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Review article

ЕТИЧКИ АСПЕКТИ НА КЛИНИЧКИ ИСТРАЖУВАЊА НА ДЕЦА

ETHICAL ASPECTS IN CLINICAL TRIALS IN CHILDREN

Ivana Arnaudova Danevska[1], Dimitar Arnaudov[2], Tatjana Jakjovska[1], Katerina Boshkovska[1], Sonja Momchilovikj[1] and Elena Gjinovska Tasevska[1]

[1]Institute for Respiratory Diseases in Children - Skopje, [2]General Hospital – Ohrid, Republic of North Macedonia

Abstract

Conducting clinical trials in children has particular importance as they strive to provide optimal care and therapy for this specific group of patients, but their implementation is still complex. This is often due to the lack of agreed-upon research objectives, unresolved questions about informed consent, and the general perception that children are a vulnerable group of subjects. For providing the best clinical care for pediatric subjects, it is important to conduct careful, effective research that has a scientific basis to answer important clinical questions. The main challenge of pediatric research is the risk and therefore the question arises whether the purpose of the research justifies the risks associated with the study? The four ethical principles should serve as a framework in the process of conducting clinical trials in children: benefacere, justice, respect for personality and confidentiality. One principle is no more or less important than the others, so all four must be taken into account when conducting a research. The ethical principle of "respect for personality" is the basis for the process of informed consent. It is a legal document by which the patient voluntarily agrees to participate in a research. The informed consent must be signed by child's legal guardian(s) and this step cannot be delegated to other family members or friends unless there is a legal basis. However, with proper planning and monitoring the study process, a research can be conducted in children even though they are considered as a vulnerable population.

Keywords: clinical research, informed consent, children, participation

Апстракт

Спроведувањето на клинички истражувања кај деца-та е особено важно бидејќи истите имаат за цел обезбедување на оптимална нега и терапија кај оваа специфична група на пациенти, но спроведувањето е сепак сложен процес. Тоа често се должи на недостатокот на договорени крајни цели на истражувањето, неразјаснетите прашања околу информираната согласност како и генералната перцепција дека децата се вулнерабилна група на субјекти. За обезбедување најдобра клиничка нега на педијатриските субјекти, важно е да се спроведе внимателно, ефикасно истражување кое има научна основа за да се одговори на важни клинички прашања. Основниот предизвик на педијатриските истражувања е ризикот и затоа се наметнува прашањето дали целта на истражувањето ги оправдува ризиците поврзани со студијата? За вклучување на децата во клинички истражувања како рамка треба да служат четирите етички принципи: benefacere, правда, почитување на личноста и доверливост. Еден принцип не е повеќе или помалку важен од другите, според тоа сите четири мора да се земат во предвид при спроведување на истражувањето. Етичкиот принцип „почитување на личноста" е основа за спроведување на процесот на информирана согласност. Информираната согласност е законски документ со кој пациентот доброволно се согласува да учествува во истражувачка студија. Информираната согласност мора да биде потпишана од законскиот старател(и) на детето и овој чекор не може да се делегира на други членови на семејството или пријатели, освен ако тоа нема законска основа. Но сепак со соодветно планирање и надзор над студијата, истражувањето може да се спроведе кај децата иако се сметаат за вулнерабилна популација.

Клучни зборови: клиничко истражување, информирана согласност, деца, учество

Introduction

Conducting clinical trials in children is crucial for providing optimal care and therapy in this specific group of patients. Although the need for pediatric research is

Correspondence to: Ivana Arnaudova Danevska, Institute for respiratory diseases in children, Kozle bb, 1000 Skopje, R. N. Macedonia; Phone: +389 71 25 26 82; E-mail: aivana77@yahoo.com

great, their implementation is still complex. This is a result of a number of factors including the lack of agreed-upon research objectives, informed consent issues and the general perception that pediatric patients are a vulnerable group of subjects. This vulnerability is based on several factors: [1] insufficient decision-making capacity, [2] children's lives are under the protection of adults, [3] children's rights and interests are often underestimated by the society. Research is necessary and should be aimed to improve well-being and treatment, prevention and diagnosis (as defined by the WHO) in subjects, including children [1]. There are many differrences in physiology, pathology, pharmacokinetics and pharmacodynamics between children and adults. For example, in pharmacokinetics there are differences in metabolic pathways and organ function. In pharmacodynamics there are differences in the functions of the receptor, effectors systems, and homeostatic mechanisms. Side effects affect growth and development, and the dose of medication depends on body weight or surface. Furthermore, childhood has several stages and studies are performed in certain age groups such as prematurity, term infants, infants, young children, older children and adolescents [2].

For providing the best clinical care in pediatric subjects, it is important to conduct careful and effective research that has a scientific basis that should answer important clinical questions. The main challenge for pediatric research is the risk. What is the risk of conducting / or not conducting the research and who has the right to decide for the child's risk? What should the child say that he/she agrees to participate in the research? What does the legal guardian have to say in order for the child to agree to participate in the research? What is the ultimate goal of the research? Finally, does the purpose of the research justify the risks associated with the study [1]?

History of research in children

In the past there were cases in which the interest of the legal guardian of the child was contrary to the best interest of the child. The Willow Brook State School for Children with Disabilities is one such example, where legal guardians allowed their children to be involved in a suspicious study for hepatitis just to ensure that the children would be enrolled in the same school. Although legal guardians knew it was risky to allow their child to participate in such a study, they feared that refusing to participate in the study would have repercussions on enrolling in the same school where enrollment was difficult. This example shows how much children's rights can be compromised by caregivers and health communities.

The second example in the history of ethical offense is the radiation experiment on pediatric subjects conducted at the Walter E. Fernald in Waltham, Massachusetts. In this experiment, wards with boys in the institution were exposed to traces of radioactive calcium and iron, in order to detect problems with mineral absorption. The parents of the children involved in the study received incomplete information about it when they gave their consent. Parents were never told that children were exposed to any radiation, while children were told that they simply joined a science club. The experiments raised important questions about what "informed consent" really is and whether institutionalized children should eventually be included in clinical trials, given that they are by nature a vulnerable group [3].

Ethical principles

Four ethical principles should serve as a framework for involving children in clinical trials: benefacere, justice, respect for personality and confidentiality. One principle is more or less important than the other, so all four must be taken into account when conducting a research. In some circumstances, all principles may be in conflict with each other, and the researcher must choose the appropriate direction that best suits for the scientific question.

Benefacere

Benefacere is an ethical principle based on kindness and well-being. It is a moral obligation and refers to the benefit of the other, to help him in his interest and to prevent or eliminate possible harm (primum non nocere). In a pediatric research, it is important that the research does not exploit the vulnerability of minors who cannot give genuinely informed consent to participate in the study. Therefore, the researcher must know that the research is scientifically based and that "unnecessary" damage is not done to the research subject. The key aspect of harm is actually the risk. There is a serious debate about the role of benefit for the subject when he/she will not benefit directly from the research. It is widely accepted that in the adult population, a researcher may risk a small level of harm to the subject, if the subject voluntarily agrees to a treatment that will make a benefit for the humanity. The child's ability to understand the risk on human health in a wider sense and the child's willingness to accept such a risk are the issues of considerable debate.

Justice

The principle of justice is an ideal distribution of risk and benefit throughout the population in conducting a research. The choice of subjects should be fair and the vulnerable ones should not be exploited for the benefit of the general population. The inclusion and exclusion of subjects in research protocols should be based on a valid scientific question but not on the basis of discriminatory factors or ease of enrollment. One aspect of

biomedical research is to determine whether the intervention improves, does not improve or has no effect on the pathophysiological condition. The principle of justice indicates that individuals in a population should have equal access to potential benefits and potential risks. Factors that may disrupt the equitable distribution of participation include demographic differences (for example, minority and social differences, mother's language...), mental status, and coercion by researchers based on financial incentives. The last aspect of particular importance for the pediatric population is concern that researchers may force children with financial incentives to obtain consent (for example, a gift card for a toy store or a fast food restaurant). The value of such financial incentives is often reviewed by Committees in order to confirm that it is acceptable with the community standards and it is not a source of potential misconceptions.

Respect for personality

The third ethical principle of "respect for personality" emphasizes an important issue in pediatric research: paternalism *versus* autonomy. Autonomy is considered when the person has the capacity for rational decision making. However, paternalism implies that the individual is incapable for decision making and another person must make the decision in the best interest of the individual. A key principle in the research of the subject is the ability to make a decision on whether to participate despite the perceived risk. The autonomous decision of the adult is clear, but at what point can an autonomous decision be made by a child? Do children have the ability to make autonomous decisions, or should a paternalistic decision be made in the best interests of the child? Societies define the ability to make autonomous decisions according to various factors such as age, sexual development and education. It is generally accepted that a child can make an autonomous decision at the age of 18 [4].

Confidentiality

The right to confidentiality of the research results, testing and screening of children is accomplished by their parents or legal guardians. The results are given to the child's parents [5].

Informed consent

The informed consent process is based on the ethical principle of "respect for personality". The informed consent standards include: [1] providing research information and opportunity to decide whether to participate in it, [2] presenting the information in a comprehensible manner, and [3] voluntary participation of the potential subject and the opportunity to withdraw freely at any time without any repercussions. Applying these standards to pediatric patients is a challenge. For example, what is the most appropriate way to make sure that a 10-year-old understands the information presented by the researcher? Therefore, it is an imperative for researchers to meet the standards of the informed consent with the child's caregiver in the best possible way. The informed consent is a legal document and therefore the age required for consent is coming with the adulthood. Institutional Review Boards (IRBs) are responsible for assessing risk levels in collaboration with the lead researcher. This assessment is made after reviewing the research protocol. The primary responsibility of the IRB is to protect the rights of the research subject by assessing the risk and obtaining informed consent and proper review and enforcement. The informed consent is a legal document in which the patient voluntarily agrees to participate in a research study. The informed consent must be signed by the child's legal guardian(s), depending on the risk of the investtigation. This step cannot be delegated to other family members or friends unless it is legally based. The informed consent must be written in a way that meets IRB standards that "information must be provided in a form that is understandable". Adults who are the child's legal guardians must sign the informed consent. The concept of Informed Consent for Children is similar to obtaining Informed Consent for Adult Research Subjects.

Challenges in performing pediatric research

Carrying out research in pediatric populations is extremely difficult because it brings a special package of challenges.

Guardianship

Guardianship is a term used to describe someone who has been elected or appointed to make legal decisions for another person who cannot make those decisions on his or her own. Guardianship's issues can become legally complex, and can occur with or without termination of parental rights. If parental rights remain in the presence of alternative guardianship, it may not be clear to the researcher who can legally make decisions regarding the child's participation in pediatric research (who may agree, whether both parents and guardians should consent, etc.). For this reason, researchers often avoid involving pediatric patients when guardianship is unclear. Adopted children, orphans and disabled children are usually excluded from participation in a research.

The role of compensation

The role of compensation in medical research is always controversial, regardless of the age of the subjects included in the research. Opponents of participant's com-

pensation argue that compensation reduces the willingness of informed consent. Proponents of compensation, on the other hand, consider it unethical to participate in the research when the subject is not paid. Fees for pediatric research are allowed in the United States, yet many countries, including those in Europe, do not provide compensation for pediatric research subjects. Compensation for medical research subjects quickly became standard practice in the United States, and thus the problems of compensating participants in pediatric research were gradually resolved. The payment to parents must be sufficient to cover the costs for transport, medical care or food. However, the compensation of the parents or guardians must not exceed the amount that would influence the decision whether to include the child in the clinical study.

Commercial sponsorship

Insufficient involvement of pharmaceutical companies in pediatric research is a result of several obstacles. The costs associated with conducting research in children are significantly higher than the costs associated with conducting similar research in adults. In addition, fewer patients are available to participate in pediatric research, making recruiting subjects a challenge. Finally, the final product market determines whether the pharmaceutical company is willing to participate in pediatric research, as profits often dictate product development. Unsuccessful research and unexpected safety issues that may occur in younger patients can quickly increase the costs associated with conducting trial in children. The complexity of ethical issues surrounding pediatric research is often enough for pharmaceutical companies not to participate. The lack of researchers to participate in and conduct pediatric research makes it difficult for pharmaceutical companies to involve sufficient research subjects with sufficient power to obtain statistical and clinical relevance. Many strategies are used in an attempt to overcome these barriers for commercial participation in pediatric research.

Research competencies

The shortage of trained clinical researchers focused on pediatric studies still remains. Very often, pediatricians choose not to participate in clinical trials, since they believe it may jeopardize the link between them and young patients. Doctors have fear regarding the impression they will leave on parents or guardians if they offer the opportunity to involve their child in a research. On the other hand, doctors simply believe that they do not have the training and skills to participate in clinical trials. Developed countries offer a variety of training programs for physicians to conduct pediatric clinical trials. These programs consist of scholarships, training grants, continuing education, and certification programs. However, there is a shortage of physicians and clinical staff trained to conduct pediatric research. Doctors with training in conducting pediatric research often migrate to certain children's hospitals in the hope of becoming more involved in pediatric studies [6].

Good clinical practice (GCP)

The principles of Good Clinical Practice (GCP) provide a balance: subjects to be adequately protected in research studies; studies to be scientifically based, well designed and properly analyzed; and study procedures to be properly undertaken and documented. If GCP principles are not followed, participating children may be at risk, the data may be unreliable or unusable, and the study would be rejected by the Ethics Committee. A good clinical practice follows the general principles of medical ethics: respect for life, human dignity and personal autonomy, benefacere, primum non nocere and justice. From these ethical principles, general guidelines for good clinical practice in pediatric research can be drawn [7]. Trials should be focused on knowledge, treatment, relief or prevention of a disease in children. Biomedical studies must be dedicated to reduce suffering and improving disease prognosis. The expected benefit must outweigh the recognizable risks. Serious predictable risks must be avoided. Only well-designed research is ethically appropriate. Research protocols must be evaluated by Ethics Committees (Institutional Review Boards) and reviewed by pediatric experts. Ethics Committees are an effective tool for protecting subjects from inappropriate research. The Ethics Committees that review pediatric research should have members who have experience in pediatric practice. Pediatric studies should be performed by medical and scientific staff who are familiar with GCP and are capable of a confidential relationship and communication with the child and parents. Studies should be conducted in institutions that provide a child-friendly atmosphere [8-9].

