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ММР

Original article

INFLUENCE OF *CYP2D6* PHENOTYPE ON ADVERSE EVENTS IN SCHIZOPHRENIA PATIENTS ON RISPERIDONE TREATMENT

ВЛИЈАНИЕ НА *CYP2D6* ФЕНОТИПОТ ВРЗ НЕСАКАНИТЕ НАСТАНИ НА ПАЦИЕНТИ СО ШИЗОФРЕНИЈА НА ТЕРАПИЈА СО РИСПЕРИДОН

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Abstract

Antipsychotic drugs are widely used in the treatment of schizophrenia and psychotic disorder and there are large inter-individual differences in clinical response and side effects. Pharmacogenetic testing is a valuable tool in tailoring treatment based on personal genetic markers. The aim of this study was to confirm the influence of CYP2D6 phenotype in clinical response of 90 patients with schizophrenia on risperidone treatment hospitalized in the University Clinic for Psychiatry in Skopje, Republic of North Macedonia. The *CYP2D6* genotyping was performed with PGX-CYP2D6 StripAssay® according to the guidelines of the manufacturer. In line with all the published data, the most frequent adverse events are the following: agitation, anxiety, somnolence, dizziness, vertigo, suboptimal treatment response, rigor, increased body weight and non-adherence. Six poor metabolizers (PM) identified in the study population experienced statistically significant more prompt adverse reactions in comparison with patients with extensive/intermediate metabolizer (EM/IM) or extensive metabolizer (EM) phenotype. The final maintenance dose was increased with a confirmed statistically significant difference between the poor *vs.* extensive metabolisers (median 5*vs.* 4 mg, p=0.026). No statistically significant differences were confirmed in the starting doses of risperidone between the groups of patients with confirmed adverse events andpatients without adverse events. Our study has confirmed that antipsychotic treatment selection and dose adjustment should be empowered with *CYP2D6* pharmacogenetic testing in order to obtain rational approach in treatment selection, control of the positive and negative symptoms, appropriate dosing, reduced adverse events, better patient compliance, improved quality of life and cost reduction.

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Keywords: pharmacogenetics, antipsychotics, schizophrenia, *CYP2D6*, adverse events, non-adherence

Апстракт

Антипсихотичните лекови широко се користат во третманот на шизофренија и психози, при што се идентификувани бројни интериндивидуални разлики во клиничкиот одговор и несаканите ефекти кај пациентите. Фармакогенетските тестирања се важна алатка за прилагодување на третманот, врз основа на индивидуалните генетски маркери на пациентите. Целта на студијата беше да се потврди влијанието на *CYP2D6* фенотипот врз клиничкиот одговор на 90 пациенти со шизофренија на третман со рисперидон, хоспитализирани на Универзитетската клиника за психијатрија во Скопје, Република Северна Македонија.

CYP2D6 генотипизирањето беше извршено со PGX-CYP2D6 StripAssay®, според упатствата на производителот. Во согласност со сите објавени податоци, најчесто идентификувани несакани настани кај испитуваните пациенти беа: агитација, вознемиреност, сомоленција, зашеметеност, вртоглавица, супоптимален одговор кон третман, ригор, зголемена телесна тежина и непридржување кон терапијата. Кај шесте спори метаболизатори (СМ), идентификувани во популацијата на студијата, се забележани повеќе статистички значајни несакани реакции, во споредба со пациентите класифицирани во групата со фенотип на екстензивни/итермедиерни метаболизери (ЕМ/ИМ) или екстензивни метаболизери (ЕМ). Финалната доза на одржување е зголемена во испитувана популација на пациенти со потврдена статистички значајна разлика меѓу спорите и екстензивните метаболизери (медијана 5 vs 4 mg, p= 0.026). Не е потврдена статистички значајна разлика во почетната доза рисперидон меѓу групите пациенти, со и без појавени несакани настани. Нашата студија потврди дека изборот на антипсихотичен трет-

ман и прилагодување на дозата треба да бидат направени, според резултатите од фармакогенетските тестови на *CYP2D6,* со цел да се добие рационален пристап при изборот на третман, контрола на позитивните и негативните симптоми, соодветно дозирање, намалена појава на несакани настани, подобро придржување од страна на пациентот, подобар квалитет на живот и намалување на трошоците.

Клучни зборови: фармакогенетика, антипсихотици, шизофренија, *CYP2D6*, несакани настани, непридржување

Introduction

According to the World Health Organization and the World Bank, schizophrenia is the 9th most important cause of disability in persons aged 15 to 44 worldwide, and the 4th in developed countries [1]. Antipsychotic drugs are widely used in treatment of mental illness including schizophrenia, but 30-50% of treated patients fail to respond sufficiently and approximately 50% of them develop severe and long-lasting side effects. Second generation antipsychotics (SGA) have dual action. Besidesbeing dopamine antagonists, they also act as antagonists of serotonergic receptors, and even of the cholinergic, histaminergic and adrenergic receptors. Although SGA have a lower incidence of EPS, they are associated with other types of severe side effects such as metabolic syndrome (weight gain, diabetes, hypertension, obesity and dyslipidemia), sexual dysfunction and, in the case of clozapine, late onset of agranulocytosis. Atypical antipsychotics are considered first line treatment for schizophrenia in all clinical guidelines as they are equipotent as firstgeneration of antipsychotics in treatment of positive symptoms, but are superior in the treatment of negative, cognitive and depressive symptoms, with a lower risk of EPS [2]. Despite the fact that antipsychoticmedications areeffective for symptom-based psychopathology, such clinical effectiveness does not necessarily translate to improved general life satisfaction. Precision medicine and individualized approach regarding selection of appropriate pharmacological treatment and appropriate dose for each patient is the main characteristic of modern psychiatry. Pharmacogenetic tests play a significant role in drug selection in the naive patents, as well as in patient population that are treatment intolerant or treatment-resistant to evidence-based medicines. Genotyping results for pharmacokinetic and pharmacodynamic markers, epigenetic factors, along with patient's individual profile, symptom manifestation, adverse effects, family history and preferences can result in symptoms improvement, better drug tolerance, improved patients' quality of life and cost savings [3-5].

CYP2D6 and *CYP3A4* play a significant role in metabolic transformation of aripiprazole, quetiapine and risperidone. Chlorpromazine and olanzapine are metabolized with *CYP2D6* and *CYP1A2*, whereas haloperidol is metabolized by thesetwo metabolizing enzymes and *CYP1A2*. Risperidone is atypical antipsychotic drug that is widely used as first line monotherapy in schizophrenia and combined therapy in bipolar disorders [6].

*CYP2D*6 gene is highly polymorphic with over 100 allelic variants and numerous subvariants described in the Human Cytochrome P450 Database (www.cypalleles.ki.se). While the most frequent allelic variations are caused by single nucleotide polymorphisms (SNPs) and small insertions or deletions, highly homologous regions in the *CYP2D6* gene locus facilitate unequal cross-over leading to large deletions, duplications and gene conversions. The metabolic transformation of antipsychotics is determined by the genetic variations in *CYP2D6* enzyme with two or more alleles contributing to the genotype and could be transformed to phenotypes depending on the sum of activity score attributed to each allele [7-11]. This genotype-based phenotype calcifies the person in one of the four phenotypes, aspoor metabolizers (PM) without enzyme activity (activity score 0), intermediate metabolizers (IM) with reduced enzyme activity (activity score 0.5), extensive/intermediate metabolizers (EM/IM) (activity score 1) or extensive metabolizers with normal activity (activity score 1-2), and ultra-rapid metabolizers (UM) with increased enzyme activity with activity score over 2 [12].

Therapeutic plasma concentrations of risperidone and its active moiety are directly influenced by genetic variations in metabolic CYP450 enzymes (*CYP2D6* and *CYP3A4/5*) transporter (*ABCB1*) protein and additional epigenetic and environmental factors. Bork *et al.* have confirmed that the *CYP2D6* poor metabolizers have three times higher risk for adverse reactions associated with risperidone treatment in comparison with EM and IM and six times higher risk for treatment failure in comparison with EM [13,14]. 9-OH risperidone is the leading active metabolite with almost equipotent pharmacological antipsychotic activity as risperidone and the clinical response is a result of concentration of active moiety-AM (sum of risperidone and 9-OH risperidone). Psychiatry patientsusing peroral form of risperidone reveal 5 to 10 times higher plasma levels of 9-OH risperidone than risperidone. Because 9-OH risperidone crosses the blood brain barrier (BBB) to a lesser extent, it is supposed that this active metabolite is responsible for risperidone-induced prolactin secretion, probably as dopamine receptors on tuber-infundibular pathway are unprotected. Risperidone is highly bound (90%) to plasma proteins, while the 9-OH risperidone binding is on lower extent with 77% bounding. Since active metabolite is equipotent to the parent drug and contributes to a higher percentage in the pharmacologically active fraction, 9-OH risperidone is assumed that contributes

significantly to both therapeutic and adverse effects. Literature results have confirmed that maximum plasma concentrations of risperidone are reached after 1 hour in extensive metabolizers and after 3 hours in poor metabolizers. Linear elimination kinetics of risperidone results in reaching of steady state plasma concentrations in a period of one day for risperidone and five days for the active metabolite 9-OH risperidone, and both are dominantly eliminated in urine. The half-life of risperidone and 9-OH risperidone are 3 and 21 hours in extensive metabolizers, while in poor metabolizers 20 and 30 hours, respectively [15].

In addition to the genetic variability, risperidone dosage is affected by several additional factors, such as sex, age, ethnicity and body mass index, patient illness severity and concomitant therapy with *CYP2D6* inhibitors such as fluoxetine and paroxetine and *CYP3A4* inducers such as carbamazepine and phenytoin [16].

The aim of the study was to confirm the previous finding for the influence of *CYP2D6* phenotype in clinical response to risperidone treatment in schizophrenia patients hospitalized in the University Clinic for Psychiatry in Skopje, Republic of North Macedonia.

Material and methods

The study included 90 patients with diagnosed schizophrenia (41males and 49 females), aged from 20 to 63 years (37.02±11.88), unrelated and Caucasian, recruited in the University Clinic for Psychiatry at the University Clinical Center "Mother Theresa"-Skopje, Republic of North Macedonia. All subjects were adult patients hospitalized in the Acute Psychiatric Unit. The diagnosis of the patients was concluded according to the Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV, American Psychiatric Association, 1994). Written informed consent was obtained for each patient. All patients were with variable length of hospital stay and were treated with risperidone. Blood samples were obtained from patients with schizophrenia and genomic DNA was extracted from peripheral lymphocytes. This study was approved by the Ethics Committee of the Faculty of Pharmacy, Ss. Cyril and Methodius University, Skopje, Republic of North Macedonia. DNA samples were tested anonymously with previously removed personal data. All procedures were conducted in accordance with the Declaration of Helsinki.

DNA isolation

DNA isolation was performed in the Centre for Bimolecular and Pharmaceutical Analysis (CBPA) atthe Faculty of Pharmacy in Skopje.The genomic DNA was extracted from peripheral lymphocytes in the blood samples obtained in EDTA vacutainers, using Proteinase K digestion, phenol chloroform extraction and ethanol precipitation. DNA yields and purity were measured at 260 nm and 260/280nm respectively (NanoDrop 2000, Thermo Scientific) and DNA integrity was confirmed with electrophoresis on 1% agarose gels, stained with ethidium bromide.

CYP2D6 Genotyping

The genotyping was performed at the Institute for Immunobiology and Human Genetics at the Faculty of Medicine, Ss. Cyril and Methodius University in Skopje with PGX-CYP2D6 StripAssay® for *CYP2D6*with PGX-*CYP2D6*StripAssay® for *CYP2D6*[(EM=49 (CYP2D6 score 2), EM/IM=35 (CYP2D6 score 1) and PM=6 (CYP2D6 score 0)] according to the guidelines of the manufacturer (Vienna Lab Diagnostics GmbH). Bound biotinylated sequences weredetected using streptavidin-alkaline phosphatase and color substrates. The assay covers 3 polymorphic loci: 1795delT (2D6*6), 1934 G>A (2D6*4), 2637delA (2D6*3). Further genetic information is available at OMIM, Online Mendelian Inheritance in Man: www.ncbi.nlm.nih.gov/omim. The CYP2D6 alleles *1 (wild type), *3, *4, *6, and the resulting homozygous and heterozygous genotypes (*1/*1, *1/*3, etc.) weredetermined by probes for mutations 2637delA, 1934 G>A and 1795delT, respectively.

All 90 patients treated with risperidone and appropriate concomitant therapy were classified in three *CYP2D6* phenotypes. Six poor metabolizers were identified as a subpopulation highly prompt on adverse drug reactions when treated with drug therapy, and no ultra-rapid metabolizer (UR) was identified. Patient population was subdivided according to the obtained *CYP2D6* score in three groups. Extensive metabolizers-EM (*CYP2D6* score 2), extensive/intermediate metabolizers-EM/IM (*CYP2D6* score 1) and poor metabolizers-PM (*CYP2D6* score 0). The obtained results were in line with the confirmed frequency of 5-10% of poor metabolizers whereas ultra-rapid *CYP2D6* phenotype wasfound in only 1-2% of Caucasian population [17].

Data Analysis

Statistical analysis was performed using SPSS software (v. 23.0). The normal distribution of the data was tested with Kolmogorov-Smirnov andShapiro Wilk's tests. Quantitative variables are presented with arithmetical mean with standard deviation and median value presented as absolute and relative numbers. Bi-variant analysis was conducted in order to compare all phenotype groups (PMs, EM/IMs and EMs). The Pearson'schi-square test and Fisher exact test were used to test differences in the distribution of categorical variables. Analysis of Variance, Kruskal-Wallis and Mann-Whitney testswere used in order to compare the groups for the quantitative variables. The level of statistical significance was defined as $p \leq 0.05$.

Results

A complete overview of phenotype classification of *CYP2D6* based on genotyping results, sociodemographic, clinical, and pharmacological information from the patients ispresented in Table 1. The results confirmed that 6.6% or 6 patients wereclassified into the PM group (4 males*vs*. 2 females), 38.8 % or 35 patients (15 males*vs.* 20 females) into the EM/ IM group and all the remaining 49 patients or 54.4% wereclassified into the EM group (22 males*vs*. 27 females).Poor metabolizers were insignificantly older in comparison to EM/IMs and EMs (42.50±13.3 *vs.* 35.69±11.1 *vs.* 37.33±12.3 years). In the evaluated group ofpatients with diagnosed schizophrenia no ultra-rapid metabolizer was identified. A different education level had the examined patientsand it is presented in Table 1; 14.4% had finished primary school, most of the patients or 56.6 % had finished high school and 25.5% had a university degree. In all three evaluated groups of patients the high school level of education was dominant (66.7% in PMs, 52.8% in EM/IMs and 63.3% in EMs). No statistical difference was confirmed among the evaluated groups of patients based on their level of education. Thirty-five percenttage of the evaluatedpatients were married and 64% were not (60% in PMs, 61.1% in EM/IMs and 67.35% in EMs); 60% of the patients wereidentified as smokers; only 15.5% confirmed that they wereconsuming alcohol occasionally and 87.7% were on concomitant therapy. The mean age of first episode onset was atthe age of 29.95±10.24 years (29.7±7.3 years in PMs, 30.1 ±10.9 years in EM/IMs and 29.9±10.2years in EMs) and the mean number of hospitalizations was 1.454± 0.96 in the evaluated period (Table 1).

Table 1. Sociodemographic data, cigarettes and alcohol consumption, concomitant therapy and clinical data of patients at baseline according to CYP2D6 phenotype

Variable	Total	*CYP2D6* PM	EM/IM	EM	p value
N	90	6	35	49	
gender m / f	41/49	4 / 2	15/20	22/27	
age**,** (mean ±SD)	37.02±11.88	42.50±13.3	35.69±11.1	37.33±12.3	F=0.88 p=0.42
Education n(%)					
none	1(1.1)	0	1(2.78)	0	
elementary	13(14.4)	1(16.67)	6(16.67)	6(12.24)	[abc]p>0.05
high school	44(48.9)	4(66.67)	19(52.78)	31(63.27)	
Faculty	23(25.5)	1(16.67)	10(27.78)	12(24.49)	
Nationalityn(%)					
Macedonians	77(85.6)	6(100)	30(85.71)	41(83.67)	
Albanians	8(8.89)	0	2(5.71)	6(12.24)	[abc]p>0.05
Gypsies	3(3.3)	0	2(5.71)	1(2.04)	
Bosnjaks	2(2.2)	0	1(2.86)	1(2.04)	
Marital status n(%)					
yes	32(35.5)	2(40)	14(38.89)	16(32.65)	[abc]p>0.05
no	58(64.4)	3(60)	22(61.11)	33(67.35)	
Cigarettes consumptionn(%)	54(60)	4(66.67)	19(52.78)	31(63.27)	[abc]p>0.05
Alcohol consumptionn(%)	14(15.5)	1(16.67)	5(13.89)	8(16.33)	[abc]p>0.05
Concomitant therapy n(%)	79(87.7)	6(100)	30(85.7)	43(87.7)	[abc]p>0.05
First episode (mean ±SD)	29.95 ± 10.24	29.67 ± 7.3	30.08 ± 10.9	29.88 ± 10.2	H=0.16 p=0.92
No. of hospitalizations(mean ±SD)	1.454 ± 0.96	2.83 ± 1.8	9.89 ± 27.9	1.53 ± 0.7	F=2.39 p=0.098

[abc]p (PM vs EM/IM vs EM); EM-extensive metabolizers (CYP2D6 score=2); EM/M- extensive metabolizers/ intermediate metabolizers (CYP2D6 score=1); PM-poor metabolizers (CYP2D6 score=0)

Table 2 presents the adverse events that were identified in patients with diagnosed schizophrenia in our study. In line with all the published data, the most frequent adverse events werethe following: agitation, anxiety, somnolence, dizziness, vertigo, suboptimal treatment response, rigor, increased body weight and nonadherence. The obtained results have confirmed that all patients categorized as poor metabolizers had experienced statistically significant more prompt adverse reactions in comparison with patients with EM/IM or EM phenotype. In 40% of PMpatients agitation was experienced by 40%with a statistically significant difference compared to the group of extensive metabolizers where agitation was identified in only one patient or 2.04% (Fisher exact test p=0.002). Anxiety, somnolence rigor and suboptimal treatment responseoccurred in all

patients categorized as poor metabolizers with statistically significant differences in comparison to groups of EMs and EM/IMsas presented in Table 2. Dizziness was observed in 83.33% of PMs, whereas only 8.33% of EM/IMsand 6.12% of patients into the group of EMs experienced such an adverse event. Additionally, vertigo was confirmed in 80% of poor metabolizers, but only in 5.56% and 6.12% in the groups of EM/IMs and EMs, respectively. Increased body weight as an adverse event was confirmed in 60% of patients from PMs group in comparison with EM/IMs (8.33%, p< 0.016) and EMs (4.17%, p<0.004).Interesting finding

Table 2. Adverse events in patients with diagnosed schizophrenia on risperidone treatment

Variable	*CYP2D6*			p value
	PM	EM/IM	EM	
Agitationn(%)	2(40)	2(5.56)	1(2.04)	[b]p=0.002
Anxietyn(%)	5(100)	0	0	
Somnolence n(%)	6(100)	10(27.78)	11(22.45)	[a]p=0.0015 [b]p=0.00043
Dizzinessn(%)	5(83.33)	3(8.33)	3(6.12)	[a]p=0.00037 [b]p=0.00009
Vertigon(%)	4(80)	2(5.56)	1(2.04)	[a]p=0.00071 [b]p=0.00008
Suboptimal treatment responsen(%)	6(100)	2(5.56)	3(6.12)	[a]p=0.000005 [b]p=0.000003
Rigorn(%)	6(100)	10(27.78)	11(22.45)	[a]p=0.0015 [b]p=0.00043
Increased body weightn(%)	3(60)	3(8.33)	2(4.17)	[a]p=0.016 [b]p=0.004
Adherencen(%)	0	22(61.11)	33(67.35)	[a]p=0.0074 [b]p=0.0026

Fisher exact [[a]p(PM vs EM/IM), [b]p(PM vs EM)]; EM-extensive metabolizers (CYP2D6 score=2); EM/M- extensive metabolizers/intermediate metabolizers (CYP2D6 score=1); PM-poor metabolizers (CYP2D6 score=0)

Table 3. Evaluation of staring and finalmaintenance dosein schizophrenia patients

Variable	*CYP2D6*			p value
	PM	IM/EM	EM	
Starting dose (mean ±SD)	2.17±0.4	2.32±0.8	1.91±0.6	H=5.94
in (mg) (median)	2.0	2.0	2.0	p=0.051
Final dose (mean ±SD)	5.17±0.4	4.72±0.9	4.31±0.9	H=7.3 p=0.026
in (mg) (median)	5.0	4.0	4.0	[b]p=0.026

[b]p(PM vs EM),H (Kruskal-Wallis test); EM-extensive metabolizers (CYP2D6 score=2); EM/M- extensive metabolizers/intermediate metabolizers (CYP2D6 score=1); PM-poor metabolizers (CYP2D6 score=0)

Table 4. Influence of starting dose of risperidone on adverse events in patients with schizophrenia

Variable		Starting dose			p value
		n	(mean ±SD)	(median)	
Agitation	yes	5	2.0±0	2	Z=0.0 p=1.0 ns
	no	85	2.08±0.7	2	
Anxiety	yes	18	2.22±0.8	2	Z=0.77 p=0.44 ns
	no	72	2.04±0.7	2	
Somnolence	yes	27	2.04±0.5	2	Z=0.11 p=0.91 ns
	no	64	2.11±0.8	2	
Dizziness	yes	11	2.04±0.3	2	Z=0.02 p=0.98 ns
	no	80	2.09±0.8	2	
Vertigo	yes	7	2.0±0	2	Z=0.0 p=1.0 ns
	no	83	2.08±0.7	2	
Suboptimal treatment response	yes	11	2.18±0.7	2	Z=0.41 p=0.68 ns
	no	80	2.07±0.7	2	
Rigor	yes	27	2.17±0.7	2	Z=0.6 p=0.54 ns
	no	64	2.05±0.7	2	
Increased body weight	yes	8	2.37±0.7	2	Z=1.04 p=0.29 ns
	no	81	2.06±0.7	2	

Z **(**Mann-Whitney U Test)

in compliance with all the other available data from the literature is that the group of poor metabolizers are characterized with non-adherence, probably due to the previously clarified adverse events, compared to 61.11% (Fisher exact test p=0.0074) and 67.35% (Fisher exact test p=0.0026), for EM/IMs and EMs, respectively.

As presented in Table 3, the starting dose in the evaluated groups ofpatientswas at median of 2 mg [PMs (2.17±0.4), EM/IMs (2.32±0.80 and EMs (1.91±0.6), H=5.94 p=0.051] and a statistically significant difference was not confirmed among the groups. In most of the patients, irrespectively in which group they were categorized, the final maintenance dosewas increased with aconfirmed statistically significant difference between the group of poor *vs*. extensive metabolizers (median of 5 *vs*. 4 mg, p=0.026).

As presented in Table 4, no statistically significant differences were confirmed in the starting doses of risperidone between the groups of patients with confirmed adverse events andpatients without adverse events.

Discussion

Remarkable interethnic differences exist in the frequency of PM and UM phenotypes and may explain the differences in treatment response observed between different ethnicities. Frequencies are the subject of geographic variations, with 7-10% PMs in the Caucasian population and 1-2% in Asians.The most frequent nonfunctional alleles that account for 90.0-95.0% of PMs in native Europeans are CYP2D6**3, CYP2D6*4* and *CYP2D6*6*, whereas the *CYP2D6*4* contributing with an allele frequency of 12.0-21.0% is the most common allele associated with PMs [10]. A total of 11 different *CYP2D6* variant alleles have been identified in the healthy population ofthe Republic of North Macedonia with estimated prevalence of 0.324 in both inactive (**3,*4* and **6*) and decreased activity (**9, *10* and **41*) alleles and identified *CYP2D6* profile is in accordance with the frequencies reported for other European populations [18]. In line with this literature results were the data obtained in our study comprisingschizophrenia patients where four of six patients with PMs phenotype were with *CYP2D6*4* genotype and the other two were with *CYP2D6*6* genotype.

Antipsychotic drug selection and appropriate dose adjustment in schizophrenia patients is especially important in the first episode of psychosis, as the treatment is most effective during the first years of illness, and likelihood of treatment response decreases over time. Recent studies suggest that risperidone is the most frequently prescribed drug in over 36% of patients [19]. Poor metabolizers treated with antipsychotics with narrow dose ranges are more likely to develop adverse reactions to the treatment whereas ultra-rapid metabolizers will fail to respond to standard doses of antipsychotics. The results obtained in our study have confirmed that all patients categorized in the group of PMs experiencedside effects with a statistically significant difference in comparison with the other evaluated patients. Anxiety, somnolence, sub-effect and rigor were experienced in all PMs, whereas dizziness and vertigo occurred in over 80% of them. These results are in compliance with literature results where the *CYP2D6* poor metabolizers have been presented with a three times higher risk for adverse reactions associated with risperidone treatment in comparison with other patients and six times higher risk for treatment failure in comparison with EMs. The increased body weight is one of the most frequently identified adverse event in antipsychotics risperidone treatment. The PMs in our study were identified as the group with the highest risk for this adverse event and in 60% of them this adverse event was confirmed with a statistically significant difference compared to EM/IMs (8.33%, p<0.016) and EMs (4.17%, p<0.004)[13,14]. In line with this are the results for the non-adherence of PMs in our evaluated schizophrenia patients. According to obtained results, dose adjustment is necessary for these patients. Additionally, our study has confirmed that the starting dose of risperidone does not influence the occurrence of the adverse events in the patient population. No statistically significant differences were confirmed in the starting doses of risperidone between the groups of patients with confirmed adverse events andpatients without adverse events. The group of PMs arealso characterized with a statistically significant increase of maintenance dose compared to other patients and this fact is also associated with more adverse events. Although antipsychotic treatment was initiated with symptom-based psychopathology, the clinical effectiveness does not translate to improved general life satisfaction. Published data suggest that dose adjustments according to patient's genetic profile may result in around 15% efficacy improvement, more than 20% reduction in adverse effects. The results obtained in our study have confirmed that pharmacogenetic testing could help in earlier reaching the effective dosage by omission of increased risk for adverse reactions, improved patient adherence and compliance, better patients' satisfaction and reduction of hospitalization costs [5,20,21].

Conclusion

The multifactorial etiology of schizophrenia, as well as the polygenetic profile of antipsychotic treatment response which integrate both the genes involved in the pharmacokinetics (basically CYPs) and in the pharmacodynamics (receptors), have to be taken into account when tailoring the antipsychotic treatment.The results obtained in the first study evaluating the adverse events in schizophrenic patients on risperidone and their *CYP2D6* phenotype in North Macedonia have confirmed their increased occurrence, necessity of dose adjustment and

decreased adherence in PMs compared to other patients. Our study has confirmed that antipsychotic treatment selection and dose adjustment should be empowered with pharmacogenetic testing in order to obtain rational approach in treatment selection, control of the positive and negative symptoms, appropriate dosing, reduced adverse events, better patient compliance, improved quality of life and cost reduction.

Conflict of interest statement. None declared.

