulcer was still present in 1 subject i.e. 25.0% of the subjects (Table 4).

Table 4. Ulcer healing in patients with PPU after 16 weeks of follow-up

Helico group N=46			Helico + NSAID group N=66		
Healed ulcer	number	%	Healed ulcer	number	%
yes	44	95.7	yes	63	95.5
no	2	4.3	no	3	4.5
NSAID without Helico group N=5			Non Helico Non NSAID group N=4		
Healed ulcer	number	%	Healed ulcer	number	%
yes	4	80.0	yes	3	75.0
no	1	20.0	no	1	5.0

After the first and second line treatment, eradication of *H. pylori* was successful in 63 i.e. 95.5% subjects out of a total of 66 subjects with a positive serological test for *H. pylori*, while in 3 i.e. 4.5% of subjects, eradication of *H. pylori* was unsuccessful. The percentage difference was statistically significant for $p < 0.05$ ($p = 0.0000$, Difference test) (Table 5).

Table 5. Eradication of H. pylori after 1 and 2 line treatment

Eradication of H. pylori after 1 and 2 line treatment	number	%
successful	63	95.5
unsuccessful	3	4.5

Out of 75 subjects with perforated peptic ulcer, who were included in the study and who received inpatient and outpatient treatment according to the established protocol after 16 weeks of follow-up and according to the results of the upper GI endoscopy, the ulcer was healed in 70 i.e. 93.3% of the subjects, and the ulcer was still present in 5 subjects, i.e. 6.7% of the subjects. The percentage difference was statistically significant for $p < 0.05$ ($p = 0.0000$, Difference test) (Table 6).

Table 6. Ulcer healing in all participants included in the study

Ulcer healing N=75	number	**%**
yes	70	93.3
no	5	6.7

DISCUSSION

In the treatment of perforated peptic ulcer, the dilemmas posed to the surgeon are whether surgical treatment is indicated, whether the patient is stable for definitive surgical treatment, whether such treatment is necessary and which type of definitive surgical method should be used. If definitive surgical treatment is not necessary there is a question whether simple closure on the perforated opening with an omental patch is sufficient and whether the new knowledge about the peptic ulcer disease has an impact on the choice of surgical treatment [11]. With the introduction of laparoscopy, the question arises whether the surgical treatment of a perforated peptic ulcer should be by the open method or performed laparoscopically [12].

Many modalities of treatment are available ranging from nonoperative option to laparoscopic repair [13].

To date, there are still some debatable issues on the treatment of perforated peptic ulcer. Options exist in this situation, which include conservative treatment, omental plugging, closure of ulcer with free omentum, closure of perforation with use of pedicled omentum, control tube duodenostomy, definitive treatment with gastrectomy, truncal vagotomy and drainage procedures, or proximal gastric vagotomy [14,15].

It is crucial to identify parameters to assess the severity of the disease (i.e. to define if a patient is stable or unstable).

A septic condition due to a perforated peptic ulcer requires prompt diagnostic evaluation and treatment [16].

Simple closure is associated with an unacceptably high recurrence rate of duodenal ulcer, as high as 42-50 percent in some series [15].

Chemical acid suppression, initially through the introduction of H2RA, and especially with proton pump inhibitors, has reduced the rate of gastric resection and vagotomy, and the main surgical treatment for PPU has become simple suture of the perforation site with, or without the addition of an omental patch [17].

Use of H2 receptors blocking drugs and proton pump inhibitors to reduce ulcer recurrence after simple patch closure has produced conflicting results [18].

Despite the huge contribution in the treatment of peptic ulcer, the disadvantage of these drugs (H2RA and PPI) there is the need for their continuous use. Discontinuation of their use leads to recurrence of the ulcer in the majority of patients [19].

Discovery of *H. pylori* changed the concept of the management of peptic ulcer. Recurrent ulcer disease after peptic ulcer perforation, however, mainly occurs in patients with *H. pylori* infection which suggests its importance in this complication [20].

In the case of ulcers caused by the use of NSAIDs, their use should be discontinued and anti-ulcer drugs should be given. If NSAID use cannot be discontinued, administration of PPI as first-line therapy is recommended. To prevent recurrence of non-*H. pylori* and non-NSAID idiopathic ulcers PPIs or H2RAs are recommended [21].

In our study, the frequency of *H. pylori* infection in patients with perforated peptic ulcer was 88.0%. This indicate an association between *H. pylori* infection and perforated peptic ulcer.

This figure is much higher than those reported by Reinbach et al (47%) [22] and Chu et al (47%) [23], but correlates with those reported by El-Nakeeb et al (84.8%) [24], Mihmanli et al (88.8%) [25], Tokunaga et al. (92%) [26], Matsukura et al (95%) [27], and slightly higher than those reported by Rodriquez-SanJuan et al (73.9%) [28], Zahid et al (68%) [29], Bose et al (64.5%) [30], Gisbert (60%) [31].

According to some authors, modified Graham omenthopexy gives excellent results in terms of ulcer healing, morbidity, and mortality [13]. Studies show that both omentoplasty and omentopexy have similar efficacy in terms of morbidity and mortality [1,32].

Laparoscopic closure of peptic ulcer perforation has recently become more commonly used due to less pain, reduced morbidity and complications, as well as shorter hospital treatment [33].

The choice of the patient is essential for a definite decision about the type of treatment. However, based on research and experience, it seems that the laparoscopic and open approach in resolving the perforated peptic ulcer are of identical value [34].

Currently, both open and laparoscopic approaches remain within standard treatment.

Now-a-days reduction in gastric acid production with proton pump inhibitors along with eradication of *H. pylori* is recommended. Therefore, *H. pylori* eradicating treatment should be started during the immediate postoperative period [20, 24].

PPIs are suggested for the treatment of perforated peptic ulcers in NSAID users et non-*H. pylori* and non-NSAID idiopathic perforated peptic ulcers [21].

In the study of Bose et al. *H. pylori* eradication was achieved in 81.1% patients [30]. In the study by Datsis et al. eradication of *H. pylori* was successful in 78,6% of subjects, and 21,4% in whom the urea breath test was positive underwent second-line eradication therapy [35]. Metzger et al. reported 96% successful eradication of *H. pylori* [36].

In our study *H. pylori* eradication was successful in 89.4% after first-line treatment, and 95.5% after first and second line treatment.

A significantly lower recurrence of the ulcer after 1 year of follow-up was reported in a study by Søreide et al. in patients receiving eradication therapy for *H. pylori* compared with those receiving omeprazole alone (4.8% vs. 38.1%) [17].

In other studies ulcer relapse after 1 year of follow-up in patients treated with eradication therapy for *H. pylori* versus patients in the control group was also lower (6.1% vs. 29.6% in the El Nakib study) [24] and (5.2% vs. 35.2% in the Tomtitchong study) [37], while in the study of Bose et al. after 18 months of follow-up the ulcer relapse was 18.6% in the group with successful eradication for *H. pylori* compared to 70% in the group in which eradication was not successful [30].

A reduced recurrence of ulcus after 8 weeks and 1 year of follow-up in patients treated with eradication therapy for *H. pylori* compared with patients in the control group was reported by Wong et al. [38], while Datsis et al. reported that after simple closure with omental patch repair plus *H. pylori* eradication with omeprazole, clarithromycin and amoxycillin for two weeks, none of the patients had a recurrence of the ulcer [35].

In our study, the healing rate 16 weeks after discharge from hospital was 95.7% for the *H. pylori* group and 95.5% for the *H. pylori* + NSAID group compared with 75.0% for the non-Helico non NSAID group and 80% in the NSAID group without *H. pylori*.

Causes for the recurrence of peptic ulcers after successful eradication of *H. pylori* include using aspirin and NSAIDs, *H. pylori* reinfection, and smoking habits [21].

CONCLUSION

All patients with perforated gastroduodenal ulcer in hospital setting should be tested for *H. pylori* infection with a serological test for IgG specific antibodies to *H. pylori* as a unique test that is not affected by previous use of antibiotics, IPPs, H2RAs, bleeding and other factors.

In the case of perforated gastric ulcer, a biopsy of the ulcer should be performed before the perforation site is covered with an omental patch with follow-up gastroduodenal endoscopy.

H. pylori eradication therapy with acido-suppression therapy should be recommended immediately after discharge from hospital for all *H. pylori* positive patients operated by the Graham method or the modified Graham method for perforated peptic ulcer.

For NSAID users operated with simple closure with an omental patch for perforated peptic ulcer, acidosuppressive therapy for 8 weeks with follow-up gastroduodenoscopy is

recommended to verify ulcer healing. Elimination or correction of the intake of the etiological factor (aspirin, NSAIDs and/or smoking) is necessary.

For Non-Helico Non-NSAID patients with perforated peptic ulcer, acidosuppressive therapy for 12 weeks with follow up gastroduodenoscopy should be recommended immediately after discharge from hospital for those operated with simple closure with an omental patch. When making a correct diagnosis of idiopathic peptic ulcer disease, the presence of factors that lead to a false negative *H. pylori* test and correct use of NSAIDs must be considered. Serum levels of thromboxane B2 indicate the latent use of NSAIDs in a number of non-*H. pylori* non-NSAID patients.

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UDC: 616.594-006.03

CASE REPORT

TREATMENT OF PILOMATRIXOMA IN PEDIATRIC POPULATION – OUR EXPERIENCE

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ABSTRACT

Background: Pilomatrixoma is a type of benign tumor most commonly diagnosed in people under the age of twenty. It usually occurs at the passage of hairy parts of the body and is associated with hair follicles. It accounts for 1% of all benign tumors. The symptomatology varies according to the location of the tumor, however most are isolated and non-symptomatic. Rarely, pilomatrixoma can become malignant after the age of twenty. Tumor size over 10 cm increases the possibility of malignant alteration. Complete surgical excision of the tumor is recommended.

Objectives: The aim of this study is to clinically analyze three cases of pilomatrixoma and to attract attention to this tumor in the differential diagnosis and its treatment in pediatric patients.

Material and methods: In a two year period, from 2018 to 2020, pilomatrixoma was found in three patients aged 5 to 14 years (average age 6 years). Two of them were asymptomatic. Pain as a symptom occurred in one patient who had visible whitish papillary tumefaction, located at the apex of the neck and the right retroauricular region of the head, with an uneven inflamed surface fused with the surroundings. Plain radiography and ultrasound were performed routinely in all patients, and MRI was indicated in one patient. The differential diagnosis included epidermoid cysts and dermoid cyst. Wide excision of the tumor with 2cm resection margins with primary skin closure was performed in all patients. Benign pilomatrixoma was confirmed in the histopathological findings in all patients. No recurrences were reported.

Conclusion: Because of possible cancerous alteration later in life, pilomatrixoma should be diagnosed in early age and treated with complete and wide surgical excision. The prognosis for isolated pilomatrixoma is good. Unless the excision was incomplete, pilomatrixoma don't recur.