Conclusion

Pediatric clinical trials provide valuable information for physicians, giving them the guidance and knowledge in providing optimal care for their patients. With proper planning and reviewing of the study, trial can be conducted in children even though they are considered as a vulnerable population. Children are not "small adults". Compared to adults, there are differences in pharmacokinetics and dynamics, as well as side effects that are common in children. Certain consequences of medical interventions can be seen in children and can occur long after exposure. Because of the special care they deserve, children should not be the subject of clinical trials when research can be done on less vulnerable subjects such as adults. If research in children is ne-

cessary, then less vulnerable children should be included, i.e. older children [10].

Conflict of interest statement. None declared.

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ММР

Original article

LOW FREQUENCY OF CLONAL B CELL EXPANSIONS IN PATIENTS WITH IDIOPATHIC THROMBOCYTOPENIC PURPURA

НИСКА ФРЕКВЕНЦИЈА НА КЛОНАЛНА Б КЛЕТОЧНА ЕКСПАНЗИЈА КАЈ ПАЦИЕНТИ СО ИДИОПАТСКА ТРОМБОЦИТОПЕНИЧНА ПУРПУРА

Marica Pavkovic and Dimitar Efremov

University Clinic for Hematology, Faculty of Medicine, Ss Cyril and Methodius University Skopje, Republic of North Macedonia

Abstract

Idiopathic thrombocytopenic purpura (ITP) is an autoimmune disorder characterized by an increased platelet destruction caused by autoantibodies directed against platelet membrane glycoproteins (GP), most commonly against GPIb/IX, GPIIb/IIIa and GPIa/IIa. In a few recent studies it has been reported that these antibodies frequently have a restricted light chain phenotype, supporting a clonal origin. In this study we wanted to explore the hypothesis of clonal B cell expansions in chronic ITP. We investigated 40 patients (28 women and 12 men) with chronic ITP for clonal B cell expansions using sensitive RT-PCR technique for analysing Ig-gene rearrangements. RNA was isolated from peripheral blood mononuclear cells separated on a Ficoll gradient. The RNA was converted into cDNA and then amplified using FW3 and IgM or IgG specific oligonucleotides to investigate the clonality of B-cells expressing the respective Ig isotype. The PCR fragments were analyzed on sequencing polyacrylamide gels or with an ABI prism 310 DNA analyser. We detected a monoclonal B cell population in only 1 patient and polyclonal rearrangement with one prominent band in 3 patients in the analysis of IgG heavy chain mRNA. The pattern of IgM heavy chain gene rearrangements was polyclonal in all cases. Our study indicates that clonal B-cell expansions are rare in patients with ITP. Most probably, the clonal B-cell expansion responsible for the production of autoantibodies in ITP, if present, is below the detection limit.

Keywords: idiopathic throbocytopenic purpura (ITP), immunoglobulin gene rearrangement, anti-platelet autoantibodies

Апстракт

Идиопатска тромбоцитопенична пурпура (ITP) е автоимуно нарушување кое се карактеризира со зголемено уништување на тромбоцитите предизвикано од автоантитела насочени против гликопротеините на тромбоцитната мембрана (GP), најчесто против GPIb/IX, GPIIb/IIIa и GPIa/IIa. Во неколку студии беше објавено дека овие автоантитела често имаат ограничен фенотип на лесен ланец, поддржувајќи клонално потекло. Во оваа студија сакавме да ја истражиме хипотезата за присуството на клоналната Б-клеточна експанзија кај болните со хронична ИТП. Постоењето на клонална Б-клеточна популација го анализиравме кај 40 пациенти (28 жени и 12 мажи) со хронична ИТП користејќи чувствителна RT-PCR техника за анализа на преуредувањето на имуноглобулинските гени. РНК беше изолирана од мононуклеарните клетки од периферна крв одделени на градиент на Фикол. Изолираната РНК беше претворена во cDNA, а потоа следуваше амплификација на специфични сегменти од имуноглобулинските гени со употреба на специфични олигонуклеотиди FW3 и IgM или IgG со цел да се испита клоналноста на Б-клеточната популација. PCR фрагментите беа анализирани со секвенционирање на полиакриламидни гелови или со АБИ призм 310 ДНК анализатор. При анализата на mRNA на тешкиот ланец на IgG откривме моноклонална Б-клеточна популација кај само 1 пациент и поликлонално преуредување со еден подоминатен фрагмент кај 3 пациенти. Преуредување на генот за тешкиот ланец на IgM беше поликлонален кај сите пациенти. Нашата студија покажува дека присуството на моноклонална Б клеточна популација е ретко кај пациенти со ИТП. Една од можните причина е дека клоналната Б-клеточна популација, одговорна за производство на автоантитела кај ИТП, доколку е присутна, е под детектибилните границата.

Correspondence to: Marica Pavkovic, University Clinic for Hematology, Faculty of Medicine, 1000 Skopje, R. N. Macedonia; E-mail: pavkovicm@yahoo.com

Клучни зборови: идиопатска тробоцитопенична пурпура (ИТП), преуредување на имуноглобулинските гени, анти-тромбоцитни автоантитела

Introduction

Chronic immune thrombocytopenic purpura (ITP) is the most common autoimmune hematological disorder characterized by a decreased survival of platelets due to the production of anti-platelet autoantibodies that results in increased platelet destruction by the reticuloendothelial system [1-5]. ITP can be primary idiopathic autoimmune disorder, and may occur together with other immune and nonimmune disease like SLE, chronic lymphoproliferative diseases like chronic lymphocytic leukemia (CLL), AIDS, reactions to drugs etc.
The first evidence that pathogenic anti-platelet autoantibodies are specifically directed against certain platelet surface glycoproteins was provided by a study of Leeuwen *et al.* [6] and Stockenberg *et al.* [7]. They showed that the most common platelet surface antigens are the glycoprotein complexes GPIIb/IIIa and/or GPIb/IX.
In the past the clonality of the autoantibodies in ITP was investigated in several studies. Most of these studies showed that in almost 70% of analyzed patients anti-platelet autoantibodies were kappa or lambda light chain restricted by employing the light-chain specific MAIPA assay [8,9]. In order to further investigate the hypothesis of clonality of autoantibodies and the presence of clonal autoreactive B cells in patients with ITP, Van der Harst *et al*. [10] analyzed eleven patients with ITP for the presence of a clonal excess using κ-λ flow cytometry and DNA analysis of immunoglobulin gene rearrangements. In 10 of 11 patients, clonal B-cell populations were found by one or both tests. These findings could not be confirmed in other studies [7]. Therefore, further analysis must investigate the clonality of the B-cell population in patients with ITP with more sensitive DNA assays.
The aim of this study was to explore the hypothesis of clonal B cell expansions in chronic ITP by the sensitive method of immunoglobulin gene fingerprinting.

Materials and methods

We investigated 40 patients (28 women and 12 men) with chronic ITP for clonal B cell expansions using sensitive RT-PCR technique for analysing Ig-gene rearrangements. The average age of the group was 46.8±6.8 years. The diagnosis of ITP was established based on the standard clinical and laboratory parameters in our department.
RNA was isolated from peripheral blood mononuclear cells, separated on a Ficoll gradient, by the method of Chomcynski and Sacchi [11]. The RNA was reverse transcribed (RT) into cDNA using random hexamers and the GeneAmp RNA/PCR kit (Perkin Elmer Cetus, Norwalk, CT, USA), following the procedure recommended by the manufacturer. The RT product was then amplified using specific oligonucleotides FW3 (hV2 5'-CTG AGG ACA CGG CCG TGT ATT ACT G-3') and IgM (hM 5'-GGA AAA GGG TTG GGG CGG AT-3'), or IgG (hGc 5'-GGA AGA CCG ATG GGC CCT TG-3'). The PCRs were performed with 50 pmol of each primer using 35 cycles of denaturation at 95°C for 1 min., annealing at 64°C for 1 min. and synthesis at 72°C for 1 min. and 30 sec. [12], 2 μl of each reaction were analyzed on denaturing 6M urea 6% polyacrylamide gel or with an ABI prism 310 DNA analyser.
We also analyzed the presence of anti-platelet antibodies in 15 patients with ITP by the indirect MAIPA method [10]. For the detection of anti-platelet antibodies in patients with ITP we used modified indirect MAIPA (Monoclonal antibody-specific immobilisation of platelet antigens) method. For that purpose we used microtiter plates coated overnight at 4°C with 100 μl of Sigma goat antimouse IgG (light chain specific) antibodies (C.No. M 1397) with concentration of 1 μg/ml in 0.05 M carbonate buffer pH 9.6. After four washes with 200 μl of PBS-Tween solution, the remaining binding sites were blocked for 60 minutes with 200 μl 2% BSA (bovine serum albumin) in phosphate buffer saline (PBS)-Tween. After this step plates were incubated for two hours with DAKO monoclonal anti-CD41 (M 7057), anti-CD42b (M 0719) and anti-CD61 (M 0753) antibodies (0.5 μg/ml) in PBS/1% BSA. After this incubation, plates were again washed with PBS-Tween solution 4 times. These plates were ready for use.
For the Indirect MAIPA method we used serum from patients and already prepared test platelets form blood group O, Rh+. Test platelets were obtained from peripheral blood with EDTA from blood donors. After centrifugation of peripheral blood at 190 g for 10 minutes platelet rich plasma (PRP) was obtained. This platelet rich plasma was recentrifuged at 1600 g for 10 minutes. Test platelets ($1x10^8$) were washed three times with 1% disodium EDTA in phosphate-buffered saline (PBS) and incubated with 100 μl patient serum for one hour at room temperature. After this, platelets were washed for three times with PBS and solubilized with 1000 μl solubilizing solution (50 mM Tris, 145 mM NaCl, pH 8.2, 1%TritonX 100 with 100 μg/ml Leupeptin) for 30 minutes at 4°C. After this step, they were centrifuged for 30 min. at 4°C at 13 000 rpm. 100 μl of the supernatant was mixed with 300 μl PBS buffer and 100 μl of this solution was incubated in already prepared microtiter plates for 90 min. at 4°C in duplicate. Plates were then washed four times with PBS, 1% TritonX 100 and 0.1 Tween20 solution. 100μl of anti-human IgG-horseradish peroxidase (Sigma, A 0170) antibodies (1:10000) were added to the wells and incubated for 120 minutes at 4°C. When kappa/

lambda specific MAIPA was performed we used anti-human kappa (Sigma, A 1764) or anti-human lambda-HRP antibodies (Biosource Int. AHI 0904) (1:2000). After this step, plates were washed for six times with washing buffer and 200 μl of OPD (Sigma substrate for peroxidase) was added for 30 min. and incubated in dark. Reaction was stopped with 50 μl of 2M $H_2SO_4$for 15 min. at room temperature. OD (optical density) values were read with Elisa reader. Positive results were wells in which OD was two times higher than negative controls.

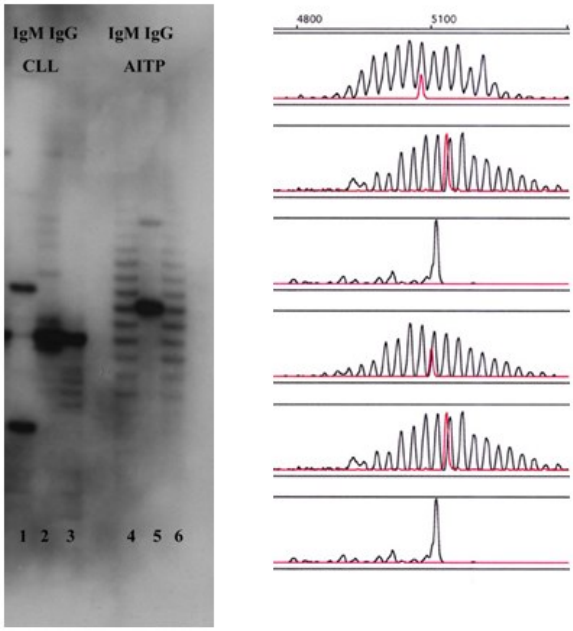

Fig. 1. Ig gene rearrangement on 6% polyacrilamide gels and analyzed by ABI Prism 310

Results

In our study we analyzed 40 patients with ITP and detected polyclonal immunoglobulin gene rearrangements in IgM heavy chain in all patients. In the analysis of IgG heavy chain mRNA we detected monoclonal Ig gene rearrangement in only 1/40 patient (Figure 1) and polyclonal rearrangement with one prominent band in 3/40 patients. Average count of platelets at the time of analysis was $20.8x10^9$/L±22.3 and the median was $12x10^9$/L (Table 1). Table 2 presents OD value for indirect kappa/lambda specific MAIPA in ITP patients with already detected anti-platelet antibodies specific for CD 61 (GP IIIa).