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Original article

ASSOCIATION BETWEEN FIRST TRIMESTER OBESITY AND SOME MATERNAL CHARACTERISTICS

АСОЦИЈАЦИЈА НА ПРИСУТЕН ОБЕЗИТЕТ ВО ПРВ ТРИМЕСТАР ОД БРЕМЕНОСТА СО ОДРЕДЕНИ МАЈЧИНИ ОБЕЛЕЖЈА

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Abstract

Introduction. Obesity in pregnant women has substantial negative impact on both pregnancy and labor/delivery. The aim of this article was to determine first trimester obesity-frequency, and its association with some maternal history data.
Methods. In this cross-sectional study, 809 first trimester pregnant women were evaluated for obesity defined as BMI ≥30. The data were collected via a questionnaire.
Results. BMI ≥30 had17.3% of all participants, 17.0% of them were Macedonian and 17.5% Albanian. BMI ≥30 had 23.9%of those with completed primary school, 16.4% of those with completed high school, and 14.8% of those with university degree; 18.7% of those with total family income less than 350 euros, 14.9% of those with total family income between 350-700 euros, and 14.8% of those with total family income above 700 euros. BMI ≥30 had 13.2% of nulliparous, and 20.6% of parous women. Among smokers, BMI ≥30 had 28.8%, but only 15.3% in nonsmokers. Regarding the IPI (interpregnancy interval), 26.2% in those with IPI ≤12 months had BMI ≥30, and 19.6% of those with IP I>12 months. Women aged40 years and over had BMI ≥30 in 40% of cases*versus* 16.8% of pregnant women younger than 40 years.
Conclusion. Obesity showed statistically significant negative association with level of education (p=0.010), positive correlation with smoking (p=0.003), age of the mother (p=0.019) and parity (p=0.006); obesity showed negative, but statistically insignificant association with IPI and family wealth, and showed no association with nationality.

Keywords: obesity, pregnancy, smoking, parity, age

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жена има значително негативно влијание врз бременоста и породувањето. Целта на овој труд е утврдување на честотата на обезитетот во првиот триместар од бременоста и неговата асоцијација со некои податоци од мајчината историја
Метод. Во пресечна студија, 809 трудници во прв триместар од бременоста беа евалуирани за обезитет, дефиниран како ВМІ≥30. Податоците за мајчините обележја беа собрани преку прашалник.
Резултати. ВМІ ≥30 имаа 17.3% од сите испитанички, вклучувајќи 17.0% Македонки и 17.5% Албанки. ВМІ ≥30 имаа 23.9% од испитаничките со основно образование, 16,4% од тие со средно, и 14.8% од испитаничките со високо образование. 18.7% од трудниците со вкупни семејни примања до 350 евра имаа ВМІ ≥30, наспроти 14.9% трудници со приход меѓу 350-700 евра, односно 14,8% трудници со семеен приход над 700 евра.
Обезни беа 13.2% од нулипарите, наспроти 20.6% жени со породување зад себе. Меѓу пушачите, обезни беа 28.8% од нив, додека само 15.3% непушачи имаа ВМІ ≥30. Што се однесува до ИМБ (интервалот меѓу бременостите), 26.2% од тие со ИМБ≤ 12 месеци и 19.6% од тие со ИМБ>12 месеци, имаа ВМІ ≥30. Трудниците на возраст ≥ 40 години беа обезни во 40% од случаите, наспроти 16.8% трудници помлади од 40 години.
Заклучок. Обезитетот покажува статистички значајна: негативна асоцијација со степенот на образование (p=0.010), позитивна асоцијација со пушењето (p=0.003), возраста на мајката (p=0.019) и паритетот (p=0.006). Обезитетот покажува негативна, но статистички незначајна асоцијација со ИМБ и семејните приходи, но не покажува асоцијација со националноста.

Клучни зборови: обезитет, бременост, пушење, паритет, возраст.

Introduction

According to WHO, obesity is considered as one of the most serious global health problems of the 21st century [1]. Defined by using its most frequent indicator (body-mass index, BMI), as a BMI equal or greater than 30 [2], obesity shows substantial negative impact on human health and well-being.

The prevalence of obesity in pregnancy varies from 1.8 to 25.3% (WHO) and represents major risk factor for both maternal and fetal complications [3-5].

Regarding the expectant mother, obesity doubles the risk of thrombosis [6,7], double to triple the risk of pre-eclampsia [4,8]; by other systematic reviews the risk can be 3-10 times higher [3], and puts obese pregnant women to two to three-fold higher risk to develop gestational diabetes [5,9,10]. Furthermore, obese mother-to-be have two-fold increase in the risk of cesarean section even without additional risk factors [11,12], which is of great concern in terms that these women are prone to postoperative complications, such as excessive blood loss, deep venous thrombosis, wound infection etc.

Regarding the fetus, maternal obesity is linked to fetal demise. According to some studies, these is up to five-fold increase in fetal death in obese women [13-15]. Excessive maternal body fat leads toinability to display the fetal anatomy on ultrasonogram, which might be the reason for a higher incidence of congenital anomalies in fetuses with obese mothers [5,16]. Finally, macrosomia and its connection with maternal obesity has been well documented in many studies [5,17,18].

The aim of this study was to find out whether there was an association between some maternal characteristics and coexistent obesity, in terms of clarifying the causes that to be identified in fighting obesity.

Material and methods

This was a cross-sectional study which took place in the Special Hospital for Obstetrics and Gynecology "Mother Tereza"- Skopje, between 02.2018-03.2019. All pregnant women in the first trimester of pregnancy attending the hospital for aneuploidy-screening were enrolled in the study. The sample size was 809 participants. The data from patients' maternal history were collected by a questionnaire. The height and weight were measured, and the body-mass index was calculated as kg/m^2. Subjects with BMI equal or more than 30 were considered obese.

Results

The subjects' maternal history data and BMI are summarized in Table 1.

Table 1. Maternal characteristics and BMI in enrolled population

	n= 809	100 %
BMI, kg/m2		
BMI≥ 30 kg/m2	140	17.3
BMI<30 kg/m2	669	82.7
Nationality		
Macedonians	229	28.3
Albanians	536	66.3
Others	44	5.4
Age (years)		
≤ 40	794	98.1
> 40	15	1.9
Parity		
Nulliparous	363	44.9
Parous	446	55.1
IPI (inter-pregnancy interval) months		
IPI ≤ 12	42	9.5
IPI > 12	397	90.5
Smoking		
Yes	118	14.7
No	691	85.3
Level of education		
Primary school	176	21.7
High school	275	34.0
University degree	358	44.3
Chronic hypertension		
Yes	22	2.7
No	787	97.3
Family income		
< 350 euros	300	37.0
350-700 euros	309	38.1
>700 euros	200	24.7

As shown above, from all 809 participants 28% were Macedonians, 66% were Albanians and 6% were with other ethnical background; 98% were 40 years or younger, and only 2% were older than 40; nulliparous were 45%*versus* 55% of parous women. Among the latter, 9.5% had interval between pregnancies equal or shorter than 12 months, opposite to 90.5% of the rest with IPI longer than a year; 15% of participants declared themselves as smokers, and 85% denied smoking. Regarding the level of education: every fifth subject had completed only a primary school, every third had completed a secondary school, and 44% were with a university degree. Positive history for chronic hypertension had less than 3% of subjects and 97.3 % had no such condition. Finally, 37% of women lived in a family with an income lower than 350 euros, 38% had family income between 350-700 euros, and only every fourth had more than 700 euros income.

The same variables were evaluated in subgroup of obese patients and the difference with a statistical significance, expressed as a p value <0.05 (when confronting with control i.e. non-obese subgroup) was searched for. The results are summarized in Table 2.

Table 2. Maternal characteristics in obese subgroup

	Total sample	Obese population	%	p-value
Nationality				
Macedonians	229	39	17.0	0.87 (*vs.* A)
Albanians	536	94	17.5	
Others	44	7	15.9	
Age * (years)				
<= 40	794	134	16.8	**0.019**
>40	15	6	40.0	
Parity *				
Nulliparous	363	48	13.2	**0.0056**
Parous	446	92	20.6	
IPI (inter-pregnancy interval) (months)				
IPI <= 12	42	11	26.2	0.31
IPI>12	397	78	19.6	
Smoking **				
Yes	118	34	28.8	**0.0035**
No	691	106	15.3	
Level of education *				
Primary school	176	42	23.9	**0.049** (*vs.* P)
High school	275	45	16.4	**0.010** (*vs.* P)
University degree	358	53	14.8	
Chronic hypertension				
Yes	22	11	50	**0.00004**
No	787	129	16.4	
Family income				
< 350 euros	300	56	18.7	
350-700 euros	309	46	14.9	0.212
>700 euros	200	38	14.8	0.925

Chi square test: * p<0.05; ** p<0.01

The prevalence of obesity in the first trimester of pregnancy was 17.3%. The prevalence among pregnant Macedonians *vs.* Albanians showed no difference (17.0 *vs.* 17.5, respectively). Although higher in obese subgroup, IPI ≤12 months failed to show a statistical significance in distribution. With 37% in general population and 40% in obese-one, low family income also failed to show association with the first trimester obesity.

Among the variables that showed such association, chronic hypertension (Chr. HTA) had the strongest impact, as presented in Table 3.

Twenty-twosubjects experienced Chr. HTA, but 11 of them were among 140 obese patients, and 11 among 669 non-obese, which created a difference with a statistical significance of p=0.00004.

Table 3. Presence of chronic hypertension

BMI (kg/m^2)	Chr. HTA +	Chr. HTA -	
>30	11	129	
≤ 30	11	658	p=0.00004
Chi-square test			

In other words, (Figure 1), only 2 % of pregnant womenin non-obese population experienced chronic hyper-

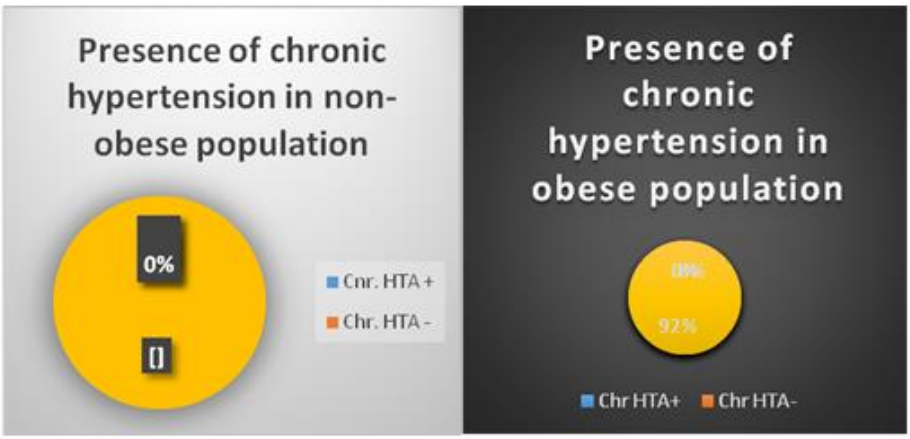

Fig. 1. Chronic hypertension presence: non-obese *vs.* obese population

tension *vs*. 8% of obese-one.
Smoking as a variable, with a statistical significance of p=0.035 also did demonstrate anassociationwith the first trimestral obesity (Table 4).

Table 4. Smoking habits in obese/ non-obese subgroup

BMI (kg/m^2)	Smokers	Non-smokers	
>30	34	106	
≤ 30	84	585	**p=0.00349**

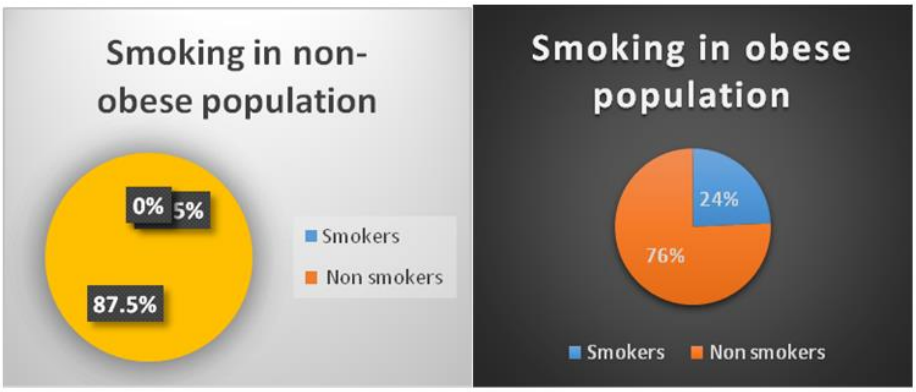

Fig. 2. Smoking: non-obese *vs*. obese population

With 24.3% smokers in obese patients, and only 12.5 % of those with BMI lower than 30 (Figure 2), smoking wasassociated with the first trimester obesity.
Parity, with p=0.0056, also wasstatistically significantly associatedwith obesity in favor of pluriparity (Table 5). 65.7% of those with BMI ≥ 30*versus* 52.9 % of non-

Table 5. Parity in obese/non-obese subgroup

BMI (kg/m^2)	Nulliparous	Parous	
>30	48	92	
≤ 30	315	354	**p=0.005623**

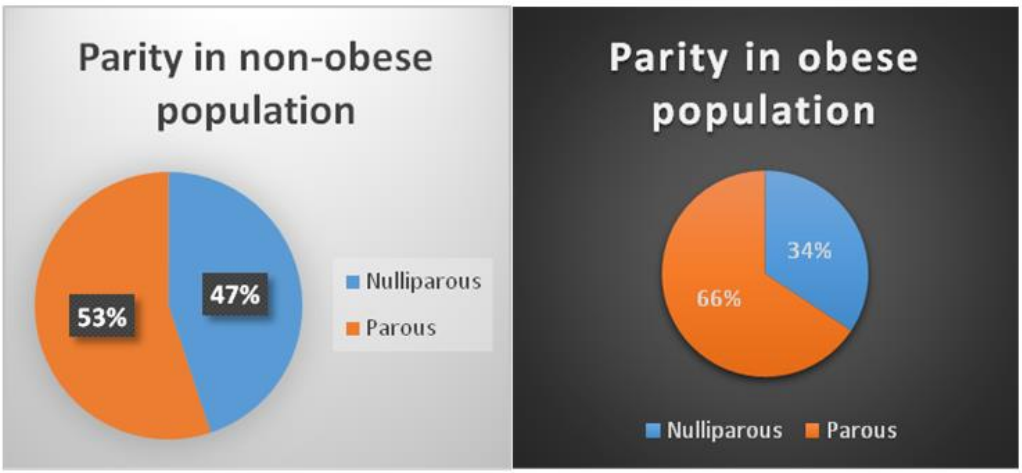

Fig. 3. Parity: non-obese *vs*. obese population

obese patients, or 55% in general population (Figure 3) were women with previous childbirth.
Advance age of the mother wasmore frequently and statistically significantly (p=0.019) observed in obese subgroup of subjects (Table 6).
With 1.3% of non-obese, or 2% ofgeneral population, but 4.3% ofobese population (Figure 4) pregnant women older than 40 years weremore likely to have increased weight from the first trimester of their pregnancy than younger mothers-to be.

Table 6. Advanced mother's age in obese/ non-obese subgroup

BMI (kg/m^2)	Age≤ 40 years	Age> 40 years	
>30	6	134	
≤ 30	9	660	**p=0.0190**

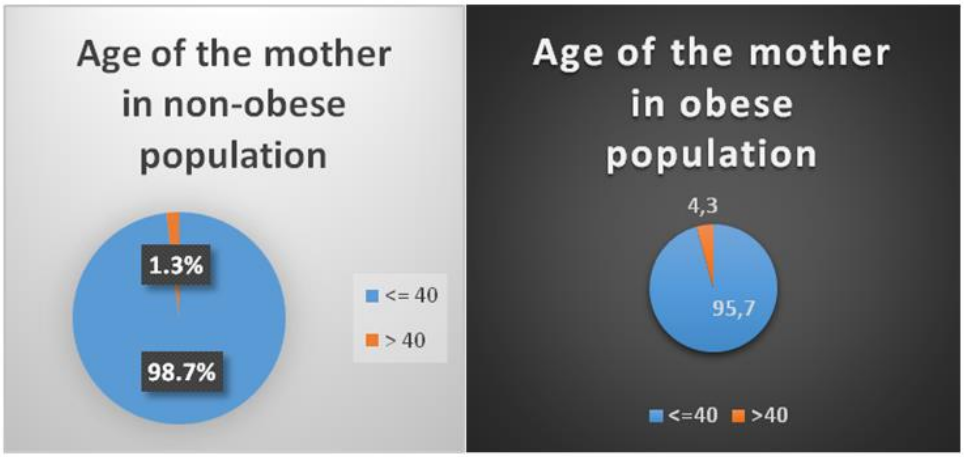

Fig. 4. Age of the mother: non-obese *vs.* obese population

Table 7. Level of education in obese/ non-obese subgroup

BMI (kg/m^2)	Primary school	High school	University degree
>30	42	45	53
≤ 30	134	230	305
		p (primary *vs.* high) = 0.049	**p (primary *vs.* university) = 0.010**

Finally, thelevel of education analysis revealed that there wasa correlation between lower level of education and obesity appearance (Table 7).

The statistical significance increasedwiththe higher level of education (p=0.049) when we compared obesity presence in women with completed primary school with those with completed secondary school; but p fell to p=0.01 when we compared the first subgroup with those with university degree. As shown in Figure 5, 30% among obese patients had the lowest level of education *vs.* 20% in non-obese population. Atthe same time, patients with university degree were outnumbered in population-obese population (46%)*vs.* obese-ones(38%).

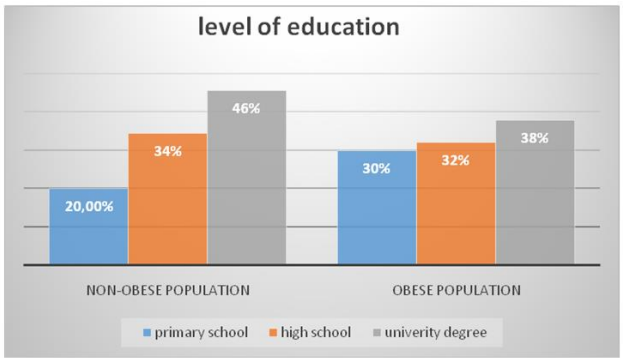

Fig. 5a. Level of education: non-obese *vs.* obese population

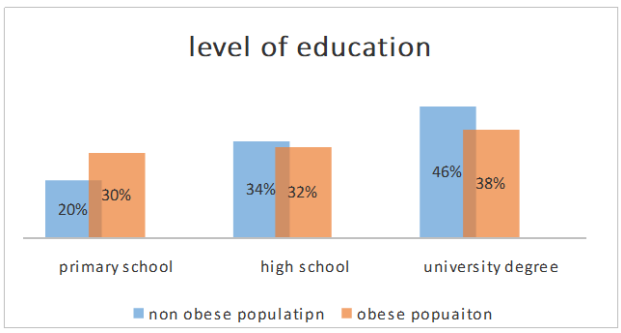

Fig. 5b. Level of education: non-obese *vs.* obese population

Discussion

The first trimester obesity is tightly associatedwith the presence of chronic hypertension. The latter is almost 4-fold more frequently observed in obese than in non-obese population. This finding is consistent with some other authors' work [19], and implicate the pre-existence of a metabolic syndrome. Smoking also shows a connection with obesity being twice more present in obese population. Coexistence of smoking and obesity might be interpreted as an oral addiction problem. Not family income [19], but rather patient's level of education is in negative correlationwith BMI: patients with lower education level had a greater possibility of being obese. On contrary, advanced maternal age and pluriparity are risk factors for being obese, which might be connected with woman's motivation of staying fit, her less physical activity during ageing, or presence of some medical issues as time goes.

On the other hand, nationality in terms of having local culture toward eating habits did not show association with the first trimester obesity. Interestingly, even when there was ashort interval between two consecutive pregnancies, obesity did not appear more frequently in this subgroup of patients.

Conclusion

The first trimester obesity shows association with some maternal characteristics, which puts into light that formal education of women as well as informal teaching them about responsibility to their own health, childbirth control, and building up their confidence and self-esteem can be a good starting point in fighting obesity.

Conflict of interest statement. None declared.

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Original article

CORRELATION OF *FLNA* GENE EXPRESSION WITH MAJOR CLINICOPATHOLOGICAL PARAMETERS IN COLON CANCER

КОРЕЛАЦИЈА ПОМЕЃУ FLNA ГЕНСКАТА ЕКСПРЕСИЈА И МАЈОРНИТЕ КЛИНИЧКО ПАТОЛОШКИ ПАРАМЕТРИ КАЈ КАРЦИНОМ НА КОЛОН

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Abstract

The distinction of the genetic landscape in the malignant cells of colorectal cancer may contribute to closer determination of the progression, treatment and prognosis of this heterogeneous group of neoplastic diseases. The aim of this study was to examine the possible association between the expression of *FLNA* gene and the basic clinicopathologicalparameters found in such patients. The study included 116 patients with verified colorectal cancer and signed consent for inclusion. Detailed clinicopathological data was gathered including location of the tumor (proximal, distal colon and rectum) and differentiation grade. RNA was extracted from the cancer tissue and normal surrounding mucosa of each patient. Gene expression levels were determined by reverse transcription and quantitative real-time amplification (qRT-PCR). The statistical differences were analyzed using Mann-Whitney-Utest, Kruskal-Wallistest, Fisher's exact test, the multivariate Logistic regression analysis and Wald's test.

In terms of location, *FLNA* gene expression was higher in tumors located proximally and reduced in tumors located in the distal parts of the colon and rectum. Furthermore, *FLNA* expression was found to be inversely correlated with grade, as seen in tumors with higher grade of differentiation with reduced expression.

The results indicate that the expression of *FLNA* in patients is consistent with its tumor-suppressor role and supports the potential use of this gene as a marker for diagnostics of colorectal cancer after confirmation on a larger group of patientsand data validation.

Keywords: colorectal cancer, gene expression, *FLNA*

Апстракт

Одредување на генетскиот пејсаж во малигните клетки на колоректалниот карцином, може да придонесе до поблиско предвидување на прогресијата, третманот и прогнозата кај оваа хетерогена група на неопластични заболувања.

Целта на оваа студија е да се испита можната поврзанот помеѓу експресијата на FLNA генот и базичните клиничко патолошки параметри кај пациентите со колоректален карцином.

Во студијата беа вклучени 116 пациенти со верифициран колоректален карцином, и потпишана писмена согласност за учество во студијата.

Беа собрани детални клиникопатолошки податоци за локација на туморот (проксимален, дистален колон или ректум) и диференцијалниот стадиум. Од карциномското ткиво и околната здрава мукоза на секој пациент вкучен во студијата, беше екстрахирана РНК. Нивото на генска експресија беше одредено со ревезна транскрипција и квантитативна амплификација во реално време (q RT-PCR).

Статистичката анализа беше изведена со соодветни тестови; Ман Витни У тест, Круска Валистер Фишер тест, мултиваријатна логистичка регресиона анализа и Валдов тест.

По однос на локацијата, експресијата на FLNA генот беше посилно застапена кај проксимално лоцираните тумори, додека кај дисталните тумори и туморите во ректумот беше послабо застапена.

Експресијата на FLNA генот беше обратно корелирана со градацијата на туморот, со намалена експресија на генот кај тумори со повисок степен на диференцијација.

Резултатите укажуваат дека експресијата на FLNA генот кај пациентите е конзистентна со неговата тумор супресивна улога, и покажува потенцијал на овој ген како маркер за дијагностика на колоректален карцином.

Клучни зборови: колоректален карцином, генска експресија, FLNA

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Introduction

Colorectal cancer (CRC) is the most common malignant transformation concerning the gastrointestinal tract. The cancer originates from the epithelial cells located in the colon or rectum. The etiology of this transformation is multifactorial including the genetic basis, environmental factors, inflammatory bowel syndrome and many other epigenetic factors leading to aseveral-year process of accumulation of genetic and epigenetic alterations.

CRC is the third most commonly diagnosed cancer (17,2/100 000) worldwide, following the lung and breast cancer. According to data from the Institute of Public Health of the Republic of North Macedonia, the estimated mortality rate for colorectal cancer in the country for 2014 was 21.3 for males and 9.5 for females, standardized per 100 000 population [1]. The precancerous lesions that lead to almost 95% of colorectal cancers are the neoplastic polyps - tubular and villous adenomas. However, it takes 5 to 10 years for development of themalignancy.

Studies have emphasized that sporadic colorectal cancer is a heterogeneous group of neoplasms that can be significantly differentiated according to the molecular profile of the gene disorders. Furthermore, meta-studies have found that molecular-genetic changes functionally affect a relatively small number of intracellular pathways responsible for regulating critical cell processes: division, apoptosis, cell invasion of surrounding tissue, cell motility, and other relevant features for the malignnancy [2]. Since the dominant malignant clone of the tumor mass contains a unique combination of molecular disorders leading to malfunctionof the cells, this combination can be determinant at certain point for the biological characteristics of the neoplasm including the clinical course, therapeutic response and prognosis.

The *FLNA* gene is located on chromosome Xq28, and encodes for the protein filamin A (FLNA), in literature also referred to as ABP 280 (from actin-binding protein 280). This protein is involved in the reorganization of the actin cytoskeleton in cells [3]. Abnormal expression of filamin A, under certain conditions, has been found in some types of malignant neoplasms, but the levels, timing, duration, and distribution of the protein product appear to influence in a stimulating or inhibitory manner on the cell proliferation [4]. One study published in February 2015 has detected reduced levels of expression of *FLNA* gene in CRCs [5].

Our study was designed to determine the quantitative expression values of *FLNA* in pairs of samples from cancerous tissue and normal mucosa of the colon or rectum in each individual patient from the study group and compare these values with the clinical and pathological parameters: anatomic location (proximal colon, distal colon and rectum), and grade of differentiation of the tumor. The aim was to determine their statistical association if found and thereby evaluate the potential utility of this gene expression as a molecular marker for diagnostic and other clinical purposes.

Materials and methods

In this observational, prospective study the relevant clinicopathological data weregathered in a database for of a group of 116 patients with colorectal adenocarcinoma. The molecular analysis was performed to quantitatively determine the levels of *FLNA* gene expression in RNA isolates from tissue samples, after which the correlation with the clinical and pathological parameters was calculated. Selection of the patients was performed according to established inclusion criteria (histopathologically proven colon or rectal adenocarcinoma, hand-signed patient consent, availability of appropriate clinical data, etc.). For the RNA extraction, after resectiontissue fragments of less than 1 gram of tumorwere takenfrom each patient, and a control tissue sample of similar size of the non-malignant mucosa at least 5 cm from the tumor edge. The whole cell RNA was isolated using the commercial Tri reagent following Chomczynski and Sacchi protocol [6]. Reverse transcription and complementary DNA synthesis (cDNA) was performed with the Invitrogen SuperScript® III First-Strand Synthesis SuperMix for qRT-PCR kit, according to the manufacturer's instructions. Oligonucleotide primers for PCR amplification of the selected *FLNA* region as well as the double labeled TaqMan fluorescent probes were from Thermo Fisher Scientific. The sequences of the oligonucleotide primers for amplification are available upon request.