Keywords: benign tumor, hair follicles, pilomatrixoma, pediatrics

INTRODUCTION

Pilomatrixoma is a benign cutaneous tumor differentiating towards the hair matrix of the hair follicle. It was first described by Malhebre as benign calcifying Epithelioma [8]. It accounts for 1% of all benign skin tumors. Approximately 50% of the lesions occur on the head and neck area, especially the cheek and the preauricular area, and are not associated with symptoms other than local. Lesions can also occur on upper and lower extremities in 22%. This tumor can occur in any age group, but the most common is around 20 years of age or younger [1]. Most studies report a slight predominance in females.

Usually is presented as firm, non-tender subcutaneous mass adherent to the skin but not fixed to the underlying tissue. It's covered by normal or hyperaemic skin and varies in size from 0.5 to 3cm, but rarely giant lesions up to 15cm are reported. Pathognomonic clinical signs for pilomatrixoma diagnosis are "tent sign" (multiple facets and angles in stretched skin over

the tumor due to calcification in the lesion) and "teeter-totter" sign (pressing on one edge of the lesion causes the opposite edge to protrude from the skin).

The exact cause for its occurrence is not known. Pilomatrixomas are not inherited. Mutations de novo in CTNNB1 gene are found in most cases. The CTNNB1 gene provides instructions for production of beta-catenin protein that among its many different functions, appears to be necessary for the normal function of the hair follicles. This protein is active in the matrix cells. The mutations produce an overactive protein that triggers matrix cells to divide in uncontrolled way, leading to tumour formation. Rarely, certain genetic syndromes inherited in an autosomal dominant manner, such as Gardner Syndrome, Myotonic dystrophy, Rubinstein-Taybi Syndrome, Turner Syndrome, Goldenhar Syndrome, Churg-Strauss Syndrome, can be associated with pilomatrixoma [2].

In the histologic findings, the lesion is usually found in the lower dermis and subcutaneous fat, sharply demarcated and surrounded by connective tissue capsule. Irregularly shaped islands of epithelial cells are seen and can be recognized as either basophilic cells or shadow cells. Calcium deposits are seen in 75% of lesions [5].

Rarely, pilomatrixoma can alter to pilomatrix carcinoma. Tumor size over 10 cm increases the possibility of malignant alteration. Pilomatrix carcinomas occur in patients older than 20 years, they are locally aggressive, can cause visceral metastases and have tendency to recur.

The diagnosis is made by physical examination, plain radiography often shows nonspecific calcification of the lesion, ultrasonography and tumor biopsy. CT or MRI is recommended in suspected cases to further diagnose the disease [4].

In the pediatric population this type of tumor is rare, asymptomatic, solitary, slow-growing and usually non-cancerous. Spontaneous regression has never been observed. The recommended treatment for pilomatrixoma is complete surgical excision with or without skin transplant depending on the size of the tumor. Wide resection margins 1-2cm have been recommended to minimize the risk of recurrence [6, 7]. The prognosis in patients with isolated pilomatrixoma is good. There are usually no serious complications. In most cases it does not recur if the excision is complete [3]. We have mentioned that large pilomatrixomas can become carcinogenic in rare cases. In accompanying syndromes the prognosis varies and depends on the associated symptoms.

CASE REPORT I (1)

In the outpatient department in our clinic (year 2019) a 13 year old male was admitted with a non-tender solid tumorous formation, with a size of a small walnut, located behind the right auricle with normal skin color surrounded by hair (Fig.1). It was first noticed by the parents 2 - 3 months before the exam. There was no positive family history. Status by systems within normal limits. An ultrasonography findings describes a tumor formation measuring 5cm x 3cm, avascular and with irregular borders. MRI findings describe an irregular heterogeneous tumor mass measuring 50mm x 36mm, without lesion of the skeletal system. Biopsy was not performed. Before the scheduled surgical intervention, the patient fell ill with pneumonia, and the intervention was postponed for two months. During this time the tumor grew to the size of a mandarin. Inflammation and pain were present. Complete surgical excision with 2cm wide margin under general anesthesia was performed. The wound was primary closed with non-absorbable sutures. Operative and postoperative period passed without complications. The histopathologic findings described macroscopically elliptical skin excision with light brown nodules on the surface cross-sected with yellow discoloration and with firm consistency. The microscopic findings showed fibromatous tissue and connective tissue with numerous cholesterol crystals, shadow cells and numerous calcifications. The finding corresponds to Pilomatrixoma, calcifying Epithelioma of Malherbe (ICD-D23).

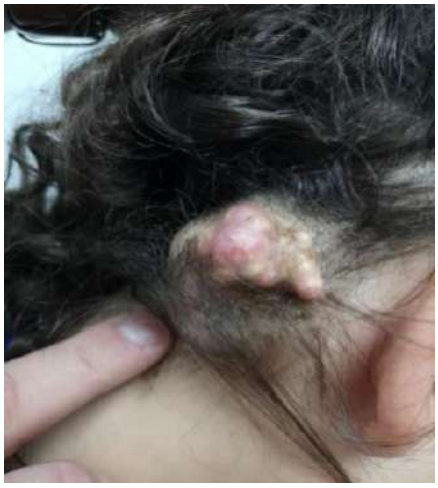

Fig.1. Pilomatrixoma located in the right retroauricular region. Firm, non-tender subcutaneous mass adherent to the skin but not fixed to the underlying tissue, with normal or hyperaemic skin coloration surrounded by hair.

CASE REPORT II (2)

In the outpatient department 5 year old male was admitted due to tumor formation in the right temporal area at the border of the hairy part of the face. The change was noticed by the parents 3 months before the exam. The child had no pain or other accompanying symptoms. X-ray diagnostic and ultrasound was performed. The findings described avascular formation measuring 0.5cm x 2cm located superficially without lesion of the skeletal system. Under general anesthesia complete wide surgical excision of the tumor with primary wound closure was performed. We had no complications during the operative and postoperative period. The histopathologic findings suggested calcifying Epithelioma of Malherbe (ICD-D23).

CASE REPORT III (3)

A 6 year old male was admitted to our Clinic due to a tumor formation in the right upper arm area measuring 3cm x 2cm x 0.5cm. The change was noticed by the parents 3-4 months before the exam. Apart from the visible tumorous formation, there were no other symptoms. There were no signs of pathological skeletal lesions on the X-ray. The ultrasound examination showed cystic formation without pathological vascularization. Complete wide surgical excision of the tumor with primary wound closure was performed. Regular operative and postoperative course. The histopathologic findings described tumor formation with a grayish-white paste consistency. The microscopic findings showed a benign neoplasm with squamous cells, calcifications and inflammatory cells seen on the periphery. The finding corresponds to Pilomatrixoma, calcifying Epithelioma of Malherbe (ICD - D23).

DISCUSSION:

Pilomatrixoma is usually a solitary lesion affecting young individuals. They most commonly involve the head and neck region followed by upper extremities, trunk and lower extremities [9]. Clinical features as documented by Duran et al [10] and later also by Perez and Nicholson [11] should arise clinical suspicion. The diagnosis is made by physical examination, plain radiography, CT and MRI. They usually present with subcutaneous red to blue mass, firm to palpation and freely movable. The differential diagnosis includes epidermoid cysts, dermoid cyst, sebaceous adenoma or carcinoma, juvenile xanthogranuloma, capillary hemangioma, chalazion and rhabdomyosarcoma [11]. Also they can resemble keratocanthoma if they grow rapidly. Rarely, they undergo malignant transformation into pilomatrix carcinoma [12].

The histopathologic microscopic examination shows islands of epithelial cells with characteristic arrangement of basophilic cells in the periphery and shadow cells in the center. Calcification is seen in 75% of the cases. Histopathologic differential diagnosis includes basal or squamous cell epitheliomas as well as a variety of skin and subcutaneous cysts [13].

Spontaneous regression has never been observed. The management includes complete surgical excision biopsy with minimum of 1-2cm wide margins with or without skin transplant depending on the size of the tumor. Various aesthetic approaches can be used.

Because of possible cancerous alteration later in life, pilomatrixoma should be diagnosed and treated in early age. The prognosis is good. Unless the excision was incomplete, pilomatrixoma don't recur.

Although pilomatrixoma is an uncommon benign tumor it has some distinctive clinical features that suggest the correct diagnosis.

CONCLUSION

The aim of this study is to show that in the pediatric population there are tumor formations that are nonspecific for age, such as pilomatrixomas, which account for about 1% of the total benign tumors. Their surgical treatment is imperative because they undergo malignant transformation later in life especially in tumors larger than 10 cm. Wide surgical excision is the recommended treatment.

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UDC: 616.36-002.951.21

CASE REPORT

ABDOMINAL ECHINOCOCCOSIS WITH LESSER SAC HYDATID CYST RUPTURE IN THE DUODENUM: CASE REPORT

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ABSTRACT

Introduction: Hydatid disease (HD) is a zoonotic disease, most commonly caused by *Echinococcus granulosus*, and is present in all continents with a more pronounced presence in certain areas. The most commonly involved organ in the abdominal cavity is the liver. The hydatid cyst may compress adjacent organs, the contents of the cyst may become infected, or the hydatid cyst may rupture into the abdominal cavity or digestive tract. Primary abdominal echinococcosis and perforation in the hollow abdominal organs is rare.

Case presentation: We present the case of a 25-year-old male patient with abdominal echinococcosis complicated with a large hydatid cyst in the lesser sac ruptured in the duodenum, omental hydatid cysts and a large hydatid cyst in the pelvic cavity.

The patient had severe epigastric pain, fever, vomited and defecated grape-shaped cystic grains (hydatidemesis and hydatidenteria).

Complete removal of omental cysts and hydatid cyst from the lesser pelvis was performed. Gastric resection was performed according to Hofmeister Finsterer to resolve the rupture of the lesser sac hydatid cyst in the duodenum, while the duodenum was closed with the Nissen procedure.

Conclusion: Hydatid cyst of a lesser sac is an extremely rare complication even in endemic areas and to our knowledge, this is the first reported case of abdominal echinococcosis with lesser sac hydatid cyst rupture in the duodenum.

Hydatidemesis and/or hydatidenteria are certainly a specific sign of gastrointestinal communication.

Keywords: hydatid disease, abdominal echinococcosis, hydatidemesis, hydatidenteria

INTRODUCTION

Echinococcosis is a parasitic disease caused by an infection with small cestodes (tapeworms) of the genus *Echinococcus* and has a great general and economic health importance in both developed and developing countries [1]. Cystic (CE) and alveolar echinococcosis (AE) are of medical importance to humans.

Cystic echinococcosis (CE), also called hydatidosis, or hydatid disease, is caused by an infection with the larval stage of *Echinococcus granulosus*, a tapeworm ~ 2 to 7 mm long found in dogs (definitive host) and in sheep, cattle, goats, and pigs (intermediate hosts).

Alveolar echinococcosis (AE) is caused by an infection with the larval stage of *Echinococcus multilocularis*, a tapeworm ~ 1 to 4 mm long found in foxes, coyotes, and dogs (definitive hosts), while small rodents are intermediate hosts for *E. multilocularis*.

In addition to these two forms, there are *Echinococcus vogeli*, which causes polycystic forms, and *Echinococcus oligarthrus*, which causes an extremely rare unicystic form.

More than 95% of human cystic echinococcosis are caused by *E. granulosus*.

E. granulosus is a universal parasite, has not race and sexual predilection and is present in all continents with a more pronounced presence in certain areas, the so-called endemic regions in which the incidence rate in humans for cystic echinococcosis can reach more than 50 per 100,000 people per year.