Table 1. Platelet number at the time of analysis

Number of patients (n=40)	Platelet number
Average (Mean) number	$20.8x10^9$/L
Median	$12x10^9$/L
Standard deviation	$22.3x10^9$/L
Minimum value	$1x10^9$/L
Maximum value	$86x10^9$/L
Range	$85x10^9$/L

Table 2. OD value for indirect kappa/lambda specific MAIPA in ITP patients with already detected anti-platelet antibodies specific for CD 61 (GP IIIa)

	Anti-human kappa Ab			Anti-human lambda Ab		
	CD 41	CD 42 b	CD 61	CD 41	CD 42 b	CD 61
Negative controls	0.094	0.087	0.076	0.086	0.075	0.91
Patient No 3 (negative results)	0.112	0.123	0.105	0.114	0.108	0.098
Patient No 5 (positive results)	0.102	0.113	**0.614**	0.108	0.098	**0.496**

Discussion

Several studies have investigated the clonality of the platelet autoantibodies in patients with ITP by a light-chain-specific MAIPA assay (8-10,13) and showed that clonal restriction of autoantibodies was present in a proportion of patients with ITP. The study of Stockenberg *et al*. [8] showed that 75% of analyzed patients by a light-chain-specific MAIPA assay had light chain restricted autoantibodies. We also analyzed the presence of anti-platelet autoantibodies using the modified indirect MAIPA method in 15 patients with ITP and we detected anti-platelet autoantibodies in only 5 of 15 patients (33%). In patients with positive indirect MAIPA we performed κ/λ specific MAIPA in order to analyze the light chain restriction of autoantibodies. This analysis showed polyclonal anti-platelet antibodies in all 5 MAIPA positive patients.

The study performed by Roark *et al*. [14] constructed phage display libraries from splenocytes from 2 patients with chronic ITP, and competitive cell-surface selection was used to isolate several dozen unique IgG platelet-specific autoantibodies. Platelet-reactive Fabs in both patients were associated almost exclusively with rearrangements of a single Ig heavy-chain variable-region gene (V(H)3-30), despite an apparent diversity of antigen specificities. Comparative analysis of platelet-reactive Fab Ig gene rearrangements from each patient suggested that they evolved from a restricted number of B-cell clones through somatic mutation with high replacement-to-silent mutation ratios. Although V(H)3-30-encoded heavy chains were found with light chains encoded by several different Ig genes, molecular repairing experiments showed an exquisite restriction on the specific heavy- and light-chain pairings that permitted platelet reactivity. Together, these data su-

ggest that the development of platelet-reactive antibodies associated with ITP is driven by an encounter with diverse platelet antigens through the clonal expansion of B cells using genetically restricted and highly specific combinations of heavy- and light-chain gene products.

Conclusion

In conclusion, our study indicates that clonal B-cell expansions are rare in patients with chronic ITP. Most probably, the clonal B-cell expansion responsible for the production of autoantibodies in ITP, if present, is below the detection limit.

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Conflict of interest statement. None declared.

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Original article

EVALUATION OF THE ANALYTICAL VALUE OF SARS-CoV-2 ANTIGEN TEST IN RELATION TO Ct-VALUES OF RT-qPCR IN PATIENTS SUSPECTED OF COVID-19

ЕВАЛУАЦИЈА НА АНАЛИТИЧКАТА ВРЕДНОСТ НА SARS-CoV-2 АНТИГЕНСКИ ТЕСТ ВО ОДНОС НА Ct ВРЕДНОСТИТЕ НА РТ-ПВР КАЈ ПАЦИЕНТИ СУСПЕКТНИ ЗА КОВИД-19

Gorica Popova[1], Katerina Boskovska[1], Ivana Arnaudova Danevska[1], Katerina Blagoevska[2]

[1]Institute of Respiratory Diseases in Children, Skopje, [2]Faculty of Veterinary Medicine, Ss Cyril and Methodius University Skopje, Republic of North Macedonia

Abstract

Introduction. COVID-19 pandemic threatens global human health. Reverse-transcription quantitative polymerase chain reaction (RT-qPCR) is a reference test for identification of acute SARS-CoV-2 infection, but it is associated with results delay. There is a need of fast and reliable tests which can improve the efforts of controlling the transmission of SARS-CoV-2.
Aim. The aim of this study was to determine the analytical value of the rapid SARS-CoV-2 Ag-test in relation to the Ct values of the RT-qPCR.
Methods. The study group comprised outpatients suspected for COVID-19, sampled twice, first for the routine RT-qPCR, and second for SARS-CoV-2 antigen testing. The results obtained by the rapid antigen test (Panbio™ COVID-19) were evaluated in relation to Ct values of the SARS-CoV-2 E-gene, obtained by RT-qPCR Allplex 19-nCoV multiplex assay platform.
Results. SARS-CoV-2 prevalence, based on RT-qPCR, was 50.8% (186/366). Specificity of the Panbio™ COVID-19 Ag Rapid Test was 100%. Test sensitivity was 73.8%. Restricting RT-qPCR to Ct-values<30 increased test sensitivity to 91.2%.
Conclusion. The findings underscored the epidemiological value of the rapid Ag-test since it reliably identifies contagious SARS-CoV-2 infected individuals who actively spread the virus in the community.

Keywords: COVID-19, RT-qPCR, rapid SARS-CoV-2 Ag test, sensitivity, specificity

Апстракт

Вовед. Здравјето на луѓето, на глобално ниво, е загрозено поради пандемијата со КОВИД-19. Референтен тест за идентификација на акутна инфекција со SARS-CoV-2 е РТ-ПВР, но ова тестирање е поврзано со доцнење на резултатите. Од тука произлегува потребата од брзи и сигурни тестови кои ќе помогнат во контрола на ширењето на SARS-CoV-2.
Цел. Да се одреди аналитичката вредност на SARS-CoV-2 антигенски тест преку споредба со Ct-вредностите добиени со РТ-ПВР.
Методи. Испитувана група беа амбулантски пациенти суспектни за КОВИД-19, од кои беа земени примероци, прво за рутинско РТ-ПВР тестирање и второ за SARS-CoV-2 антигенски тест. Резултатите добиени од брзиот антигенски тест (Panbio™ COVID-19) беа компарирани со Ct-вредностите на Е-генот добиени со мултиплекс РТ-ПВР (Allplex 19-nCoV assay).
Резултати. Преваленцата на SARS-CoV-2, заснована на РТ-ПВР, изнесуваше 50,8% (186/366). Специфичноста на брзиот Panbio™ COVID-19 Ag тест беше 100%. Сензитивноста на тестот изнесуваше 73,8%. При ограничување на Ct-вредностите на РТ-ПВР на <30 сензитивноста на тестот се зголеми на 91,2%.
Заклучок. Резултатите ја потенцираат епидемиолошката вредност на брзиот антигенски тест кој со сигурност ги детектира инфицираните лица со SARS-CoV-2 кои се заразни и активно го шират вирусот во заедницата.

Клучни зборови: КОВИД-19, РТ-ПВР, Брз SARS-CoV-2 Аг тест, сензитивност, специфичност

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a novel corona virus, emerged in December 2019 in Wuhan, China [1], and within a few months had spread worldwide. To date, 89.9 million have been infected with SARS-CoV-2, and 1.9 million have died from coronavirus disease 2019 (COVID-19) [2]. In this pandemic situation, early diagnosis of infectious patients is especially important for implementation of relevant epidemiological measures for discontinuation of the SARS-CoV-2 transmission chain. Reverse-transcription quantitative polymerase chain reaction (RT-qPCR) is a reference test for identification of

Correspondence to: Gorica Popova, Institute of Respiratory Diseases in Children, 1000 Skopje, R. N. Macedonia; E-mail: gorica.popova@yahoo.com

acute SARS-CoV-2 infection and it is routinely used in clinical practice [3]. Despite its high sensitivity and specificity, RT-qPCR test typically takes 4-5 h for results and requires specialized laboratory equipment and skilled technicians. Therefore, the need of inexpensive, reliable tests for detection of SARS-COV-2 was recognized by the WHO [4]. Lateral Flow Assay (LFA)-based point of care tests (POCT) for rapid antigen detection seems to be a good choice. They do not require special equipment or specially trained staff and generate results within 20 minutes [5]. Considering short turn around times, this testing system enables expanding of the testing and therefore detection of a larger number of contagious people. However, the diagnostic value of the rapid tests should be based on comparing the test results with the results obtained by the RT-qPCR as a gold standard. There are rapid SARS-CoV-2 Ag detecting tests with a different specificity and sensitivity [6-9].

Aim

The aim of this study was to determine the analytical value of the Panbio™ COVID-19 Ag rapid test in relation to the Ct values of the SARS-CoV-2 E-gene, obtained by RT-qPCR Allplex 19-nCoV multiplex assay platform, in outpatients suspected for COVID-19.

Material and methods

During the one-month period, from 1st to 30th of December 2020, a total of 366 outpatients visited the COVID-19 testing center, situated at the Institute of Respiratory Disease in Children, Skopje, RNM. Patients were referred by their general practitioners (GPs) due to high suspicion of COVID-19 aiming to be PCR tested to detect SARS-CoV-2 infection.
Eighty-four of them were sampled twice, first for the routine RT-qPCR testing, using a combined throat/nasopharyngeal swab, and second for SARS-CoV-2 antigen testing, using additional nasopharyngeal swab.

Diagnostic tests
RT-qPCR

PCR was conducted in a certificated clinical laboratory situated at the Institute of Respiratory Diseases in Children, Skopje, RNM. After collection, swabs were transferred into 2 ml PBS (Dulbeco's Phosphate Buffered Saline, Sigma, Life Science) and transported to the laboratory which is located within 2 min of walking distance from the sampling location. All specimens were processed in biosafety level-2 (BSL-2) facilities with full personal protective equipment. Nucleic acid extraction, RT-qPCR and results interpretation were performed according to the instructions of the manufacturer. Briefly, RNA was isolated and purified using the STARMag 96 ProPrep extraction kit (Seegene, South-Korea) on an automatic nucleic acid extractor SEEPREP 32 (Seegene South Korea). Amplification was performed in a single tube assay using the Allplex 19-nCoV multiplex platform which targets three SARS-CoV-2 genes [envelope gene (E) of *Sarbecovirus*, RNA-dependent RNA polymerase (RdRp) and nucleocapsid (N) genes which are specific of SARS-CoV-2], according to the manufacturer's instructions (Seegene, South Korea). Amplification and detection were performed on a CFX-96 real-time thermal cycler (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The conditions consisted of 1 cycle of 20 min. at 50 °C, 15 sec. at 95°C and followed by 45 cycles of 15 s at 94 °C, 30 s at 58 °C. The results were interpreted with Seegene Viewer data analysis software, in which the threshold Cycle (Ct) was automatically determined, and a positive result was defined as amplification of any of the three SARS-CoV-2 genes, within the cut-off values $\leq$40.

LFA (Lateral Flow Assay)

The Panbio™ COVID-19 Ag rapid test device by Abbott (Lake Country, IL, U.S.A) is a membrane-based immunochromatography assay which detects the nucleocapsid protein of SARS-CoV-2 in nasopharyngeal samples. Collected swabs were transferred into dedicated sample collection tubes containing a sampling buffer and transported to the same laboratory where the RT-qPCR was conducted. All samples were analyzed within a maximum of 30 minutes after collection, during which time the samples were kept at ambient temperature. Collected samples were subsequently processed in a level 2 biosafety cabinet. Test results were recorded after 15 min. of assay initiation by two independent observers (blinded to each other and to the PCR results). Intensities of the test bands were compared to the control bands and designated as "++" if the test and control bands intensity were similar or "+" if the test band intensity was weaker than the control band.

Results

During December 2020, a total of 366 outpatients were RT-qPCR tested because of high suspicion of Covid-19. According to the results interpreted by Seegene Viewer data analysis software (in which a positive result is defined as amplification of any of the three SARS-CoV-2 genes within the cut off $\leq$ 40) 50.8% (186/366) of all tested samples were recorded as positive. In 16.1% of all positive samples (30/186), test result indicated amplification of only one or two genes. In these cases (previously categorized as inconclusive results) the most often detected was N gene with mean Ct-value equal to 38.01 (35.49-39.5).
Of all double tested patients (n=84), 31 were tested positive by both test methods-RT-qPCR and rapid antigen (Ag) detecting test, with mean Ct-value of the E

gene 22.27 95% CI [20.52-24.02] (Figure 1). According to the intensity of the test band compared to the intensity of the control band, 20 of them were designnated as ''++'', and the corresponding mean Ct-value of the E gene was 19.6 95% CI [17.97-21.23]. Eleven had test band intensity weaker than the control band and were designated as ''+'' with corresponding mean Ct value of the E gene equal to 27.1 95% CI [24.84-29.37] (Figure 1).

Discrepancy between both test methods was observed in 11 cases.

Six cases tested positive by RT-qPCR with amplifycation of only one or two genes (N gene was detected in all six cases with mean Ct-value of 38.4, and in two cases, the E gene was detected along with the N gene with mean Ct-value of 35.4) were tested negative by the rapid antigen (Ag) test.

In addition, 5 cases tested positive with RT-qPCR by amplifying all 3 genes within the cut-off values, were also tested negative by the rapid Ag test. These cases had a corresponding mean Ct value of the E gene of 30.3 (29.17-32.30) (Figure 1).

All specimens tested negative by the rapid Ag test (n=42) were also tested negative by the RT-qPCR.

When the RT-qPCR was used as a reference, the antigen test diagnosed SARS-CoV-2 infection status with sensitivity of 73.8% (31/42), and specificity of 100% (42/42) (Table 1).

False negative Ag test results were observed in subjects with high RT-qPCR Ct-values (including inconclusive results), reflecting low viral levels in nasopharyngeal material. When defining RT-qPCR Ct positivity on a cut-off Ct-value of 30, Ag test sensitivity increased to 91.2% (31/34) (Table 1).