Determination of gene expression was performed by quantitative reverse transcriptase real-time polymerase chain reaction (qRT-PCR) using the relative comparison method. In each sample of tumor and healthy tissue, the RNA expression of the *BACT* (β-actin) genewas simultaneouslydetermined, as a referent gene whose expression is balanced in almost all cell types. In the transcript analysis for *FLNA*, fluorescent dye SYBR Green (Applied Biosystems) was used. The real-time fluorescence amplification was performed on the Step One RT-PCR System (Applied Biosystems) and the data was processed with Step One (Applied Biosistems) software. The specificity of the amplification was checked by post-PCR melting curve analysis (MCA). The mean of the triplicate valueswas used.

The gene expression values were expressed as normalized to the reference gene. A comparison with a control sample of healthy tissue was used to determine the differences in the tumor tissue. The ΔΔCt calculation by Livak *et al.* [7], as well as Rao *et al.* [8] was used. The formulaused to compare how many times higher or lower was the expression when normalized to the reference gene and compared to healthy tissue was: $2^{-\Delta\Delta Ct}$. Furthermore, to express the relative

concentration (RQ) values we used the formula: RQ = $\log_{10}$ $2^{-\Delta\Delta Ct}$. Positive RQ values indicate increased values for expression relative to healthy tissue, while negative values indicate decreased expression of the gene of interest. The unchanged values of RQ or those of healthy tissue have a value of 1.

Preliminarily, the quantitative values wereanalyzed with the Shapiro-Wilk test in order to check their normal distribution.The statistical differences between gene expression levels of stratified patients' data were analyzed using Mann-Whitney-U and Kruskal-Wallis tests. The gene expression levels were categorized into the binary categories (high and low) and the association of these values with the clinicopathological data of each patient was calculated by the two-sided Fisher's exact test. The association of expression values of all genes was estimated using them ultivariate Logistic regression analysis and Wald's test.

Results

The analysis of the results of our study showed that *FLNA* expression was higher in cancers located in the proximal colon and reduced in the distal colon and rectum. The correlation between the expression of *FLNA* and the degree of differentiation wasinversely proportional, the values decreasedas the grade increased and this was statistically highly significant when calculated with different statistical calculations in which the reduced expression of *FLNA* was clearly associated with the degree of differentiation. These results support the tumor-suppressor role of this gene in colorectal carcinogenesis.

Table 1. The expression levels of FLNA gene categorized as decreased and increased in patients with colorectal cancer

***FLNA* expression**	**Mean value**	**Min. value**	**Max. value**	**Variance**	**Standard deviation**	**Frequency of patients**	
						n	**%**
Decreased	-0.56	-1.96	0.41	0.27	0.52	101	87.07
Increased	1.65	0.54	3.80	1.08	1.04	15	12.93
Total/whole group	-0.28	-1.96	3.80	0.92	0.96	116	100.00

The patient samples were divided by expression levels into two groups according to the expression levels: the first was with decreased and the second with increased values. The categorization was performed according to the cut-off value calculated from the standard deviation of the levels of the whole group of subjects (0.957), divided by 2. The analysis of *FLNA* gene expression in our study showed that values were decreased in 87.07% of patients, while increased in 12.93%. The distribution of patients according to the categorized values of gene expression is shown in Table 1 and Figure 1.

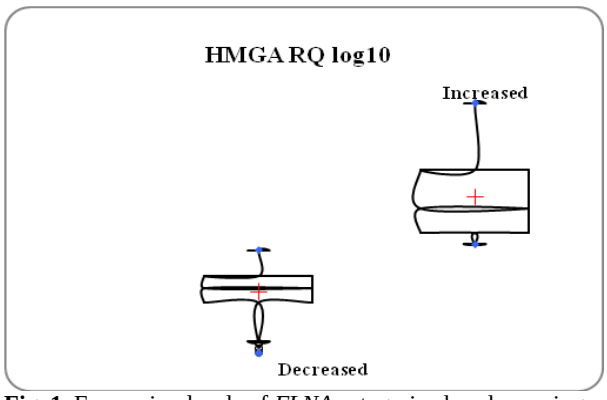

Fig. 1. Expression levels of *FLNA* categorized as decreasing and increasing

Table 2. Comparison of *FLNA* gene expression levels at two sites of colorectal cancer

Anatomical location	**Mean value**	**Min. value**	**Max. value**	**Standard deviation**	**Mann-Whitney test (two-sided) *p***
Proximal	0.04	-1.85	3.80	1.13	**0.038**
Distal colon and rectum	-0.38	-1.96	3.03	0.88	

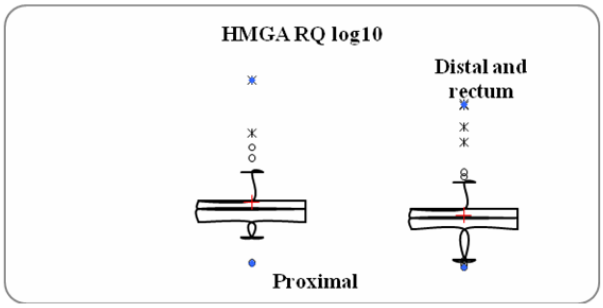

Fig. 2. *FLNA* gene expression levels at two different sites of colorectal cancer

A comparison of the expression levels of *FLNA* gene relative to the proximal *versus* distal colon and rectum is shown in Table 2 and Figure 2.

These analyses have shown that there is a correlation between the levels of gene expression and the anatomical location of the cancer or that the expression is statistically significantly higher ($p<0,05$) in the proximal colon than in the distal colon and the rectum where it is lower.

Table 3. Comparison of *FLNA* gene expression levels with grades of differentiation of the colorectal cancer G1, G2 and G3

Grade	Mean value	Min. value	Max. value	Standard deviation	Kruskal-Wallis test (two-sided) *p*
G1	1.87	0.11	3.80	1.25	
G2	-0.35	-1.75	3.02	0.67	**< 0.0001**
G3	-1.37	-1.96	-0.33	0.51	

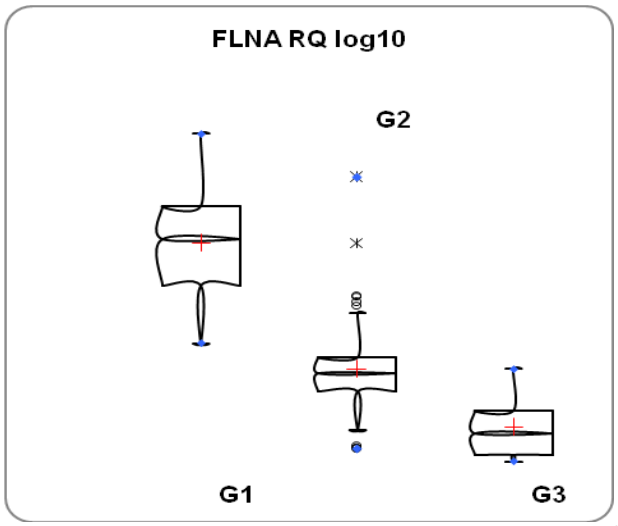

Fig. 3. *FLNA* gene expression levels with respect to grades of differentiation of colorectal cancer G1, G2 and G3

The comparison of *FLNA* gene expression levels with the histological grades of differentiation of CRC is shown in relation to each of the three grades (Table 3 and Figure 3). The expression levels of *FLNA* gene were analyzed with respect to the anatomical location of CRC in patients. According to the results of the statistical analyses, there is a clear negative correlation between *FLNA* gene expression levels and CRC grades of differentiation, which is statistically highly significant ($p < 0.01$).

To confirm this correlation with respect to grade, the expression levels of *FLNA* were divided into two groups: decreased and increased values, and the standard deviation of the values of the whole group of subjects was also used to categorize the two groupsas shown in Table 4.

Table 4. Association of decreased *versus* increased levels of *FLNA* gene expression with respect to G1, G2, and G3 grades

Grade	Expression of *FLNA*				Fisher's exact test *p*
	Decreased		Increased		
	n	%	n	%	
G1	2	1.98	6	40.00	**< 0.0001**
G2	90	89.11	9	60.00	
G3	9	8.91	0	0.00	
Total	101	100.00	15	100.00	

The analysis has confirmed that *FLNA* gene expression levels decrease with the increasing of the histological grade. We registered a very high statistical significance ($p < 0.0001$), using all four types of statistical comparisons. Thus, we can conclude that the correlation between *FLNA* expression and the grade is clear.

Discussion

Filamin A is a large cytoskeletal noncontractile protein that binds to actin and stabilizes the delicate three-dimensional actin networks by binding them to the cell membranes [9]. The actin cytoskeleton is of an essential importance for the dynamic regulation of the cell morphology, cell movement and migration in response to the external stimuli. The functions associated with the regulation of the cell movement and migrationhave a potentially direct and indirect role in the process of metastasis of the malignant cells. The FLNA protein interacts with and binds to a number of other protein molecules, and this complex interaction is still not clarified. Furthermore, FLNA participates in the regulation of cell proliferation and intracellular signal transduction, which plays an important role in the formation and development of neoplasms [10,11]. It was found that in activated cancer cells *FLNA* gene is overexpressed together with some oncogenes and growth factors, such as *c-MET* [12]. The biological role of this coexpression of *FLNA* and *c-MET* may have a great significance for tumor biology and potentially lead to the development of new therapeutic approaches for the treatment of human cancers.

There are not many research studies including the expression of *FLNA* gene and its role in colorectal carcinogenesis. In a study by Tian *et al.* [5], the expression of *FLNA* gene was determined in 46 tumor and normal tissue samples from the same patients using three parallel methods: immunohistochemistry, semiquantitative RT-PCR and Western blotting, after which levels of expression were compared in terms of clinical, pathological and prognostic parameters. The immunohistochemical results showed that positive expression was detected in 47.83% (22/46) of samples ofcolorectal adenocarcinomas, of which 26.09% (12/46) with mild positive expression and 21.74% with strong positive expression (10/46). In normal colorectal tissue specimens, the positive expression of filamin A was identified in 91.30% (42/46), of which low expression was found in 10.87% (5/46) and strong positive expression in 80.43% (37/46). The expression was higher in the normal mucosa compared to the cancerous specimens, and the difference was statistically significant ($p < 0.001$). The semiquantitative RT-PCR method, using *GAPDH* gene as a reference gene, showed that *FLNA*specific mRNA levels in tumor tissues were lower than in normal tissues (0.24 ± 0.03 *vs.* 0.95 ± 0.04), and the differences between the two groups were statistically significant ($p = 0.017$). *GAPDH* was also

used as an internal reference when quantifying protein-filamin A by Western-blotting. Results showed that filamin A levels in tumor tissues were lower than in normal tissues (0.15±0.02 *vs.* 0.76±0.04). The difference between the two groups was statistically significant (p=0.013).

Conclusion

In conclusion, the results of our study indicate the significance of the quantitative change in transcriptional activity of *FLNA*gene in colorectal carcinomas, suggesting a potential role in the process of colorectal carcinogenesis. Following a larger study and validation, the expression of this gene could play a role as a molecular marker in accordance with the existing clinical and pathological parameters and in order to facilitate the screening, diagnosing, monitoring and planning of the individualized therapeutic strategies.

Conflict of interest statement. None declared.

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Original article

МАЈЧИНАТА ДЕБЕЛИНА, ВОЗРАСТА И ТИРОИДНИОТ СТАТУС-ИНТРИГАНТНА ВРСКА СО НЕОНАТАЛНИОТ ИСХОД

MATERNAL OBESITY, AGE AND MATERNAL THYROID STATUS - AN INTRIGUING CONNECTION TO THE NEONATAL OUTCOME

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Abstract

Introduction. Thyroid dysfunction is the second most common endocrine disorder affecting women of reproductive age. Higher body mass index (BMI>30 kg/m^2) is linked with many endocrine abnormalities, including thyroid dysfunction. Gestational age at birth (GAB) and birth weight (BW) are important predictors of neonatal mortality and morbidity. The objective of this prospective study was to determine the adverse neonatal outcomes of women [small for gestational age (SGA), intrauterine growth restriction (IUGR) and others] complicated with impaired thyroid function and obesity, when compared with those with normal function.

Methods. Dried blood spot and urine samples were analyzed for thyroid and iodine status in 358 pregnant women in any gestational week, without known thyroid disorders. They gave birth at the University Clinic of Gynecology and Obstetrics-Skopje. The blood samples were analyzed with time-resolved fluoroimmunoassay in Zurich, and UIC was analyzed by mass spectrometry in Helsinki.

Results. We found a significant positive correlation between total thyroxine (TT4) and GAB (p=0.045) and UIE and mother age (p=0.007), but a significant negative correlation between GAB and UIE (p=0.051), GAB and mother's age (p=0.01), GAB and BMI (p=0.02). There was an inverse correlation between BW and maternal age (βst=-0.0641, P=0.010) and between BW and maternal TT4 (βst=-3.3640, P=0.0016). We found a positive correlation between BW and maternal BMI (βst = 21.847, P = 0.006).

Conclusion. Overweight, obese and older women are at increased risk of thyroid dysfunction during pregnancy considered as high-risk pregnancies for adverse neonatal outcomes. We can use maternal TT4, BMI and age for predicting the BW.

Keywords: obesity, thyroid status, small for gestational age (SGA), intrauterine grow restriction (IUGR), neonatal outcome

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Апстракт

Вовед. Тироидната дисфункција е второто најчесто ендокрино нарушување, кое ги зафаќа жените во репродуктивна возраст. Повисокиот BMI (>30 кг/м^2) е поврзан со многу ендокрини абнормалности, вклучително и тироидна дисфункција. Гестациската возраст при раѓање (GAB) и родилната тежина се важни предиктори за неонаталниот морталитет и морбидитет. Целта на оваа проспективна студија беше да се утврди евентуалниот неповолен неонатален исход на жените, мали за гестациската возраст (SGA), интраутерин застој во растот (IUGR) и други комплицирани случаи, со нарушена функција на тироидната жлезда и дебелина, во споредба со тие со нормална функција.

Методи. Исушените капки крв и примероците на урина беа анализирани за тироидниот и јодниот статус кај 358 бремени жени во која било гестациска недела, без познати тироидни нарушувања. Тие се породија на Универзитетската клиника за гинекологија и акушерство-Скопје. Крвните примероци беа анализирани со флуороимунолошка анализа во Цирих, а уринарната јодна концентрација (UIC) беше анализирана со масна спектрометрија во Хелсинки.

Резултати. Откривме значајна позитивна корелација меѓу TT4 и GAB (p=0.045), UIE и возраста на мајката (p=0.007), но значајна негативна корелација меѓу GAB и UIE (p=0.051), GAB и возраста на мајката (p=0.01), GAB и BMI (p=0.02). Варијаблите на тироидната жлезда T4 (p=0.119) и UIE (p=0.367) не се разликуваат значајно меѓу предвремените и

повеќе предвремените бремености, освен TSH (p= 0.0394). Постои инверзна корелација меѓу BW и возраста на мајката (βst=-0.0641, P=0.010) и меѓу BW и мајчиниот TT4 (βst=-3.3640, P=0.0016). Најдовме позитивна корелација меѓу BW и мајчиниот BMI (βst=21.847, P=0.006).

Заклучок. Жените со прекумерна телесна тежина и обезните, се со зголемен ризик од тироидна дисфункција за време на бременоста, како и постарите жени, и затоа треба да се сметаат како високоризични бремености со неповолни акушерски исходи. Можеме да ги користиме TT4, BMI и возраста на мајката како предиктори за родилната тежина.

Клучни зборови: дебелина, тироиден статус, мали за гестациската возраст (СГА), интраутерин застој во растот (ИУГР), неонатален исход

Introduction

Thyroid hormones and their most important component, iodine are essential for fetal growth and development and play a vital role in the early growth and development of most organs, especially the brain [1,2]. Thyroid dysfunction is the second most common endocrine disorder affecting women of reproductive age. The recent studies have found an association between impaired maternal thyroid status and pregnancy outcome, but only few studies have studied the correlation between body mass index (BMI) and maternal thyroid dysfunction.
Higher BMI (>30 kg/m^2), according to the World Health Organization (WHO) [3], or obesity, especially central obesity, is linked to many endocrine abnormallities, including thyroid dysfunction. This is not uncommon, because triiodothyronine (T_3) regulates the energy metabolism and thermogenesis, playing a key role in glucose and lipid metabolism as well as the fatty acids oxidation [4].
Fat cells produce leptin and are considered as an active endocrine organ. Leptin physiologically regulates energy homeostasis by informing the central nervous system about adipose tissue reserves and is also an important neuroendocrine regulator of the hypothalamic-pituitary-thyroid axis [5]. Evidence suggests that slight variations in thyroid function contribute to the development of regional obesity and the tendency to gain weight, although this has not been confirmed by all studies [4,5].
Gestational age at birth and birth weight are important predictors of neonatal mortality and morbidity and evidence showed an increased risk of preterm birth in relation to maternal hyperthyroidism and hypothyroidism [6]. Some studies reported an increased risk of low-birth weight or small-for-gestational age (SGA) in mothers with hypothyroidism [7,8], while others found no association [6,9].

SGA is defined as weight below the 10th percentile or 2 standard deviation (SD) for the gestational age [10]. The term intrauterine growth restriction (IUGR) is used to describe a fetus that cannot reach its growth potential due to placental insufficiency. Preterm delivery was defined as delivery before 37 completed gestational weeks. Low Apgar score was considered if Apgar score at 1st and 5th min was less than 7.
The objective of this prospective study was to determine the adverse neonatal outcomes of women (SGA, IUGR and others) complicated with impaired thyroid function and obesity, when compared with those with normal function and to find the predictive impact of maternal demographic characteristics and thyroid parameters on neonatal outcome.

Material and methods

Studied population

This prospective study conducted in the period from April to July 2017 included 358 healthy pregnant women in any gestational week, without known thyroid disorders (mean age 30.15±5.26 years) who gave birth at the University Clinic of Gynecology and Obstetrics - Skopje.

Inclusion criteria

Inclusion criteria were singleton pregnancy in any gestational age without previous history of thyroid disease of the mother or treatment with thyroid drugs.

Exclusion criteria

The study did not include: mothers who smoke cigarettes, mothers with any chronic diseases, especially diabetes mellitus or hypertension, and any fetal anomaly diagnosed with amniocenteses or ultrasound.

Ethical Approval and informed consent

This study was approved by the Ethics Committee of the Faculty of Medicine in Skopje, Ss. Cyril and Methodius University in Skopje and an informed consent was obtained from all individual participants included in the study.
A sample of 2 mL (milliliter) of urine and five drops of heparinized blood of 5 μL (microliter) was taken from each participant and applied to a special type of filter paper. The samples were dried for 24 hours at room temperature and then they were frozen at -20° C, until sending them to the Department of Health Sciences and Technology in Zurich (Departement Gesundheitswissenschaften und Technologie-ETH Zürich). TSH and TT4 were analyzed with time-resolved fluoroimmunoassay with GSP 2021-0010; PerkinElmer, Turku, Finland [10].

Urine iodine concentration (IUC) in urine samples was analyzed by mass spectrometry (MS) using Agilent 7800 ICP-MS system, with the Pinell-modified Sandell Kolthoff method, at the National Institute for Health and Welfare (THL) in Helsinki (ICP) [11].
The data about maternal age, parity, obstetric history, gestational age at the time of birth as well as the way of birth were noted from the medical history. Birth weight and length were measured by the midwife attending the birth, while condition of the newborn after delivery and Apgar score was given by the neonatologist.

Statistical analysis

Data analyses were performed using MedCalc Statistical Software, version 19.1.3 (MedCalc Software bv, Ostend, Belgium). The results are presented as mean± SD, median and percentages (%). Bivariate Pearson's (parametric test) or Spearman's (non-parametric test) were used to measure the strength and direction of variables relationships. The comparison of pregnancy outcomes between the normal and abnormal groups was analyzed by Student's *t* test with equal or unequal variances. A *p* value of <0.05 was considered statistically significant. Multiple regression analysis was used to show predictable values of independent variables (age, BMI, TT4 and TSH) on the dependent variable BW.

Results

Demographic and clinical characteristics

The maternal demographic characteristics (Body Mass Index) and nationality, maternal thyroid function values (TSH, TT4, Tg and UIC), gestational week on birth, mode of delivery, birth characteristics and other essential data are shown in Table 1.

Table 1. Demographic, clinical and other characteristics of neonatal outcome

	Normal thyroid status n = 220	**Impaired thyroid status n = 139**	Range	P
Maternal age (years)	28.96 ± 5.22	29.75 ± 6.04	14 - 52	0.189
BMI (kg/cm^2)	26.7 ± 4.61	27.71 ± 5.00	17 - 47	0.081
Nationality				0.744
- Macedonian	130 (59.09%)	74 (53.23%)		0.275
- Albanian	61 (27.72%)	51 (36.69%)		0.074
- Gipsy	28 (12.72%)	12 (8.63%)		0.231
Gestational week at birth	38.46 ± 2.30	38.38 ± 2.89	20 - 42	0.798
Mode of delivery				
- Spontaneous, eutocic	127 (57.72%)	67 (48.20%)		0.078
- Emergency Cesarean Section	23 (10.45%)	11 (7.91%)		0.424
- Dystocic Cesarean Section	28 (12.72%)	13 (9.35%)		0.329
Preterm				
Yes	26 (11.81%)	15 (10.79%)		0.766
No	194 (88.18%)	124 (89.20%)		
Birth weight (g)	3093.47 ± 523.09	3184.14 ± 619.16	555 - 4470	0.153
Birth weight categories				0.289
SGA/IUGR	9 (4.09%)	3 (2.15%)		0.319
Sex				
- male	122 (55.45%)	62 (44.60%)		0.045
- female	98 (44.54%)	77 (55.39%)		
Apgar score (1 min)			5 - 9	
< 5	7 (3.18%)	8 (5.75%)		0.599
> 5	213 (96.81%)	131 (94.24%)		
Apgar score (5 min)			4 - 10	
< 5	5 (2.27%)	2 (1.43%)		0.575
> 5	215 (97.72%)	137 (98.56%)		
Thyroid function values				
TSH (mU/L)	0.555 ± 0.293	0.515 - 0.400	0.1-3.7	0.282
TT4 (nmol/L)	118.33 ± 23.51	80.956 ± 19.935	46.2 - 195.2	<0.0001
Tg (□g/L)	11.40 ± 7.645	11.909 ± 10.823	0.141 - 80.241	0.634
Iodine status				
UIC (μg/L)	261.83 ± 683.31	198.759± 121.687	13.5 - 558.120	0.183

The results are expressed as mean ± SD (standard deviation), mean and n (%), number (percent); BMI, body mass index; LBW, Low Birth Weight; IUGR, Intrauterine Growth Restriction; UIC, Urinary Iodine Concentration; P, value of significance

According to the guideline of the American Thyroid Association (ATA) for the diagnosis and management of thyroid disease during pregnancy and postpartum (13) the reference values for TSH and FT4 range from 0.1 to 3.7 mIU/L and 65/97.5-165-247.5 nmol/L, respectively. We compared our TSH and TT4 results in accordance with ATA reference, and we made two groups of participants (those with normal thyroid parameters-Group 1) and Group 2 with impaired thyroid function (participants with different levels from the reference ranges).

The mothers with normal thyroid status were of Macedonian nationality predominantly with lower BMI and lower age compared to the second group. The number of preterm births was greater in the group with normal thyroid status. All mentioned and the other results presented in Table 1 did not significantly differ between the two groups. We found a statistical significance only in the sex and TT4 of the neonate.

T – test for independent samples

Thyroid variables T4 (p=0.119) and UIE (p=0.367) did not differ significantly between premature and very premature pregnancies, except for TSH (p=0.0367).

Box plots of the mean and the results for 95% Confidence Interval (CI) of the mean, range, median, and the 25th and 75th percentiles for both subgroups [Spontaneous premature pregnancies (SPP) and Spontaneous very premature pregnancies (SVPP) are shown in Figure 1. The mean TSH of the SVPP (0.358±0.254 mU/L) was higher than the mean SPP (0.545±0.294 mU/L). The results from the unpaired t-test (for equal variances and unequal sample size): Test statistic t (d), Difference, P-value and F-test (that determines the equality of the variances) are shown in Figure 1, too.

There was a high statistical significance (P = 0.0394)

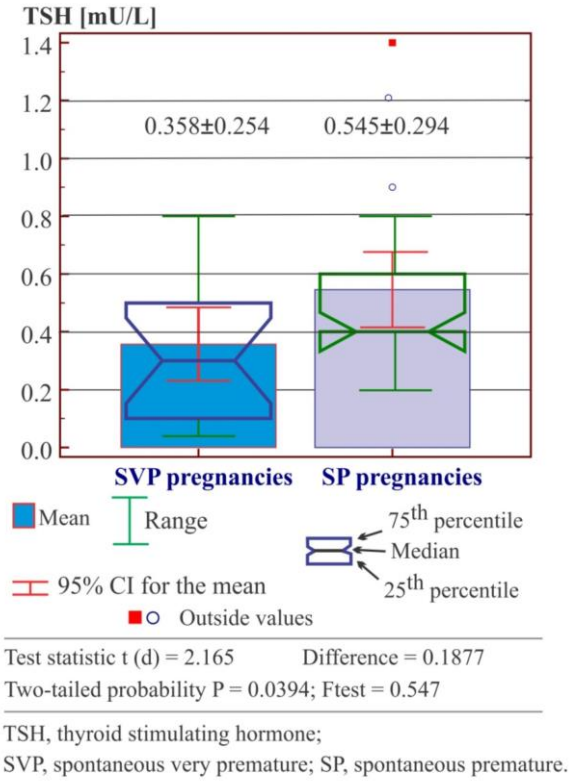

Fig. 1. Notched Box-and-Whisker diagram of SVP and SP pregnancies according to TSH values (independent samples t- test)

between TSH values among SVP and SP pregnancies.

Bivariate Pearson or Spearman correlation

Appropriate correlation coefficients [(Pearson's (r) or Spearman rho (ρ)] as measure of the strength for linear relationship according to distribution of the variables are shown in Table 2.

Table 2. Bivariate correlation between thyroid and clinical parameters

	Mother age	BMI	GAB	Baby weight	Apgar 5 min	SGA	IUGR
TSH	r = - 0.249	r = - 0.107	r = 0.286	r = 0.286	r = 0.162	r = - 0.075	r = 0.146
	p = 0.1166	p = 0.506	p = 0.092	p = 0.069	p = 0.311	p = 0.642	p = 0.363
TT4	r = - 0.004	r = - 0.206	r = 0.314	r = 0.265	r = 0.198	p= 0.178	p = - 0.175
	p = 0.979	p = 0.071	p = 0.045	p = 0.094	p = 0.214	p = 0.264	p = 0.272
UIE	r = 0.413	r = 0.183	r = - 0.373	r = 0.265	r = - 0.247	ρ = 0.214	ρ = 0.096
	p = 0.007	p = 0.252	p = 0.051	p = 0.094	p = 0.119	p = 0.178	p = 0.552
GAB	r = - 0.395	r = - 0.359	/	r = 0.592	r = 0.238	ρ = 0.198	ρ = 0.287
	p = 0.01	p = 0.02	/	p< 0.0001	p = 0.134	p = 0.214	p = 0.068

BMI, body mass index; GAB, gestational age on birth; SGA, small-for-gestational age; IUGR, intrauterine growth restriction; TSH, thyroid stimulating hormone; TT4, total thyroxine; UIE, urinary iodine excretion

The positive value of product-moment correlation coefficient (r, ρ) as the measure of the strength of linear dependence between two variables indicated a *significant positive correlation* between T4 and GAB (r=0.314, p =0.045), UIE and mother's age (r=0.413, p=0.007) and between GAB and baby weight (p<0.0001). A *significant negative correlation* was found between GAB and UIE (r=-0.373, p=0.051), GAB and mother's age (r=-0.395, p=0.01) and GAB and BMI (r=-0.359, p=0.02).