Hydatid cyst can affect all organs of the human body, but the most affected are the liver (63%), lungs (25%), muscles (5%), bones (3%), kidneys (2%), spleen (1%) and other sites (1%) [2].

Infections with *Echinococcus granulosus* are generally asymptomatic until they reach a sufficient volume to cause a mass effect on the surrounding organs.

Symptoms depend on the size of the cyst, its location, and its relationship to adjacent structures, while complications include rupture of the cysts in the biliary and digestive tract and abdominal cavity [3].

Rupture of a hydatid cyst in the abdominopelvic cavity can cause abdominal pain, systemic anaphylactic reactions or development of new hydatid cysts. This can be a life-threatening condition [4].

The diagnosis is confirmed by radio-imaging studies (ultrasonography and computed tomography), as well as serological tests (complement fixation test, indirect haemagglutination test and ELISA) [5].

CASE PRESENTATION

We present a 25-year-old male patient who 5 days before the admission at the University Clinic for Digestive surgery in Skopje suddenly felt sharp pain in the abdomen after eating chocolate. The patient was previously in good general condition and healthy.

The patient called the primary care doctor, who gave him a pain injection and sent him home. The pain continued the next day and intensified, which made the patient call the surgical department of the local hospital. The surgeon sent him to a hospitalist at the Regional Medical Center for an outpatient upper gastrointestinal endoscopy (Figure 1).

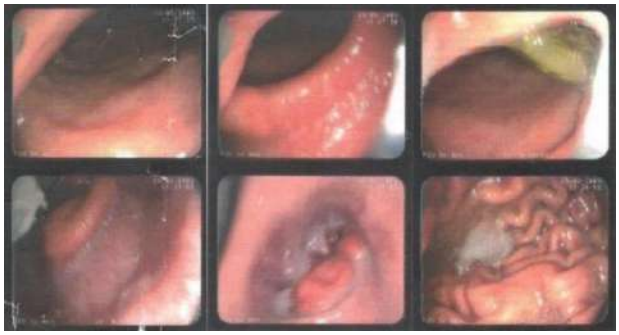

Fig. 1. Upper GI endoscopy finding: Sliding hiatal hernia, mucosal erosions of the upper duodenum, and chronic gastroduodenitis.

In the following day, in addition to the pain, the patient suffered from fever, sweating and vomited a white grain in the form of a grape or a small balloon, due to which he called his primary care doctor again. The family doctor saw the sample and said it looked like a cyst and sent him to the hospitalist in the local hospital where he was admitted in the internal ward and received infusion therapy. At the local hospital, the patient noticed a large sluggish balloon in the stool with grains similar to the grain he had previously vomited. The next day, the hospitalist from the local hospital sent the patient to the University Clinic for Gastroenterohepatology in Skopje, where outpatient ultrasound examination was performed.

Ultrasonography reveals multiple hydatid cysts in the upper abdomen with a thick and double-contoured wall and a large hidatyd cyst, retrovesical in the true pelvis, which is also filled with numerous daughter cysts (Figure 2).

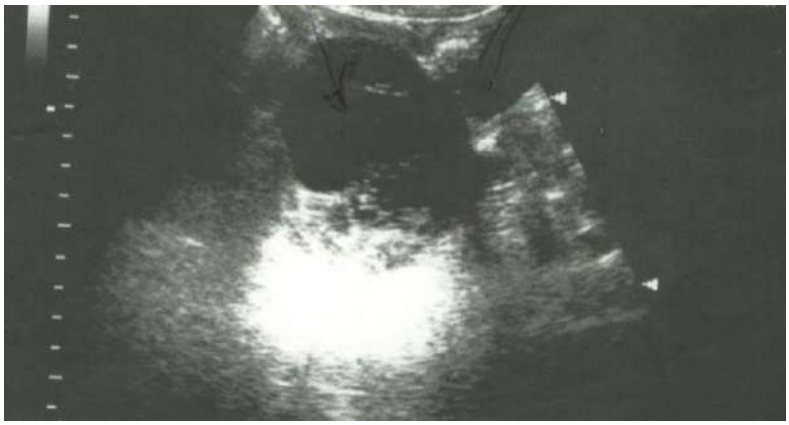

Fig. 2. US finding: Retrovesical hydatid cyst with numerous daughter cysts

Due to this finding the patient was transferred to the University Clinic for Digestive surgery. The pain subsided in meantime. On admission in our Clinic, the physical examination was with mild tenderness in the epigastrium. The fecal specimen also contained a small balloon.

Laboratory examination noted: WBC $10.5x10^9$/L; ALP 309 (U/L); AST 74 (U/L), ALT 79 (U/L), CPK 278 (U/L), CK-MB 12 (U/L), LDH 331 (U/L), GGT 221 (U/L), Total bilirubin 36 (μm/L), Direct bilirubin 8 (μm/L), Indirect bilirubin 28 (μm/L), Na 137 (μm/L), K 3,9 (μm/L).

Non contrast and contrast-enhanced computed tomography of the abdomen performed the following day, detected three different multilocular cystic formations (with septa), somewhat laterally and posteriorly from the left lobe of the liver and very close to the hepatic hilus, as well as a similar change without capsules, septal (unilateral) change visible in the caudal images to the left and in front of the transverse colon and clearly demarcated multilocular cystic change (10x8.5 cm) located ventrally from the rectum and dorsally from the urinary bladder that suppresses the surrounding anatomical structures without calcifications (Figure 3 and 4). CT changes indicate echinococcal cysts.

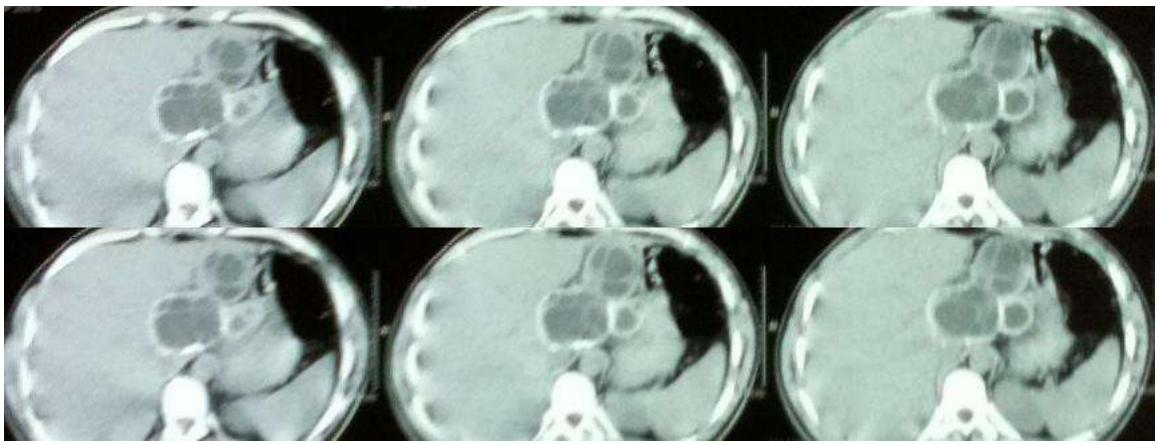

Fig. 3. Laterally and posteriorly from the left lobe of the liver and very close to hepatic hilus, three different multilocular (with septa) cystic formations are detected. A similar capsule-free, septal (unilocular) change is seen on the postural scans to the left in front of the transversal colon.

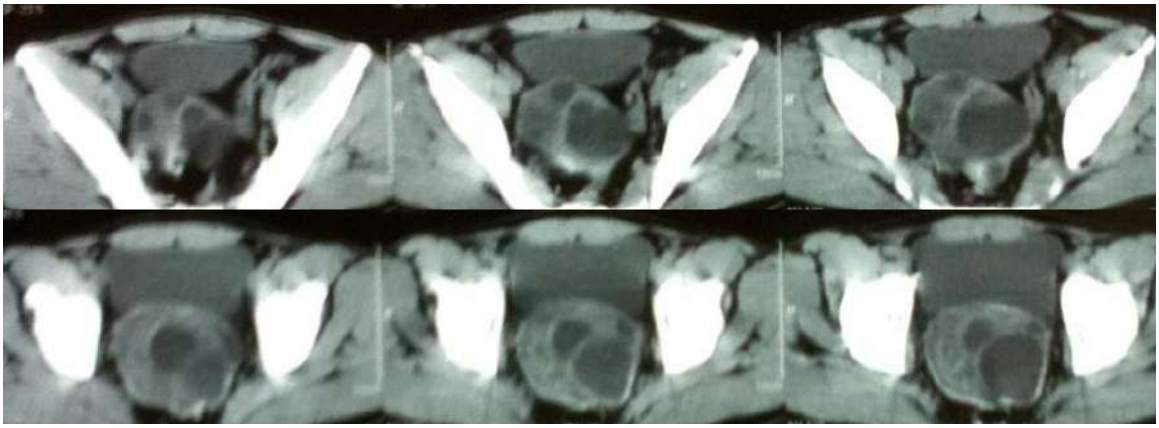

Fig. 4. Ventrally from the rectum, and dorsally from the urovesica, clearly demarcated multilocular cystic change

An indication for surgical treatment was set two days after admission. Approached by midline laparotomy (Figure 5).

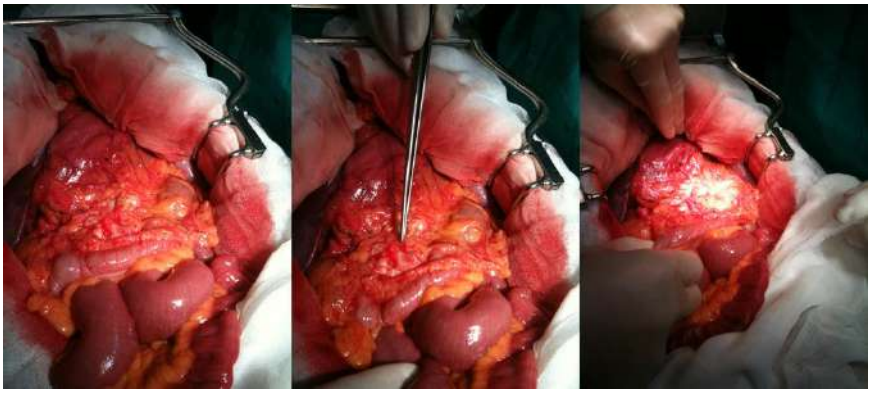

Fig. 5. Midline laparotomy

Intraoperatively, a large hydatid cyst was found in the lesser sac, with the presence of hydatid fluid and daughter hydatid cysts. Extensive communication of the lesser sac hydatid cyst with the posterior wall of the upper duodenum was seen after evacuation of the hydatid contents (Figure 6).