Table 1. Sensitivity and specificity of the antigen detection test in comparison with RT-qPCR

		Antigen test			
		Negative	**Positive**	**Sensitivity**	**Specificity**
RT- qPCR	Negative	42	0		100%
	Positive	11	31	73.8%	
Ct < 30	Negative	42	0		100%
	Positive	3	31	91.2%	

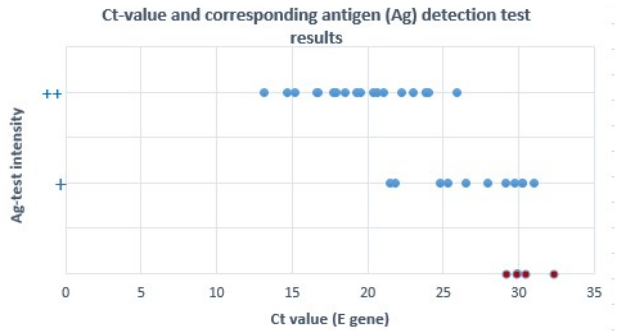

Fig. 1. Ct-value and corresponding antigen (Ag) detection test results

Cycle threshold (Ct) value of the E-gene and corresponding antigen (Ag) detection test results [blue circles positive (n=31), red circles negative (n=5) for each RT-qPCR positive sample with amplification of the all tree genes (n=36)].

Intensities of the test bands were compared to the control bands and designated as "++" if test and control bands intensity were similar or "+" if the test band intensity was weaker than the control band.

Discussion

In this study the Panbio™ COVID-19 Ag rapid test by Abbott (Lake Country, IL, U.S.A) was compared with the Allplex 19-nCoV multiplex platform RT-qPCR as a confirmatory test. Both different testing methods were performed in the same settings, and the samples for the two tests were collected at the same time, as it is recommended by WHO [4]. The tested population was outpatients highly suspected for COVID-19, and this could be the explanation for the high percentage of positivity (50.8%). The positive samples with no amplification of all three SARS-CoV-2 genes (inconclusive results) always corresponded to high Ct-values (the most often detected was N gene with mean Ct-value 38.01). In this context, Bhattacharya and colleagues [10] stated that the inconclusive results were probably due to different analytical sensitivity of individual viral gene PCR and were probably more subject to stochasticity which can result in positive results in only one or two targets especially at low viral load levels.

According to data of this study, Panbio™ COVID-19 Ag rapid test, has 100 specificity and overall, 73.8% sensitivity compared to Allplex 19-nCoV RT-qPCR. The manufacturer reported sensitivity of 93.3%, which is probably resulted from testing individuals with symptoms for less than seven days in high-endemic settings in Brazil [11]. In another study with cohort of 257 patients, the overall sensitivity was 73.3%, and 86.5% among individuals with symptoms for less than seven days [12]. Gremmeles and colleagues reported sensitivity of 72.6% and 81.0% in community-dwelling mildly symptomatic subjects in a medium- and high-endemic area [13].

In this study, the rapid Ag detecting test reliably identified SARS-CoV-2 infected individuals with Ct-values lower than 30 cycle by RT-qPCR. The overall positive samples by Ag detecting test had a mean Ct value of the E gene equal to 22.27. The intensity of the test

bands correlated with the Ct values of the RT-qPCR. Those with test band intensity similar to the control band had a corresponding mean Ct-value of the E gene equal to 19.6, and those with test band intensity weaker than the control band had a corresponding mean Ct value of the E-gene equal to 27.1 (95% Confidence Interval, CI: 24.8-29.4). Hence, this study demonstrates that the Panbio™ COVID-19 Ag rapid test has limit of detection of viral antigen near to the viral load which corresponds to 30 Ct value of the E gene detected by Allplex 19nCoV RT-qPCR. On the other hand, there are studies which undoubtedly revealed that high viral RNA load was independently associated with shedding of infectious virus [14,15]. Using cycle threshold (Ct) values as a quantitative measure for viral RNA load, Bulland and colleagues [16] reported that infectious virus could not be isolated from diagnostic samples when Ct values were above 24. These reports point out that from an epidemiological point of view most important is to detect persons with SARS-CoV-2 RNA load associated with spreading of infectious viruses. Furthermore, they recommend the use of quantitative viral RNA load assays as a part of test-based strategies for infection prevention and control measurements.

False negative Ag test results were observed in subjects with high RT-qPCR Ct-values (including inconclusive results), reflecting low viral levels in nasopharyngeal material. Intending to single out clinically significant cases, as well as in accordance with the results from previously mentioned viral culture studies [14-16], the lowering of Ct cut-off to 30 cycles increased the sensitivity of the rapid antigen test to 91.2%. Hence, the results suggest that Panbio™ COVID-19 Ag rapid test can detect SARS-CoV-2 infected individuals who are infectious and can potentially transmit the virus.

Conclusion

The results underscore the epidemiological value of the Panbio™ COVID-19 Ag rapid test. Positive samples indicate persons who are highly contagious, and this should be taken into consideration when implementing strategies aiming to prevent the spread of the virus in the community. Despite the lower sensitivity comparing to RT-qPCR, these quick and inexpensive tests should be especially helpful for low income countries where the availability and cost of RT-qPCR tests are limiting factors.

Conflict of interest statement. None declared.

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Original article

ROBSON CLASSIFICATION OF CESAREAN SECTION IN NORTH MACEDONIA - CURRENT TRENDS

КЛАСИФИКАЦИЈА НА ЦАРСКИ РЕЗОВИ ВО СЕВЕРНА МАКЕДОНИЈА ПО РОБСОН – МОМЕНТАЛЕН ТРЕНД

Ivo Kjaev, Ana Daneva Markova, Pajtim Asani, Viktorija Jovanovska, Adelina Dalipi, Martina Mladenovska, Romir Kadriu, Irena Aleksioska Papestiev, Dafina Karadjova, Katerina Nikoloska, Durim Asani, Rosa Spasova, Daniel Milkovski and Goran Kocoski

University Clinic for Gynecology and Obstetrics, Faculty of Medicine, Ss Cyril and Methodius University Skopje, Republic of North Macedonia

Abstract

Introduction. Over the last few decades, the global cesarean section rate has significantly increased and reached an unprecedented level. The World Health Organization (WHO) has advised that cesarean section (CS) rates should not rise above 15%. Several classifycation systems have been proposed to tackle the increased cesarean section epidemic. Most of the countries have adopted and started using the Robson (10 groups) classification as the best and the one that is the easiest internationally applicable CS classification.
Aim. To present the Robson classification as a way to start better classification of cesarean section and hence to reduce the number of unnecessary cesarean section deliveries.
Methods: This study was realized at the University Clinic for Gynecology and Obstetrics in Skopje, North Macedonia. It is a retrospective study where two years were compared.
Results. The rate of cesarean sections for 2017 was 38.5% and for 2019 42.6%. Categorization of deliveries according to Robson criteria showed a different rate of cesarean section for each subgroup.
Discussion. The implementation of the Robson classification in most countries has shown a reduction in the number of cesarean deliveries and thus a reduction in overall maternal and neonatal morbidity and mortality. The analysis has shown that group 5 had the largest number of cesarean section deliveries in both years, 2017 and 2019; these were patients with previous cesarean sections. They were followed by group 1 and 2, or primipara with spontaneous onset and induced delivery.
Conclusion. The goal of Robson clasification is to identify the target groups that contribute most in the percentage of cesarean sections and to act on these target groups through appropriate education and training to reduce the percentage of cesarean sections. The reduction of cesarean section will also decrease the number of hospital days and lower the health care cost of each delivery.

Keywords: cesarean section, groups, classification

Correspondence to: Ivo Kjaev, University Clinic for Gynecology and Obstetrics, 1000 Skopje, R. N. Macedonia; E-mail: ivo_kaev@yahoo.com

Абстракт

Вовед. Во тек на последниве неколку децении, глобално процентот на царски резови значително се зголеми и достигна невидени размери. Според светската здравствена организација (СЗО) процентот на царски резови не треба да биде повеќе од 15%. Повеќе класификациони алгоритми се предлоќени со цел да се намали стапката на зголемениот број на царски резови. Повеќето земји ја прифатја и почна да ја применуваат класификацијата по Робсон (10 групи) како најдобра и најлесна за апликација интернационално.
Цел. Да се презентира Робсоновата класификација како начин преку кој подобро ќе може да ги класифицираме царските резови и со тоа да се намали бројката на непотребни царски резови.
Методи. Студијата беше изведена на Универзитетската Клиника за Гинекологија и Акушерство во Скопје, Северна Македонија. Претставува ретроспективна студија каде две години беа споредени.
Резултати. Стапката на царски рез за 2017 година изнесува 38,5% а за 2019 година 42,6%. Категоризација на породувањата по Робсон покажа различни стапки на царски рез во секоја подгрупа.
Дискусија. Имплементацијата на Робсоновата класификација во повеќе земји покажа редукција во стапката на царски резови како и редукција во севкупниот мајчин и неонатален морбидитет и морталитет. За време на анализата на студијата најдовме дека најголем стапка на царски резови во 2017 и 2019 имавме во групата 5, тоест во групата на пациентки со претходни царски резови, потоа во гру-

па 1 кај првородтки со спонтан почеток на раѓањето и во групата 2 кај трудници каде раѓањето беше индуцирано.
Заклучок. Целата на Робсоновата класификација е да ги идентификува целните групи кои најмногу допринесуваат за стапката на царски рез и да се делува на тие групи преку соодветна едукација и тренинг со цел намалување на стапката на царски рез. Редукцијата на стапката на царски рез ќе допринесе во намалување на престојот во болница и да се намали цената на секое породување.

Клучни зборови: царски рез, групи, класификација

Introduction

Cesarean section rates continue to increase worldwide while the reasons appear to be multiple, complex and, in many cases, country-specific. Over the last few decades, the global cesarean section rate has significantly increased and reached an unprecedented level [1]. The World Health Organization (WHO) has advised that cesarean section (CS) rates should not rise above 15%. [1]. Some evidence suggests that cesarean section rates above 15% do not improve the reduction of maternal and neonatal morbidity and morbidity [1]. Recently, cesarean sections have been performed without medical reasons or with imprecise indications such as obstructed labor, with intact membranes. Several classification systems have been proposed to tackle the increased cesarean section epidemic. Most of the countries have adopted and started using the Robson (10 groups) classification as the best and the one that is the easiest internationally applicable CS classification [1]. The Robson classification is currently endorsed by WHO, [2] International Federation of Gynecology and Obstetrics, [2] and European Board and College of Obstetrics and Gynecology [3].
The Robson classification criteria have so far been adopted and used in more than 50 countries. No large-scale studies about cesarean rates in North Macedonia have been done or published so far. The idea and aim of this study were to implement the 10-group classification model and to reduce the number of cesarean sections and still to have a good maternal and neonatal outcome. The ten Robson categories are mutually exclusive, totally inclusive and can be applied prospectively since each woman admitted for delivery can be classified immediately based on a few variables that are generally routinely recorded. This system helps institutions specific monitoring and auditing and offers a standardized comparison method between institutions, countries and time point.

Materials and methods

This study was realized at the University Clinic for Gynecology and Obstetrics in Skopje, North Macedonia. It is a retrospective study where two years were compared. Our institution has an average of 4000 deliveries per year, which represents 20% of all live births in the country. It is the only tertiary center for early preterm delivery. Deliveries are categorized according to Robson criteria.

Table 1. Robson criteria

Groups	Clinical characteristics
1	Nulliparous, singleton, cephalic, ≥ 37 weeks, spontaneous labor
2	Nulliparous, singleton, cephalic, ≥ 37 weeks, induced labor or cesarean section before labor
3	Multiparous without previous cesarean section, singleton, cephalic, ≥ 37 weeks, spontaneous labor
4	Multiparous without previous cesarean section, singleton, cephalic, ≥ 37 weeks, induced labor or caesarean section before labor
5	Multiparous with prior cesarean section, singleton, cephalic, ≥ 37 weeks
6	All nulliparous breeches
7	All multiparous breeches (including previous cesarean section)
8	All multiple pregnancies (including previous cesarean section)
9	All pregnancies with transverse or oblique lie (including those previous cesarean section)
10	Singleton, cephalic, ≤ 36 weeks (including previous cesarean section)

Results

The total number of deliveries in 2017 was 4249, of which 1637 were cesarean sections and in 2019 there were 4103, of which 1747 were cesarean sections. The rate of cesarean sections for 2017 was 38.5% and for 2019 42.6% (Table 2). Categorization of deliveries according to Robson criteria showed a different rate of cesarean section for each subgroup. The fifth group contributed with the largest number of cesarean sections for the two years, 2017 and 2019. The first group was second in the contribution for both 2017 and 2019. The third in contribution to the number of cesarean sections was the fifth group.

Табела 2. Robson classifications of cesarean sections deliveries in Republic of North Macedonia

Year	2017				2019			
	2019 1637/4249 C/S %	**Size of group %**	**C/S rate in group %**	**Contr of each gp 38.5%**	**2019 1747/4103 C/S %**	**Size of group %**	**C/S rate in group %**	**Contre of each gp 42.6%**
1 Nulliparous single cephalic >=37 weeks sponeus labour	278/1046	24.6	26.6	6.5	287/1028	25.1	27.0	7.0
2 Nulliparous single cephalic >=37weeks induced or CS before labour	257/383	9.0	67.1	6.0	277/361	8.8	76.7	6.8
3 Multipara (excluding previous caesarean sections) single cephalic >=37 weeks spontaneus labour	47/1220	28.7	3.9	1.1	55/1175	28.6	4.7	1.3
4 Multipara (excluding previous caesarean sections) single cephalic >=37wks induction. or CS before labuor	35/132	3.1	26.5	0.8	55/111	2.7	49.5	1.3
5 Previous caesarean section single cephalic >= 37 weeks	471/570	13.4	82.6	11.1	480/552	13.5	87.0	11.7
6 All nulliparous breeches	100/123	2.9	81.3	2.4	113/119	2.9	95.0	2.8
7 All multiparous breeches (including previous caesarean sections)	76/94	2.2	80.9	1.8	80/92	2.2	87.0	1.9
8 All multiple pregnancies (incl previous caesarean sections)	113/147	3.5	76.9	2.7	121/139	3.4	87.1	2.9
9 All abnormali s (including previous caesarean sections)	54/55	1.3	98.2	1.3	54/55	1.3	98.2	1.3
10 All single cephalic <= 36 weeks (includig previous caesarean sections)	206/479	11.3	43.0	4.8	220/471	11.5	46.7	5.4

Discussion

The rate of cesarean sections has increased in recent years. Different countries show different rates of cesarean sections. While the World Health Organization recommends that the number of cesareans should be below 15%, many countries have significantly higher birth rates, for example, Italy 36%, USA 32%, Turkey 50%, Chile 45%, while there are countries in which the rate of cesarean section is close to the recommendation of the World Health Organization, for example, Iceland 15%, Israel 15%, Sweden 16% and Norway 17%.