Multiple regression analysis

Assessments [standardized coefficient β (βst), standard error of βst (Std. Error), t, and p-value] of the depen-

dent predictor "baby weight" or determinants (age, BMI, TT4, and TSH) for prognosis of the birth weight in group with impaired thyroid status after backward multiple regression analysis and the results from Analysis of Variance (F-ratio, degrees of freedom and significance level) are shown in Table 3.

Table 3. Multiple backward regression analysis in mothers with impaired thyroid status

Multiple regression (backward)				
Sample size	139			
Enter variable if P < 0.05, Remove variable if P > 0.5				
Dependent Y	Baby weight			
Coefficient of determination R^2	0.06690			
Multiple correlation coefficient	0.2587			
Regression equation				
Independent variables	*βst coefficient*	*Std. Error*	*t*	*P*
Age	-0.06401	0.02484	- 2.577	0.010
BMI	21.847	6.3473	3.442	0.006
TT4	-3.3640	1.0600	- 3.174	0.0016
TSH	98.2086	88.4056	1.111	0.2674
Variables thyroglobulin and urinary iodine excretion were not included in model				
Analysis of Variance	F -ratio	Significance level	DF	Residual
	6.13	P = 0.0001	2	136

βst, beta standardize; Std. error, standard error of the βst, DF, Degrees of freedom; BMI, body mass index; TT4, total thyroxine; TSH, thyroid stimulating hormone

There was an inverse correlation (negative βst coefficient, βst=-0.0641) between birth weight and maternal age (P=0.010) and inverse correlation (βst=-3.3640) between birth weight and maternal TT4 (P=0.0016). We found a positive correlation (positive βst=21.847) between birth weight and maternal BMI (P=0.006). The correlation between birth weight and maternal TSH was positive, but not statistically significant (P=0.2674).

The multiple correlation coefficient (0.2587) is a measure of how well a given variable (baby weight) can be predicted using linear function of a set of other variables (BMI, age and TT4). Only 25.87% from birth weight changes was dependent on BMI, age and TT4 as the predictors. The remaining from the total variability between them were not explained (74.13% of baby weight were dependent on other factors, which were not covered with the regression model).

The predictive impact of maternal demographic characteristics and thyroid parameters in predicting of birth weight were statistically significant (TT4, P=0.0016; BMI, P=0.006 and age, P=0.0100).

Discussion

In our study, similar to that of Ajmani SN *et al*. [14], women with impaired thyroid function had higher maternal age (29.75±6.04 *vs*. 28.96±5.22) compared to women in the normal group. Hence, the increased maternal age was associated with a higher incidence of thyroid dysfunction. The increase in prevalence of thyroid dysfunction in the older age group is due to current trend of becoming pregnant in older age.

Mannisto *et al*. [15] in their study found that maternal BMI was related to thyroid function: obese pregnant women had higher serum concentrations of TSH and TT4, which is consistent with the results of our study, in which we found a significant negative correlation between gestational age at birth (GAB) and BMI (r=0.359, p=0.02). Patients with higher BMI delivered earlier and that is connected to a lower birth weight.

According to the multiple regression results in our study we could predict the birth weight by maternal demographic characteristics and thyroid parameters such as TT4, BMI and age. We found an inverse correlation of birth weight with maternal age and TT4, and a positive correlation between birth weight and maternal BMI. The results presented by Brynhildsen *et al*. [16] suggested that BMI can explain trends in infants' weight, which is agreement with the results obtained in our study. Gois *et al.* [17] in a regression analysis predicted the risk of low birth weight in advanced maternal age. They found βst=1.8 for mothers which age was > 40 years.

This study has the following limitations. We have not taken into consideration the TPO Ab (Thyroid Peroxidase antibodies) as a possible factor of mother's impaired thyroid status. Also, missed deliveries outside the University Clinic of Gynecology and Obstetrics in Skopje reduced the accuracy of the statistical model further. Thirdly, the neonatal cord blood TSH levels were not examined, which can be done in further studies.

Conclusion

Based on the results of previous studies, and alongside with the results of our study, we can conclude that overweight and obese women are at an increased risk of thyroid dysfunction during pregnancy, as well as older women, and thus these should be considered as high risk pregnancies for adverse neonatal outcomes. We can use maternal TT4, BMI and age for predicting the birth weight.

Conflict of interest statement. None declared.

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ММР

Original article

SOME ASPECTS OF FUTURE MARKERS FOR EVALUATION OF RHEUMATOIDARTHRITIS

НЕКОИ АСПЕКТИ НА ПРОГНОСТИЧКИ МАРКЕРИ ЗА ЕВАЛУАЦИЈА НА РЕВМАТОИДНИОТ АРТРИТИС

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Abstract

Introduction. Reactants of the acute phase like ESR and CRP indirectly reflect synovitis, but at the same time they are sensitive tools which enable objectivization and measurement of immune-mediated inflammatory response in RA. Simultaneous testing especially of ESR, CRP and RF (that are reversible measures of inflammation) together with clinical variables of inflammatorysynovitis are recommended for evaluation of disease activity.
Aim. Evaluation of the activity of rheumatoid arthritis (RA) with the reactants of the acute phase-erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) and rheumatoid factor (RF) and their assessment as prognostic markers for disease activity in patients with early RA treated with methotrexate.
Methods. This study included 35 patients (pts) with early RA, while 35 pts were in the healthy control group. Pts were treated with methotrexate at an average dose of 10mg once weekly. For clinical evaluation of disease activity at certain time intervals (0 time, after 6, 9 and 12 months) we analyzed ESR, CRP and RF in every patient.
Results. RA was evaluated following the dynamics of changes of the mean values of ESR, CRP and RF. Statistical analysis showed statistically significant differences among mean values of ESR in the four time intervals (p=0.00002). In regard of CRP there were statistically significant differences among mean values in all four time intervals (p=0.0428) (standard deviations were with great variations). There were no statistically significant differences of RF in the four time intervals (p=0.573). We found high values of CRP and RF in most of the patients.
Conclusion. In spite of the therapy with methotrexate, disease progression continues especially in patients with elevated values of ESR, CRP and RF, which are shown as predictors for aggressive course of disease. This enables selection of high-risk groups of patients for aggressive course of disease and point the need for early and aggressive treatment.

Keywords: rheumatoid arthritis, reactants of the acute phase, rheumatoid factor.

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Апстракт

Вовед. Реактаните на акутната фаза, како ЕСРи ЦРП, индиректно го одразуваат синовитисот, но истовремено се и сензитивниалатки за објективизирање на имери имунолошко-медииранот инфламаторен одговор кај РА. Се препорачуваат симултани тестирања, особенона СЕ, ЦРП и РФ, коишто претставуваат реверзибилни маркери на инфламацијата со клиничките варијабли на инфламаторниот синовитис,со што може да се процени активноста на болеста.
Цел. Евалуација на активноста на ревматоидниот артритис (РА) со реактантите на акутната фаза-еритроцитна седиментациска рата (ЕСР), Ц-реактивен протеин (ЦРП) иревматоиден фактор (РФ)и нивна процена како прогностички маркери за исходот на болеста кај пациенти со ран РА, лекувани со метотрексат.
Методи. Проследени беа 35 пациенти со ран РА, како и 35 пациенти, какоконтролна здрава група. Пациентите беа лекувани со метотрексат со средна дозаод 10 мг,еднаш седмично. За клиничката проценка на активност на болеста, воодредени временски интервали (во 0-време, по 6,9,12 месеци), кај секој поединечен пациент од групата беше анализиран ЕСР, ЦРПи РФ.
Резултати. РА беше евалуиран следејќи ја динамиката на променитена средните вредности на ЕСР, ЦРПи РФ. Статистичката анализа покажа статистички значајни разлики меѓу средните вредности на ЕСР во четирите временски интервали (p=0,00002). Во поглед на ЦРП постоеја статистички сигнификантни разлики меѓу средните вредности во сите че-

тири временски интервали (p=0,0428) (стандардните девијации беа со големи варијации). Не постоеја статистички значајни разлики меѓу средните вредности на РФ во четирите временски интервали (p= 0,573). Кај поголем број од пациентите беа регистрирани високи вредности на РФ и ЦРП

Заклучок. Прогресијата на болеста продолжува, и покрај терапијата со метотрексат, особено кај пациентите со високи вредности на ЕСР, РФ и ЦРП, кои се покажаа како предиктори за агресивен тек на болеста. Тие овозможуваат селектирањена високоризични групи за агресивен тек на болеста и укажуваат на потребата од рано и агресивно лекување.

Клучни зборови: Ревматоиден артритис, реактанти на акутна фаза, ревматоиден фактор

Introduction

RA is a chronic systemic inflammatory disease with mainly joint manifestation. In the disease course every synovial joint could be affected, but the most affected are small hand joints. Chronic synovitis is usually the reason for irreversible destructive changes of the joint cartilage and subchondral bone [1-7]. Reactants of the acute phase like ESR and CRP indirectly reflect synovitis, but at the same time they are sensitive tools which enable objectivization and measurement of immune-mediated inflammatory response in RA. Simultaneous testing especially of ESR, CRP and RF (that are reversible measures of inflammation) together with clinical variables of inflammatorysynovitis are recommended for evaluation of disease activity. Considering the changeable course of the disease, for credible evaluation of RA the most appropriate are serial measurements of ESR and CRP (timely integrated) [8]. Reports from studies are paradoxical in terms of joint damage and inflammatory synovitis expressed with the reactants of the acute phase. Although there is a correlation between radiographic progression and the reactants of the acute phase, some studies showed that progression of the erosion continues despite suppression of the joint inflammation [9].

From laboratory tests anemia and thrombocytopenia also reflect the inflammation in RA. Rheumatoid factor (IgM-RF) is a serological indicator included in ARA criteria for RA and represents anti-immunoglobulin antibody that directly acts on Fc fragment of immunoglobulin G. RF is detected in 75-80% of patients. High titer of RF is associated with a foudroyant course of the disease. Clinically, high activity in the beginning of the disease and positive RF after one year of treatment with drugs that slow down disease course-SAARD are the best predictors for bad prognosis, especially in patients with early RA. However, from clinical point of view, one could not predict the disease outcome in individual patients with early RA [10].

Aim

The aim of this study was to evaluate the activity of RA with radiographic assessment of hand joints, reactants of the acute phase (ESR, CRP) and rheumatoid factor (RF). They were analyzed as prognostic markers for disease outcome in patients with RA, treated with methotrexsate.

Material and methods

The disease diagnosis in patients included in the study wasmade upon the revised diagnostic criteria for classification of RA proposed in 1987 by the American Association for Rheumatism (ARA).

In order to be includedin the RA group the patient had tosatisfy at least 4 of the proposed 7 criteria. The criteria from 1 to 4 should be present at least 6 weeks.

The study included 35 pts (28 women, 7 men) with RA and 35 pts (18 women, 17 men) as a healthy control group. The mean age in the group with RA was 56.68 years (±6.79) (40-65years), while in the healthy control group the mean age was 46.2 years (±12.49) (9-65). The mean disease duration from the beginning, expressed in months was 43.97 (±45.23 months), in the interval (6-168 months). Examinations were made in several time points-in 0 time, 6, 9 and 12 months. For the first time immunomodulatory therapy with methotrexate was indicated (average dose of 10mg once weekly), in addition tothe non-steroidal antirheumatic therapy. None of the patients included in the study had previous or actual history of the disease.

Clinical evaluation of the disease activity

Clinical evaluation was made by a subspecialist in the field of medicine. Disease activity was evaluated using DAS 28 index (Disease Activity Score (DAS 28)). The Index uses mathematical formula to obtain unique composite quantitative score, which consists of palpatory pain joints (maximal number 28), swollen joints (maximal number 28), Westergren ESR and patient's global assessment for disease activity (0-100 mm Visual Analogue Scale-VAS), as well as morning stiffness (minutes). DAS 28 index ranges between 0-10, and the score <3.2 qualifies the disease as low active.

Inclusion criteria:

The study included patients with RA, aged 18-65 years, newly diagnosed and untreated for RA.

Exclusion criteria:

The study did not include all patients with diseases or conditions that could directly or indirectly affect the results, such as:

1. Pts with previous history of diseases of spleen, thyroid gland, liver, kidney, hematological, cardio-

vascular, neurological, lung disorders, autoimmune diseases, aged <18 years.
2. Pts with diabetes, acute infections, malignant diseases, febrile conditions.
3. Pts with uric arthritis, urine infections, SLE, mixed connective tissue disease, vasculitis.
4. Pts with history of blood transfusion, as well as overweight.
5. 5.Pts that at 0-time were detected with hyperglycemia, or elevated degradation products like serum and urine creatinine, serum urea, arterial hypertension, CBC disorder and enzymes disorder.

All participants voluntarily participated in the study, so the ethical criteria for realization of this study were fulfilled.

Laboratory evaluation

For clinical evaluation of the disease it was necessary to take into account the following laboratory variables: complete blood count (CBC), differential blood count, reactants of the acute phase, ACPA antibodies, C-reactive protein (CRP), rheumatoid factor (RF), erythrocyte sedimentation rate (ESR), alkaline phosphatase (AP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatine kinase (CK), lactate dehydrogenase (LDH), serum urea and creatinine.

CRP was determined with agglutination test (Latex CRP test) (BioSystem S.A. reagent&instruments Costa Brava 30, Barcelona (Spain). Reference values <6 mg/L CRP in serum.

RF was determined with agglutination test (Latex CRP test) (BioSystem S.A. reagent&instruments Costa Brava 30, Barcelona (Spain). Reference values <30 IU/ml RF in serum.

Westegren method was used as a quantitative method for determination of ESR, and normal values for men were 7-8 mm, and for women 11-16 mm.

ACPA antibodies weredetermined by the manufacturer DIA-STAT^{TM}Anti-CCP (Axis-Shield Diagnostics). The test is semi-quantitative/qualitative ELISA test, based on detection of IgG autoantibodies in human serum or plasma, directed towards synthetic cyclic citrullinated peptides (CCP) that comprise modified arginine residues. Calculation and interpretation of the results for quantitative protocol is estimated from the absorbent value (optic density) from positive and negative control, as well as for every sample.

Absorbent value	Interpretation of the results
<0.95	negative
≥0.95≤1.0	borderline
From the absorbent value	positive

Statistical analysis

Data analysis was made with the statistical package Statistica, 7.0.

For data processing we used the following statistical methods: for testing the significance of the differences among more arithmetic means in the groups (independent samples) we used Friedman's analysis of variance: for testing the significance of differences between two arithmetic means (dependent samples) Wilcoxon Matched Pairs Test was used.P-value between 0.05 and 0.1 was considered statistically significant.

Results

RA was evaluated following the dynamics of changes of the mean values of RI score, mean values of ESR, CRP and RF (Table 1).

1. Wilcoxon matched pairs test showed statistically significant differences in mean values of ESR in time intervals between 0 time and 6 months (p= 0.00014); between 0 time and 9 months (p=0.00014); 0 time and 12 months (p=0.00010). Friedman's analysis of variance showed statistically significant differences among mean values of ESR in the four time intervals-Frχ^2=19.485 (p=0.00002) (Table 1 and Figure 1).
2. Friedman's analysis of variance showed that there were statistically significant differences among mean values of CRP in the four time intervals - Fr χ^2=2.804 (p=0.0428) (standard deviations showed great variations). Analysis with χ2-test showed that the number of pts in whom values of CRP were negative increased within the course of time, and the differences were statistically significant (χ2= 11.35df = 3 p=0.0099).
3. Friedman's analysis of variance showed that there were no statistically significant differences among the mean values of RF in the four time intervals- Fr χ^2=1.017 (p=0.3875) (standard deviations showed great differences).

Table 1. Radiographic index in patients with RA with mean values of tight joint space, erosive score and total score

Time intervals in RA up to 1 year	Mean values of RF JU/ml RF< 30JU/ml (neg)	Mean values of ESR (mm/h)	Mean values of CRP mg/l CRP< 6 mg/l(neg)
0 time	195.5±289.9	59.9 ± 27.7	26.3 ± 28.8
After 6 months	194.4±366.1	31.6 ±16.9	19.0 ± 24.0
After 9 months	89.3±157.9	31.4 ±17.4	10.6 ± 11.9
After 12 months	126±311.7	25.0 ±11.6	13.4 ± 22.1

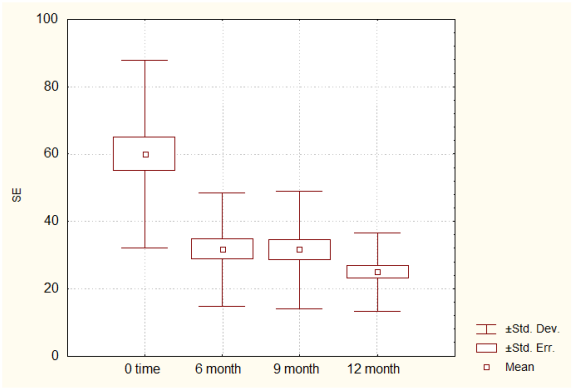

Fig 1. Mean values of sedimentation in patients with RA

Analysis with $\chi 2$-test showed that the number of pts in whom values of RF were negative increased within the course of time, but the differences were not statistically significant ($\chi 2$=1.99 df=3 p=0.573).

4. Friedman's analysis of variance showed that there were no statistically significant differences among mean values of hemoglobin Fr ($\chi 2$x2=1.715; p= 0.1677), mean values of erythrocytes Fr ($\chi 2$=0.872; p=0.4578), mean values of leukocytes Fr($\chi 2$= 1.0276; p=0.4751), among mean values of hematocrit Fr ($\chi 2$=1.1028; p=0.3509) in the four time intervals in the group of RA patients.

Discussion

Results obtained by following the dynamics of changes of the reactants of the acute phase enabled us to evaluate disease activity and treatment efficacy from methotrexate [11-15].

The statistical analysis showed statistically significant differences among mean values of CRP in all four time intervals. Distribution of pts according to CRP values over and bellow 6mg/l showed that the number of pts in whom values of CRP were negative increased over time, but, however, some pts had elevated values of CRP (standard deviations showed great variations). Decrease in CRP values in the consecutive controls in the four time intervals confirmed the resultsfrom other studies for CRP sensitivity in terms of joint inflammation. The statistical analysis showed that there were no statistically significant differences among mean values of RF in the four time intervals (standard deviations showed great variations). Some patients had enormously high values of RF. According to the values of RF, patients were distributed over and bellow 30 JU/ml. The number of patients in whom values of RF were negative had increased over time, but the differences were not statistically significant. In patients with high values of RF we noticed a greater progression of the joint damage atcertain time periods of follow-up of RA activity.

The methotrexate therapy achieved clinical suppression of RA after 6 months sincethe beginning of treatment.

In the consecutive time intervals of follow-up of patients we could confirm the reports from several studies that there was a mutual correlation between variables of inflammation and reactants of the acute phase [16-19].

Many clinical studies have reported that it is very difficult to assess which factors are the most significant predictors for treatment outcome and that difficulties are due to the different definitions of disease outcomes from therapy and examinations of different disease predictors in different clinical studies. Also, there are different approaches to RA treatment with DMARD alone or in combination with several DMARD [20,21].

Studies dealing with disease activity as a predictor in treatment outcome are inconsistent in their reports. Some studies consider that inflammatory markers manifested in the beginning of the disease (RA), when therapy was started, had no predictive significance in the treatment outcome.

On the contrary, recent studies report that positive RF is a predictor of disease activity and radiographic progression, i.e. high value of RF is a predictor of consecutive joint damage [22,23]. This wasalso confirmed in our study.

In one study patients with very early disease and intensive therapy, positive RF in the beginning of the study proved to be prognostically significant intreatment outcome [10]. In some studies RF is an indicator for disease severity, while other clinical variables measured in the beginning of the disease that evaluate disease activity by counting of inflammatory joints as well as reactants of the acute phase vary in their influence inthe latter joint damage [24].

Chronically active disease-RA is a reflection of elevated values of CRP and ESR [25,26]. The superiority of CRP in regard to ESR as a predictor for radiographic progression shows a greater correlation of CRP than of the number of sensitive joints. Superiority of CRP in regard to ESR could be explained with better sensitivity of CRP compared to inflammation. Also, CRP compared to ESR is resistant to the influence of gender, age, anemia and other serum proteins. The combination of CRP and ESR has no additive predictive value.

CRP is the best indicator for detectible damage because it is a direct and sensitive marker that gives a rapid answer tothe changes of the inflammatory synovitis compared toESR, which is an indirect marker of inflammation. CRP more precisely and in shorter time reflects the changes in disease activity compared to ESR that registers the changes in RA activity after few weeks.

Conclusion

In spite of the therapy with methotrexate, disease progression continues especially in patients with elevated values of ESR, CRP and RF, which are shown to be predictors of aggressive course of the disease. This enables selection of high-risk groups of patients for

aggressive course of disease, and point the need for early and aggressive treatment.

Conflict of interest statement. None declared.

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Original article

HYDROSALPINX AND DISTRIBUTION OF PREGNANCIES AMONG LAPAROSCOPICALLY TREATED PATIENTS

ХИДРОСАЛПИНКСОТ ВО КОРЕЛАЦИЈА СО СТАПКАТА НА БРЕМЕНОСТИ КАЈ ЛАПАРОСКОПСКИ ТРЕТИРАНИТЕ ПАЦИЕНТКИ

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Abstract

Introduction. Hydrosalpinx is a common medical condition encountered among female population with infertility issues. One or both fallopian tubes can be filled with a substantial amount of fluid, making them dilated and dysfunctional, usually as a result of an injury or infection. Damages of different degrees can be seen on the mucosal lining, which compromise the processes of normal fertilization and zygote passage.
Methods. We retrospectively analyzed clinical records from a period of five years (2013-2017), selecting patients diagnosed as having unilateral or bilateral hydrosalpinx. Clinical files were divided by years and according to the treatment protocol used. Our scope of interest was focused on patients who were treated surgically, with different types of laparoscopic interventions. A total of 74 patients met our criteria for selection.
Results. Two groups were related to conservative laparoscopic approaches and one to complete radical surgical treatment, unilateral or bilateral salpingectomy. Patients in the first group, 9(16.36%), were treated with a laparoscopically-guided creation of salpingostomy. The second group comprised patients treated only with bilateral chromopertubation and consisted of 17 (30.91%) patients. The third, and the most numerous group comprised 29(52.7%) patients treated with salpingectomy. Bilateral salpingectomy was performed in 9(31.03%) and unilateral in 20(68.97%) patients, showing that majority of patients were treated with unilateral salpingectomy, after intraoperative evaluation of tubal patency of the contralateral uterine tube.
A total number of 30 patients (54.5%) had successful pregnancies, which ended up with a desired outcome, healthy live birth. Procedures for artificial reproduction and successful pregnancies achieved with IVF were recorded in 11(36.67%) patients and in the remaining 19(63.33%) patients pregnancies were achieved via spontaneous conception.
Conclusion. Hydrosalpinx management is mainly influenced by the local tubal changes evaluated laparoscopically and can be surgically treated, either conservative or radical. Conservative approaches lead to fair chances of spontaneous conception and successful pregnancies. An integrated management of hydrosalpinx with bilateral salpingectomy and postsurgical usage of artificial reproduction techniques also leads to a substantial cumulative pregnancy rate.

Keywords: hydrosalpinx, surgical approach, pregnancy rates

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Апстракт

Вовед. Хидросалпинксот е многу честа дијагноза во клиничката гинеколошка пракса, особено кај пациентките со проблеми поврзани со инфертилитет. Едната или обете Фалопиеви туби може да се исполнат со значителна количина на течност, што доведува до нивна дилатација и ги прави нефункционални, обично како резултат на повреда на ткивото или инфекција. Оштетувања со различен степен може да се видат долж мукозата и тие доведуваат до нарушување на процесите на нормална фертилизација и транспорт на зиготот.
Методи. Направена е ретроспективна студија на клинички материјал од период од 5 години (2013-2017 година), притоа селектирајќи пациентки кои биле оперативно третирани, под дијагноза за унилатерален или билатерален хидросалпинс. Податоците потоа се поделени по години, според типот на третман кој бил употребен кај пациентките. Нашата цел беше да се детектираат пациентките кои биле третирани хируршки со различни типови на лапароскопски интервенции. Вкупен број од 74 пациентки влегоа во студијата, задоволувајќи ги сите критериуми.

Резултати. Во групата на пациентки третирани со лапароскопски конзервативен пристап, со презервација на тубите, зависно од типот на третман пациентките ги поделивме до две подгрупи. Дополнително се креираше трета група во која се опишани пациентки третирани со лапароскопски дефинитивен третман, унилатерална или билатерална салпингектомија. Во првата група, 9(16.36%) се спаѓаат пациентки кои се третирани лапароскопски, со формација на салпингостома (тубостома). Втората група на пациентки се третирани со билатерална хромопертубација, 17(30.91%). Третата, а притоа и најголема група на пациентки, се состои од 29 (52.7%) пациентки, од кои билатерална салпингектомија била изведена кај 9(31.03%), додека пак унилатерална била направена кај 20 (68.97%). Јасно е од резултатите најголем дел од пациентките биле третирани со унилатерална салпингектомија, секако по интраоперативна потврда за комплетна проодност на контралатералната утерина туба.
Кај вкупен број од 30 пациентки (54.5%) биле забележани успешни бремености кои завршиле со раѓање на живородени деца. Од тој вкупен број на пациентки кои остариле бременост кај 11 пациентки (36.67%) биле употребени некоја од процедурите за артефициелна репродукција и биле постигнати успешни бремености. Кај останатиот број на пациентки 19(63.33%) остварена била спонтана концепција.
Заклучок. Третманот на хидросалпинкост, како честа состојба кај пациентките што се борат со инфертилитет, главно зависи од степенот на локални тубарни промени, кои истовремено можат да бидат и евалуирани и третирани лапароскопски. Конзервативниот хируршки пристап доведува до задоволителна можност за спонтана концепција и остварување на успешна бременост. Модерниот интегриран хируршки пристап подразбира пак билатерална салпингектомија и постоперативна употреба на некоја од техниките за артефициелна репродукција, пристап што исто така води до задоволителна кумулативна стапка на успешни бременост.

Клучни зборови: хидросалпинкс, хируршки пристап, стапка на бременост

Introduction

Hydrosalpinx is a common medical condition encountered among female population with infertility issues. One or both fallopian tubes can be filled with a substantial amount of fluid, making them dilated and dysfunctional, usually as a result of an injury or infection. Often the affected area can become substantially swollen and grow to even as big as few centimeters in diameter. Damages of different degrees can be seen on the mucosal lining, which compromise the processes of normal fertilization and zygote passage. The pathophysiology of this condition is unique, usually related to pelvic inflammatory disease or excessive tissue buildup due to endometriosis. Inflammation results in destruction of the mucosal lining and fimbria, fusing them together until complete distal obstruction of the tubes. Hydrosalpinges can be divided in three groups, hydrosalpinx simplex, follicularis and sactosalpinx, depending on the anatomical changes. Destruction of the tubal epithelium leads to loss of membrane polarity, expression of membrane transporters, reabsorption and release of a large amount of inflammatory mediators and increase of serosa to mucosa fluid flow [1].