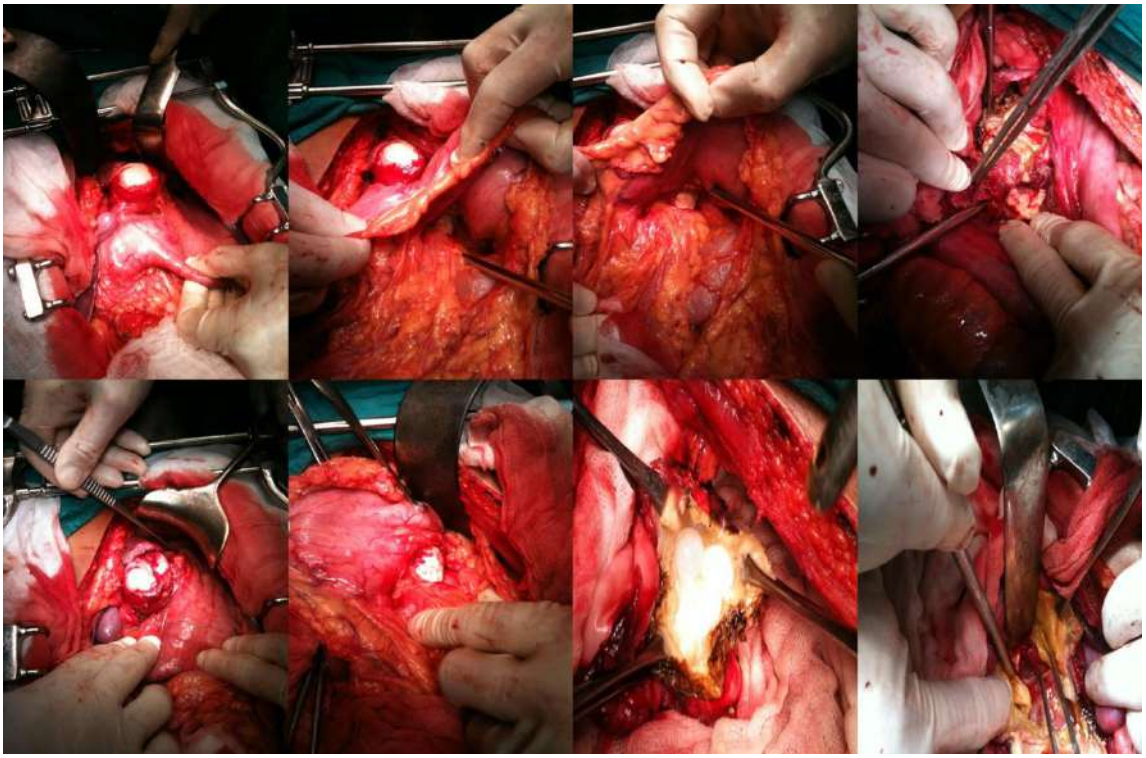

Fig. 6. Large hydatid cyst in the lesser sac, with the presence of hydatid fluid and daughter cysts. Extensive communication of the hydatid cyst with the posterior wall of the upper duodenum.

There is also a calcified omental and peritoneal hydatid cysts and a large hydatid cyst that fills the lesser pelvis and has fused with the rectosigmoid colon and urinary bladder (Figure 7).

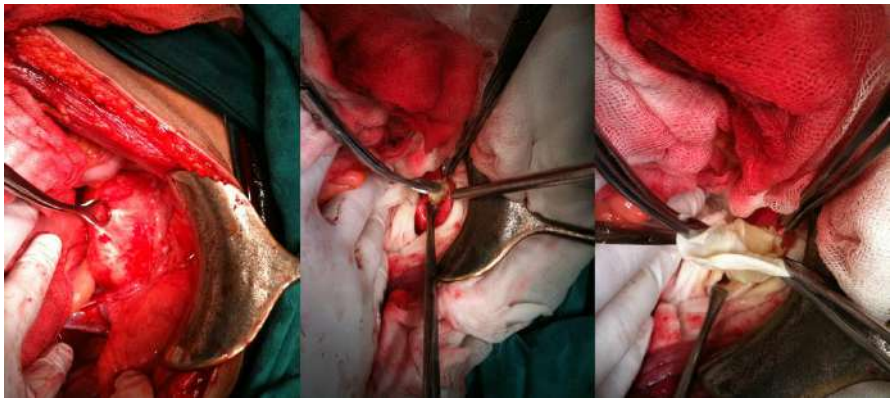

Fig. 7. Large hydatid cyst in the lesser pelvis with the presence of germinative layer.

We performed evacuation of the content of the lesser sac hydatid cyst cavity and partial pericystectomy and filling of the residual cavity with omentum (omentoplasty). Due to the high risk of duodenal suture, it was decided to perform Hofmeister–Finsterer partial gastrectomy. The duodenum was closed by Nissen procedure. The hydatid cyst in the true pelvis was completely removed. Omental hydatid cysts were completely removed (Figure 8).

Two abdominal drains were placed, one in the Douglas space and the other under the liver.

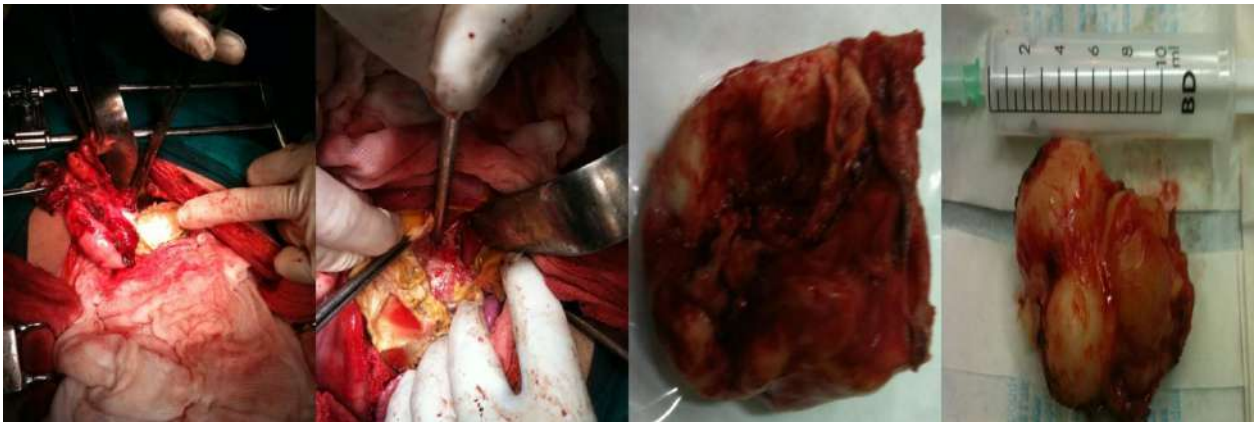

Fig. 8 Partial pericystectomy and omentoplasty, Hofmeister-Finsterer partial gastrectomy, completely removed hydatid cyst in the true pelvis and omentum majus.

Postoperatively, the patient developed enterocutaneous fistula, which was cured over a period of 4 months. The patient had no recurrence of hydatid disease after one year of follow-up (Figure 9).

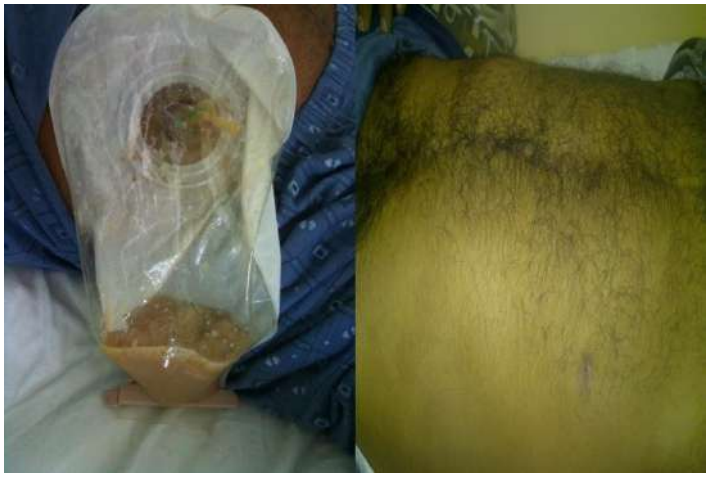

Figure 9. Fistula and complete heal of fistula after 4 months

DISCUSSION

Humans may become intermediate hosts through ingestion of parasitic eggs in contaminated food, water or soil, or after direct contact with a definitive host (dogs or other canines). Ingested ova hatch in the small intestine into oncospheres that penetrate through the intestinal wall to reach the portal venous system or lymphatic system. Oncospheres can reach the liver which acts as the first line defense and is therefore the most frequently involved organ [5].

The initial stage of hydatid cyst is similar to a fluid-filled cyst (type 1). In further development, daughter cysts and/or matrix may develop in it (type 2) or it may become hyper-mature and starve to death and become an inert calcified cyst (type III) [6].

The parasitic endocyst may rupture, and the contents may be restricted to the pericyst (contained echinococcal cyst), the contents of the echinococcal cyst may leak into the biliary tract via an open bile duct (communicating echinococcal cyst), or both the endocyst and the pericyst may rupture and spill the cyst contents into the abdominal cavity or other structures (direct echinococcal cyst) [7].

The most common complication faced by the surgeon is hydatid cyst fistulas with the bile ducts [8].

The walls of the digestive tract are highly resistant to fistula formation between the cystic cavity and the gastrointestinal lumen, making rupture of a hydatid cyst in the digestive tract less common. Chronic aseptic pericystic inflammation causes adhesions and a fistulisation between the cyst and the adjacent organs, such as the stomach, pancreas, colon, and others [9].

Intraperitoneal hydatid cysts are usually secondary to spontaneous or iatrogenic rupture of hepatic, splenic or mesenteric cysts [5].

Primary peritoneal echinococcosis is rare and accounts for 2% of all abdominal hydatidoses without evidence of cysts in other intra-abdominal organs. Dissemination occurs by either lymphatic or systemic circulation. Cysts may be multiple and located anywhere in the peritoneal cavity [10].

Imaging methods (US, CT, MRI) are used in the diagnosis of hydatid cyst in the abdominopelvic cavity. US also allows detection of cystic membranes, septa and hydatid sand. CT may show an air-level cyst, calcification of the cyst wall, and cyst infection, as well as a fistula between the cyst and the hollow organ [11].

Hydatid serological testing (enzyme-linked immunosorbent assay, the indirect immunofluorescence antibody test, immunoelectrophoresis, immunoblotting) help to make the diagnosis [12].

Depending on the location and size of the echinococcal cyst, the type of treatment is determined. Therapeutic options are drug anti-infective therapy (albendazole at a dose of 10-15 mg/kg/day), percutaneous treatment with PAIR technique (associated with a high recurrence rate), "watch and wait" and surgical treatment [13].

Abdominal hydatid disease outside the liver is uncommon, with the most common being in the peritoneum. Rupture of an abdominal hydatid cyst in the lumen of the digestive tract is very rare [14].

In our report we present a case of a patient with a hydatid cyst located in the bursa omentalis, three omental cysts and a hydatid cyst in the small pelvis. The hydatid cyst in the bursa omentalis perforated the posterior wall of the upper duodenum and is in extensive communication with it. The patient had hydatidemesis and hydatidenteria.

There are reports for cases of primary peritoneal echinococcosis [15,16], hydatid cyst in the lesser sac [17,18], hydatid cyst in omentum [19,20] and hydatid cyst in pelvic cavity [21,22]. Spontaneous rupture of the hepatic hydatid cyst in the duodenum is a rare complication [23]. Fistulization of the abdominal hydatid cysts in the intestinal lumen is less frequent complication [24]. Peritoneal hydatidosis with rupture of the abdominal hydatid cyst in the stomach and duodenum is extremely rare with very few cases reported in the existing literature [14].