The implementation of the Robson classification in most countries has shown a reduction in the number of cesarean sections and thus a reduction in overall maternal and neonatal morbidity and mortality. Examples are several countries which have implemented the Robson criteria:

In Brazil, cesarean section rates in groups 1 and 2 decreased from 34.6 to 13.5% in a 10-month observation period. The authors did not observe changes in APGAR score less than seven at 5 minute and perinatal mortality at 10 months [11].

Sweden had a reduction in the cesarean sections rate in group 1 from 10% in 2006 to 3.1 in 2015%. No changes in neonatal outcomes and patient satisfaction were observed [12].

In Italy in 2012-2013 there was a reduction in cesarean section rates from 17.2% to 11% during the implementation of the Robson classification. There were no statistically significant changes in APGAR score less than seven at 5 minute or the rate of instrumental vacuum deliveries [18]. In Northern Italy there was a decrease from 28.8% in 2008 to 25% in 2009. There were no significant changes in the APGAR score or stillbirth rate [13].

Increasing the rate of cesarean section doues not reduce maternal, neonatal morbidity and mortality, and also does increases the complications for mother and newborn and is associated with an increased number of infections, haemorrhages, adhesions, bleeding, lacerations, prolonged hospitalization and drug reactions and other [4-6]. For the newborn, the increased cesarean section rate increases respiratory complications, low APGAR score, fetal injury, allergic rhinitis, food allergy, asthma, type 2 diabetes compared to spontaneous vaginal delivery [7-10].

Estimating the number of cesareans is simple; however, it is difficult to standardize the indications for cesarean sections. Categorizing of deliveries according to the Robson criteria allows us to find which of the subgroups has the greatest contribution and accordingly to analyze that subgroup, and to find a solution which would reduce the number of cesarean sections. The same solutions can be followed for efficiency over time and share with other institutions to achieve a reduction in the number of cesarean sections.

The analysis in this study has shown that group 5 had the largest number of cesarean section deliveries in both years, 2017 and 2019, and these were patients with previous cesarean section. This group was followed by group 1 and 2; they were primipara with spontaneous onset and induced delivery.

In groups 1 and 2, the most common indication for cesarean section was a non-reactive NST record, arrest of labour and fetomaternal disproportion. To reduce the rate of cesarean section, the focus should be on educating medical staff for the proper interpretation of NST, timely admission of patients in the delivery room when they are already active from stage 1 of delivery, which is 5 cm for primipara and 6 cm for multipara. This can help in reducting the number of cesarean sections in groups 1 and 2 that are delivered by SC for arrest labour and fetomaternal disproportion.

Group 5, which has the largest contribution to cesarean section, are patients with previous cesarean section, as much as 1 third of cesarean sections are indicated for the previous cesarean section. In this regard, it is necessary to educate medical staff for spontaneous vaginal delivery after a previous caesarean section.

Groups 6-10 are the smallest but have the highest percentage of cesarean sections; almost all studies show similar results in terms of percentage of cesarean sections in these groups.

This is the first time in our institution and in the Republic of North Macedonia that deliveries are categorized according to the Robson criteria in order to achieve a reduction in the percentage of cesarean sections and to reach the WHO recommendation of 15% of cesarean sections.

Conclusion

Although the rate of cesarean section in our tertiary facility during 2017 and 2019 is close to most western and developed countries, it is still necessary to make efforts to reduce the percentage of cesarean section, especially the primary cesarean section. The purpose of the Robson clasification is to identify the target groups that contribute most in the percentage of caesarean sections and to act on these target groups through appropriate education and training to reduce the percentage of cesarean sections.The reduction of cesarean section will also decrease the number of hospital days and lower the health care cost of each delivery.

Conflict of interest statement. None declared.

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Original article

ASPECTS OF NEPHROTOXICITY OF THE MOST USED NONSTEROIDAL ANTI-INFLAMMATORY DRUGS IN PATIENTS WITH RHEUMATOID ARTHRITIS

АСПЕКТИ НА НЕФРОТОКСИЧНОСТ ОД НАЈЧЕСТО УПОТРЕБУВАНИ НЕСТЕРОИДНИ АНТИИНФЛАМАТОРНИ ЛЕКОВИ КАЈ ПАЦИЕНТИ СО РЕВМАТОИДЕН АРТРИТИС

Dejan Spasovski[1], Emilija Sandevska[1], Sonja Genadieva Stavrikj[2], Tatjana Sotirova[2], Svetlana Krstevska-Balkanov[2], Slavica Subevska Stratrova[3] and Julijana Brezovska-Kavrakova[4]

[1]University Clinic for Rheumatology, [2]University Clinic for Hematology, [3]University Clinic for Endocrinology, [4]Department of preclinic biochemistry, Clinical Center "Mother Therese", Skopje, Republic of North Macedonia

Abstract

Introduction. Microalbuminuria is used as a marker for glomerular damage and urinary excretion of N-acetyl-β-D-glucosaminidase (NAG) as an indicator of proximal tubular damage.
Aim. The aim of the study was to quantify the toxicity of these drugs by measuring the enzyme excretion that correlates with the degree of damage of the tubular epithelium. It was also our aim to determine the effects of the initial therapy with Etoricoxib and Diclofenac on glomerular and tubular integrity in patients suffering from rheumatoid arthritis (RA).
Methods. By using the colorimetric method for determination of NAG, as well as the immunoturbidimetric method for detection of microalbuminuria, tests were performed in 70 participants (35 RA patients treated only with Etoricoxib, 35 RA patients with Diclofenac) in four-time intervals within the course of eight weeks.
Results. There was a moderate correlation between NAG and microalbuminuria (r=0.21) in the group of patients treated with Etoricoxib, while there was a statistically significant correlation (r=0.28) in the group treated with Diclofenac. NAG enzymuria, in volume, by the number of participants in whom it was registerred and the time of its occurrence was much faster during the use of Diclofenac compared to Etoricoxib.
Conclusion. Diclofenac is a potent NAG-inductor and gives a larger tubular enzymuria in comparison with Etoricoxib.

Keywords: N-acetyl-β-D-glucosaminidase, microalbuminuria, rheumatoid arthritis, Etoricoxib, Diclofenac

Correspondence to: Dejan Spasovski, University Clinic for Rheumatology, University Clinical Center "Mother Therese", Skopje, Republic of North Macedonia; E-mail: sdejan36@yahoo.com; Phone: +389 2 31 47 668

Апстракт

Вовед. Микроалбуминуријата е употребен како маркер за гломеруларно оштетување, а уринарната екскреција на Н-Ацетсл-β-Д-Глукозаминидаза (НАГ) како индикатор за проксимално тубуларно оштетување. Да се квантифицира токсичноста на овие медикаменти преку мерење на ензимската екскреција која колерира со степенот на оштетувањето на тубуларниот епител; Да се одреди ефектот на иницијалната терапија со Еторицохиб и Диклофенак врз гломеруларниот и тубуларниот интегритет кај пациенти кои боледуваат од Ревматоиден артритис (РА),
Методи. Користејќи ја колориметриска метода за одредување на НАГ, како и имунотурбидиметриска метода за детекција на мукроалбуминурија, испитани се примероци на 70 партиципанти (35 РА третирани само со Еторицохиб, 35 РА пациенти со Диклофенак), проследени во четри временски интервали во тек на 8 недели.
Резултати. Постои умерена корелација помегу НАГ и микроалбуминуријата (p=0,21) кај групата пациенти третирани со Еторицохиб, додека статисти сигнификантна корелација (p=0,28) кај групата со диклофенак. НАГ ензимурија, по обем, побројот на испитаници кај кои се регистрира и по времето на појавување е поголема и многу побрзо се јавува при употребата на Диклофенак во однос на Еторицохиб.
Заклучок. Диклофенакот е попотетен НАГ-индуктор и дава поголема тубуларна ензимурија од Еторицохиб.

Клучни зборови: N-acetyl-β-D-glukozaminidaza, mikroalbuminurija, revmatoiden artritis, Еторицохиб, Диклофенак

Introduction

Microalbuminuria is used as a marker for glomerular damage and urinary excretion of N-acetyl-β-D-glucosaminidase (NAG) as an indicator of proximal tubular damage. These tests indicate that there is no specific indicator, tracer, marker, which detects nephrotoxicity that occurs within the course of the therapy. Efforts are made to detect such side effects by analyzing the activity of certain enzymes in the urine.
Many uses of certain groups of drugs for therapeutic purposes (NSAIDs, drugs that modify disease activity - DMRADs and immunosuppressive cytotoxic drugs), may have a specific nephrotoxic effect. The given dose is often not suitable for the patient's condition; it can cause side effects, i.e., it can lead to reduction of the kidney function, as a result of accumulation in the kidneys' cells. This is usually found in the long-term therapy of rheumatoid arthritis.

Urinary enzymes to assess nephrotoxicity

Albumin in urine (Microalbuminuria). Albumin (molecular weight of 66 KDa) is the most important protein in plasma, as well as in urine. Approximately 30% of the protein in the urine belongs to albumin and presents a good indicator for assessing the change in glomerular permeability. Urinary albumin excretion has a high individual variability and depends on physical activity or food variations. From a pathophysiological point of view, microalbuminuria can be caused by an increased glomerular permeability for albumin, by an increased glomerular pressure and/or by a reduced tubular albumin reabsorption [1,2].
From all the urinary enzymes, U-NAG (urinary) is the most examined one. This enzyme from the hydrolase class is abundantly present in lysosomes in proximal tubular cells. In human tissue and biological liquids there are two main enzyme forms: A (Acid) and B (Basic) [3-5]. The percentage of isoform A (U-NAG-A) is highest in normal urine [3,4]. Therefore, its excretion is related to the exfoliative turnover and is known as a functional enzymuria. The isoform B (U-NAG-B) is dependent on the maturation and is closely related to the basement membrane in which it is present. Due to this localization of the B isoform, NAG is massively released in the tubular lumen only in the case of cytolytic tubular lesions. Its presence in the urine is in correlation with cell lysis and is known as lesion enzymuria [5,6]. NAG can also be detected in the circulation. However, NAG plasma could not pass through the intact glomerular membrane due to its large molecular weight (140,000 daltons). Therefore, in the healthy urinary tract NAG is representative of the total amount released from the renal tubular cells [7] and is a very sensitive marker for renal tubular damage [8-14].

Materials and methods

In patients included in this study, the disease diagnosis was based on the revised diagnostic criteria for classification of rheumatoid arthritis proposed in 1987 by the American Rheumatism Association (ARA) [15]. In order to include patients in the RA group, it was necessary to meet at least 4 of the predicted 7 criteria.
Criteria from 1 to 4 were present for at least 6 months. The study included 35 patients with RA (20 females, 15 males) treated with Etoricoxib, as well as 35 patients with RA (22 females, 13 males) treated with Diclofenac. The mean age was 50.43 years (±6.42) (38-65 years) in the group treated with Etoricoxib, while 50.13 years (±8.36) (39-65 years) in the group treated with Diclofenac. The average disease duration from the onset of disease was 8.11 (±10.23), in the interval of 1-15 months. None of the patients included in the study had a history of previous or current renal disease. None of the patients had previously used NSAID. The others did not use other drugs before taking the test, especially gold salts or antibiotics or diuretics. The samples were collected over a period of 2 months.

Inclusion criteria

The study comprised patients with RA at the age of 18-65 years, who were not previously treated with NSAIDs or DMARDs.

Exclusion criteria

The study did not include patients with symptoms or conditions that can directly or indirectly affect the results, such as:

1. Patients with a history of gonorrhea, mild to moderate hepatic, renal, hematologic, cardiovascular, neurological diseases, nausea, vomiting, autoimmune disease.
2. Patients with diabetes mellitus, acute infections, malignant neoplasms, febrile conditions.
3. Patients with urinary tract arthritis, urinary tract infections, SLE, mixed connective tissue disease, vasculitis.
4. Patients with a history of blood transfusion, and excessive body weight.
5. Patients who received baseline therapy.
6. Patients with a history of glycemia or increased levels of product degradation in the 0 range: serum creatine and urine, serum urea, hypertension, arterial hypertension. and hematological and enzyme status.
7. Patients previously treated with salicylates, antibiotics, gold salts, or diuretics.
8. All patients took part in this study on a voluntary basis.

Clinical assessment with disease activity score (DAS 28) index

Clinical assessment was made by a subspecialist in the given area Disease Activity Score (DAS 28) index [16-18]. Indexes use mathematical formula to obtain unique composite quantitative score consisting of palpable sensitive joints (maximum number 28), and swollen joints (maximum number 28). Westergren's erythrocyte sedimentation rate (ESR) and patient's global assessment of disease activity (0-100 mm Visual Analogue Scale-VAS) as well as morning stiffness (minutes) were used.
DAS 28 indexes range from 0 to 10 and score below 3.2 qualifies the disease as low active.