Some cases of hydrosalpinx can be easily repaired with the surgical procedure of neosalpingostomy or tubostomy, which allows pregnancy to occur naturally. Recovery from this procedure is relatively rapid and tubal function is completely resumed within a few days. Pregnancy rates following surgical tubal reconstructtion are 10% in the year following neosalpingostomy. Due to this fact and also facing with the increased risk of ectopic pregnancies in these patients most women with hydrosalpinx and extensive tubal damages are advised to move directly to salpingectomy and in vitro fertilization (IVF). Major debates have been conducted upon the impaired IVF outcome in patients with hydrosalpinx, mainly focused on the embryotoxic properties of the fluid, distorted endometrial receptivity and low percentage of successful implantations [2].

Tubal factors responsible for infertility issues account for an approximately 25% of the cases, whereas the most severe manifestation is hydrosalpinx. 10-30% of all tubal diseases can be described as unilateral or bilateral hydrosalpinges, with variable degrees of tubal distension or dilatation and presence of distal tubal occlusion. Mainly these patients present with mild lower abdominal pain and concerns related to infertility problems [3]. Surgical interventions, both conservative and definitive, are very successful and thought to improve the chances of fertilization, zygote transport, implantation and pregnancies in the future. The majority of pregnancies occur during the first year postoperatively [4].

Diagnostic evaluation and decision on how to treat tubal diseases, especially hydrosalpinx, can be very difficult. It comprises of many surgical, medical, social, emotional and economic factors [5]. Unilateral or bilateral salpingectomy has been one of the most frequently employed surgical interventions during the past years, mainly due to the theories for the possible effect of hydrosalpinx fluid on the human embryos. The majority of poor IVF results have been tracked in the group of patients with hydrosalpinx, compared to women with other tubal factor-related infertility [6]. Hydrosalpinges are mainly related to *Chlamydia trachomatis* infection, which is the main cause for pelvic inflammatory disease and 30% of these patients undergoing IVF have unilateral of bilateral hydrosalpinx present [7]. Labo-

ratory investigations were conducted in order to find the correlation between hydrosalpinges and reduced pregnancy rates in this population of patients. Changes as impaired ovarian function, endometrial damage, dilutional effect on essential nutrients and substrates, direct cytotoxic effect on gametes and embryos, inflammatory responses, mechanical washout of embryos, effect on endometrial receptivity and implantation rates were recorded and directly correlated with undesired outcomes [8-12].
The aim of this study was to find the correlation between different approaches in the surgical treatment of hydrosalpinx and post-treatment pregnancy rates.

Material and methods

We retrospectively analyzed clinical records from a period of five years (2013-2017), selecting patients diagnosed as having unilateral or bilateral hydrosalpinx. Clinical files were divided by years and according to the treatment protocol used. Our scope of interest was focused on patients who were treated surgically, with different types of laparoscopic interventions. A total of 74 patients met our criteria for selection. Inclusion criteria that we used during records' assortment were complete data aboutduration of infertility, microbiological analyses and status, complete data aboutdiagnostic procedures, treatment protocol selection, surgical protocol and detailed information related to postoperative period. All patients were contacted and data related to pregnancy rates were collected. Due to a lack of complete and detailed information 19 patients were excluded from the study, making the final group comprising of 55 patients.
All patients were evaluated at the University Clinic for Obstetrics and Gynecology, where they went through a standardized diagnostic protocol for hydrosalpinx. From the collected clinical records we collected data related to ultrasound exams, hysterosalpingogram, past and present microbiological status, presence of additional gynecological conditions, reproductive history and surgical treatment employed. After a proper preoperative preparation a total number of 55 patients was surgically treated at our surgical unit, using a KARL STORZ laparoscope. Additionally, they were divided in two groups comprising patients treated with more conservative surgical methods and those treated with radical surgical treatment, unilateral or bilateral salpingectomy.
Patients were directly contacted and asked about their postoperative period. They provided us with data related to achieved conceptions, either spontaneous or via methods for artificial reproduction and number of successful pregnancies.

Results

The final group of analyzed clinical records and patients being directly contacted consisted of 55 patients. We analyzed a period of five years (2013-2017) and divided the patients according to the year of surgical treatment of hydrosalpinx. Yearly distribution of patients showed that 8 (14.55%) were treated during 2013, 10 (18.18%) during 2014, 18(32.73%) during 2015, 7(12.73%) during 2016 and 12(21.81%) during 2017. Minimal age recorded was 21 years and maximal age was 46 years (interval 33±12, average age 33.09). Analysis of admission diagnosis showed that 17 (30.9%) patients were admitted as having bilateral salpingeal involvement and 38(69.1%) as having unilateral involvement.
Hysterosalpingography (HSG) scans revealed information upon tubal anatomy distortion and tubal patency. All 55 patients were with HSG results for hydrosalpinx, which in 23(41.8%) patients was described as bilateral and in 32(58.2%) patients as unilateral. During analysis of HSG results we recorded additional gynecological conditions, described as septum uteri and peritubal adhesions. A total number of 20 (36.3%) patients had septum of different degree and they received additional treatment, hysteroscopic resection of septum, while 9(16.36%) patients had an additional HSG changes explained as peritubal adhesions.
Data related to reproductive history were extracted from the collected clinical records. We analyzed all pregnancies reported, abortions, ectopic pregnancies and successful pregnancies, which resulted in a live birth. The analysis of these data showed that 45(81.8%) patients were nulliparous and 10(18.2%) patients were parous. In the second group all patients reported one or more live births. All patients had a complete documentation about their past microbiological status and 21 (38.2%) patients had a previous history of *Chlamydia trachomatis* infection.
All clinical records were analyzed in details regardingdata related to infertility status. Patients had been asked to give information about infertility problems, duration of infertility and treatments used in the past. Minimal duration of infertility, after which patients asked for evaluation and treatment, was one year and maximal duration was 20 years (average years of infertility 6.4 years).
Surgical protocols of laparoscopic treatment were extracted from clinical records and based on that data patients were divided in three groups. Two groups were related to conservative laparoscopic approaches and one to complete radical surgical treatment, unilateral or bilateral salpingectomy. Patients in the first group, 9 (16.36%), were treated with a laparoscopically guided creation of salpingostomy. The second group comprised patients treated only with bilateral chromopertubation and consisted of 17(30.91%) patients. The third, and the most numerous group consisted of 29(52.7%) patients treated with salpingectomy. Bilateral salpoingectomy was performed in 9(31.03%) and unilateral in 20(68.97%) patients, showing that majority of patients were treated with unilateral salpingectomy, after intraoperative evaluation of tubal patency of the contralateral uterine tube.

Data collected from the postoperative period, with a mean follow-up of 15 months, provided us with information related to additional infertility treatment and conceptions achieved. A total number of 30 patients (54.5%) had successful pregnancies, which ended up with a desired outcome, healthy live birth. Procedures for artificial reproduction and successful pregnancies achieved with IVF were recorded in 11(36.67%) patients and in the remaining 19 (63.33%) patients pregnancies were achieved via spontaneous conception (Table 1). Methods of delivery were also analyzed and the distribution of spontaneous deliveries and cesarean sections was equal (15 patients, 50% in both groups).

Table 1. Distribution of pregnancies among patients in correlation with methods of treatment

Treatment	Number of pregnancies
Chromopertubation	9, from which 7 spontaneous and 2 ART
Creation of tubostoma	6, from which 4 spontaneous and 2 ART
Unilateral salpingectomy	9, from which 8 spontaneous and 1 ART
Bilateral salpingectomy	6, all ART

Our special field of interest was the group of patients in which the laparoscopic chromopertubation probe showed that one tube was overall healthy and patent, while contralateral was distorted, pathologically changed and obstructed. This group consisted of 20 patients, who were surgically treated with unilateral salpingectomy. From the total number of successful pregnancies achieved (30), nine were recorded in this group. Interestingly, pregnancies among these patients were mainly spontaneous conceptions, with only one pregnancy achieved via IVF (Figures 1, 2 and 3).

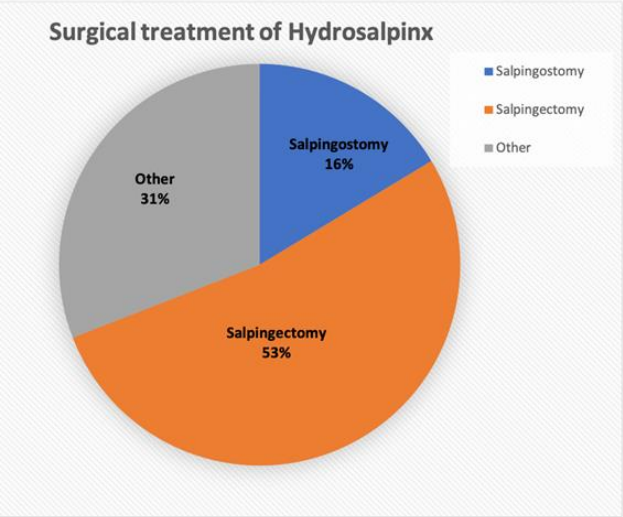

Fig. 1. Distribution of different surgical treatment options in patients with hydrosalpinx

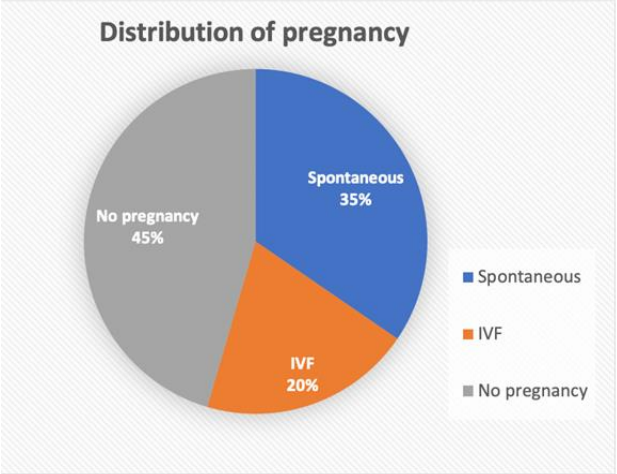

Fig. 2. Pregnancy rates in treated patients

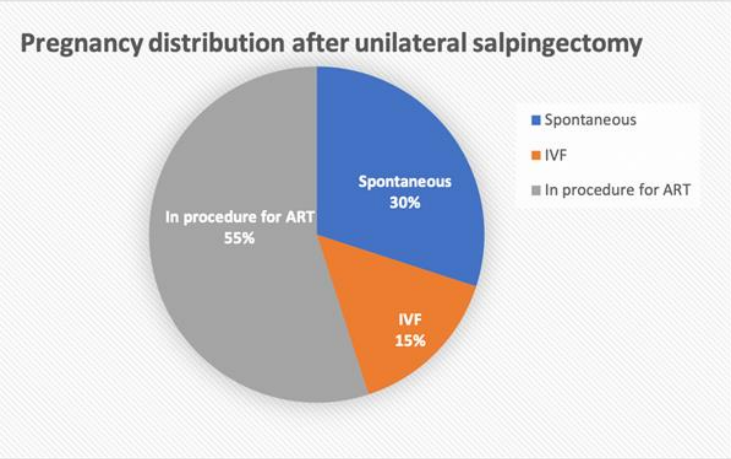

Fig. 3. Distribution of pregnancies in the group of patients treated with unilateral salpingectomy

Discussion

Increasing rates of gynecological infections and incidence of pelvic inflammatory diseases among young fertile population lead to a considerable rise in patients diagnosed with hydrosalpinx. This condition is mainly recorded among infertile young female population. Many debates and researches have been conducted during the past decade in order to detect and explain factors of hydrosalpinx that influence conception rates. Also, hydrosalpinges have been related to IVF success rates. Several studies showed that presence of hydrosalpinx can reduce the probability of achieving a pregnancy by 50% and at the same time double the rate of spontaneous abortions [13]. Hydrosalpinx fluid is the main reason for lower pregnancy rates in these patients. Any surgical intervention that interfere the communication between uterus and tube would remove the leakage of hydrosalpinx fluid and restore pregnancy rates particularly seen in patients treated with salpingectomy. Many studies which were conducted during the past ten years showed that hydrosalpinx fluid had major embryotoxic properties, which was proved *in vitro* on human embryos. Undiluted hydrosalpinx fluid resulted in a 50% reduction in blastocyst development compared with control medium. Mainly patients did not exhibit presence of pathogenic microorganisms at the time of evaluation, but slightly elevated concentrations of endotoxins have been demonstrated in individual fluids as a sign of previous *Chlamydia trachomatis* infection. For these patients there is still a debate with regard tothe effectiveness of aspiration of hydrosalpingeal fluid before initiation of IVF protocol [14]. Oxidative stress, which is defined as an elevated concentration of reactive oxygen species on cellular level, has been employed in the function of various reproductive conditions, such as hydrosalpinx, proposing that these products can change the environment for fertilization and decline its success [15]. Hydrosalpingeal fluid has been related to improper embryo development, due to low energy substrate distribution, mainly related to glucose and pyruvate transport. Glucose concentration is generally found to be very low in hydrosalpinx fluid, compared tolevels in normal tubal fluid. Lack of nutrients leads to impaired development of blastocysts and impaired implantation capacity of the embryo [16-18]. A substantial number of studies have evaluated the impact of hydrosalpingeal fluid on endometrial receptivity, due to a high concentration of cytokines. Some of them focused on the role of fluid leakage through the uterine cavity, causing simple embryo disposal, or wash out [19-21].

Tubal diseases have been reported to be responsible for 20-25% of infertility issues and the main discussion is focused on how to treat them, especially hydrosolpinges. Many studies increased the awareness of tubal factors for infertility and pointed out the main protocols for diagnosis and treatment of hydrosalpinx. One review compared tubal surgery, spontaneous conception, tubal surgery and IVF. The first line treatment offered for women aged less than 35 years, especially in those with unilateral tubal pathology, should be tubal surgery. IVF should be offered to patients older than 35, with additional factors responsible for the couples' infertility, if severe tubal disease is present or if there has been more than 12 months post-surgery without successful conception. Many authors evaluated the outcome of pregnancies in patients with hydrosalpinx treated with minimally invasive alternative therapies, due to the dense pelvic adhesions. They compared laparoscopic treatments such as salpingectomy, tubal occlusion, hysteroscopic insertion of devices for tubal occlusion and aspiration of hydrosalpingeal fluid and concluded that laparoscopic surgical treatment should be considered in all patients diagnoses with hydrosalpinx, especially in those preparing for IVF [22].

Analyzing the results of natural conception rate after treatment of hydrosalpinx with laparoscopic salpingecto-

my showed that clinical pregnancy rate was 27%, in the hands of experienced surgeons who most often publish their results. During the past years, due to the high successful rates and advent of assisted conception treatment, tubal surgery is not commonly offered to women with hydrosalpinges, which has been counterproductive because there is a detected rise in the number of natural conceptions following surgical laparoscopic treatment of hydrosalpinx. One systematic review and meta-analysis of 22 observational studies revealed that the cumulative pregnancy rate after laparoscopic salpingostomy was 8.7% at 6 months, 13.3% at 9 months, 20% at 12 months, 21.2% at 18 months and 25.5% at 24 months post-surgery. The findings of this systematic review suggest that salpingostomy can be an effective alternative treatment strategy in patients with hydrosalpinx which are poor candidates for salpingectomy, due to dense per tubal adhesions [23].

Eighty-one patients, who were infertile and diagnosed with uni- or bilateral hydrosalpinx, were planned for surgical treatment, during a period of five years. During laparoscopy a systematic evaluation of the tubes was firstly conducted and the local management protocol was based on validated tubal prognostic values. Surgery was conservative, neosalpingostomy, or radical, salpingectomy. Neosalpingectomy was performed in 35 patients and salpingectomy in 46 patients. The overall cumulative pregnancy rate was 61% for couples who completed the treatment. Among patients with at least one functional tube, the overall cumulative pregnancy rate was 63.3%, with a spontaneous pregnancy rate of 30.4% [24].

Hydrosalpinx has been tracked as a major problem related to tubal factors of infertility and its treatment is of a great importance. Some studies compared different approaches such as salpingectomy, tubal occlusion and neosalpingostomy, with an accent on the cumulative pregnancy rates post-surgery. Additional analyses were conducted to point out the impact of different treatment protocols on IVF. Almost similar responses to controlled ovarian stimulation and pregnancy outcomes were observed in patients treated with salpingectomy, tubal occlusion and neosalpingostomy [25].

In patients with tubal obstruction, but intact endothelium morphology, we can choose the creation of a tubostoma as the most appropriate treatment with substantial success in achieving spontaneous implantation (in our study 66.6% among pregnant patients).

Presence of hydrosalpinx undoubtedly impairs the outcome of IVF treatment. It can be improved by simple removal of the tube, but some concerns rose regarding its feasibility and safety. One meta-analysis evaluated the efficiency of hydrosalpinx aspiration with or without sclerotherapy, compared with salpingectomy and compared to no treatment. Authors were mainly focused on recurrence rate, fertility outcomes and adverse events. The overall recurrence rates of hydrosalpinx after aspiration with or without sclerotherapy were 21.7% to 30.5% and 21.8% to 32.5%, respectively. Clinical pregnancy rates were similar between group treated with salpingectomy and those treated with aspiration and sclerotherapy. Compared to no intervention, treatment with tubal aspiration led to a significant increase in the clinical pregnancy rates. In selected cases aspiration and scelotherapy can be used as an alternative to salpingectomy [26]. Recent studies have raised awareness aboutthe effect of salpingectomy on ovarian function. Salpingectomy, as the most aggressive form of tubal surgery, may be performed for various tubal diseases, including hydrosalpinx, as a method to enhance the fertility. Although salpingectomy has an overall positive effect on the spontaneous conception rates (when it is unilateral) and the successful outcomes with IVF treatment (when it is bilateral) meta-analysis showed that it was not statistically related to the ovarian function. Various authors have stated that in limited cases salpingectomy was related to an unaware removal of a functional ovarian tissue, leading to a reduction of the ovarian function. There are conflicting studies that show some slight impairment in the parameters of ovarian function and reserve, but they are mainly in patients who underwent salpingectomy for an ectopic pregnancy. This field of research is still wide and opened for conducting of new studies [27].

To test the hypothesis that IVF pregnancy rates for patients with tubal factor infertility were improved after surgical treatment, a group of authors retrospectively evaluated IVF program success in patients treated in a private infertility clinic. A total of 160 patients undergoing 238 cycles of IVF were evaluated and surgical intervention improved implantation and pregnancy rates in patients with more than one prior failed cycles (16.1% and 37.5%). The type of selected surgery did not affect success rates in this study [28].

Some authors suggest that surgical treatment should be considered for all women with hydrosalpinges prior to IVF treatment. Previous protocols supported only unilateral salpingectomy for unilateral hydrosalpinx and bilateral salpingectomy for bilateral hydrosalpinx. Recent reviews of literature provide evidence that laparoscopic tubal occlusion provides equal rates of success [29]. Ovarian response in patients with hydrosalpinx undergoing IVF treatment was also evaluated. One study evaluated patients aged <37 years, undergoing IVF 12 months post-surgery. Differences found among these patients showed that pregnancy rate was not only influenced by the type of surgery employed but also by the factors such as clinical status, selection of stimulation protocol and total number of oocytes retrieved [30].

When we face cases with destroyed tubal endothelium morphology, tubal obstruction and hydrosalpinx, but at the same time the contralateral ovarian tube is morphologically normal, we can choose laparoscopic unilateral salpingectomy as a choice of successful treatment.

With this approach we can reach appreciable percentage of cases with spontaneous pregnancies (89% in our study, which means eight of nine patients achieved spontaneous implantation).

A study for evaluation of differences in uterine and ovarian blood flows prior and after surgery for hydrosalpinx was conducted. In a university teaching hospital 60 patients were treated surgically and parameters as uterine and ovarian artery pulsatility index, resistance index, flow index, vascularization flow index, 3D Doppler vascularization index, endometrial and ovarian volume were recorded. They concluded that hydrosalpinx was majorly correlated with impaired endometrial and ovarian blood flows, which in return may adversely affect endometrial receptivity and oocyte quality [31].

Conclusion

Hydrosalpinx management is mainly influenced by the local tubal changes evaluated laparoscopically and can be surgically treated, either conservative or radical. Conservative approaches lead to fair chances of spontaneous conception and successful pregnancies. An integrated management of hydrosalpinx with bilateral salpingectomy and post-surgical usage of artificial reproduction techniques also leads to a substantial cumulative pregnancy rate.

Conflict of interest statement. None declared.

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ММР

Case report

SUCCESSFULLY TREATED INFERTILITY IN A PATIENT WITH TURNER SYNDROME WITH IN VITRO FERTILIZATION WITH DONATED EGG – A CASE REPORT

УСПЕШНО ТРЕТИРАН ИНФЕРТИЛИТЕТ КАЈ ПАЦИЕНТКА СО ТАРНЕР СИНДРОМ СО ИН ВИТРО ФЕРТИЛИЗАЦИЈА, СО ДОНИРАНА ЈАЈНА КЛЕТКА-ПРИКАЗ НА СЛУЧАЈ

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Abstract

We present a case of a 34-year-old woman, diagnosed with Turner syndrome at the age of 18 because of short stature and irregular menstrual cycle. After the diagnosis had been established, she started with hormonal therapy for regulation of the menstrual cycle. When she got married at the age of 32 she wanted to conceive, but it was not successful and after one year she decided to make *in vitro* fertilization with a donated egg. It was successful in the first attempt. Her high-risk pregnancy was followed up by her gynecologist and obstetrician in atertiary level institution. The pregnancy was normal, without complications. During the pregnancy examinations at the University Clinic for Cardiology in Skopje were made and cardiovascular diseases were excluded. In 39 week of gestation an elective Caesarean section was made at the University Clinic for Gynecology and Obstetrics in Skopje, R. N. Macedonia.
On 28th of February 2019 she delivered ahealthy baby boy with birthweight of 2950 gr and birthlength of 48 cm, with Apgar scores 8 and 9 at 1 and 5 min, respectively. No severe complications occurred during the postpartum period.
By application ofnew assisted reproductive techniques such as *in vitro* fertilization with a donated egg, women with Turner syndrome and infertility can resolve their problem and fulfill their wishto have a baby.

Keywords: Turner syndrome, pregnancy, *in vitro* fertilization, donated egg

Апстракт

Презентираме случај на 34 годишна жена, дијагностицирана како Тарнер синдром на возраст од 18 години, заради низок раст и нерегуларен менструален циклус. Потоа, таа почнала со хормонска терапија за регулација на менструалниот циклус. Кога се омажила на 32 години сакала да забремени, но тоа било неуспешно и по една година одлучила да направи вештачко оплодување со донирана јајна клетка. Тоа било успешно од првпат. Бременоста била следена како високоризична од нејзиниот гинеколог и акушер од институција од терциерно ниво и таа била нормална, без компликации. За време на бременоста биле направени иследувања на Универзитетската клиника за кардиологија во Скопје и биле исклучени кардиоваскуларни заболувања, при што во 39-та гестациска недела бил направен елективен царски рез на Универзитетската клиника за гинекологија и акушерство во Скопје.
Таа се породила на 28 февруари, 2019 година, со здраво машко новороденче со родилна тежина од 2.950 грама и родилна должина од 48 сантиметри, со Апгар скор од осум и девет, во првата и во петтата минута. Немало посериозни компликации во постпарталниот период.
Со новите техники на асистирана репродукција, како вештачко оплодување со донирана јајна клетка, жените со Тарнер синдром и инфертилитет можат да го решат нивниот проблем и да ја исполнат нивната желба да имаат дете.

Клучни зборови: Тарнер синдром, бременост, вештачко оплодување, донирана јајна клетка

Introduction

Turner syndrome (TS) is one of the common chromosomal abnormalities in women.
It occurs in one of 2500 live female births [1]. This syndrome is characterized by abnormal meiotic division with missing or partial missing of one of the X chromosomes (sex chromosomes). The genetic alteration of Turner syndrome may be different, such as:

1. Monosomy with complete absence of an X chromosome. This occurs because of an error in the father's sperm or in the mother's egg and results in every cell in the body having only one X chromosome.

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2. Mosaicism, with an error in cell division during early stages of fetal development. This results in some cells in the body having two complete copies of the X chromosome. Other cells have only one copy of the X chromosome.
3. X chromosome abnormalities with abnormal X chromosome or missing parts of one of the X chromosomes. Cells have one complete and one altered copy. This situation occursdue toproblems with the sperm or the egg, and sometimes the loss or alternation of the X chromosome happens early in fetal development. In early fetal development only some cells contain the abnormal or missing parts of one of the X chromosomes (mosaicism). This causes errors during fetal development and after birth short stature, ovarian insufficiency and heart defects.
4. The presence of Y chromosome material. In this situation some cells have one copy of the X chromosome and other cells have one copy of the X chromosome and some Y chromosome material. These individuals develop biologically as female, but the presence of Y chromosome material increases the risk of developing a type of cancer called gonadoblastoma [2,3].

Turner syndrome can cause a variety of medical and developmental problems and affect psychosocial development of the women [4].

These are the signs of Turner syndrome at birth and infancy: wide and weblike neck, broad chest with widely spaced nipples, cardiac defects, short fingers and toes, slightly smaller than average height at birth, low-seat ears, slowed growth, low hairline at the back of the head, high and narrow roof of the mouthetc. In childhood, teens and adulthood they may havethese signs and symptoms: slowed growth, adult height less than might be expected for the female memberof the family, failure of sexual changes expected during puberty, sexual development that stalls during teenage years, inability to conceive without fertility treatment. Patients with Turner syndrome may have other problems: ear malformations and hearing problems, vision problems (strabismus, nearsightedness), heart defects (constriction of the aorta, malformations of the aortic valve), high blood pressure, abnormalities with their kidneys and increased risk ofurinary tract infections, high risk of type 2 diabetes, higher risk ofosteoporosis, thyroid disorders (hypothyroidism), gluten intolerance (celiac disease), risk ofautoimmune disorders (Hashimoto's thyroiditis), skeletal problems (scoliosis), learning disabilities, mental health issues with an increased risk of attention-deficit or hyperactivity disorder.

Mosaic cases of TS present milder phenotypic abnormalities compared to those with 45, X karyotype. Premature ovarian failure (POF) can be expected in most of Turner women. Hormonal replacement therapy (HRT) is necessary to achieve the development of normal sexual characteristics and to prevent cardiovascular complications and osteoporosis. Very often amenorrhea can be presented in thesegirls as aresult of an accelerated loss of oocytes during the second half of fetal life or over a few postnatal months or years. About 40-50% of girls with TS have some spontaneous pubertal development and 5-10% have menstrual periods [5].

Most of the women with TS are infertile. Spontaneous pregnancies occur in about 2-10% [6]. Most of these pregnancies occur in women with mosaic type of TS and only 0.4% were reported in women with non-mosaic type of TS. Very few pregnancies in which the fetus has Turner syndrome result in live births. Most of them end in early pregnancy loss [7,8].

With the improvement of theassisted reproductive techniques, oocyte donation has becomea new treatment option for infertility of these women. When *in vitro* fertilization (IVF) with donated oocytes is performed in patients with Turner syndrome, only a single embryo should be transferred to avoid complications in pregnancy [9].