Typically, the communication is not discovered until surgery, although in some cases it is found at radiology.

CONCLUSION

Abdominal echinococcosis with hydatid cyst in the lesser sac are very rare and only a few cases have been described in the literature.

Rupture of the lesser sac hydatid cyst in the duodenum is an extremely rare condition, perhaps not at all previously described in the literature.

Hydatid cysts and membranes in the vomitus (hydatidemesis) and/or passage of hydatid membranes in the stools (hydatidenteria) is certainly a specific sign of rupture and gastrointestinal communication.

Surgery is the treatment of choice for peritoneal echinococcosis complicated with rupture of intraabdominal cyst in digestive tract.

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UDC: 616.13/.14-002.7:[616.98:578.834

CASE REPORT

POSSIBLE RELATIONSHIP BETWEEN WEGENER-GRANULOMATOSIS WITH POLYANGITIS (VASCULITIS) AND COVID-19 INFECTION AND THEIR COMPLEX TREATMENT- CASE REPORT

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ABSTRACT

Introduction: Granulomatosis with polyangiitis (GPA),previously known as Wegener's granulomatosis (WG) is a rare, long-term systemic disease with the formation of granulomas and inflammation of blood vessels (vasculitis). It is an inflammation that affects the small and medium-sized blood vessels, primary the upper respiratory tract, lungs and kidneys.

Coronavirus (COVID-19) infection is an infectious disease caused by severe acute respiratory syndrome (SARS-CoV-2).

Case report: In order to find out the connection of granulomatosis with polyangitis and Coronavirus (COVID-19) infection and the complications that may occur, we observed the history of the disease in a 64-year-old patient, who suffered from vasculitis and polyangitis wich was treated with doses of corticosteroids, cyclophosphamide, and plasmapheresis.The patient had terminal chronic kidney disease and started treatment with intermittent hemodialysis 6 months ago. For an unknown reason, he discontinued the corticosteroid treatment and developed symptoms of active vasculitis. After several medical examinations, he was hospitalized at the University clinic for Pulmonology - Skopje, where they started examinations in the direction of reactivation of the basic disease-granulomatosis with polyangitis. Results were obtained for a positive Coronavirus-19 patient who had a previous renal disease. Accepted for hospitalization and further treatment in the University clinic for Nephrology with Covid-19 positive patients. Protocol therapy was applied, but without success. Fatal outcome occurs due to the difficulties of the overall complications of the disease.

Conclusion: Coronavirus (COVID-19) infection is often associated with vasculitis in terms of endothelial cell inflammation.

Keywords Coronavirus (COVID-19) infection, SARS-CoV-2, granulomatosis with polyangitis- Wegener

INTRODUCTION

Coronavirus (COVID-19) infection is an infectious disease caused by severe acute respiratory syndrome (SARS-CoV-2), which begins as an epidemic in December 2019 in Wuhan, China . It has since developed rapidly to become a pandemic disease as declared by the World Health Organization on March 12, 2020, with a mortality rate of more than 3.5 million worldwide by June 2, 2021.

The mode of transmission of this disease is through the respiratory route, especially indoors, when sick patients are coughing and sneezing, touching surfaces almost contaminated with the causative agent and the like [1,2].

Risk groups include patients of advanced age, diabetics, males, obese people and those affected by chronic diseases, heart and lung diseases [1,3]. Immunosuppressive status was not introduced as a cause of this disease. On the contrary, immunosuppressive drugs affect the

hyperactive effect of cellular and humoral immunity in this disease, which is not a common case for other diseases of this nature.

The diagnosis consists in defining all the clinical signs and symptoms [3,4,5,6]. Diagnosis of COVID-19 requires detection of SARS-CoV-2 RNA or antigen in respiratory specimens. The detection of SARS-CoV-2 viral RNA is more effective in the nasopharynx samples compared with throat samples[6]. Lower respiratory samples may have better viral yield than upper respiratory samples. SARS-CoV-2 antigen tests can also be used in a variety of testing strategies.

Two types of tests are mostly used for coronavirus infection, one is the so-called rapid test, and the second is the PCR method, which is more reliable.

There is still no specific treatment for patients with Covid-19. Symptomatic therapy is used, corticosteroids in patients who worsen and decrease in oxygen saturation, oxygen substitution through masks (nasal, highly concentrated oxygen masks), antibiotics to prevent bacterial superinfection, antipyretics, fluids, and good antidepressants are sometimes prescribed as well.

According to the world treatment protocol [7,8], the treatment starts with: antibiotic therapy which does not have to consist of too strong antibiotics, prevention of bacterial super-infection, vitamin substitution (of vitamin C, vitamin D, etc. but in usual doses, monitoring for hypervitaminosis), hydration of patients, oxygen support and substitution depending on the stage of disease development, antipyretics, monitoring of parameters for inflammatory markers such as CRP, monitoring of blood count (predominantly lymphocyte count),monitoring of enzyme values in laboratory parameters, values of D dimers that can grow up to 35,000 and more, treatment with corticosteroids when the value of oxygen saturation begins to fall below normal values (especially critical days between the 5th and 10th day), anticoagulant therapy that is administered by bodyweight protocol and depending on hemostasis with D dimers and whether it is for therapeutic or preventive purposes, monitoring of serum glycemia values and treatment with insulin therapy.

Granulomatosis with polyangiitis (GPA), previously known as Wegener's granulomatosis (WG) is a rare, long-term systemic disease with granuloma formation and vascular inflammation (vasculitis) [9,10]. It is an inflammation that affects the small and medium blood vessels, primarily the upper respiratory tract, lungs and kidneys [11,12].

Both males and females are equally affected by this disease.

The cause of its occurrence is unknown, except that bacteria and viruses are mentioned as possible causes as well as the genetic factor.

Signs and Symptoms

Kidneys cause rapidly progressive glomerulonephritis (75%). Eyes and ears are affected from the upper airways (hearing loss and scleroceratitis of the eyes), gingivitis occurs in the oral cavity, the trachea is affected by form changes-stenosis[11,13].

Lungs with coin nodules, pneumonia, bleeding, etc[14]. Other affected systems include the joints, with arthritis, pain, or swelling (60%), often initially diagnosed as rheumatoid arthritis. The skin is affected by subcutaneous nodules (granulomas) of the elbow, purpura, and various others changes (see cutaneous vasculitis)[13], and the nervous system is occasionally hurt by sensory neuropathy (10%) and less often by mononeuritis multiple. Heart, gastrointestinal tract, brain, other organs are rarely affected.

It is important to have ANCA immunoassay to make a diagnosis. The determination of the ANCA positive test does not fully confirm the diagnosis, but the negative test does not rule out the possibility that the disease is present.

If the person has signs of kidney involvement or cutaneous vasculitis, it could be proven by a kidney biopsy. In rare cases, thoracoscopic lung biopsy is required. On histopathological examination, the biopsy will show leukocyte vasculitis with necrotic changes and

granulomatous inflammation (lumps of typically distributed white blood cells) under microscopy. But biopsies are 57-75% sensitive. [14].

Involvement of the ears, nose, and throat is more common in granulomatosis with polyangitis than in a similar condition with microscopic polyangitis.

Regarding Granulomatosis with polyangiitis (GPA), there are several criteria according to the American College of Rheumatology 1990, according to which 2 or more present criteria are with over 85% susceptibility to the presence of the disease. And those are:

-Nasal or oral inflammation: painful or painless oral ulcers or purulent or bloody discharge from the nose

-Lungs: abnormal chest X-ray with nodules, infiltrates or cavities

-Kidneys: urinary sediment with microscopic haematuria, with extracapillary glomerulonephritis on renal biopsy

Treatment consists use of immunosuppressive drugs such as corticosteroids, cyclophosphamide or Rituximab [15].

1. Corticotherapy - Methylprednisolone 500e1000 mg intravenously daily for 3 days is followed by oral prednisolone 0.5e1 mg/kg/d for at least 4 weeks.

2. Oral and intravenous cyclophosphamide are effective in inducing GPA remission. Oral cyclophosphamide at a dose of 2 mg / kg / day has been standard treatment for many years; this regimen has resulted in complete remission in more than 75% of people with GPA, but is associated with significant toxicities, including infertility, inflammation and bleeding from the bladder, and bladder cancer [15].

In contrast, administering pulsed doses of intravenous cyclophosphamide is equally effective in causing remission, resulting in a lower cumulative dose, and reducing the incidence of abnormally low white blood cell counts by one third.

However, pulsed intravenous cyclophosphamide may be associated with a higher risk of GPP relapse when compared to oral cyclophosphamide. Due to the high frequency of abnormally low white blood cell counts observed with cyclophosphamide treatment, pneumocystis pneumonia is a common complication and prophylaxis against this pathogen is recommended.

3. Rituximab can be used as a medicine because it is also effective in the treatment of this disease. Increasingly, the disease is being brought under control, as soon as remission occurs, the goal is to maintain this condition.

Given the similar symptoms of the disease and the findings obtained, there is a possibility of a related effect on the overall condition of the patients. Therapy for the underlying disease is known, especially when the disease is activated. In cases where COVID pneumonia occurs as a complication, the same therapy is recommended in higher doses of corticosteroids. As with Wegener's granulomatosis, COVID 19 infection can present with acute respiratory distress syndrome, which is fatal. In addition, due to impaired coagulation and changes in hemostasis, there is a risk of thrombosis, which leads to a number of complications and strokes, as the actual cause of death.

In our patient there was a sharp deterioration in general condition with loss of consciousness in addition to cerebrovascular stroke despite the use of appropriate therapy with low molecular weight heparin.

CASE REPORT

We report on a 64 year-old patient, with granulomatosis with polyangitis, who was hospitalized in University clinic for Nephrology-Skopje, section for Covid-19 positive patients due to Coronavirus (COVID-19) infection. Previously, the patient was admitted to the University clinic of Pulmonology, due to shortness of breath, coughing up blood, as a suspicion of progression and activation of Wegener disease. It was basically a patient with Wegener's

disease- granulomatosis with polyangitis and chronic renal failure, on dialysis treatment for 6 months. The patient has complained of coughing up blood and shortness of breath in the period of one month before the day of admission. The day before hospitalization, he consulted the emergency center due to the worsening of the symptoms, and he was sent to the University clinic for Pulmonology where a lung ultrasound was effected with evacuation of about 1500 ml of fluid. Heteroanamnestic data-patient has discontinued corticosteroid therapy.

After the investigations, a positive finding was obtained for Coronavirus (COVID-19) infection, and the patient was hospitalized at the University clinic for Nephrology, a cohort of Coronavirus (COVID-19) positive patients ward, for further treatment and examination.

The patient is a 64-year-old man, a former smoker, who has stopped smoking for 8 months before. On the day of hospitalization the patient is febrile, has dyspnea, rhythmic heart action (95/min), with normal blood pressure 120/80mmHg. Respiratory signs with bilaterally crepitations at the middle and basal parts of the lungs.