Laboratory assessment

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

Determination of microalbuminuria by immunoturbidimetric method (Randox laboratories limited)

Reference values: Microalbuminuria 2.0-20.0 mg / L

Determination of N-acetyl-α-D-glucosaminidase (NAG) activity: colorimetric method (Roche) Principle

3-Cresolsulfonphthaleinyl-N-Acetyl-α-D-glucosaminide, as sodium salt, is hydrolyzed by NAG to release 3-cresol-sulfonphthalein, sodium salt (3-cresol purple) which iphotometrically is measured at 5 nm (Roche mancheim kits). The urine that has been examined previously is centrifuged and supernatants are separated.
Reference values: NAG in urine 0.27-1.18 U / mmol creatine.

Statistical analysis

For testing the significance of the differences between two arithmetic means, i.e., the corresponding proportions, the Student's t-test was used, when comparing the mean values of the given number of parameters between two groups, such as Wilcoxon-matched test for independent samples. Sensitivity and predictivity for positive and negative tests of the examined markers was determined with tests for sensitivity and specificity. The P value between 0.05 and 0.1 was considered statistically significant. The data analysis was made with the statistical package Statistica 7.0.

Results

Pearson's analysis of $\chi 2$ test showed that there was a moderate correlation between NAG and microalbuminuria (r=0.21) in the four samples tested during the period of 8 weeks in patients group treated only with Etoricoxib, while there was a statistically significant correlation (r=0.28) between increase in NAG and microalbuminuria values in the four samples in the period of 8 weeks in the group treated with Diclofenac (Figure 1).

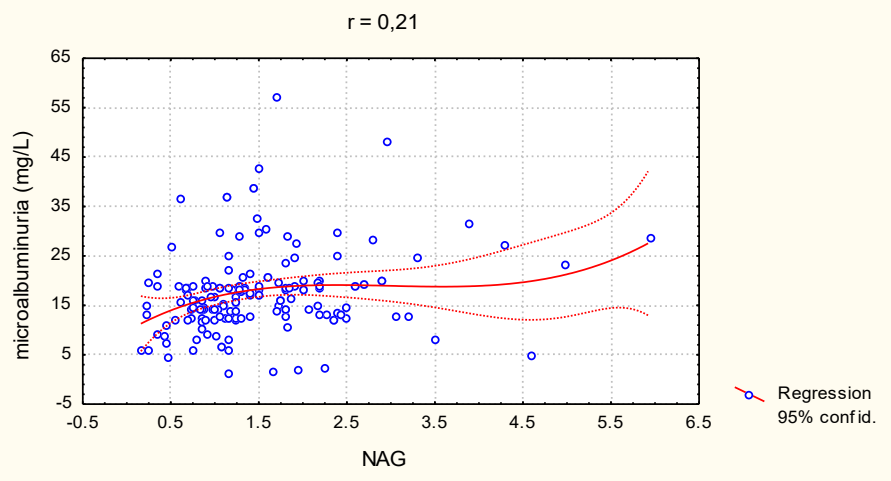

Fig. 1. Pearson's coefficient of correlation (r) between NAG and microalbuminuria values in the groups treated with Etoricoxib. There is correlation between NAG and microalbuminuria (r=0.21).

When testing the significance of the differences in the two groups in the null probe, in the group of patients treated with Etoricoxib, the mean value of microalbuminuria was 0.46±0.37, while in the group of patients treated with Diclofenac it was 0.56±0.41. This explains why Etoricoxib gives almost an identical value of microalbuminuria compared to Diclofenac.
Analyzing the group of patients treated with Etoricoxib in relation to the distribution of patients according to the values of NAG in the four groups, it was concluded that NAG was registered in 4 patients in the 3rd week, when the mean value of NAG urinary induction was highest (1.12±0.13).
Analyzing patients distribution according to NAG values in the four probes in the group of patients treated only with Diclofenac, it was concluded that NAG was registered in 6 patients in the 3rd week, when the mean value of NAG urinary induction was highest (1.41±0.31).

Discussion

Approaches for the assessment of nephrotoxicity of drugs are possible only with drugs or medications that have a dominant proximal tubular excretion, such as Methotrexate, Etoricoxib, Diclofenac, Acetaminophen and gold salts. This approach for the assessment of nephrotoxicity of drugs is not possible with other medications or drugs from the baseline which are used in the treatment of RA, such as resorhin, sulfazalazine and leflunomide, due to predominantly hepatic excretion. For these preparations, there are no literarature data on the occurrence of proximal tubular dysfunction.
Traditional treatment of RA includes nonsteroidal anti-inflammatory drugs (NSAIDs), disease-modifying drugs (DMARDs) and immunosuppressive cytotoxic drugs. Methotrexate in the low-dose regimen is the most commonly used drug from the DMARDs group, while from the NSAIL group the most commonly used drug is Diclofenum (Diklofenak^{R}), as well as Etoricoxib (Arcoxia^{R}).
In the non-treated RA tubular apparatus is primarily damaged and to a very small extent the glomerular apparatus [19]. A significant increase in the activity is due to the changes in cellular synthesis and not always the enzymuria may result in lytic or necrotic processes. Etoricoxib did not cause a significant damage to the renal proximal tubules in most of the observed patients. The nephrotoxicity during the use of Diclofenac was greater in comparison to Etoricoxib. Diclofenac was discretely more potent NAG inductor than Etoricoxib. Our observations correspond with those presented by other authors [20,21].
Early detection of increased NAG enzymuria or occurrence of microalbuminuria before exposure to drugs may be used for prediction of possible toxicity associated with renal impairment.
There was no change in the clinical findings of the renal function in relation to degradation products of nitrogen metabolism (serum creatine, urea/serum, GFR) during the follow-up.

Conclusion

Determination of urinary NAG together with urinary creatinine excretion may be considered as a more sensitive test for renal lesions in patients with RA, as a complementary diagnostic tool.
The results obtained in some studies confirmed the safety of Etoricoxib and Diclofenac in the treatment of RA.

Conflict of interest statement. None declared.

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Case report

CARDIOVASCULAR COMPLICATIONS IN COVID-19 INFECTED PATIENTS: MASSIVE PULMONARY EMBOLISM AND MYOCARDIAL INFARCTION WITH ACUTE HEART FAILURE - CASE SERIES REPORT

КАРДИОВАСКУЛАРНИ КОМПЛИКАЦИИ КАЈ COVID-19 БОЛНИ ПАЦИЕНТИ: МАСИВНА БЕЛОДРОБНА ЕМБОЛИЈА И МИОКАРДЕН ИНФАРКТ СО АКУТНА СРЦЕВА СЛАБОСТ – ПРИКАЗИ НА СЛУЧАИ

Irena Mitevska, Oliver Busletikj, Elena Grueva. Irina Kotlar and Elma Kandic

University Clinic for Cardiology Diseases, Faculty of Medicine, Ss Cyril and Methodius University Skopje, Republic of North Macedonia

Abstract

Patients with diagnosed COVID-19 infection have an increased risk of thrombotic events and complications, which highly contribute to raised morbidity and mortality rate. Inflammation and hypercoagulability caused by infection are responsible for pulmonary embolism and acute myocardial infarction in infected patients. Bedside focus echocardiography is a very useful noninvasive imaging method for fast diagnosis in critically ill patients suspected of being infected with COVID -19. We report two cases with acute thrombotic complications as a manifestation of COVID-19 infection. Echocardiography and pulmonary CT angiography helped us to properly proceed with patient's management. Prompt diagnosis and evidence-based management of these conditions are lifesaving. Echocardiography plays an important role in the bedside management of critically ill patients during the COVID-19 pandemic.

Keywords: COVID-19 infection, echocardiography, pulmonary embolism, CT angiography, ST segment elevation myocardial infarction

Абстракт

Пациентите со дијагностицирана COVID-19 инфекција имаат зголемен ризик од тромбемболиски компликации, кои значајно доприносуваат за зголемен морталитет и морбидитет. Инфламацијата и хиперкоагулабилната состојба причинети од оваа инфекција се одговорни за појава на белодробна емболија и миокарден инфаркт кај овие пациенти. Фокус ехокардиографијата изведена кај пациенти во легло е многу корисна неинвазивна визуализациона метода за брза дијагноза на суспектна тромбемболија или можна коронарна болест кај критично болни пациенти кои се хемодинамски нестабилни со суспектна COVID-19 инфекција. Презентирани се два прикази на случаи со акутни тромботични компликации кај пациенти со COVI-19 инфекција, кај кои ехокардиографијата и плуќната CT ангиографија ни помогнаа соодветно да продолжиме со третманот на пациентите. Брзата дијагноза и третманот базиран на докази при овие состојби ги спасуваат животите на пациентите. Фокусираната ехокардиографија има важна улога во дијгнозата и третманот на витално загрозени болни со тешка клиничка слика во услови на COVID-19 пандемија.

Клучни зборови: COVID-19 инфекција, ехокардиографија, белодробна емболија, CT ангиографија, CT сегмент миокарден инфакркт

Introduction

Inflammation and hypercoagulability are one of the mechanisms responsible for thrombotic complications in patients with COVID-19 infection [1]. We report two cases with acute thrombotic complications as a manifestation of COVID-19 infection. The first patient was admitted because of sudden chest pain and dyspnea. After excluding coronary artery disease, with CT angiography the diagnosis of massive pulmonary embolism (PE) complicated by right heart failure and cardiogenic shock was established. The second patient was a 47-year-old man with acute chest pain and dyspnea with ECG showing ST segment elevation myocardial infarction (STEMI). Urgent focus echocardiography was performed which detected reduced left ventricular ejection fraction (LVEF) of 35%. Both patients had polymerase chain reaction (PCR) test positive for COVID-19. These are two cases of thrombotic cardiovascular complications as first manifestations of COVID-19-infection where cardiovascular imaging adds to fast diagnosis and successful treatment. Focused

Correspondence to: Irena Mitevska, Intensive Care Unit, University Clinic for Cardiology, 1000 Skopje, R. N. Macedonia; E-mail: peovskai@yahoo.com

echocardiography is a very useful noninvasive widely available method particularly when evaluating patients with undifferentiated hypotension or acute dyspnea.

Cases

A 73-year-old man presented to our emergency outpatients department with first episode of dyspnea, syncope and chest pain. The patient had diabetes mellitus type 2 and was receiving therapy for hypertension. He was afebrile (36.4° C). Physical examination showed irregular heart rhythm, with peripheral and basal crepitation on the right lung. ECG showed atrial fibrillation with heart rate 120-130 bpm and right bundle branch block. Blood pressure was 85/45 mmHg with cold periphery. The patient denied any provocable PE risk factors (absent history of injury, surgical treatment, bed-rest over 72h, cancer history, no signs of DVT).

Bedside echocardiography was performed immediately after coronary angiography in order to evaluate the cause of patient symptoms and hemodynamic instability. Examination showed increased right ventricle (RV) size and D-shaped left ventricle, increased RV to LV ratio >1, reduced RV function (TAPSE 13, TDI S' 9), presence of McConnell's sign, severe tricuspid regurgitation with dilated non-collapsible v. cava - 23 mm, and signs of pulmonary hypertension (SPAP 54 mmHg); LV function was normal, with left ventricular ejection fraction 65%. Bedside transthoracic echocardiography findings with parasternal short-axis view of the heart showed dilated right ventricle as presented in Figure 1A. Doppler echocardiographic view of severe tricuspid regurgitation is shown in Figure 2B.

Due to confirmed PE in the patient with cardiogenic shock fibrinolysis, Alteplase 100 mg infusion for two hours was applied, based on PE protocol guidelines for high risk patients. The patient hemodynamically stabilized after the 60 minutes of Alteplase 100mg dose infusion, with BP normalization to 110/70 mmHg, HR 100 bpm, 0_2 92% on room air, respiratory rate of 11/min. The treatment continued with Heparin 25.000 IE infusion for 24h hours and the patient remained clinically stable with no further episodes of dyspnea or hypoxia.

He was referred for pulmonary CT angiography the next day, which showed large intraluminal thrombi in the right pulmonary artery with dimensions 50x12 mm. Thrombotic mases were found riding over the pulmonary trunk and multiple filling defects involving lobar and segmental branches of the right pulmonary artery, as well as linear saddle pulmonary embolus extending to the left pulmonary artery up to subsegmental level (Figure 2A and B). Lung parenchyma showed 4-5 pneumonic focuses with ground glass pattern located subcostally in the upper and basal paracardial zones, as shown in Figure 2C. After the PE was confirmed, nasopharyngeal smear for SARV CoV-2 was taken and the result was positive for virus RNK (real-time fluorescence polymerase chain reaction-PCR). Evaluated sPESI score was 2, which indicated elevated 30-day death risk (10.9%).

Laboratory results showed increased leukocytes level of $15x10^9$/l (ref 4-$9x10^9$/l), lymphopenia (6.8%)-range 15-50%, elevated lactate dehydrogenase of 436 U/l-range (up to 248 U/L), C-reactive protein of 99.5 mg/l (ref. up to 6 mg/l), creatinine 188 ng/l (range 45-109 nmol/l), ferritin 476.51 mg/l (ref. up to 300 mg/l). Hs-Troponin I (ABBOT Essay) was elevated – 420.6 ng/l (reference values for men 0-34.2 ng/l), D-dimer levels were increased-10.000 ng/ml (cut off value <500 ng/ml). Gas analyses were within normal limits, with slightly decreased pCO2 levels.

The next hospital day the patient was transferred to the Clinic for infectious disease in a clinical stable condition. The patient was clinically stable and treated at Clinic for additional two weeks. He was discharged with a recommendation for oral anticoagulant therapy with Rivaroxaban of 20mg one daily for the next three months. The patient was called for outpatient visit at the Cardiology clinic after four months. He was in a stable condition with completely normalized echocardiography result and no additional thrombotic complications.