Pregnancies in women with TS, conceived spontaneously or with donated oocytes, are considered high risk because of the associated miscarriages and life-threatening cardiovascular complications (aortic dilatation, aortic valve disease, aortic dissection, severe hypertension) and elevated risk of pregnancy-induced hypertension, preeclampsia, eclampsia and maternal and fetal complications [10].

Therefore, it is recommended to conduct full preconception evaluation such as cardiac assessment with Holter blood pressure monitoring, echocardiography, thoracic MRI. Abnormal findings (aortic dilatation) could contraindicate pregnancy in thesepatients.

Women with a Turner mosaic karyotype appear to have a lower risk of obstetrical and cardiovascular complications, but should nevertheless undergo the full preconception evaluation [11,12].

A case presentation

We present a case of a 34-year-old primaparous woman. She had her first menarche at the age of 11. After that her menstrual cycle was irregular (at three or six months), a type of oligomenorrhea and secondary amenorrhea. She started with medical examination sat the age of 14 at the University Clinic for Pediatric Disease in Skopje because of short stature and irregular menstrual cycle. Her height was 143 cm and body weight 42 kg, with body mass index (BMI) of 20.5 kg/m^2. After that she was transferred to the University Clinic for Gynecology and Obstetrics in Skopje. Karyotype showed that she had Turner syndrome (karyotype 45, XO), sex chromatine was negative (Figure 1 and Figure 2). The examination for kidney diseases showed normal findings. Also, echocardiography was made and it was with a normal finding. Hormonal analyses were done, with a finding of hypothyroidism (Hashimoto thyroidi-

tis). She started with supplementation therapy (Tbl. Euthyrox 50 mg/per day). Because of osteoporosis and amenorrhea secundaria she started with hormonal therapy, a combination of estrogens andprogestagenes (Drag. CycloProgynova, Scherring). After that she had regular menstrual cycle. She got married at 32 years and she wanted to conceive. After one year of attempts to conceive without success,she decided to make *in vitro* fertilization with egg donation, which was successful in the first attempt.An adequate hormonal replacement therapy prior to IVF was given. She started with estradiol 5 days before the donor's oocyte retrieval and progesterone was started on the same day of oocyte retrieval. One embryo was transferred to her uterine cavity the second day after oocyte retrieval. The serum concentration of choriongonadotropin (HCG) was measured 15 days after embryo transfer and the result was positive. Pregnancy was carefully followed-up byher gynecologist in coordination with obstetricians from a tertiarylevel institution.

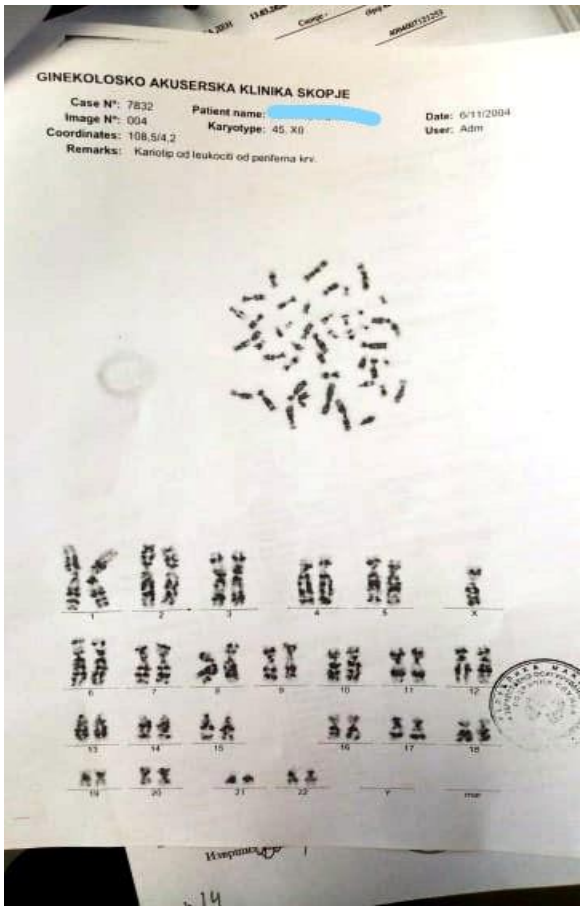

Fig. 1. Karyotype of the patient 45,XO (Turner syndrome)

The pregnancy was normal, without complications. During pregnancy, examinationsat the University Clinic for Cardiology in Skopje were made and cardiovascular diseases were excluded. In 39 week of gestation an elective Caesarean section was made at the University Clinic for Gynecology and Obstetrics in Skopje.

On 28th of February 2019, she delivered a healthy baby boy with birthweight of 2950 gr and birthlength of 48 cm,

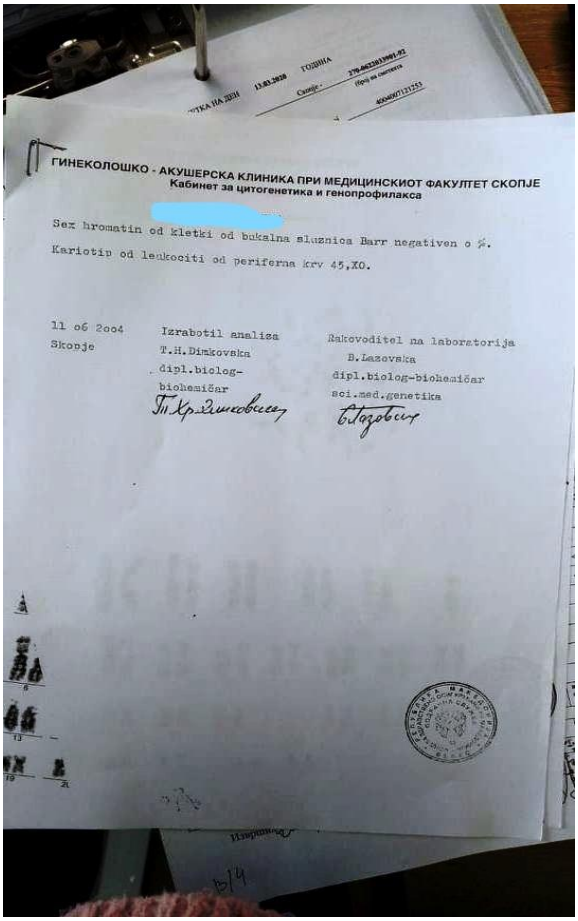

Fig. 2. Karyotype 45, XO, Sex Hromatin Barr negative 0%

with Apgar scores 8 and 9 at 1 and 5 min, respectively. No severe complications occurred during the postpartum period.

The baby was on breastfeeding from the delivery and has hada normal development in the first year of his life.

Discussion

Modern techniques of artificial human reproduction can help in infertility treatment of patients with TS. *In vitro* fertilization with donated eggs is one possible option for these patients [13]. All women with TS are at risk during pregnancy, without distinction between those with 45, X karyotypes, partial X-chromosome deletions or mosaic karyotype. Complete pre-pregnancy medical evaluation is mandated, including cardiovascular assessment with cardiac imaging with echocardiography and cardiac magnetic resonance imaging (MRI). It is necessary to make consultations with specialists in cardiovascular diseases, endocrinology and high-risk obstetricians to ensure the best chance for a successful pregnancy and delivery of a healthy infant [14].

In our case IVF with a donated egg was offered for fertility treatment and was successful in the first attempt.

According to a recommendation only a single embryo was transferred. Our patient was followed during her pregnancy in atertiarylevel institution (University Clinic for Gynecology and Obstetrics in Skopje, R.N. Macedonia). There were no complications during pregnancy. We advised her to check her blood pressure at home during pregnancy and the values were in normal range. Because of hypothyroidism she received higher doses of supplementation therapy (Tbl. Levothyroxin-75 µgrto 100µgr/per day). Pregnancy was terminated in 39 week of gestation with Caesarean section.

Because of multiple medical conditions associated with Turner syndrome, it is very important women considering pregnancy to be appropriately counseled for potential complications during pregnancy and about adequate screening before and during pregnancy [15]. Pregnancy is contraindicated in patients with TS in presence of difficult hypertension and structural cardiac diseases (bicuspidal aortal valve, aortic coarctation, aortic dilatation) [16]. In cases where assisted reproductive techniques are required, a single embryo should be transferred to avoid complications, such as multiple gestation with an increased risk ofspontaneous abortion or preterm delivery.

During pregnancy, a close follow-up is needed, with periodic echocardiography or MRI, monitoring of thyroid function, glucose metabolism and blood pressure.

In absence of cardiovascular abnormalities, vaginal delivery may be attempted. In cases with increased aortal diameter Cesarean section should be done [16]. Because of their short stature, women with TS usually have small pelvis, which predisposes them to fetopelvic disproportion. That is why there is a high percentage of deliveries with Caesarean section in this group of patients compared to the general population. Hagman *et al.* reported in their study that among 122 pregnancies in patients with Turner syndrome with IVF with an egg donation, 100 patients (82%) were delivered by Caesarean section [11]. Our patient was with short stature and small pelvis, and hence we decided to deliver her with Caesarean section.

Women with TS must be informed about the increased chance of delivery with Caesarean section. In patients with contraindications for pregnancy, alternative methods including adoption or surrogacy, should be offered [17].

Conclusions

- By application ofnew assisted reproductive techniques, women with Turner syndrome and infertility can resolve their problem and fulfill their desire to have a baby.
- Medically-assisted pregnancies with donor oocytes should only be attempted in TS women without cardiovascular diseases.
- Only one embryo should be transferred, and close medical follow-up during pregnancyshould be warranted.
- Because of many risks associated with these pregnancies adequate follow-up is mandatory, especially by acardiologist, an endocrinologist and a high-risk pregnanciesobstetrician.
- We must think about many risks during pregnancy and during delivery of these patients and choose the best option for them.

Conflict of interest statement. None declared.

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ММР

Case report

MENAGEMENT OF BUTTONHOLE RECTAL INJURY AFTER VAGINAL DELEVERY - A CASE REPORT

МЕНАЏМЕНТ НА BUTTONHOLE РЕКТАЛНА ПОВРЕДА ПОСЛЕ ВАГИНАЛНО ПОРАЃАЊЕ - ПРИКАЗ НА СЛУЧАЈ

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Abstract

Introduction. A special form of higher degree perineal tear is a laceration of the anorectal epithelium with intact external anal sphincter muscle ("buttonhole tear"). This is very rare but, when not treated, carries the risk of a rectovaginalfistula.
Case Report. We present a case of a nullipara, in which a spontaneous vaginal delivery of a fetus in the vertex presentation was complicated by a buttonhole rectal tear with a partial lesion of the sphincters. It was recognized on the 4th postpartum day, and a reconstruction of the lesion with a diversing colostomy was made soon. Upon returning the colostoma, the patient at 6 months postpartum has good continence and is in a good condition.
Discussion. There are very few cases reporting an isolated rectal lesion during parturition. Several factors may play a role in the etiology of these lesions, including instrumentation, birth weight of more than 4 kilograms, midline episiotomy, persistent occipitoposterior presentation, nulliparity, tissue factors, and second stage >1 hour. Obstetric anal sphincter and rectal injuries can be missed if rectal examination is not carried out as a standard procedure prior to suturing. This can have a devastating effect on the physical and emotional well-being of women.
Conclusion. Careful examination of the vagina and the rectum should be performed in all cases of perineal tears following a vaginal delivery. Buttonhole injuries, although rare, should be considered as severe traumas similar to the 4th degree lacerations and managed promptly by experienced surgeons.

Keywords: buttonhole rectal tear, vaginal delivery

Апстракт

Вовед. Buttonhole rectal tear претставува повреда од повисок степен и означува лацерација на аноректалниот епител со интактен надворешен и внатрешен сфинктер. Ваквите акушерски повреди се многу ретки, но кога не се третирани носат ризик од ректо вагинална фистула.
Приказ на случај. Презентираме случај на нулипара, кај која спонтаното вагинално породување на фетус во главична презентација се комплицира со buttonhole ректална повреда со парцијална лезија на сфинктерите. Повредата клинички се манифестира на четвртиот постпартален ден, а наскоро потоа е направена реконструкција на лезијата, и креирана е диверзирачка биполарна колостома. По враќањето на колостомата, на евалуацијата на шестиот постпартален месец пациентката е со уредна континенција и во општа добра состојба.
Дискусија. Во литературата е објавен само мал број изолирани интрапартални ректални повреди. Во етиологијата на овие повреди се вбројуваат: оперативно вагинално породување, родилна тежина над четири килограми, медијална епизиотомија, перзистентна окципитопостериорна презентација, нулипаритет, локални ткивни фактори, второ родилно време, подолго од два часа. Акушерските анални и ректални повреди може да бидат пропуштени, доколку стандарден ректовагинален преглед не се направи пред да се почне со сутурирање на породилните повреди. Ваквите повреди можат да имаат катастрофален ефект врз физичката и емоционалната добро состојба на пациентката.
Заклучок. Неопходен е внимателен преглед на вагината и ректумот кај сите повреди на перинеумот по вагинално породување. Иако ретки, buttonhole ректалните повреди се повреди од висок степен слично на перинеалните повреди од четврт степен и потребно е што побрзо да бидат менаџирани, и тоа од искусни хирурзи.

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Клучни зборови: buttonhole ректална повреда, вагинално породување.

Introduction

Obstetric perineal and anal sphincter injuries have been well described. Perineal tears are divided into four grades according to the extent of the lesion [1]. A special form of higher degree perineal tear is a laceration of the anorectal anterior wall with laceration of posterior vaginal wall and at the same time with intact external anal sphincter muscle ("buttonhole tear"). This is very rare but, when not treated, carries the risk of a rectovaginal fistula and can be diagnosed by anal palpation in the postpartum period [2-4]. Rectal lesions with or without anal sphincter trauma in childbirth are only sporadically described in literature. There is no separate classification for obstetrical rectal tears without injury to the perineum. The prevalence of these injuries has not been reported in the literature. Failure to recognize and repair perineal and rectal lacerations is associated with short and long-term morbidity as hemorrhage, perineal pain, dyspareunia, rectovaginal fistulae, perineal abscess, and incontinence of stool or flatus [5]. With this case report, we would like to raise the awarness that a rectal lesion is a potential but rare complication of vaginal delivery.

Case report

A 29-year-old primigravida was admitted to the labour room at 39 5/7 weeks amenorrhea for delivery with cephalic presentation. The patient had a history of three miscarriages, and the current pregnancy was achieved by artificial insemination. During this pregnancy, due to a short cervix in the 20th gestational week, a cervical cerclage was placed.
The first stage of labour progressed normally with no support of oxytocin. Only spasmolytic agents were administered. The second stage of labour started after full dilatation was reached with the occiput at level Hodge 3. A left mediolateral episiotomy was placed when the fetal head remained visible in the vaginal introitus between contractions. A boy of 3620 grams was born with an Apgar score of 8 after 1 minute and 9 after 5 minutes and umbilical artery pH of 7.31. Rectovaginal examination immediately after delivery showed both the internal and external sphincters to be intact. The episiotomy was repaired routinely according to our local protocolunder local field anesthesia in the operating room. The patient remained hospitalized longer due to neonatal infection of the newborn. On the third postpartum day, fecal contents of the hygiene pad were observed during the examination. A vaginal speculum examination revealed the presence of fecal matter in the vagina, while a rectovaginal examination revealed communication between the vagina and rectum. The sphincter function seemed preserved. According to local practice, the patient was transferred to the Clinic of Digestive Surgery for further treatment. There the detailed examination showed a rupture of the posterior wall of the vagina and anterior wall of the rectum approximately 6 cm in length, occupying only a small portion of the upper sphincter. An indication for operative treatment was established on the 7th postpartum day. First a laparoscopic bipolar colostomy for diversion was made. Then a complete evaluation of the perianal region under anesthesia was performed, which concluded the following: the external sphincter was preserved 2 cm in length, with the finger collapsing into a large cleft of the rectum and vagina of approximately 6 cm.
The mediolateral episiotomy was completely opened in the operating room, giving the impression that it was not the cause of the rupture, but a classic buttonhole rectal tear (Figure 1). Given the size of the cleft, as well as the non-inflammatory state of the tissue, it was decided to do the reconstruction in this act.

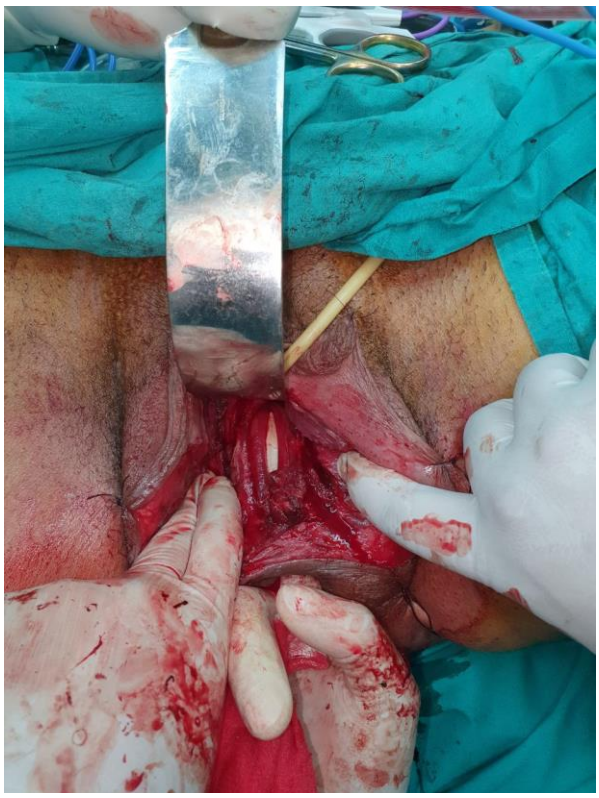

Fig. 1. Big laceration of posterior vaginal wall with rectal communication (finger inserted through anal canal)

An incision was made between the vagina and the rectum, accessing the rectovaginal septum. The posterior wall of the vagina was carefully dissected from the rectal muscle layer to obtain all the necessary layers. The vagina and rectum edges were debridged, followed by suturing of the rectum mucosa with Vicryl 2-0 (Image2). The rectal muscle layer was then sutured with monosyn 2-0 continuous suture (Image 3),

followed by suturing the vagina with Monosyn 2-0 continuous suture. A full-length drainage drain was installed in the rectovaginal space. The external anal sphincter was partially sutured with "U" sutures without overlapping. Then a laparoscopic diverting colostomy was performed.

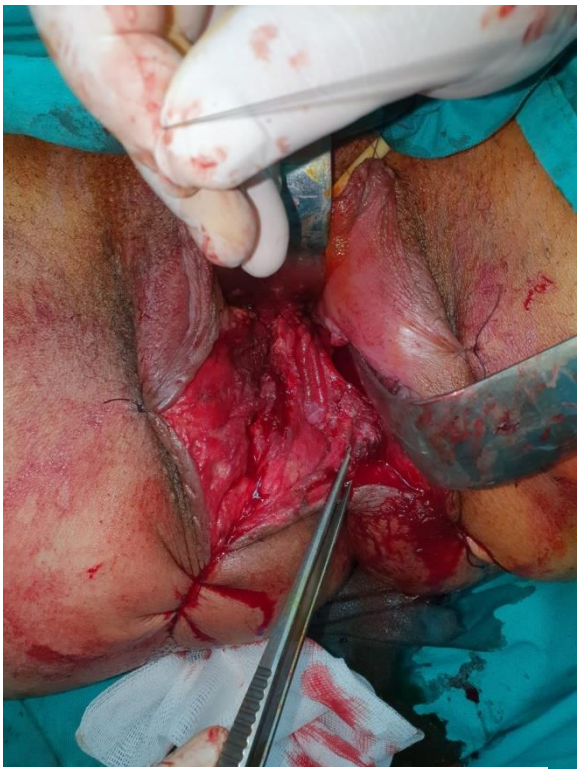

Fig. 2. Suture of rectal mucosa layer with continuous 2-0 Vicryl suture

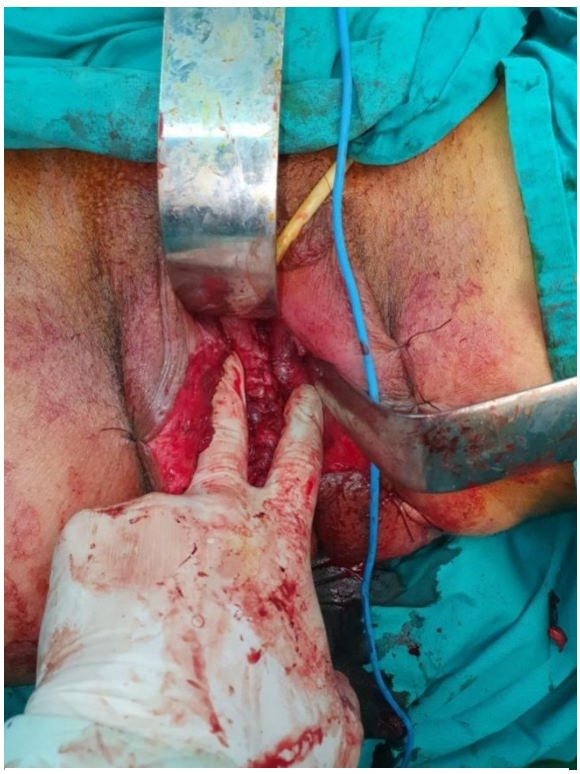

Fig. 3. Suture of rectal muscular layer with continuous 2-0 Monosyn suture

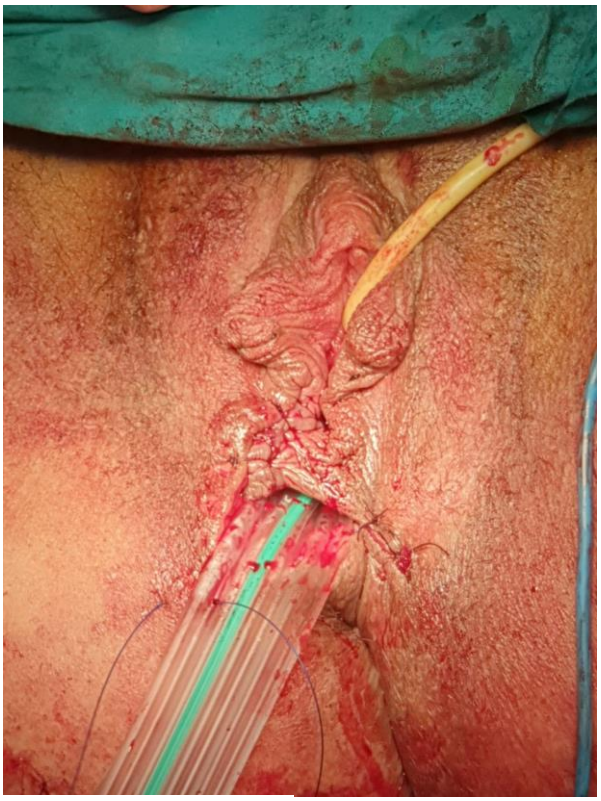

Fig. 4. Final result after repair

The postoperative course was uneventful.

Three months after this surgery, a colostomy return was made and intestinal continuity was established by creating a T-T-anastomosis. At 3-month follow-up check-up, the patient was in good condition, with a good continence and with no additional complaints.

Discussion

A review of the literature revealed very few cases reporting an isolated rectal lesion during parturition [6-10]. This may be because of its rarity, but the most possible explanation is underreporting of the problem. Few isolated cases of rectal lesions during parturition of different causes have been reported [6,9,10]. Three were after vacuum delivery, three during spontaneous delivery, and one prior to the first traction in vacuum delivery. All these cases concerned deliveries with the child in vertex presentation [6,9,10]. A rectal lesion due to perforation of the rectovaginal septum by the foot of the child in cases with breech presentation has been very rarely described. Lesh [7] described a case in 1952, and thesecond case wasdescribed as “hear-say” in the textbook on Operative Obstetrics by Myers-cough [11]. The third case was reported by Verges Spooren and de Leeuw in 2011 [12].

It is important to recognize obstetric anal sphincter and rectal injuries. These lesions can be missed if rectal examination is not carried out as a standard procedure prior to suturing. This can have a devastating effect on the physical and emotional well-being of women.
Rosenhein *et al.* [13] have madea classification of lesions (not necessarily obstetric in origin) and have given a possible protocol for treatment. The lesion in our patient was not recognized in the acute period, hence it was not possible to repair it directly.
There are several factors that may play a role in the etiology of these lesions, including instrumentation, birth weight of more than 4 kilograms, midline episiotomy, persistent occipitoposterior presentation, nulliparity, tissue factors, and second stage >1 hour [14]. It is also postulated that rapid descent of the fetal head gives inadequate time for tissues to adjust to the passage of the fetus. The only apparent predisposing factor in our patient was nulliparity.

Conclusion

Careful examination of the vagina and the rectum should be performed in all cases of perineal tears following a vaginal delivery. Buttonhole injuries, although rare, should be considered as severe traumas similar to the 4th degree lacerations and managed promptly by experienced surgeons. An appropriate surgical technique and postoperative management ensures optimal results minimizing long-term risks. Our case has shown that even in cases where this complication of vaginal delivery is later diagnosed and manifested, proper assessment and adequate surgical treatment can result in almost complete recovery without consequences.

Conflict of interest statement. None declared.

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ММР

Case report

DOUBLE, VERY SHORT CYSTIC ARTERY: ANATOMIC VARIATION REVEALED DURING LAPAROSCOPIC CHOLECYSTECTOMY: A CASE REPORT

ДВОЈНА, МНОГУ КРАТКА ЦИСТИЧНА АРТЕРИЈА: АНАТОМСКА ВАРИЈАЦИЈА ЗАБЕЛЕЖАНА ВО ТЕК НА ЛАПАРОСКОПСКА ХОЛЕЦИСТЕКТОМИЈА

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Abstract

Introduction. The cystic artery (CA) is the key structure sought to be clipped or ligated during laparoscopic or conventional cholecystectomy. In up to 25% of subjects, the superficial and deep branches of the CA have separate origins and Michels called them double CA.
Case report. We are presenting a 38-year-old female with one-year history of chronic gallbladder inflammation. During the laparoscopy dissection in the region of the Calot's triangle, we revealed an anatomic variation of the cystic artery-a double cystic artery. The more important thing was that both branches were extremely short, or at the lower limit of the published lengths of this blood vessel-approximately 3mm each. By doing so, the surgicalcourse further took the standard course-laparoscopic clips were placed on both branches.
Conclusion. The incidence of double CA ranges from 15 to 25%. Such arteries usually arise from RHA and frequently replace the deep branch of the CA. Anatomic variations in and around Calot's triangle are frequent. Therefore, careful dissection of Calot's triangle is necessary for both conventional and laparoscopic cholecystectomy. Hemorrhage could be a problem during search of the CA if these variations are overlooked and that increases the rate of conversion to open surgery. It also hasto be kept in mind that during laparoscopic visualization anatomical relations are seen differently compared to conventional cholecystectomy.