From the investigations of University clinic for Pulmonology:

-Blood count: Hgb = 97 g / L Hct = 29% Rbc= 3.51x10 ^ 12 / L Wbc = 6.1 x10 ^ 9 / L Plt = 353 x10 ^ 9 / L CRP 181 Glycemia: 7.44 mmol / L, Ionogram Na = 134 mmol / L,K = 4.7mmol / L Ca = 2.15 mmol / L, Serum Urea = 8.8mmol / L Serum Creatine = 649 umol / L

-CT of the lungs with the finding of infiltrative changes bilaterally in the parenchyma in terms of ground glass opacity-GGO, and in some places micronodular changes. Bilateral pleural effusion. There is a significant progression of changes compared to the previous finding.

-Acid-base analysis: in addition to hyposaturation Hemostasis with D-dimers 8000.

-Antigen and PCR for COVID 19 negative.

- SARS COV 2-IgM 3.82 g/L (refer range -0,4-2,5), IgG 3.42g/L (ref range 6-16).

During his hospitalization at the University clinic for Nephrology, the patient was, treated with dual antibiotic therapy, corticosteroid, electrolyte, gastroprotective therapy and low molecular weight heparin. Set to oxygen support 11L / min, with gradual reduction to the stadium where the patient does not need oxygen therapy. Due to reduction in changes of blood counts and hemoptysis, CT angiography was performed indicating regression of changes, predominantly on the right, but we had the previously described changes on the left side. There was pleural effusion at the right side, from middle to basal parts. The heart was enlarged, with left ventricular wall hypertrophy-predominantly followed. Pericardial effusion was up to 8 mm.

On several occasions the patient was substituted with units of erythrocytes and protein derivatives. Regular hemodialysis was performed. After 2 weeks of admission, there is a sharp deterioration of consciousness, with appearance of spasms. Immediate CT scan of the brain was performed in consultation with a neurologist and the patient was transferred to the Clinic of Neurology for further treatment.

From the examinations performed by the University clinic for Nephrology:

-Haemostasis with D-dimer is regularly monitored and therapy is applied on the recommendation of a transfusiologist (Fraxiparine 0.1ml s.c. at 10kg / TT divided into two daily doses), with adjustment of D-dimer values from 29112 to 8778

-The value for c- ANCA 167 and 180 (Negative: ≤19 AU/mL. Equivocal: 20-25 AU/mL) was checked on 2 occasions in a period of one week.

Table 1. Biochemical parameters of the patient

Date	12.01.21	15.01.21	22.01.21	25.01.21	unit	Reference parameters
RBC	2.47	2,8	3,2	3,1	10^12/L	4.20-5.50
HGB	73	80	96	93	g/L	120-180
HCT	0.1	0,2	0,2	0,2	rv	0.37-0.54
WBC	7.1	8,6	9,6	8,9	10^9/L	4.00-9,\.00
PLT	342	472	231	203	10^9/L	150-450
Albumin	28	29	29	26	g/L	35-50
Total Protein	54	46	48	45	g/L	63-83
CRP	29.8	16.7	26,2	34	mg/L	6
LDH	265	244	227	219	U/L	<248
ALT	8	3,9	13	9	U/L	10-45
AST	10 8,4	14	8		U/L	10-34
AP	32	35	37	36	U/L	36-126
Ca	1.9	1,9	1,8	1,8	mmol/L	2.1-2.6
K	4.6	4,3	3,4	3,9	mmol/L	3.8-5.5
Na (S)	134	135	140	137	mmol/L	137-145
Ac. Uricum	245	256	242	259	umol/L	150-450
Serum Kreat	590	605	676	731	umol/L	45-109
Serum Urea	14.7	20	23	22	mmol/L	2.7-7.8
Serum Glyc	4.3	4,4	4,4	4,5	mmol/L	3.5-6.1

DISCUSSION

Can it be said that Covid-19 is closely related to vasculitis, the skin changes, kidney involvement, stroke? Will science take an even more interesting direction? The complexity in the treatment of these two diseases is significant, which can be seen in their symptomatology, physical changes and consequences.

Covid 19 infection has a different course of the disease. Symptoms include headache, body and muscle pain, fatigue and malaise, dry throat, runny nose, loss of smell and taste, fever, cough, blood clotting and respiratory problems, and so-called ground glass opacity changes [1,3,4].

On the other hand, the activation of Wegener's -granulomatosis with polyangtis emphasizes the symptoms of the type of hemoptysis, shortness of breath. The patient is febrile at the beginning of follow-up and treated according to protocol, after which regression of the lung finding occurs [15].

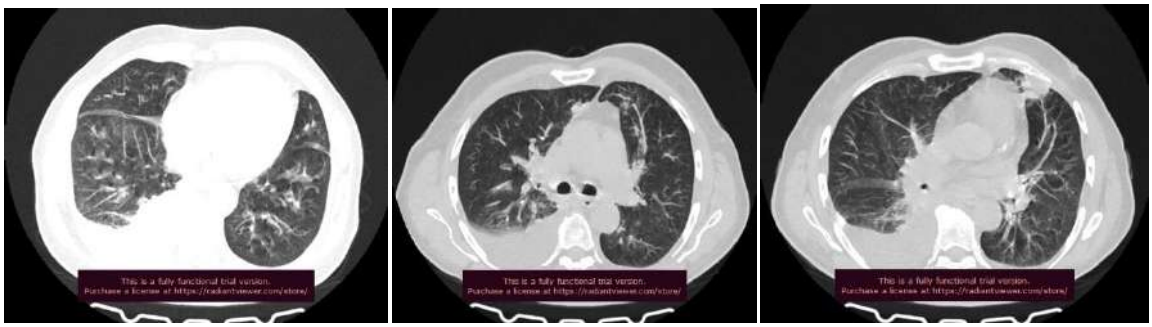

Fig. 1. CT showing coronavirus infection- from our patient explained before.

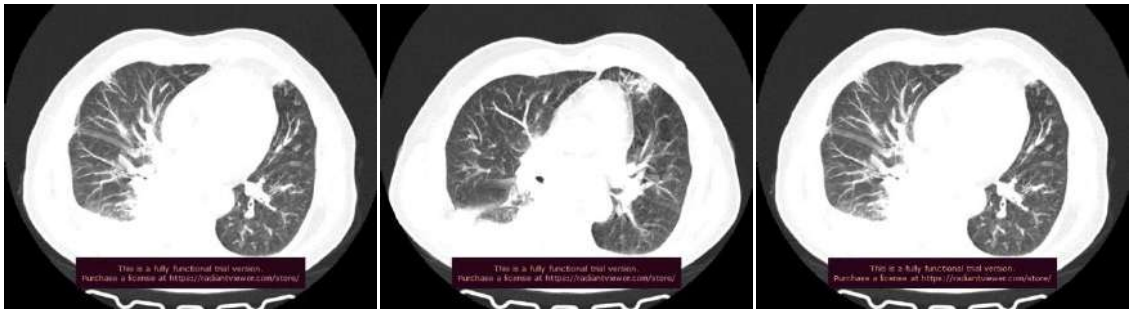

Fig.2. Progressive stage of Coronavirus infection from our patient explained before.

CONCLUSION

Covid-19 is often associated with vasculitis. Patients with severe COVID-19 pneumonia may develop vasculitis-like skin lesions and systemic arterial and venous thromboembolism, including stroke and other features of vasculopathy. The underlying mechanisms for these severe manifestations include immunodeficiency, dissemination of viruses with direct systemic endothelial infection, viral RNA anemia with immunothrombosis, activation of the clotting pathway mediated by hypoxaemia, and immobility. At this point, we emphasize the world's postmortem findings of patients with COVID-19, indicating that this mechanism represents a new mock vasculitis associated with COVID-19 that can lead to cryptogenic strokes across multivessel areas, acute renal injury with haematuria, and skin imititis, intestinal ischaemia, as well as other organ ischemic manifestations. This finding is supported by worldwide pathological reports of extensive pulmonary venous thrombosis and peripheral organ thrombosis with pauci-immune cell infiltrates.

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UDC: 616.329-009.1-053.2

CASE REPORT

ACHALASIA OF THE CARDIA - A PEDIATRIC CASE REPORT

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ABSTRACT

Introduction: Achalasia is an esophageal motor disorder characterized by aperistalsis of the esophageal body and lack of relaxation of the lower sphincter in response to swallows. It affects both sexes and all age groups. The exact etiology of this degeneration is unclear though many theories have been proposed. Possible etiology of achalasia can be familial, infection or autoimmune. Patients often present with progressive dysphagia to solids and liquids, heartburn, chest pain, regurgitation, and varying degrees of weight loss or nutritional deficiencies.

Case report: In January 2020, a 10 years old boy was admitted to our hospital due to vomiting, chest pain during swallowing, and prolonged cough. A lot of investigations were performed. Fyberbronchoscopy showed that the trachea, the right main bronchus and the left main bronchus were with anteroposterior narrowed lumen. Computed tomography of thorax and abdomen showed dilated esophagus with largest diameter of 45 mm subcarinal with retained content.There is a compression of the right hillus. The finding indicates achalasia of the cardia. One month after the hospital stay, the child was admitted at the Clinic for pediatric surgery. Laparoscopic Heller Myotomy (LHM) with antireflux Dor fundoplication was performed. The following period the boy has no gastric or respiratory symptoms.

Conclusion: We can conclude that it is worth to suspect achalasia in a pediatric patient with prolonged cough and recurrent respiratory infections refractory to conventional treatments such as bronchodilators, especially if the patient also has a history of vomiting and dysphagia.

Keywords: achalasia, esophagus, pediatric

INTRODUCTION

Achalasia is an esophageal motor disorder characterized by aperistalsis of the esophageal body and lack of relaxation of the lower sphincter in response to swallows. It affects both sexes and all age groups [1,2]. Achalasia was first described by Willis [3] in 1674 as “food blockage in esophagus”. He treated these patients successfully with a dilator made of whale bone and sponge [3].

Achalasia (AC) is a major primary esophageal motor disorder with a reported incidence of approximately 1.63/100,000 of population [4]. Recent studies have shown that the actual incidence is far higher than that previously reported [5,6].

Most of cases are idiopathic, but the syndrome can be associated with malignancy (especially involving the gastroesophageal junction) and as a part of the spectrum of Chagas disease. Rarely, achalasia is genetically transmitted [7].

Achalasia is thought to occur from the degeneration of the myenteric plexus and vagus nerve fibers of the lower esophageal sphincter. [8,9] There is a loss of inhibitory neurons containing vasoactive intestinal peptide (VIP) and nitric oxide synthase at the esophageal myenteric plexus, but in severe cases, it also involves cholinergic neurons [10,11].

The exact etiology of this degeneration is unclear though many theories have been proposed. Possible etiology of achalasia can be familial, infection or autoimmune.

The existence of familial cases may suggest that in some achalasia is an inherited disease [12-15]. Such familial cases have been mostly seen in the pediatric population, between siblings and in a few cases in monozygotic twins [12,13], and there are also a few reports of a parent-child association for achalasia [14].