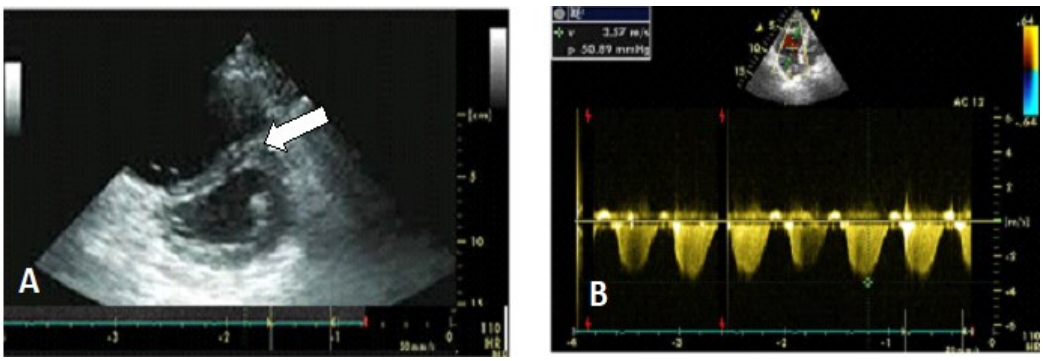

Fig. 1. Bedside Transthoracic Echocardiography. (A) Parasternal short-axis view of the heart showing a dilated right ventricle. Doppler echocardiographic view of severe tricuspid regurgitation (B)

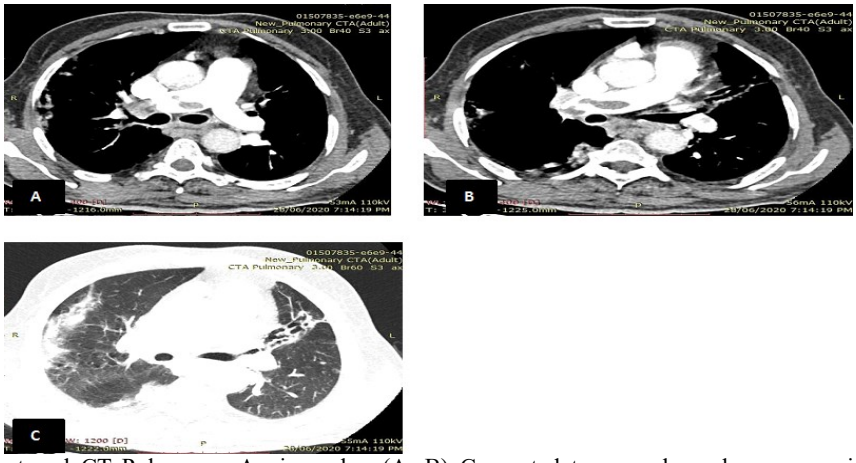

Fig. 2. CT of the chest and CT Pulmonary Angiography. (A, B) Computed tomography pulmonary angiography demonstrates multiple filling defects involving lobar and segmental branches of the right pulmonary artery and a linear saddle pulmonary embolus. (C) Axial unenhanced chest computed tomography (CT) scan shows areas of ground-glass interstitial opacities in the subcostal upper and basal paracardial zones

Our second case was a 47-year-old man who presented at our emergency department with chest pain and dyspnea. He was febrile with temperature of 37.8ºC one day before hospital admission. His previous medical history included hypertension, smoking, diabetes type 2 treated with Metformin, hypercholesterolemia and increased body weight. Admission blood pressure was 110/65 mmHg and heart rate 130 bpm. Physical examination showed basal lung crepitations and raised heart rate.

Admission ECG showed signs of acute anterior ST segment elevation myocardial infarction. The patient received 300 mg aspirin, 600 mg Clopidogrel, 40 mg Rosuvastatin, 70 IE/kg bolus Heparin (5000 IE) and Furosemide intravenous bolus. The patient had Killip Class II and GRACE Score 108.

Laboratory analyses showed serum Hs-Troponin-I 6385 ng/mL (normal range for men 0-34.2 ng/ml ABBOTT essay), N-terminal proBNP 6097 pg/ml (Abbot Diagnostics), increased leukocytes level of 16 x10^9/l (ref. 4-9x10^9/l), lymphopenia (7.1%)-range 15-50%, elevated lactate dehydrogenase of 517 U/l (range up to 248 U/L) and C-reactive protein of 115.5 mg/l (ref. up to 6 mg/l). Nasopharyngeal smear for SARV CoV-2 was taken and result positive for virus RNK (real-time fluorescence polymerase chain reaction-PCR) was received.

Focus bedside transthoracic echocardiography (TTE) showed increased left ventricular volumes with severely reduced left ventricular function (LVEF) and EF 35%, akinesia of the apex, anterior wall, mid and apical septal wall as shown in Figure 3. Coronary angiography was performed through right radial access and showed distal occlusion of the left circumflex artery (LCx), with TIMI flow 0-chronic total occlusion (CTO), proximal 95% stenosis of right coronary artery (RCA) with TIMI flow 3, 1st obtuse marginal artery stenosis of 100% with TIMI flow 0-CTO. Culprit lesion was proximal left anterior descending coronary artery (LAD) with 99% stenosis and TIMI flow 0, shown in Figure 6A. A signifycant stenosis of the proximal RCA is presented in Figure 4C. Direct coronary percutaneous procedure with stenting (PCI) of the mid LAD lesion with drug eluting stent (DES) was performed (Figure 4A). The final result after stenting of proximal LAD is shown in Figure 4B. An additional stage procedure with PCI and stenting to proximal RCA stenosis was performed on the third day of hospitalization with final results shown in Figure 4D. The patient was discharged after seven days of hospitalization, in a clinically stable condition and NYHA Class II. Control echocardiography was recommended in 6 weeks in order to assess the need for ICD implantation for primary prevention of sudden cardiac death. He was discharged with the following therapy: Aspirin 100mg od, Clopidogrel 75 od, Rosuvastatin 40mg od, Ramipril 5mg od, Carvedilol 6.25mg 2 x1/2, Elprenone 25mg od.

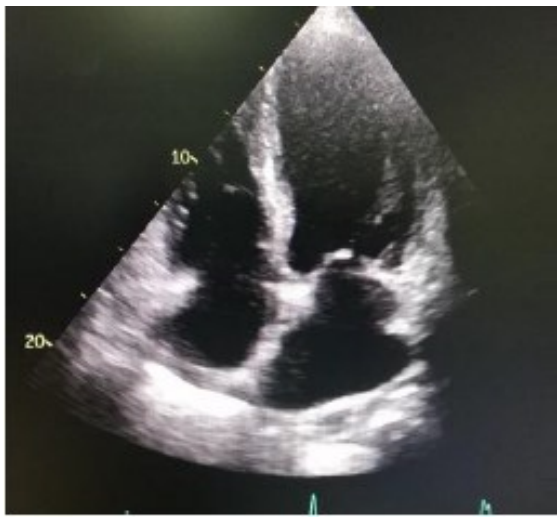

Fig. 3. Bedside echocardiography showing increased left ventricular cavities and volumes with reduced left ventricular function

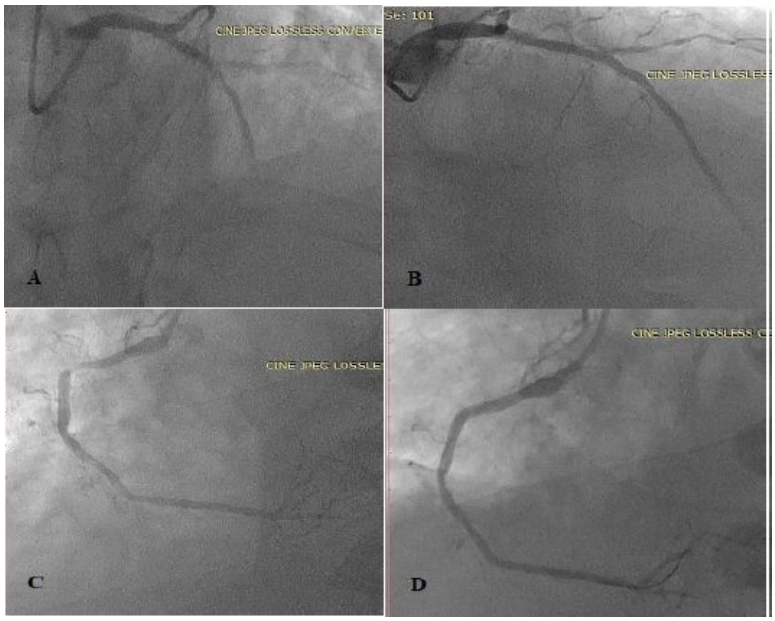

Fig. 4. (A) Significant stenosis of proximal left anterior descending coronary artery. (B) Final result after stenting of the proximal left anterior descending coronary artery. (C) Significant stenosis of the proximal right coronary. (D) Final result after stenting of the proximal right coronary artery

Discussion

The pandemic caused by the severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) has increased patients' risk of thrombotic complications due to enhanced activation of several inflammatory and prothrombotic mechanisms as well as endothelial dysfunction and blood stasis [2]. The induction of an abnormal inflammatory state may lead to cytokine storm, resulting from disbalance of T cell activation, abnormal interleukin (IL)-6 and other cytokines release. Several reports indicate that abnormal immune system activation may lead to plaque instability as a cause of acute coronary events [2,3]. Early reports suggest incidences of DVT and PE up to 30% in COVID-19 infected patients. The clinical experiences since the start of the pandemic report that approximately 50% of infected patients have elevated D-dimer levels during the disease course, which is associated with increased thrombosis risk and worse prognosis [4].

In the presented cases, the first patient had elevated C-reactive protein and D-dimer levels with no other risk factors for pulmonary embolism, indicating a COVID-19–related hypercoagulable state as a possible cause of PE. The use of focused echocardiography allowed us to better evaluate the patient's shock etiology after excluding coronary artery disease (CAD), facilitating early decision-making regarding ongoing management strategies. It should be recognized that acute RV strain may be a complicating feature of COVID-19 infection and may not always be due to PE [5]. Pulmonary hypertension due to hypoxic vasoconstriction has been proposed as a plausible physiological mechanism for acute RV dysfunction in patients with SARS-CoV-2 infection. An Italian prospective study evaluating chest CT results in patients with COVID-19 showed a high prevalence of sub-segmental pulmonary vessel enlargement, which may be caused by pro-inflammatory factors [6]. Generalized inflammatory endotheliopathy might be the cause of microthrombi risk and one of the explanations of pulmonary hypertension or multiorgan failure in COVID-19 patients. Focus echocardiography in our first patient revealed typical signs of RV dysfunction and pulmonary hypertension. The diagnosis of massive PE was confirmed by CT angiography of the pulmonary artery.

Our second patient had several comorbidities and an increased risk of myocardial infarction, although the presence of COVID-19 infection might additionally add to hypercoagulability state, severe inflammation and plaque instability. In our second case bedside echocardiography showed a severe left ventricular dysfunction due to CAD. Diagnosis of PE or STEMI in patients with COVID-19 infection might be quite challenging. ST-segment elevation in the COVID-19 era may mimic several heart conditions such as myocarditis, microvascular thrombosis, cytokine-induced injury, and stress cardiomyopathy [7]. Noninvasive imaging modalities such as echocardiography and CT angiography are of great value in these cases, helping to obtain fast diagnosis. European Society of Cardiology recommendations for imaging in COVID-19 patients state that focus echocardiography should be used in clinical situations were obtained information is expected to significantly add to patient's diagnosis and treatment. Focus echocardiography addresses specific questions and it is especially useful in hemodynamically unstable patients in intensive care units. Some of the key echocardiogra-

phic features of RV dysfunction include RV dilatation, flattening of the interventricular septal wall ("D-sign" on parasternal short-axis view) and impaired longitudinal RV systolic function denoted by reduced tricuspid annular plane systolic excursion (TAPSE) [8]. It has been proposed that acute right heart failure in COVID-19 may also be caused by an increased pulmonary vascular resistance secondary to hypoxic vasoconstriction. In the state of pandemic it is particularly important to consider other differential diagnoses for acute respiretory symptoms, acute heart failure and hemodynamic instability. Point-of-care ultrasound (POCUS) can be effective in helping to discriminate between the important and life-threatening causes of acute dyspnea, which is especially relevant in emergency department population [8].

The number of STEMI complications in COVID-19 pandemic increased mostly due to a higher rate of acute heart failure caused by severe left ventricular dysfunction. Late call for medical help is one of the explanations for the ischemia-induced left ventricular dysfunction in those patients. Based on the latest European guidelines, reperfusion therapy is a treatment of choice in patients with symptoms of ischemia below 12 h duration and persistent ST-segment elevation [9]. Initiation of anticoagulation is recommended in patients with high or intermediate clinical probability of PE while diagnostic work-up is in progress or while COVID-19 preventive measures are being implemented. Fibrinolytic therapy is lifesaving for management of PE in patients with cardiogenic shock [1,10].

Conclusion

Pulmonary embolism and acute myocardial infarction can be the first manifestation or complication of COVID-19 infection. Point-of-care ultrasound provide on time diagnosis and help us categorize the type of shock and prompt assessment of LV dysfunction caused by myocardial injury. CT angiography remains a gold standard for diagnosis of pulmonary embolism in these patients. It is of vital importance to understand COVID-19 cardiovascular complications and practice evidence-based medicine to avoid this infection becoming the trigger for a new cardiovascular pandemic.

Conflict of interest statement. None declared.