Keywords: cystic artery variations, laparoscopic cholecystectomy

Апстракт

Вовед. Цистичната артерија (ЦА) е клучната структура, која се лигира или на која и се поставуваат клипси во тек на лапароскопската или на конвенционалната холецистектомија. Стапката на јавување двојна цистична артерија-засебен почеток на површните и длабоките гранки на ЦА, според класификацијата на Michels е до 25 проценти.
Приказ на случај. Презентираме 38 годишна пациентка со едногодишна анамнеза за инфламација на жолчното ќесе. Во тек на лапароскопската дисекција на елементите во Калотовиот триаголник, наидовме на анатомска варијација на цистичната артерија-двојна цистична артерија. Позначајно, пак, беше тоа што двете гранки беа екстремно кратки, на долниот лимит на објавените должини на овој крвен сад низ литературата-секоја по три милиметри, апроксимативно. По навременото идентификување на оваа варијација, оперативниот тек продолжи по стандардна процедура, беа поставени клипси на двете гранки.
Заклучок. Инциденцата на двојна ЦА се движи од 15 до 25 проценти. Ваквите артерии обично потекнуваат од десната хепатична артерија ДХА и често ја заменуваат длабоката гранка на ЦА. Чести се анатомските варијации на елементите на Калотовиот триаголник. Оттука, потребна е внимателна дисекција во овој регион, како при конвенционалната, така и при лапароскопската холецистектомија. Крварењето може да е проблем при пристапот кон ЦА, особено доколку се превидат нејзините варијации. Тоа ја зголемува стапката на конверзија кон отворена хирургија. При лапароскопската хирургија, дополнително, анатомските релации изгледаат поинаку, споредено со класичната холецистектомија. Оттука се наметнува неопходноста за познавање на анатомските варијации на цистичната артерија, со цел изведување безбедна холецистектомија.

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Клучни зборови: цистична артерија, варијации, лапароскопска холецистектомија.

Introduction

Minimally invasive surgery hasevolved over the last three decades. Nowadays, laparoscopic cholecystectomy is widely accepted as the golden standard in treatment of cholelithiasis. A good knowledge of Calot's triangle is very important in both open and laparoscopic biliary surgery. In 1980 Rockoput attention topossible variations in the region of Calot's triangle bordered by cystic duct, common hepatic duct and lower edge of the liver. Bleeding of the cystic artery is a troublesome complication during laparoscopic surgery which increases conversion rate to open surgery and appearance of different complications. The reported incidence of conversion to open surgery because of blood vessel injury is approximately up to 1-2% during laparoscopic surgery. Knowledge of variations in biliary anatomy increases safeness in laparoscopic interventions in biliary surgery.
The most common origin of cystic artery is from right hepatic artery. The cystic artery is the key structure sought to be clipped or ligated during laparoscopic or conventional cholecystectomy. The possible complications like hemorrhage or hepatobiliary injury are always centered on the search, dissection, and clipping or ligation of the cystic artery, mainly because of possibility of variations in its course and relations to the biliary ducts. The CA usually arises from the right hepatic artery (RHA) to the right of common hepatic duct (CHD) in Calot's triangle. On reaching the gallbladder neck, it divides into superficial and deep branches to supply free peritoneal surface and attached nonperitoneal surface of the gallbladder (GB), respectively. The branches anastomose over the surface of body and fundus of the gallbladder and give off numerous twigs to the liver substance. In up to 25% of subjects, the superficial and deep branches of the CA have separate origins and Michels called them double CA.

Case report

We are presenting a case of a 38-year-old female with one-year history of chronic gallbladder inflammation, operated at the University Clinic for Digestive Surgery. During the laparoscopy dissection in the region of the Calot's triangle, a blood vessel was found in a very close ratio tothe gallbladder (Figure 1), apparently resembling the cystic artery, but considering itsdiameter, the preparation carefully continued, with further dissection revealing that it was the right hepatic artery from which the deep and superficial branch of the cystic artery originated. It was clear that this was an anatomic variation- a double cystic artery. The more important thing was that both branches were extremely short, or at the lower limit of the published lengths of this blood vessel - approximately 3 mm each. By doing so, the surgical course further took the standard course - laparoscopic clips were placed on both branches. The operative and postoperative course were uneventful.

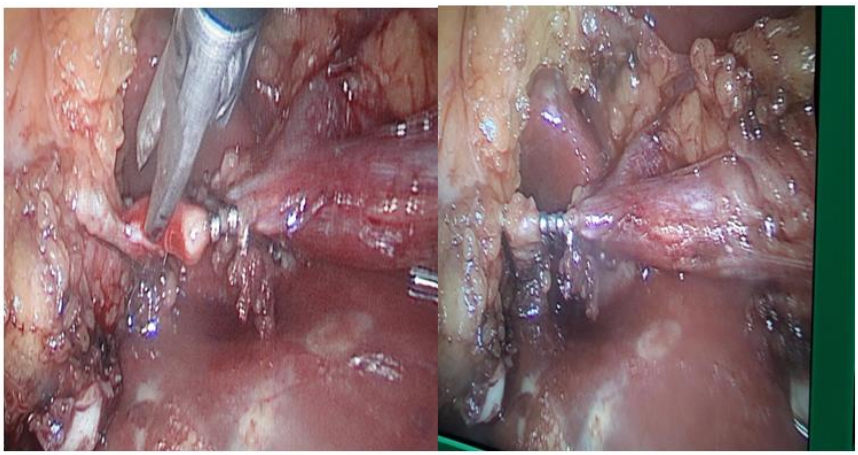

Fig. 1. Clipping to cystic artery

Discussion

A largenumber of papers on this issue have been published, but still some ambiguityexists because of the diversity of data and classification. There have been few reports on laparoscopic anatomy of the Calot's triangle, especially of the cystic artery. Michels [1] quoted that, according to Lahey, "cholecystectomy is a dangerous operation unless one realizes that variations are very common". The CA varies in number, origin, course, and its relations to biliary ducts. These abnormalities have been mentioned from time to time in the literature [2-8]. The variations of the cystic artery draw the attention of thesurgeons to recognize an error, often leading to complications such as hemorrhage.The incidence of double CA ranges from 15 to 25% [9]. This indicates that in 1/4th of cases there is a possibility of double CAs suggesting that one should have a high

index of suspicion for double CAs while operating in this region. Such arteries usually arise from RHA and frequently replace the deep branch of the CA. Anatomic variations in and around Calot's triangle are frequent, found in approximately in 20-40% of patients. Therefore, a careful dissection of Calot's triangle elements is necessary. The absence of a deep branch close to the gallbladder may be a clue that doubling of the CA is present. Since anatomic variations in and around Calot's triangle are frequentcareful dissection of Calot's triangle is necessary for both conventional and laparoscopic cholecystectomy. Hemorrhage could be a problem during search of the CA if these variations are overlooked and that increases the rate of conversion to open surgery. It also hasto be kept in mind that during laparoscopic visualizationanatomic relations are seen differently compared to conventional cholecystectomy due to the methods of retraction used in the laparoscopic procedure. Thus, the term 'laparoscopic anatomy' has been introduced. This emphasizes the importance of cystic arterial dissection and necessity of thorough knowledge of cystic arterial variations for a safe performance of cholecystectomy.

Variations in the CA are very tricky and surgeons must be cautious during realization of laparoscopic cholecystectomy.

Conclusion

The cystic artery is a key anatomic structure to be isolated and ligated during laparoscopic or conventional cholecystectomy. It is crucial for the surgeon to give careful attention, identify, and confirm the cystic artery before clipping or ligation (Figure 2).

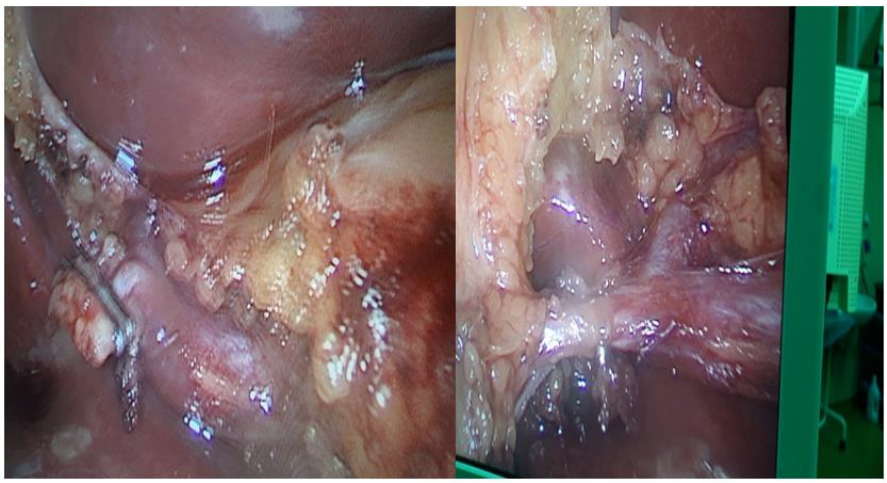

Fig. 2. Anatomic variation of cystic artery

Thus, it is essential from the surgeon's viewpoint to have a thorough knowledge and awareness of variations of cystic artery which will contribute to the safety of cholecystectomy. Hence, a detailed knowledge of the arterial system of the extrahepatic biliary ductal system along with its variations is necessary to prevent iatrogenic biliary injuries. Cystic artery bleeding is considered to be one of the main reasons for converting laparoscopic cholecystectomy to open cholecystectomy. The incidence of conversion to open surgery due to vascular injury is reported to be 0-1.9% and its mortality 0.02% 3, hence these variations should stay in surgical conscience to prevent procedure-related morbidity.

Conflict of interest statement. None declared.

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ММР

Case report

TRANEXAMIC ACID FOR PREVENTION OF POSTPARTUM HEMORRHAGE IN AVERY YOUNG PRIMIPARA WITHVON WILLEBRAND DISEASE

ТРАНЕКСЕМИЧНА КИСЕЛИНА ЗА ПРЕВЕНЦИЈА НА ПОСТПАРТАЛНА ХЕМОРАГИЈА КАЈ МНОГУ МЛАДА ПРВОРОТКА СО ВОН ВИЛЕБРАНДОВА БОЛЕСТ

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Abstract

Von Willebrand disease (vWD) is an inherited disorder in the coagulation process. It occurs due to the quantitative or qualitative lack of the von Willenbrand Factor (vWF) coagulation factor. It is clinically presented by mucous bleeding, most commonly nasal, gingival, prolonged and heavy menstrual bleeding, and only in severe forms of the disease, there can be bleeding in the joints and muscles. Treatment of patients with vWD focuses on stopping or preventing episodes of bleeding using medications. With good treatment, patients can have a normal, healthy life.

Treating pregnant women with vWD is a particular challenge in many respects: pregnancy bleeding, bleeding in the course of delivery and 4-6 weeks after delivery. vWD may have varying degrees of clinical severity. Serious bleeding may occur in patients with miscarriage or intentional abortion in the first three months of pregnancy due to insufficiently high levels of factor VIII (FVIII). Bleeding in pregnant patients occurs less frequently due to increased concentrations of FVIII and vWF during pregnancy. Their values drop sharply during delivery and after delivery, so they can cause heavy bleeding. Postpartum hemorrhage can cause serious problems. Antifibrinolytic medications are also used to control postpartum bleeding in patients with vWD. Tranexamic acid (TxA) belongs to that group of medications and has a wide range of effects in hemorrhagic conditions.

We present a case of vaginal delivery and postpartum hemorrhage controlled by TxA in a very young primipara (14 years old) who was diagnosed with vWD and completed the pregnancy at 36th gestational week.

Keywords: tranexamic acid, delivery, postpartum hemorrhage, von Willebrand diseas

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Апстракт

Вон Вилебрандова болест (vWD) е наследно пореметување во процесот на коагулација. Настанува поради квантитативен или квалитативен недостиг од вон-Вилебрандовиот фактор (vWF) на коагулација. Клинички се манифестира со крварење од слузници, најчесто од нос, гингиви, продолжено и обилно менструално крварење, а само во тешки облици на болеста има крварење во зглобови и мускули. Третманот на пациентките со vWD се фокусира на запирање или спречување на епизодите на крварење со помош на лекови. Со добриот третман пациентите можат да водат нормален, здрав живот.

Водењето на бремените пациентки со vWD е посебен предизвик од повеќе аспекти: крварење во бременоста, во тек на породувањето и четири до шест недели по породувањето, при што vWD може да има различен степен на клиничка сериозност. Сериозни крварења може да се јават кај пациентки со спонтан или со намерен абортус во првите три месеци од бременоста, поради недоволно високи нивоа на фактор VIII (FVIII). Крварења кај бремените пациентки се јавуваат поретко, а тоа се должи на зголемената концентрација на FVIII и vWF за време на бременоста. Нивните вредности нагло опаѓаат за време на породување и по породувањето, така што можат да предизвикаат обилни крварења.

Постпарталната хеморагија може да предизвика сериозни проблеми. За контрола на постпартално крварење кај пациентки со vWD се користат и антифибринолитични лекови. Транексемичната киселина (TxA) спаѓа во таа група лекови и има широк спектар на делување во хеморагични состојби.

Прикажан е случај на вагинално породување и постпартално крварење, контролирани со TxA кај многу млада прворотка (14 години) со дијагностицирана vWD кај која бременоста е завршена во 36 гестациска недела.

Клучни зборови: транексемична киселина, породување, постпартална хеморагија, Вон Вилебрандова болест

Introduction

Peripartum hemorrhage (PPH) accounts for about one-quarter to one-third of all maternal deaths. Worldwide, 7 women die of PPH every hour [1]. Von Willebrand disease is the most common inherited bleeding disorder among American women, with a prevalence of 0.6-1.3% [2]. The overall prevalence is even greater among women with chronic heavy menstrual bleeding, and ranges from 5% to 24% [3,4]. Among women with heavy menstrual bleeding, von Willebrand disease appears to be more prevalent among Caucasians (15.9%) than African Americans (1.3%) [5,6]. Von Willebrand disease (vWD) is the most common congenital hemorrhagic disease. It is presented in different types in 1% of the total population, equally in both genders [7]. It is classified into three types depending on the age and degree of vWF coagulation deficiency. The most common is type 1 (70-80%), while type 2 (20-30%) is less frequent. Both are inherited autosomal dominant. Type 3 occurs less frequently (1%) and is inherited autosomal recessively [8]. vWD occurs due to a quantitative (type 1 and type 3) or qualitative (type 2A, 2B, 2M, 2N) deficiency of vWF. vWF allows for easier platelet adhesion at the site of injured blood vessel and platelet aggregation. vWF is a carrier and stabilizer of factor VIII (FVIII) in the circulation, i.e. vWF forms a covalent complex with FVIII and prolongs its life in the blood plasma [9]. vWF is produced in megakaryocytes and endothelial cells. vWF, which occurs in endothelial cells, circulates in the plasma, and the other is found in the platelet alpha granules. FVIII is produced in hepatic cells [10]. vWD is characterized by a disorder of primary hemostasis, i.e. prolonged bleeding due to decreased platelet aggregation. vWD is clinically presented by mucosal bleeding. The main symptoms are nasal bleeding, gingival bleeding, bleeding of gums, heavy and prolonged menstrual bleeding, whereas joint and muscle bleeding is present only in severe forms of the disease with reduced FVIII values. In some patients the condition is completely asymptomatic, before serious bleeding following surgery or traumatic injury [11]. During pregnancy, the concentration of coagulation factors increases, including the FVIII complex, so that progression of gestation within the patient can lead to gradual decrease in bleeding time [12]. Severe bleeding may occur in patients with miscarriage or intentional abortion in the first three months of pregnancy due to insufficiently high levels of FVIII. Postpartum hemorrhage (PPH) can cause severe problems [13].

Treatment of vWD is only performed during active bleeding or before invasive procedure (surgical interventions) and only after the bleeding time is determined. At prolonged bleeding time in patients Desmopressin (vazopressin) for vWD type 1 is prescribed or FVIII/vWF concentrates for vWD types 2 and 3 [14,15]. Antifibrinolytic drugs are also used to control PPH in patients with vWD. Tranexamic acid (TxA) belongs to that group of medicines and has a wide range of effects in hemorrhagic conditions. TxA does not systematically affect coagulation, does not alter platelet count and function, nor it affects activated partial thromboplastin time (aPTT) and prothrombin time (PT) [16,17].

Clinical case

In December 2016 at the Clinic for Gynecology and Obstetrics in Skopje, a 14-year-old patient was admitted with vWD and first pregnancy at 36th gestation week. Information on family history could not be obtained due to separation of the parents andthe patient was raised by guardians. Personal history: vWD diagnosed in January 2015. Gynecological history: menarche at 11 years of age.

Medical history: due to prolonged menstrual bleeding (juvenile metrorrhagia) in a period of 3 months in January 2015, the patient was admitted and hospitalized at the University Clinic for Gynecology and Obstetrics-Skopje (UCGO). After ordination of hormone therapy (tabl. Microgynon 1x1/day) the bleeding stopped. After being discharged, the patient bled again despite the intensive regime with oral contraceptive (tabl. Microginon 21+7). Afterwards, she was sent to the Clinic for Children Diseases-Skopje. After performed children's hematologic examination in January 2015 she was diagnosed with vWD. The patient was recommended to use TxA (Tranexamic acid 500 mg to 6-8 hours) during bleeding (prescribed by a pediatric hematologist) and oral contraceptive Microgynon 21+7 (prescribed by a gynecologist-endocrinologist).

In April 2016 the patient's selected gynecologist after an ultrasound examination diagnosed the patient with pregnancy in second lunar month. During the ultrasound examination the patient was also diagnosed for uterus bicornis unicolis showingin left hemi-uterus GS with fetus with SA+ and right hemi-uterus decidually altered. Due to symptoms and signs of threatening miscarriage, a gestational therapy was provided (amp. Progesteron 1x1/week and tabl. Utrogestan 200 mg 2x1/day). The pregnancy was controlled by the patient's selected gynecologist and the patient was regularly examined by a pediatric hematologist. Due to the risk arising from the age and accompanying disease, the patient was sent to the UCGO-Skopje before her due date, in 36th gestational week. After the patient was admitted and examined with an ultrasound, the findings showed pelvic presentation of the fetus, with SA+, fetal biometry appropriate to the gestational age, good flow of feto-placental unit, CTG record without uterine activity, vaginal examination showed complete amniotic sac, without bleeding.

Before delivery, laboratory tests were made in the Center for Hemophilia within the Institute of Transfusion Medicine, Department: Hemophilia. The following tests

Table 1. Tests for diagnosis and monitoring of vWD – before delivery

Analysis (description of status)	Reference values	Results
Tests of hemostatic system		
Thrombocyte count [10exp9/L]	150-450	208
Hematocrit[%]	35-50	33.2
PT[sec)	9.8 (14) 14.2	11.33
aPTT[sec]	27.9(33) 37.7	27.25
TT[sec]	16.1(20) 24.1	15.42
Quantitative determination of coagulation factors		
Antihemophilic globulin (f.VIII)[%]	50-150	128.61
Von Willebrand factor Ris with activity [%]	50-150	126.91
Von Willebrand factor vWF Ag [%]	50-150	59.42
Tests for fibrinolytic system		
D-dimers [ng/mL]	0-500	1416.19
Aggregation and monitoring of anti-aggregation therapy		
ADP [%]	78-97	71
Collagen [%]	70-94	78
Ristocetin [%]	87-102	89
Blood test – White blood cells		
WBC [109/L]	3.5-10	8.5
LYM [109/L]	0.5-5	1.5
MID [10exp9/L]	0.1-1.5	0.5
GRAN [10exp9/L]	1.2-8	6.5
LYM% [%]	15-50	17.7
MID% [%]	2-15	5.5
GRA% [%]	35-80	76.8
Blood test – Red blood cells		
RBC [10exp12/L]	3.8-5.8	3.91
HGB[g/L]	110-165	12.1
MCV[fl]	80-97	85
MCH[pg]	26.5-33.5	30.9
MCHC [g/L]	315-350	36.4
RDW% [%]	10-15	15
RDWa [fl]	30-150	60.3
Blood test – platelets		
MPV[fl]	6.5-11	8.6
PCT[%]	0.01-9.99	0.17
PDW[fl]	0.1-99.9	13.5
LPCR[%]	0.1-99.9	21

were made: hemostatic system, quantitative determination of coagulation factors: FVIII and vWF (Ris with activity, vWFAg), tests for fybrinolitic system, aggregation and monitoring anti-aggregation therapy and overall blood test (Table 1).

After the results were obtained, a specialist in transfusion medicine provided an opinion on D-dimers appropriate for the gestational age, border value of vWF and RisCo activity, good aggregation of thrombocytes.

After a spontaneous start, with spontaneous breaking of the amniotic fluid sac, the early delivery of live fetus with footling breech presentation was completed vaginally with classic liberation of hands and extraction of head following the Smelli-Wheit method. Placenta and umbilical cord without irregularities. The episiotomy incision was sutured.

The delivery and postpartum bleeding were controlled with TxA, dosed by a pediatric hematologist (amp. Tranexemic acid 15mg/kg body weight (BW)/6-8 hours intravenous). The patient with BW=62 kg postpartum received1 g TxA (i.v.).

After delivery on the puerperium department the patient was examined consiliary by a pediatric hematologist and a treatment was given of TxA (amp. Tranexemic acid 3x1 g in the course of seven days, amp. Tranexemic acid 2x1 g after alleviation of bleeding and amp. Tranexemic acid 1x1 g until total termination of bleeding).

Uterotonic therapy was administered (amp. Methylergometrin 2x1 i.m. in the course of seven days, afterwards sol. Oxytocin 2x10IU/ml i.v. until termination of bleeding).

Two days after delivery at the Institute of Transfusion Medicine,Department for Hemostasis and Thrombosis, control laboratory analyses were made (Table 2).

According tothe results, a specialist in transfusion medicine said that there wasa hyper-coagulation condition with a strong hypo-agregability of thrombocytes with ADP and collagen.

Table 2. Tests for diagnosis and monitoring of vWD – after delivery

Analysis (description of status)	Reference values	Results
Tests of hemostatic system		
Thrombocyte count [10exp9/L]	150-450	220
Hematocrit[%]	35-50	32
PT[sec]	9.8 (14) 14.2	13
aPTT[sec]	27.9(33) 37.7	33
TT[sec]	16.1(20) 24.1	22
Quantitative determination of coagulation factor		
Antihemophilic globulin (f.VIII)[%]	50-150	145
Von Willebrand factor Ris with activity [%]	50-150	66
Von Willebrand factor vWF Ag [%]	50-150	114
Tests for fibrinolytic system		
D-dimers [ng/mL]	0-500	799.0
Aggregation and monitoring of anti-aggregation therapy		
ADP [%]	78-97	24
Collagen [%]	70-94	1
Ristocetin [%]	87-102	4
Blood test – White blood cells		
WBC [109/L]	3.5-10	9.4
LYM [109/L]	0.5-5	1.4
MID [10exp9/L]	0.1-1.5	0.3
GRAN [10exp9/L]	1.2-8	7.7
LYM% [%]	15-50	15.0
MID% [%]	2-15	3.5
GRA% [%]	35-80	81.5
Blood test – Red blood cells		
RBC [10exp12/L]	3.8-5.8	3.75
HGB[g/L]	110-165	117
MCV[fl]	80-97	84.8
MCH[pg]	26.5-33.5	31.4
MCHC [g/L]	315-350	370
RDW% [%]	10-15	14.9
RDWa [fl]	30-150	60.0
Blood test – platelets		
MPV[fl]	6.5-11	9.1
PCT[%]	0.01-9.99	0.20
PDW[fl]	0.1-99.9	14.1
LPCR[%]	0.1-99.9	23.4

The control tests for hemostasis made before discharge resulted in border values of vWF and vWFAg, hypo-aggregability with ADP, normal values for collagen and ristocetine and secondary activated fybrinolisis (Table 3).

The bleeding of the patient was regularly monitored clinically with gynecological controls, ultrasound examinations, complete blood test analyses, and analysis of hemostasis and dosing of vWF. Postpartum ultrasound results: uterus bicornis with low retained contents in one hemicavum. After administering uterotonic therapy the control ultrasound examination was good - without residual contents in both cavums and thin endometrium.

During her stay in thehospital, the patient was monitored by a multidisciplinary team consisting of gynecologist-obstetrician, gynecologist-endocrinologist, and pediatric hematologist, specialist in transfusion medicine, social worker and psychologist.

Before discharge, a control hematological examination was made and a recommendation was given for TxA therapy (tabl. Tranexamic acid 500 mg/2x1 until termination of bleeding).

The hospitalization of the patient until delivery lasted 23 days. She was discharged in generally stable condition and advised for therapy and control of bleeding up to 6 weeks by the selected gynecologist. Before discharge, the guardian was informed in detail for the condition of the minor patient and the recommendations, and due to the bad socio-economic status of the patient, the social service for good implementation of doctor's recommendations was informed.

Table 3. Tests for diagnosis and monitoring of vWD – before discharge

Analysis (description of status)	Reference values	Results
Tests of hemostatic system		
Thrombocyte count [10exp9/L]	150-450	268
Hematocrit [%]	35-50	31
PT[sec]	9.8 (14) 14.2	13
aPTT[sec]	27.9(33) 37.7	30
TT[sec]	16.1(20) 24.1	18
Quantitative determination of coagulation factor		
Antihemophilic globulin (f.VIII)[%]	50-150	110
Von Willebrand factor Ris with activity [%]	50-150	44
Von Willebrand factor vWF Ag [%]	50-150	49
Tests for fibrinolytic system		
D-dimers [ng/mL]	0-500	1064
Aggregation and monitoring of anti-aggregation therapy		
ADP [%]	78-97	64
Collagen [%]	70-94	109
Ristocetin [%]	87-102	125
Blood test – White blood cells		
WBC [109/L]	3..5-10	6.3
LYM [109/L]	0.5-5	1.4
MID [10exp9/L]	0.1-1.5	0.4
GRAN [10exp9/L]	1.2-8	4.5
LYM% [%]	15-50	22.9
MID% [%]	2-15	6.4
GRA% [%]	35-80	70.7
Blood test – Red blood cells		
RBC [10exp12/L]	3.8-5.8	3.81
HGB[g/L]	110-165	110
MCV[fl]	80-97	82.0
MCH[pg]	26.5-33.5	28.9
MCHC [g/L]	315-350	353
RDW% [%]	10-15	14.9
RDWa [fl]	30-150	61.4
Blood test – platelets		
MPV[fl]	6.5-11	8.5
PCT[%]	0.01-9.99	0.22
PDW[fl]	0.1-99.9	12.5
LPCR[%]	0.1-99.9	18.6

Discussion

vWD is a rare disease that is given particular importance in gynecology and obstetrics because of the risk of PPH. According to Holmgren the incidence of severe primary PPH is approximately 3.5% for vaginal delivery, 8% for instrumental vaginal delivery and 13% for caesarean section [18].

The incidence of PPH is higher in all forms of delivery, and is particularly high in instrumental vaginal delivery, with three out of seven deliveries (43%) resulting in blood loss of more than 1000 ml. Although there is insufficient data to make generalizations, these results underscore the recommendation to avoid instrumental vaginal delivery in patients with vWD, in order to prevent lacerations of the delivery routes and episodes of severe bleeding [19].

The Nordic Hemophilia Council gives several recommendations regarding the treatment, which differ from those given by other authors. Thus, TxA treatment is recommended in all births where the patient has vWD, and other authors recommend TxA only when it is found that vWF activity levels are low during the third trimester of pregnancy [20-22].

A study done at Karolinska University in Sweden analyzed birth data from 14 different obstetric units over a period of 18 years (1995-2012). Results showed that low levels of FVIII in the third trimester of pregnancy may predict PPH [23-25].