Several studies, where measles and varicella zoster virus antibodies were found to be higher among a number of achalasia patients, have suggested a possible association between viral infections and achalasia [16,17]. Strong piece of evidence in favor of infection in the pathogenesis of achalasia, however, is the fact that Chagas disease, caused by Trypanosoma cruzi, very closely mimics the pathophysiology of primary achalasia [18].

An autoimmune etiology for achalasia has been considered because of the presence of neural inflammation in absence of conclusive evidence of infection. Studies have demonstrated inflammatory cell infiltrate of the myenteric plexus in 90%-100% of esophageal specimens from achalasia patients [19,20]. All of these evidences are not sufficient to conclude what is the etiology of achalasia. More studies are needed to explore the exact cause of this enigmatic disease.

Patients often present with progressive dysphagia to solids and liquids, heartburn, chest pain, regurgitation, and varying degrees of weight loss or nutritional deficiencies [21,22]. Classically, achalasia presents as progressive dysphagia to solids and liquids. Heartburn may present in 27% to 42% of patients with achalasia, and thus, patients are frequently misdiagnosed with gastroesophageal reflux disease (GERD) and treated with proton pump inhibitor (PPI) therapy [23] Younger patients are more likely to have chest pain and heartburn. Some achalasia patients may also experience respiratory symptoms such as cough, wheezing, and hoarseness. Respiratory symptoms are observed in over 40% of patients with achalasia. Most of these occur at least daily and may be secondary to retention of food with regurgitation, the mass effect of a dilated esophagus, or both [24].

CASE REPORT:

In January 2020, a 10 year old boy was admitted to our hospital due to vomiting, chest pain during swallowing, and prolonged cough.

From the anamnestic data, the child was healthy until 8 years of age, when he started to vomit after every meal, and lost 6 kg of his weight. He also had respiratory symptoms such as cough and wheezing, and was investigated for asthma, with positive skin prick tests for *Dermatophagoides*. He started taking inhaled topical corticosteroid fluticasone several months before admission to hospital. Because of the vomiting the patient was first sent at the University pediatric clinic at the gastroenterohepatology department. Many investigations, such as abdominal ultrasound, tests for celiac disease, coproculture, testing for Helicobacter pylori were made, and all of them were with normal finding. Two months ago, he was treated with Azythromycin because of *Mycoplasma pneumoniae* infection.

At admission, the boy was afebrile, pale, with frequent productive cough, moderate dyspnea, and auscultatory on the lungs with pneumonic finding and moderate bronchoopstruction. Heart sounds were rhythmic. Heart rate was 70 beats per minute. Arterial blood pressure was 100/70 mmHg. Palpation revealed that abdomen was soft and painless in all areas.

Diagnostic findings: Complete blood count – hemoglobin 140g/L, erythrocytes $4,5 \times 10^{12}$ leukocytes $7,9 \times 10^{9}$, Platelets 207×10^{9}, ESR 20/50 mm/h, CRP 2,5 mg/l.

Blood biochemistry: total protein 60 g/L, albumin 38 g/L, Na 139 mmol/l, K 4,07 mmol/l Cl 107 mmol/l, Hepatal enzymes: AST 21 u/l, ALT 19 u/l, blirubin 14 mcmol/l, gamma GT 8 u/l.

Urine- all findings were within normal range. Serology for *Helicobacter pylori* was negative.

Because of the prolonged cough investigations for tuberculosis were realized - Mantoux test, Quantiferon TB gold test, ARB and Lowenstein-Jensen culture, all of them were normal.

Chest X-ray showed accentuated lung pattern on the right side, without parenchymal consolidation (Figure 1).

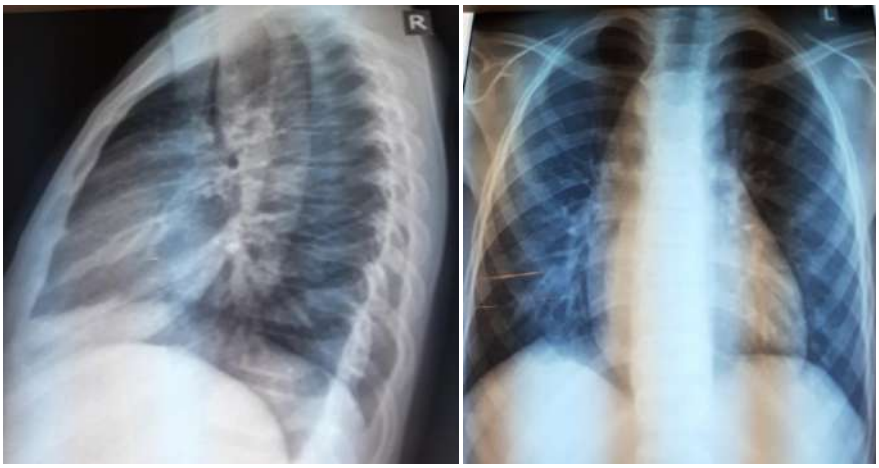

Fig. 1. Chest X-ray

Abdominal ultrasound was with normal findings.

Fyberbronchoscopy showed that the trachea, the right main bronchus and the left main bronchus were with anteroposterior narrowed lumen. The finding suggested malformation of the tracheobronchial tree with compression from the outside.

Because of this finding, computed tomography of thorax and abdomen was performed. It showed dilated esophagus with largest diameter of 45 mm subcarinal with retained content. There is a compression of the right hillus. The finding indicates achalasia of the cardia. (Figure 2)

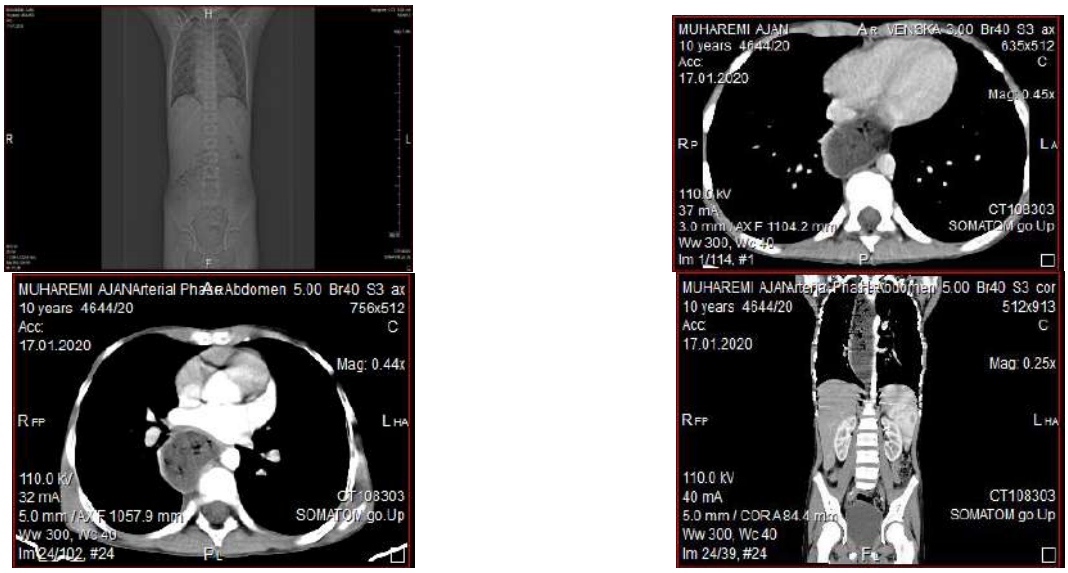

Fig. 2. CT of thorax and abdomen

The patient was treated with antibiotics, inhaled bronchodilator, and corticosteroids in the first several days, proton-pump inhibitor. During the hospital stay he had gastric pain and vomiting after meal in the first week, and the symptoms withdrew after we started the therapy with omeprazole and diet with small but more frequent meals. The auscultatory lung finding was getting better every day, until it was completely withdrawn. He was discharged from hospital with proton pump inhibitor therapy, inhaled corticosteroid Fluticasone, and with advice for diet. One month after the hospital stay, esophagogram with act of swallowing was performed, the diagnosis of achalasia was confirmed, and the child was admitted at the Clinic for pediatric surgery. Laparoscopic Heller Myotomy (LHM) with anti-reflux Dor fundoplication was performed. In the postoperative period the child was in good condition, with antibiotic and analgetic therapy, and after 2 weeks he was discharged from hospital. We were following the child's condition after the surgical treatment. In this period, he had no gastric or respiratory symptoms.

DISCUSSION:

This case report represents a 10 years old boy with idiopathic achalasia of the cardia, who had gastric symptoms and prolonged cough. The findings of tracheal and bronchial compression on fyberbronchoscopy leaded us to further investigations and finally to the diagnosis of achalasia.

Although respiratory symptoms are unlikely to precede the more typical symptoms of achalasia such as dysphagia and regurgitation, cough as a primary presenting symptom has been reported in the pediatric population [25].

Laparoscopic Heller Myotomy **(**LHM) performed with or without antireflux fundoplication (Dor) is a highly effective treatment for achalasia. Originally performed as an open thoracotomy and laparotomy, the less-invasive laparoscopic approach has similar efficacy rates but decreased morbidity [26,27]. Patients also need to understand necessary lifestyle changes following myotomy, such as the need to eat small food boluses in an upright position, which allows gravity to assist with food transit and never to lay flat but rather at 30 to 45 degrees due to increased risk for aspiration.

CONCLUSION: From this case report we can conclude that it is worth to suspect achalasia in a pediatric patient with prolonged cough and recurrent respiratory infections refractory to conventional treatments such as bronchodilators, especially if the patient also has a history of vomiting and dysphagia.

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UDC: 617.751.6-085.214

REVIEW ARTICLE

EFFECTS OF CITICOLINE ON AMBLYOPIA

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ABSTRACT

Amblyopia is a decrease in visual acuity, which cannot be attributed to any structural abnormality of the eye or visual pathway, and leads to partial loss of vision due to inadequate stimulation in early childhood. It is still a challenge for ophthalmologists working in pediatric ophthalmology. Several methods of solving have been tried in the past, but the occlusion of the leading eye remains the most effective today.

The aim of this paper is to emphasize the importance of citicoline in the treatment of amblyopia, as a single or as an adjunct treatment.

Citicoline (cytidine-5'-diphosphocholine) is an intermediate by-product involved in the biosynthesis of cell membrane phospholipids. After systemic administration, it degrades into its constituents cytidine and choline. Once absorbed, it crosses the blood-brain barrier and is incorporated into phospholipids in the cell membrane. Increases norepinephrine and dopamine levels in the CNS, thus contributing to neuroprotection in hypoxic and ischemic conditions.

The effect of citicoline in amblyopia is attributed to the modification of neuronal mechanisms in the genital body and visual cortex, where functional and morphological changes occur in amblyopia.

A significant number of published studies indicate the efficacy of citicoline in the treatment of amblyopia, even in adults and older children, outside the plastic period of vision development.

Keywords: amblyopia, citicoline, neuromodulators, CNS, pediatric ophthalmology

INTRODUCTION

Amblyopia is a decrease in visual acuity that can not be attributed to any structural abnormality of the eye or visual pathway, and is characterized by partial or complete loss of vision due to inadequate stimulation in early childhood [1].

Although in the past there was a general belief that the treatment of amblyopia over a certain age is ineffective, finally there is promising treatment and research that indicates that age is not an obstacle to successful treatment of anisometric amblyopia. Some authors believe that after the age of six or seven the response to treatment is less successful, while others believe that by the age of nine or ten successful treatment can be achieved. There are many studies involving older children and adults with amblyopia that respond to occlusion treatment as the most successful treatment to date [2, 3].

The prevalence of amblyopia ranges from 1 to 5% of the general population and is the most common cause of preventable vision loss. Various treatments have been tried such as refractive correction, occlusion (full-time and part-time), penalization and pharmacological therapy. Refractive correction alone improves visual acuity in only one-third of patients with anisometric amblyopia. Various drugs have also been tried, including carbidopa and levodopa. Most of these agents are still in the experimental phase, although levodopa-carbidopa combination therapy has been extensively studied in human amblyopia with good results. Levodopa therapy may be considered in cases of residual amblyopia, although treatment with occlusion remains the primary treatment of choice [4].

The most accepted theory of amblyopia is that it is a consequence of competition in the "input" of each eye to the visible cortical cells. This phenomenon of binocular competition, in addition to age, has been shown to depend on the presence of certain neurotransmitters and neuromodulators in the brain [4].

Citicoline is an intermediate by-product involved in the biosynthesis of cell membrane phospholipids. After systemic administration, it degrades into its constituents cytidine and choline. Citicoline, once absorbed, crosses the blood-brain barrier and is incorporated into phospholipids in the cell membrane. Increases norepinephrine and dopamine levels in the CNS, thus contributing to neuroprotection in hypoxic and ischemic conditions [5].

Based on these neuromodulatory properties, it may be useful in ophthalmic conditions such as amblyopia and glaucoma. In addition, it can be used in organic brain disorders such as cerebral vascular disease, head trauma, cognitive impairment, posttraumatic coma and neurological deficits, post-comotio syndrome, acute ischemic cerebral vascular disease, senile cognitive impairment diseases and chronic cerebral vascular disease, Parkinson's disease and drug addictions [5, 6].

MATERIAL AND METODS

We searched the largest database for medical publications Medline database, by entering the keywords: amblyopia, citicoline, neuromodulators, CNS, pediatric ophthalmology.

We have received data for 15 published articles in the past 10 years, however, the research shows us that Google search and Google Scholar have to offer us as much more articles than Medline.

In this review paper we showed the most important observations from the literature, as well as our observations from the research in this direction.

Mode of action of citicoline

Citicoline (cytidine 5-diphosphocholine) stimulates the dopaminergic system in the brain and retina, increasing the level of the retinal neurotransmitter (dopamine) [7].

Citicoline is an intermediate product in the production of phosphatidylcholine, a phospholipid on the cell membrane. Citicoline activates the synthesis of structural phospholipids in the membrane, increases brain metabolism and affects neurotransmitter levels. It has been shown to increase levels of acetylcholine, norepinephrine, and dopamine in the central nervous system. Thanks to these pharmacological mechanisms, citicoline has a neuroprotective effect in hypoxic and ischemic conditions, reduces the extent of ischemic lesion, and also improves learning and memory performance in animal models of brain aging. It is currently used in the treatment of Alzheimer's and stroke as a brain stimulant [5, 6, 8].

It has been shown that citicoline restores the activity of mitochondrial ATPase and membrane Na + / K + ATPase, thereby accelerating the resorption of cerebral edema in various experimental models. It has also been shown to inhibit apoptosis in neurodegenerative models, enhancing neuroplasticity [5, 6].

Citicoline is a safe and effective drug in enhancing the endogenous mechanisms of neurogenesis and neuroreparation. Therefore, it is used in the treatment of neurological diseases, with minimal side effects. Improves functional outcome and reduces neurological deficit in acute stroke [9, 10].

The effect of citicoline on amblyopia can be attributed to modifications of neuronal mechanisms in the genital body and visual cortex, where functional and morphological changes are known to occur in amblyopia [11].

Studies show that citicoline improves visual performance in amblyopia, increases visual acuity, improves contrast sensitivity, and has effects on VEP (visual evoked potentials) [12].

Citicoline is well tolerated in long-term use without significant systemic cholinergic effects [9].

Effect of citicoline in children with amblyopia

Campos and co. conducted an open-label study, started in 1991, of fifty patients with amblyopia treated with citicoline, 1000 mg daily for 15 days, in an age group outside the plastic period of the CNS. Statistically significant improvement in visual acuity was found in both amblyopic and healthy eyes in 46 of 50 patients (92%). The action was different for normal and amblyopic eyes. The improvement remained stable for at least 4 months. These results were confirmed by a double-blind study. No side effects were observed. Citicoline has been shown to improve visual acuity in amblyopic patients outside the plastic period of the visual system [13].

In a study in Indonesia, the effect of Citicoline was studied in children aged 5 and 6 years with amblyopia. They were grouped according to the severity of the amblyopia (mild, moderate, severe) and according to the duration of the therapy, 3, 4 and 6 months respectively. Citicoline has been shown to be effective in mild to moderate amblyopia for 3 to 6 months ($p < 0.05$). Other groups had significant clinical improvement, but no adequate data were available for analysis [1].

The Prabha and Lagre study was performed on a total of 378 patients aged 5 to 30 years with myopic amblyopia. Patients were divided into two groups. One group of patients was given oral citicoline (500 mg) daily for 12 weeks, while the other group of patients was given a placebo for 12 weeks. Visual acuity improved or remained stable over time after citicoline therapy compared with placebo. The maximum improvement in visual acuity was from 6/60 to 6/9. The conclusion was that citicoline could be a promising therapy for slowing the progression of myopia and myopic amblyopia in the future [7].

A randomized controlled trial conducted in India examined the effect of citicoline in amblyopia, with the dose being 250 mg for children under 5 years and 500 mg for children over 5 years. The patients were divided into two groups, and the aim was to investigate the effect of citicoline as an adjunct treatment in amblyopia, along with occlusion. Both groups received "occlusion" therapy until the plateau in phase 1 of the study was reached. Then, in stage 2, group 1 received citicoline plus occlusion, and group 2 continued with occlusion only. The results indicate that no significant difference was found in the average visual acuity of these two groups in phase 1, up to which the plateau was reached. In phase 2, for the first four months, there was no significant difference in visual acuity between these two groups, at the corresponding intervals. However, five months later, up to 12 months, there was a significant difference in visual acuity in these groups. The result was the same in younger patients (<seven years) as in older patients (> seven years of age). In phase 2, the average proportional improvement in group 1 was significantly more pronounced than in group 2, for two months and longer, at appropriate intervals. The conclusion was that in the group that received cyticoline in addition to the occlusion, there was a significant improvement in visual acuity during one year, in contrast to the group where only occlusion was performed. And the improvement was in patients under 7 years and over 7 years [8].

In a study in Pakistan of 22 children with reduced vision, in addition to visual acuity, contrast sensitivity was also studied. The children were divided into two groups of 11 children in each group. One group consisted of eleven children with impaired vision due to bilateral congenital cataract, operated on later than the first year, and other conditions that obscure the optical media, assuming it was visual deprivation amblyopia (VDA). The other group consisted of eleven children with impaired vision due to retinal or optic nerve disease, as a group with peripheral visual impairment (PVI). All children received intramuscular citicoline, 1 gram, for 10 consecutive days. The first signs of improvement in visual function after treatment with

citicoline were observed in subjective assessments. With the exception of two children in the POV group, all other children reported that the environment appeared brighter and clearer during treatment with intramuscular injections of citicoline while receiving injections and almost 30 days after the start of treatment [14].

In many children in the VDA group, visual acuity increased during the 30-day test and remained at the same level or decreased during the following period. In the PVI group the changes in vision were more inconsistent but still there were children with improvement. Cyticoline treatment had a positive effect on visual acuity when all children were examined over time, but the effect was not statistically significant. Linear contrast analysis revealed an almost significant increase in visual acuity in the PVI group and a less significant effect for the VDA group [14].

It was found that children with impaired vision experienced a subjective improvement in visual function and often showed an improvement in visual acuity after treatment with citicoline. No side effects were observed, which is consistent with previous studies in children 5 to 7 years of age, except that most children reported pain in the arm during treatment, especially at the injection site [14].

Porciati and co. examined visual acuity, contrast sensitivity, and VEP in amblyopic patients before and after citicoline administration. Citicoline was administered systemically 100 mg / i.m once daily for 15 days. Significant improvement in visual acuity was observed, on average 1.4 to 1.5 lines in the amblyopic eye, while the contrast sensitivity in both eyes increased by 3 db, VEP amplitudes increased by about 30% [11].

In Sabetti's study and co. a total of 80 amblyopic patients, with an average age of 4.5 years, were included and followed for a period of 12 months. Patients were randomly divided into two groups as follows: 41 patients underwent anti-amblyopia therapy with Bangerter filter, while 39 patients underwent anti-amblyopic therapy with Bangerter filter together with citicoline. Citicoline was administered orally once daily 5 days a week throughout the study. The results showed that patients treated with the Bangerter filter alone had a 40% improvement in visual acuity, while the other group of patients treated with citicoline had a 75% improvement in visual acuity. The results of this study show that the combined anti-amblyopic treatment of Bangerter filter and citicolines leads to a significant improvement in visual acuity [15].

DISCUSSION

Amblyopia is a major cause of preventable vision loss. The prevalence ranges from 1-5%. Occlusion is still the most effective treatment. The general belief was that with this treatment vision improvement could only occur up to a certain age, up to 6 or 7 years, and according to others up to 9 or 10 years [2, 3].

The well-known theory is that amblyopia is a consequence of binocular competition, as well as the presence of certain neurotransmitters and neuromodulators [4].

Thus, levodopa-carbidopa combination therapy has been used to treat amblyopia since 1993. Dopamine is known to play an important role in retinal function and central visual processing [16].

Study conducted by Duffy et al. indicates that GABA antagonist drugs are effective in reversing the neuronal response in the myopic eye. According to this study, bicuculin restored binocularity in 50% of the tested visual cortical neurons and naloxone to 36% of the neurons [17].

Citicoline is used to improve the level of consciousness in cerebral trauma and as a complement to levodopa in Parkinson's disease [13].

Chronic treatment with citicoline leads to an increase in the density of dopamine receptors and promotes partial recovery of dopamine receptor functions (which normally decreases with

age). Citicoline activates the biosynthesis of structural phospholipids (phosphatidylcholine) in neuronal cell membranes, which increases neurotransmitter levels and enhances neuroprotection [8].

Citicoline stimulates glutathione synthesis and facilitates the storage of sphingomyelin, which promotes signal transduction into retinal nerve cells. Reduces retinal nerve fiber layer (RNFL) damage by reducing nitric oxide synthase levels and impairs nitric oxide function in retinal and spinal cord neurons [7].

CONCLUSION

Amblyopia is still a problem for pediatric ophthalmologists. Occlusion is the method of choice, but most studies have shown that the effect is achieved only up to a certain age, in the period of plasticity of vision development.

The use of citicoline, as a single or adjunctive treatment, has been shown to be effective in the treatment of amblyopia. Improvement in visual performance, ie increase in visual acuity after its administration is present in almost all amblyopic patients, even those aged, outside the period of CNS plasticity.

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