Patients with type 3 vWD were at highest risk of severe PPH, although all were administered prophylactic treatment [26-29].

Onestudy included 59 births and wasconsidered relatively large because vWD wasa rare disease [30].

Similar sample sizes were reported in previous studies from England, Scotland and the United States [31,32].

There are cases where vWF deficiency is diagnosed after prolonged bleeding following a caesarean section. Many experts suggest that vWD patients may have vaginal delivery, while caesarean delivery is reserved only when there are obstetric indications [33,34].

Treatment

Treatment options for vWD include desmopressin, vWF-FVIII, cryoprecipitate concentrates and other therapies such as antifibrinolytic agents and estrogens.
Desmopressin (DDAVP) is a synthetic analog of vasopressin. DDAVP increases plasma FVIII and vWF levels temporarily in normal subjects and in patients with vWD. DDAVP is most effective in patients with type 1 vWD who have normal vWF. Multiple reports suggest that DDAVP is effective in preventing or controlling abortion and bleeding during delivery [35-38].

Transfusion therapy

FVIII/vWF concentrates are used to prevent or control pregnancy-related bleeding in patients with vWD unresponsive to DDAVP [39-41].
Cryoprecipitate should not be used during pregnancy because of the risk of transmitting viral or other bloodstream infections. They can be used in emergencies if vWF-FVIII concentrates are not available.
Antifibrinolytic agents are usually avoided during pregnancy, but are used to control postpartum hemorrhage in patients with vWD [42].
TxA is a synthetic derivative of the amino acid lysine. TxA is used to control or prevent bleeding from placental abruption, caesarean section or other obstetric causes, with no visible side effects in the mother or fetus [43-48]. Table 4 shows benefits and risks of TxA [49].
According to one investigation of TxA, it is shown that various fixed (0.5 g or 1 g) and adjusted to body weight (10mg/kg or 15mg/kg) doses of TxA are used for prevention.
Given the wide range of body weight in pregnant women in modern obstetric practice, it is crucial to determine the minimum effective dose of TxA to avoid undue or overdose. The rationale of this study states that the minimum effective dose of TxA required to achieve therapeutic plasma levels of TxA is 5-15mg/L, following administration of a single dose of TxA intravenously (i.v.) after the delivery of a newborn and the cutting of the umbilical cord before delivery of the placenta [50-52].

Conclusions

Prophylaxis in consultation with a hematologist is a standard way of treating vWD patients for successful termination of pregnancy.The optimal dose and duration of prophylactic therapy depend on the type and severity of vWD, mode of delivery, and vWF level at term of delivery.
If patients are not diagnosed before pregnancy and delivery, then there is a risk of life-threatening bleeding during childbirth or postpartum hemorrhage. Levels of FVIII and vWF should be determined in pregnancy, primarily in the third trimester, to facilitate delivery planning and in the event of postpartum hemorrhage.TxA has a wide range of action in hemorrhagic conditions. TxA reduces menstrual blood loss and is an alternative to menorrhagia.
TXA reduces postpartum blood loss and transfusion needs. TxA has been successfully used to control bleeding in pregnancy and delivery.

Conflict of interest statement. None declared.

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Case report

MARTORELL'S ULCER-THERAPEUTIC APPROACH

MARTORELL УЛКУС- ТЕРАПЕВТСКИ ПРИСТАП

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Abstract

In the last sixty years, as a result of numerous researches and studies, the approach in the treatment of wounds has significantly changed, which has contributed to greater efficiency of the treatment. Acute wounds by definition are those wounds that heal without complications in the estimated time period, when the structural and functional integrity of the skin is restored. Chronic woundsdo not follow a normal trajectory of healing, and no complete functional and structural integrity of the skin is achieved. Chronic wounds usually take monts to heal, leading to disruption in patients' lives and risk of amputations. They are afinancial burden for the health systems.

Martorell's ulcer is an ischemic and very painful lesion of lower limbs, associated with poorly controlled hypertension. It is located in the distal third of the lower limbs. Hyperbaric oxygen therapy has a significant place in the therapeutic approach in addition to the conventional one.

Treatment involves breathing 100% oxygen at the pressure higher than the atmospheric pressure in order to provide better oxygenation of the tissues and to enhance the wound healing processes. The aim of the study wasto evaluate the effect of 100% oxygen under pressure applied as an adjuvant treatment in a patient with Martorell's ulcer of the lower limb.

In this report, we present a patient with painful ulcers of the distal part of the lower limb and poorly controlled systemic arterial hypertension treated withadjuvant hyperbaric oxygen therapy.

The results of the investigations and the local and systemic therapy are shown in details.

The treatment improved and accelerated the process of granulation and epithelialization, and provided absence of a wound infection. Hyperbaric oxygen therapy as an adjuvant treatment is effective in accelerating the wound healing processes.

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Keywords: hyperbaric oxygen therapy, chronic wounds, Martorell's ulcer, hypertension, oxygen

Апстракт

Во последните шеесет години, како резултат на бројни истражувања, значајно се промени пристапот во лекување на раните, што придонесе за поголема ефикасност од третманот. Акутни рани се тие коишто зараснуваат без компликации во предвиден временски период, кога настапува обновување на структурниот и функционалниот интегритет на кожата. Хронични рани не ја следат нормалната траекторија на зараснување и не постигнуваат комплетен функционален и структурен интегритет на кожата. На хроничните рани, обично им се потребни месеци за зацелување, што води до нарушување на животите на пациентите, способност за работа, ризик за ампутации и финансиско оптоварување на здравствените системи.

Martorell улкус претставува исхемична и болна лезија локализирана во дистална третина на долните екстремитети, асоцирана со лошо регулирана хипертензија. Покрај конвенционалниот тераписки пристап значајно место ѝ припаѓа и на хипербарната оксигено терапија, која вклучува дишење на 100% кислород, на повисок притисок од атмосферскиот, со цел да се обезбеди подобра оксигенација на ткивата и да се подобрат процесите на зараснување на раните.

Целта на студијата е да се евалуира ефектот на 100% кислород, под зголемен притисок како адјувантен третман кај пациент со Martorell улкус на долните екстремитети. Во трудот е презентиран случај на пациентка со болни улцерации во предел на дисталниот дел на десен долен екстремитет и лошо регулирана артериска хипертензија. Детално се прикажани резултатите од инвестигациите и ординираната локална, како и системска терапија.

Третманот ги подобри и ги забрза процесите на гранулација и епителизација и овозможи побрзо справување со инфекцијата. Хипербарната оксигенотерапија, како адјувантен третман е ефикасна во за-

брзување на процесите на зараснување на хроничните рани.

Клучни зборови: Хипербарна оксигено терапија, хронична рана, Martorell улкус, хипертензија, кислород

Introduction

Chronic wounds are defined as wounds in which the order and time of reparative processes are impaired. Therefore, it is impossible to establish anatomical and functional integrity, i.e. reparative processes do not result in a satisfactory anatomical and functional result. Chronic wounds are a serious problem for patients and a burden on the health system accompanied by a high mortality [1,2].

Martorell's ulcer is an extremely painful, progressive ischemic ulcer of the lower extremities in patients with hypertension. It is a rare and often unrecognized chronic, necrotic wound of the lower extremities.

It is present in patients over 50 years of age with chronic progressive hypertension, which is uncontrolled. It is more common in the female population and is often associated with diabetes. Localization is in the lower third of the lower extremities, and is usually dorsolateral [3,4].

It was described by Fernandes Martorell in 1945. It occurs in about 5% of all ulcers of the lower extremities with different etiologies. Its frequency increases in poor living conditions, obesity and hypertension [5].

The pathogenesis of Martorell's ulcer is related to local factors that trigger dermal arteriosclerosis and subsequent hyperplasia of the media layer and elastic lamina, a process known as hyalinosis. As a result of atherosclerotic changes in the dermis and subcutis, blood flow is disrupted. Increased resistance to flow and ischemic changes (lack of adequate oxygen supply) occur, leading to skin necrosis and ulceration [6,7].

In terms of differential diagnosis it can bepyoderma gangrenosum (PG), necrotizing vasculitis and calciphylaxis [8].

Early diagnosis is important because of appropriate treatment, and thus pain relief in patients.

The diagnosis is made on the basis of the clinical picture, chronic arterial hypertension, and present-comorbidities [9].

Because it is a rare disease, few studies have been done on the treatment and rehabilitation of patients.

Occurrence of painful ulceration, solitary or multiple, usually symmetrical and in the lower third of the lower leg, deep, with irregular borders, with a bottom with fibrinous adhesions and necrosis and without response to standard therapy is an indication for this disease [10,11].

The therapeutic approach is aimed at regulating blood pressure, using calcium channel blockers, selective beta 1-blockers, and ACE inhibitors as long as there are no contraindications. A large series of investigated cases have shown that even a slight decrease in blood pressure can have a major impact on pain and healing [12].

The ulcer care usually involves surgical debridement along with compression therapy, vacuum-assisted negative pressure closure, and for large ulcers, skin grafting [13].

The combination therapy improves the general condition of the patients, reduces pain, and thus leads to a reduction or complete cessation of analgesics.

Paracetamol and other nonsteroidal anti-inflammatory drugs are usually sufficient to relieve pain, but narcotic analgesics (opiates) are included to relieve severe pain.

Antibiotics-whenever treatment of a secondary bacterial infection is required [14].

Regulation of glycemia in patients with diabetes [15].

Hyperbaric oxygen therapy involves breathing 100% oxygen at a pressure higher than atmospheric in order to provide better tissue oxygenation and improve wound healing processes.

The aim of the study was to assess the effect of 100% oxygen under pressure applied as adjuvant treatment in a patient with Martorell's ulcer of the lower extremities and to determine the optimal therapeutic modalities [16,17].

Case study

Two years ago, a female patient, 56 years of age, had painful ulcerations measuring 7x4 cm, 5x2 cm, 1x1 cm with a bottom with fibrinous adhesions and necrosis (wound bed with areas of fibrin and necrosis) in the area of the distal part of the right lower extremity, in the area of lateral malleolus and hyperaemia of the surrounding skin with signs of infection (Figure 1, Figure 2).

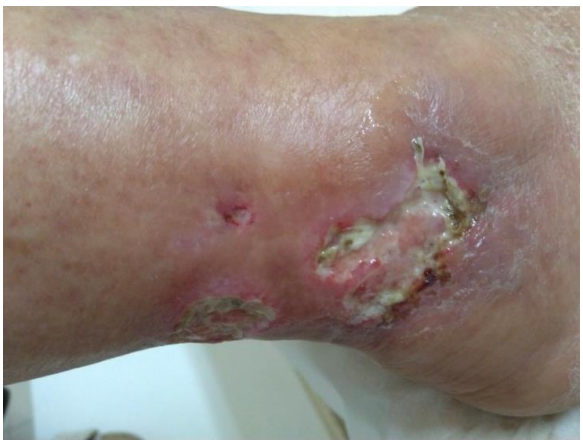

Fig. 1. Wound bed with areas of fibrin and necrosis

For 10 years the patient had suffered from poorly regulated arterial hypertension that ranged up to 220/130 mm Hg. Diabetes mellitus-insulin dependent diabetes was diagnosed 6 years ago.

Over the period of 2 years, she was treated locally with standard dressings and debridement. The rest of the therapy included oral antibiotics depending on the microbiological finding; if necessary analgesics were given, and the patient received antihypertensive thera-

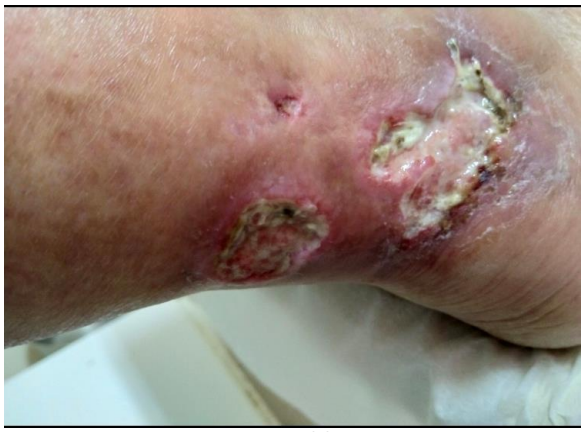

Fig. 2. Wound bed with areas of fibrin and necrosis

py irregularly that had not been corrected by a cardiologist for years. Insulin therapy was also not regularly monitored-poorly regulated diabetes. Local and systemic therapy did not significantly improve the condition.
During the examination the following tests were made-ulcer swab for microbiological examination, a complete laboratory blood analysis with CRP. Ultrasound color Doppler of lower extremities was also made. A complete cardiac examination was performed along with echocardiography. Additionally, an ultrasound of the abdomen and a native roentgenography of the lungs was performed to assess whether to include hyperbaric oxygen therapy.
The examination results showed presence of *Pseudomonas aeruginosa* in the wound, higher glucose levels 11 mmol/l and higher CRP 57. Pathohistological analysis from elliptical deep biopsy of wound to fascia, through the edge of the ulcer and necrosis confirmed the diagnosis or showed subcutaneous arteriosclerosis with thickened arterial walls, narrowed lumen and thrombosis, skin infarction, and medial calcinosis. Ultrasound color Doppler of the lower extremities showed a normal flow inarterial and venous blood vessels of the lower extremities with ABI 0.98. Cardiac examination with echocardiography showed signs of cardiac left side afterload.

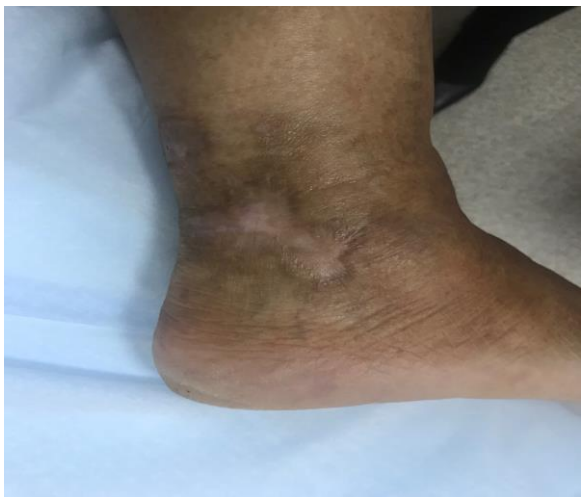

Fig. 3. Complete epithelialization of the wound

Local therapy applied-regular dressings (every day, and after improving the local finding, every other day) and surgical debridement of the ulceration. At the beginning of the treatment, an oral antibiotic according to the antibiotic sensitivity test was included, appropriate antihypertensive therapy (Skopryl a 20 mg 2x1, Furosemid 1x1/2). Oral analgesic Niflam was included, as needed.
The course of treatment included hyperbaric oxygen therapy. A total of 40 HBO exposures were performed under protocol 2.0 ATA, in duration of 90 minutes, 5 times a week, for a total of 2 months.

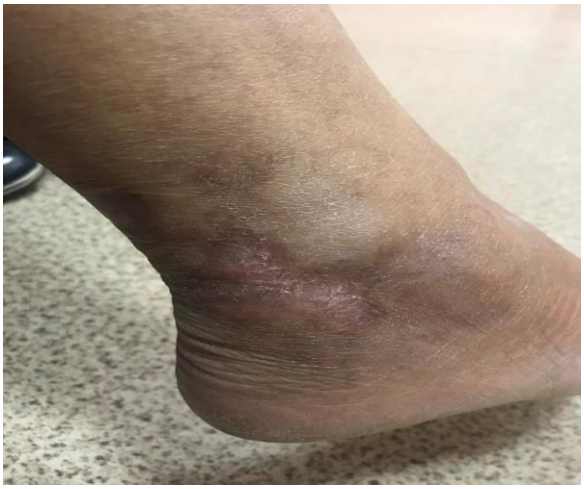

Fig. 4. Complete epithelialization of the wound

Complete epithelialization of the wound was achieved after 8 weeks (Figure 3, Figure 4). Control examination was conducted every month, during the first 6 months, and after that every 6 months throughout 2 years with a normal local finding.

Discussion

Chronic wounds are a serious socio-economic problem, a burden on patients and on the health care system of any country. Their treatment is a challenge for healthcare professionals. Due to the different etiology and associated comorbidities, a multidisciplinary approach is necessary.
In chronic wounds, infection is often present, leading to prolonged inflammatory and connective tissue damage, increased production of free radicals, and proteolytic activity. The inflammatory phase disrupts growth factors, cytokines, and chemokines, causing ischemia, edema, and inhibition of nutrients entry into the wound [18].
Martorell's hypertensive ischemic ulcer is a rare chronic wound that is often misdiagnosed and therefore it is very important to recognize and treat it in a timely manner. For the treatment, it is very important not to confuse the Martorell's ulcer with Pyoderma gangrenosum or necrotizing vasculitis since their treatment is quite different, and exposure of patients with tissue necrosis due to ischemic arteriosclerosis to systemic

immunosuppressive therapy may increase the risk of septicemia [7].

Based on the clinical picture, the presence of uncontrolled hypertension, severe pain, comorbidities, exclusion of other related diseases, Martorell's ulcer was suspected in our patient. The diagnosis was confirmed by multidisciplinary approach, antibiotic therapy, regulation of hypertension, analgesics, and improvement of the clinical picture.

In addition to conventional therapy such as regular local wound treatment (dressings and surgical debridement), adjuvant hyperbaric oxygen therapy was included to reduce pain and especially in the process of granulation and complete tissue epithelialization [17,18].

Systemic Hyperbaric Oxygen Therapy (HBOT) has been suggested as an adjuvant treatment in patients with chronic wounds. HBOT has been shown to have antimicrobial action and it increases oxygenation of chronic wounds. This improves neutrophil activity, stimulates angiogenesis, and enhances fibroblast activity and collagen synthesis.

HBOT provides ischemic tissue hyperoxygenation and hypoxia correction. Indeed, fibroblasts, endothelial cells, and keratinocytes are replicated at higher rates in an oxygen-rich environment. In addition, leukocytes kill bacteria more efficiently when supplied with oxygen [16].

The main goal of HBOT is to increase the oxygen diffusion gradient in the tissues by about 10-20 times, to allow hyperoxygenation of the ischemic tissue, thereby reducing the levels of inflammatory cytokines and stimulating growth factors production. In addition, HBOT increases antibacterial activity, and reduces the specific activation of inflammatory cells. Further, HBOT functions are to stimulate stem cell transmigration in the wound, to alter leukocyte endothelial cell adhesion, and to promote collagen formation [17-19].

Conclusion

HBOT has many effects on wound healing such as improving oxygen supply to the wound, reducing inflammation, increasing angiogenesis, and stem cell mobilization.

The treatment improved and accelerated the process of granulation and epithelialization and ensured the absence of wound infection in the case of our patient.

Hyperbaric oxygen therapy as an adjuvant treatment is effective in accelerating wound healing processes.

Conflict of interest statement. None declared.

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УПАТСТВО ЗА ПРИЈАВА НА ТРУД ОД СОРАБОТНИЦИТЕ НА ММП

"Македонски медицински преглед" (ММП) е стручно списание на Македонското лекарско друштво, првенствено наменето на лекарите од општа практика, специјалистите од одделните медицински дисциплини и истражувачите во областа на базичните медицински и други сродни науки.

Списанието ги има следниве рубрики и категории на трудови:

1. **Изворни трудови**
2. **Соопштувања за клинички и лабораториски искуства**
3. **Прикази на случаи**
4. **Од практика за практика**
5. **Едукативни статии**
6. **Вариае (**писма од редакцијата, општествена хроника**,** прикази на книги, извештаи од конгреси, симпозиуми и други стручни собири, рубриката ,,Во сеќавање,, и др).

Изворните трудови имаат белези на научни трудови, додека трудовите категоризирани во рубриките 2-5 имаат белези на стручни трудови.
Во ММП се објавуваат трудови на членовите на МЛД или на членови на други стручни здруженија. Авторите се одговорни за почитувањето на етичките начела при медицинските истражувања, а изнесените ставови, изведени од анализата на сопствените резултати, не се нужно и ставови на Редакцијата на ММП.
Редакцијата ги испраќа ракописите на стручна рецензија; рецензентот (ите) и Редакцијата ја определуваат дефинитивната категоризација на ракописот кој е прифатен за печатење. Редакцијата го задржува правото ракописите да ги печати според рецензираниот приоритет.
Упатството за соработниците на ММП е во согласност со Ванкуверските правила за изедначени барања за ракописите кои се праќаат до биомедицинските списанија.

1. ТЕКСТ НА РАКОПИСОТ

Сите ракописи се испраќаат во електронска форма на електронската адреса (е-маил) на МЛД-ММП, со двоен проред и најмногу 28 редови на страница. Трудот се поднесува на англиски јазик латиничен фонт Тимес Нењ Роман големина 12 и апстракт на македонски јазик. Лево, горе и долу треба да се остави слободна маргина од најмалку 3 см, а десно од 2,5 см.. Редниот број на страниците се пишува во десниот горен агол.
Ракописот на трудот треба да е придружен со писмо на првиот автор, со изјава дека истиот текст не е веќе објавен или поднесен/прифатен за печатење во друго списание или стручна публикација и со потврда дека ракописот е прегледан и одобрен од сите коавтори, односно со придружна декларација за евентуален конфликт на интереси со некој од авторите.

Насловната страна треба да има: наслов на македонски и англиски, имиња и презимиња на авторите, како и институциите на кои им припаѓаат, имињата на авторите и насловот на установата се поврзуваат со арапски бројки; автор за кореспондеција со сите детали (тел. е-маил); категорија на трудот; краток наслов (до 65 карактери заедно со празниот простор); како и информација за придонесот за трудот на секој коавтор (идеја, дизајн, собирање на податоци, статистистичка обработка, пишување на трудот).

Насловот треба концизно да ја изрази содржината на трудот. Се препорачува да се избегнува употреба на кратенки во насловот.

Изворните трудови и **соопштувањата** го имаат следниов формален редослед: насловна страна, извадок на македонски јазик (вовед, методи, резултати, заклучок) со клучни зборови, извадок на македонски јазик со клучни зборови, вовед, материјал и методи, резултати, дискусија и заклучоци, литература и прилози (табели, графици и слики) и легенди за прилозите во еден фајл.

Приказите на случаи треба да содржат вовед, детален приказ на случајот, дискусија со заклучок и литература со прилози.

Извадокот на македонски јазик треба да содржи најмногу 250 зборови и да биде структуриран со сите битни чинители изнесени во трудот: вовед со целта на трудот, методот, резултати (со нумерички податоци) и заклучоци. Заедно со извадокот, треба да се достават и до 5 клучни, индексни зборови.

Извадокот на англиски јазик мора да е со содржина идентична со содржината на извадокот на македонски јазик. Клучните зборови треба да се во согласност со МеСХ (Медицал Сибјецт Хеадингс) листата на Индеџ Медицус.

Воведот треба да претставува краток и јасен приказ на испитуваниот проблем и целите на истражувањето, со наведување на етичкиот комитет односно институцијата која го одобрила испитувањето (клиничка студија која се работи според принципите на Хелсиншката декларација за пациентите и нивните права).

Методите треба да бидат точно назначени, за да се овозможи повторување на прикажаното истражување. Особено е важно да се прецизираат критериумите за селекција на опсервираните случаи, воведените модификации на веќе познатите методи, како и идентификација на употребените лекови според генеричното име, дозите и начинот на администрација.

Резултатите треба да се прикажат јасно, по логичен редослед. Резултатите се изнесуваат во стандардните СИ единици. Во текстот треба да се назначи оптималното место каде ќе се вметнат табелите и илустрациите, за да се избегне непотребното повторување на изнесените податоци. Значајноста на резултатите треба да се обработи статистички, со детален опис на употребените статистички методи на крајот на делот *методи*.

Дискусијата треба да ги истакне импликациите од добиените резултати, споредени со постојните сознанија за испитуваниот проблем.

Заклучоците треба да не бидат подолги од 150 зборови.

2. ПРИЛОЗИ

Како прилог-документација на трудовите предложени за печатење, може да се достават до 5 прилога (табели, фигури,/слики - илустрации).

Табелите се доставуваат на крајот на трудот во истиот фајл. Секоја табела треба да има свој наслов и реден број кој ја поврзува со текстот. Хоризонтални и вертикални линии на табелата не се дозволени; ознаките на колоните во табелата се пишуваат скратено или со симбол, а нивното објаснување се пишува на дното на табелата, во вид на легенда.

Илустрациите се доставуваат со реден број како слика во црно-бела техника, а секоја слика треба да е придружена со легенда (опис).

Микрофотографиите може да содржат посебни ознаки во вид на стрелки или симболи. Покрај описот на сликата, мора да се наведе и зголемувањето и видот на боењето на препаратот (ако тоа веќе не е направено во секцијата *материјал и методи*).
Сите ознаки на фотографиите мора да бидат доволно големи, за да може јасно да се распознаат и по смалувањето во печатницата, при нивното вклучување во печатената страница на списанието.

3. ЛИТЕРАТУРА

Цитираната литература се пишува на крајот на трудот по заклучоците, со редни броеви според редоследот на појавувањето на цитатот на текстот на трудот ставени во средни загради и без простор меѓу нив (ако се последователни треба да се поврзани со цртичка, на пр. Ш3-6Ќ).
Литературата се цитира на следниов начин (кратенките за насловите на списанијата треба да се според листата прифатени во Индекс Медицус):
а) ***статија во списание*** (се наведуваат сите автори, ако ги има до 4 или помалку; ако ги има повеќе од 4 се наведуваат првите 3 автори и се додава: *и сор.*) Неглиа ЈП Меадоњс АТ, Робисон ЛЛ *ет ал*. Сецонд неопласмс афтер ацуте лымпхобластиц леукемиа ин цхилдхоод. Н Енгл Ј Мед 1991; 325:1330-6.
б) заеднички автор
ГИВИО (Интердисциплинарс гроуп фор цанцер царе евалуатион). Редуцинг диагностиц делас ин бреаст цанцер. Поссибле тхерапеутиц имплицатионс. *Цанцер* 1986; 58: 1756-61.
в) ***без автор*** **-** анонимно. Бреаст сцреенинг: нең евиденце. (*Едиториалл Ланцет* 1984; и :1217-8).
г) ***поглавје во книга или монографија***
Њеинстеин Л, Сњартз МН. Патхогениц пропертиес оф инвадинг мицроорганисмс. Во: Содеман ЊА Јр, Содеман ЊА, Ед. Патхогениц пхссиологс: мецханисмс оф дисеасе. Пхиладелпхиа; Њ Б Саундерс, 1974: 457-72.

Првите отпечатоци на трудовите им се праќаат на авторите за корекција: авторите се должни коригираниот отпечаток да и го вратат на Редакцијата на ММП во рок од 2 дена.

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Известување за членовите на МЛД

Сите што сакаат и натаму да го добиваат списанието треба да ја имаат уплатено членаринатa за 2019 година во висина од 600 денари и за тоа да ја информираат стручната служба на Македонско лекарско друштво, писмено или преку телефон.

Детални информации можете да добиете на телефонот на Друштвото 02 3 162 557.

Известување за рецензентите за ММП

Во склад со правилникот на УКИМ рецензентите што навремено и одговорно ќе ја одработат рецензијата ќе добијат 0.4 бода кои се собираат за унапредување во академските звања. Бодовите можат да се добијат и ретроградно преку побарување во МЛД - 3162 557.