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ММР

Case report

EXPLORATIVE LAPAROSCOPY IN THE ASSESSMENT OF MALIGNANCIES OF FEMALE GENITAL ORGANS - A CASE REPORT

ЕКСПЛОРАТИВНА ЛАПАРОСКОПИЈА - ВО ПРОЦЕНКА НА МАЛИГНИТЕТИ НАГЕНИТАЛНИ ЖЕНСКИ ОРГАНИ – ПРИКАЗ НА СЛУЧАЈ

Saso Stojcevski and Violeta Nikolov

University clinic for Ginekology and Obstetrics, Faculty of Medicine, Ss Cyril and Methodius University Skopje, Republic of North Macedonia

Abstract

Introduction. The term "peritoneal carcinomatosis" mainly refers to primary or secondary malignancy of the peritoneum, but is also used to describe metastatic changes to the peritoneal surface of cancer cells of various origins.
Case report. A 55-year-old patient with a clinical picture of heaviness and pain in the abdomen, with presence of ascites, and an orderly finding on a classic gynecological and ultrasound examination, with elevated tumor markers was admitted to our hospital. Additional investigations (upper and lower digestive endoscopy, as well as CT of abdominal pelvis) ruled out pathological changes in the digestive and urinary organs. After proper preoperative preparation, the patient underwent laparoscopic exploration. Diffuse presence of whitish changes wassuspected as metastatic deposits and registered intraoperatively. A biopsy of the omentummajus was performed and a sample of ascites fluid was taken for cytodiagnosis, with a finding for carcinoma lobulare mammae metastaticum omenti et peritonei and group IV classification. Additional examinations (mammography and thin-needle aspiration biopsy) confirmed the diagnosis-Ca lobularemammae G1(7 mm).
Discussion. Metastatic spread of breast cancer to the gastrointestinal tract, peritoneum-retroperitoneum, and gynecological organs has a higher prevalence in intralobular carcinoma than in ductal breast cancer. Occurrence of peritoneal metastases is a life-threatening condition, with a very high mortality rate, although the prevalence of peritoneal metastases from breast cancer is small (0.7%), and occurs during disease progression or it is detected as a consequence. The aim of the study is to indicate the advantage of exploratory laparoscopy over laparotomy in obtaining a definitive diagnosis.
Conclusion. Peritoneal carcinomatosis originating from breast cancer is a rare condition that develops late in the evolution of the disease. To improve the health condition of these patients, exploratory laparoscopy is recommended, with short hospitalization, accurate diagnosis and appropriate further management of the disease in a good general health of the patient.

Keywords: explorative laparoscopy, breast cancer, peritoneal carcinomatosis, metastases

Correspondence to: Violeta Nikolov, University clinic of Ginekology and Obstetrics, 1000 Skopje, R. N. Macedonia; E-mail: violeta13dr@yahoo.com

Абстракт

Вовед. Терминот „Перитонеална карциноматоза“, главно се однесува на примарен или секундарен малигнитет на перитонеумот, но се користи и за дескрипција на метастатските промени на перитонеалната површина од канцер клетки со различно потекло.
Приказ на случај. Пациентка на 55 год. со клиничка слика на тежина и болка во абдоменот и присуство на асцит, со уреден наод на класичен гинеколошки и ултразвучен преглед, со покачени туморски маркери. Дополнителните инвестигации (горна и долна дигестивна ендоскопија, како и КТМ на абдомен и мала карлица) исклучија патолошки промени на дигестивните и уринарните органи. По соодветна предоперативна припрема, пациентката беше подложена на лапароскопска експлорација. Интраоперативно е регистрирана дифузна присутност на беличасти промени суспектни за метастатски депозити. Направена е биопсија на оментум мајус и земен примерок од асцитна течност за цитодијагноза, со наод за Carcinoma lobulare mammae metastaticum omenti et peritonei parietalis, а цитолошкиот наод е од IV класификациона група. Дополнителни иследувања (мамографијата и тенкоиглената аспирациона биопсија) ја потврдија дијагнозата-Ca lobulare mammae G1(7mm).
Дискусија. Метастатското ширење на карциномот на дојка кон гастроинтестиналниот систем, перитонеум-ретроперитонеум, и гинеколошките органи има поголема преваленца кај интралобуларниот карцином, отколку кај дукталниот карцином на дојка. Појава на перитонеални метастази претставува жи-

вотно-загрозувачка состојба, со многу висок процент на морталитет, иако преваленцата на перитонеалните метастази од карцином на дојка е мал (0,7%), а се појавува за време на прогресија на болеста или се открива како негова последица. Целта е да се укаже на предноста на експлоративната лапароскопија пред лапаротомијата, во добивање на дефинитивна дијагноза.

Заклучок. Перитонеалната карциноматоза со потекло од канцер на дојка е ретка состојба, која се развива доцна во еволуција на болеста. За да се подобри здравствената состојба на овие пациентки се препорачува експлоративната лапароскопија, со кратка хоспитализација, прецизна дијагноза и соодветно понатамошно менацирање на болеста во добра општа здравствена состојба на пациентката.

Клучни зборови: експлоративна лапароскопија, рак на дојка, перитонеалната карциноматоза, метастази

Introduction

The term peritoneal carcinomatosis generally refers to the metastatic involvement of the peritoneum. The name was first coined in 1931 by Sampson for the thorough description of metastatic involvement of the peritoneal stromal surface by ovarian cancer cells. Since then, it refers to almost any peritoneal metastatic deposits. Metastatic cancer to the peritoneum is more common than a primary peritoneal malignancy. It often occurs with gastrointestinal or gynecological malignancies of advanced stages with locoregional involvement. Peritoneal involvement is most common with cancers of the gastrointestinal, reproductive, and genitourinary tracts. Ovarian, colon, and gastric cancers are by far the most common conditions presenting in advanced stages with peritoneal metastasis. Cancers involving other organs such as the pancreas, appendix, small intestine, endometrium, and prostate can also cause peritoneal metastasis, but such occur less frequently. While peritoneal carcinomatosis can arise from extra-abdominal primary malignancies, such cases are uncommon, and they account for approximately 10% of diagnosed cases of peritoneal metastasis. Examples include breast cancer, lung cancer, and malignant melanoma. Peritoneal carcinomatosis from extra-abdominal malignancy presents in only 10% of cases where metastatic breast cancer is 41%[1]. Recognition and treatment of breast cancer metastases, such as bone, liver, lung, and brain, are well documented, and their spread to the peritoneal surface is a rare clinical presentation. Abdominal carcinomatosis from breast cancer is very rare and usually occurs during the progression of the disease or is revealed as a consequence, as in the case we present. Although peritoneal metastases of breast cancer are a clinical challenge, there is not much data in the literature with particular attention to diagnostic problems due to the masked clinical picture.

Case report

The patient N.Z, a 55-year-old woman with a clinical picture of heaviness in the abdomen, due to the presence of ascites and pain in the abdomen was admitted to our hospital. The gynecological examination, under the speculum and bimanual examination, showed an orderly finding. Ultrasound examination-uterus and ovaries looked good macromorphologically, with ascites fluid present in cavum Douglasi.

PAP test showed signs of inflammation.

Laboratory analysis: platelets 657x10^9/L and D-dimers=3024mg / l.

Gynecological examinations showed no convincing signs of gynecological pathology, only elevated tumor markers: CA15.3=180 U/ml, CA19.9=184.5 U/ml, CA125=75.5 U/ml.

A gastroscopy finding indicated an oesophageal hernia and a Helicobacter pylori test was made. The endoscopic examinationof the colon showed an orderly finding of the left colon.

Auscultatory impaired vesicular respiration at the bases of the lungs. CT of the lungs and mediastinum-with findings in support of peripheral, subpleural nodular and ground glass opacifications locally confluent in addition to inflammatory pneumonic changes. Present fibroadhesive changes basal, ventral, pleurodiaphragmatic, and pleuropericardial.

CT of the abdomen and small pelvis-uterud and ovaries neat. Expressed intestinal retention, more pronounced in the small intestine with a slightly thickened wall, in addition to inflammation. In thesmall pelvis quite a small amount of free fluid. Reactive inguinal lymph nodes bilaterally with massive hilus and d=1.5 cm. in the projection of the shown skeleton, numerous punctiform hyperdence changes were observed on all segments of the spine and pelvis.

After proper preoperative preparation, the patient underwent laparoscopic exploration. Upon entering the abdominal cavity, a yellowish-free fluid was found, which was aspirated and sent for cytopathological examination. Bleach deposits were visualized on the parietal peritoneum on the anterior abdominal wall, as well as small bowel and mesentery. A biopsy of the parietal peritoneum and the large omentum was performed, with which the biopsy material was histopathologically confirmed and a definitive diagnosis was made. The pathohistological finding of the biopsy material was Carcinoma lobulare mammae metastaticum omenti et peritonei parietalis, and the cytological finding wasof group IV classification. Later, additional investigations such as mammography and thin-needle aspiration biopsy confirmed the diagnosis-Carcinoma lobulare mammae G1(7 mm). Tumor markers were in-

creased: CA15.3=339 U/ml, CEA=322 U/ml, CA125= 230.5 U/ml, He4 187.9 U/ml.

Discussion

The metastatic spread of breast carcinoma to the gastrointestinal system, peritoneum-retroperitoneum, and gynecologic organs is much more prevalent in carcinoma intralobulare than in carcinoma ductale. Comparing rates of metastasis from carcinoma intralobulare and carcinoma ductale revealed statistically significant differences to peritoneum-retroperitoneum 3.1% carcinoma intralobulare *vs*. 0.6% carcinoma ductale [2]. Peritoneal metastasis is a life-threatening condition with a very high mortality rate. This carcinomatosis may originate from extra-abdominal primary malignancies, and such cases are uncommon. They account for 10% of the diagnosed cases of peritoneal metastases, and of them as much as 41% account for breast cancer [3]. The diagnosis and treatment of metastases from breast, bone, liver, lung, and brain cancers are well documentted, but their spread to the peritoneal surface is a rare clinical presentation. Distant metastases affect organs at the following frequency: bones 67.8%, liver 47.8%, lungs 42.6%, distant lymph nodes 27%, brain 15.2%, peritoneum (peritoneal carcinomatosis) 7.6%, and elsewhere 6.9%. The prevalence of peritoneal breast cancer metastasis is 0.7% [4-6]. Abdominal carcinoma of breast cancer is very rare, usually occurring during cancer progression or being detected as a consequence, as was the case we have presented here.

The aimof this report wasto point outthe role of exploratory laparoscopy as an advantage of laparotomy in obtaining a definitive diagnosis with a histopathological finding. Thhe patient wasdischarged immediately after the intervention in a good health condition for further treatment.

It poses a challenge in cases where clinical presentation, endoscopic and radiological examinations show no convincing signs of ovarian changes or advanced stage tumorsof undefined origin.

Confirmation of such malignancies requires histopathological findings, which is why practice has shown that it is better to do explorative laparoscopy, which allows a decision to be made for proper management of the advanced stage of the patient's disease, and to avoid explorative laparotomy which has severe postoperative survival after the procedure.

Conclusion

Peritoneal carcinomatosis originating from breast cancer is a rare condition that develops late in the evolution of the disease. The development of such lesions is most commonly associated with aggressive histology (as in lobular breast cancer) and advanced stage in the diagnosis of the disease, and is mainly associated with a poor outcome [7].

A positive diagnosis of peritoneal carcinomatosis originating from breast cancer can be obtained by biopsy of the tumor nodules. In order to improve the health of these patients, explorative laparoscopy is recommendded as a diagnostic tool in the strategy for management of advanced stages of breast cancer. In this way, with a short hospitalization of a patient in good health condition, an accurate diagnosis is obtained and proper disease management can be conducted.

Conflict of interest statement. None declared.

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MMP

Case report

MACROHEMATURIA IN SARS-COV-2 PREGNANT PATIENT

МАКРОХЕМАТУРИЈА КАЈ SARS-COV-2 БРЕМЕНА ПАЦИЕНТКА

Rina Purrini, Jadranka Georgievska, Maja Pejkovska Ilieva, Vesna Livrinova, Albina Bejta and Romir Kadriu

University Clinic for Obstetrics and Gynecology, "Mother Teresa", Skopje, Macedonia

Abstract

Covid-19 is a new virus and very little research has been done regarding it. Target organs of SARS-CoV-2 are not only the lungs and the heart but other organs as well. There has been a huge focus on the effect this novel virus has on the kidneys. This is proven by the presence of proteinuria, hematuria and acute kidney injury (AKI). This has been specifically noted in pregnant women, who are a target group for a more complex COVID19 disease than in the nonpregnant ones. We have reported a case of a COVID19 positive patient with a gemellar pregnancy complicated with macrohematuria because of transitory glomerular damage due to the virus infection. The patient delivered two healthy COVID19 negative newborns, while she continued treatment in the Clinic of Infectology. Pregnant women, without any previous kidney pathology are prone to kidney damage as a result of SARS-CoV-2 infection, and should be closely monitored for the development of AKI in order to prevent it.

Keywords: SARS-CoV-2, macrohematuria, acidum uricum, kidney

Abstrakt

Ковид 19 е нов вирус за кој што има малку истражувања. Таргет органи на SARS-CoV-2 инфекцијата не се само белите дробови и срцето но и други органи исто така. Голем фокус е даден на ефектот кој овој нов вирус го има вра бубрезите. Ова е докажано од присуството на протеинурија, хематурија па се до акутно бубрежно оштетување кај тие пациенти. Оваа појава е забележана и кај гравидната популација, која што е таргет група на комплексна КОВИД 19 болест во споредба со негравидната популација. Ние имаме пријавено случај на КОВИД19 позитивна пациентка со гемеларна бременост комплицирана со макрохематурија поради транзиторно гломеруларно оштетување поради вирусната инфекција. Пациентката роди две здрави КОВИД 19 негативни новороденчиња, а таа продолжи со третман на Клиника за инфективни болести. Бремените жени, без претходна бубрежна патологија се склони на бубрежно оштетување како резултат на SARS-CoV-2 инфекција, и треба да бидат внимателно мониторирани за развојот на акутното бубрежно оштетување, се со цел да го спречат тоа.

Клучни зборови: SARS-CoV-2, макрохематурија, acidum uricum, бубрези

Introduction

The COVID19 infection in the pregnant population signifies a specific issue with not yet enough research. Pregnant women infected with COVID-19 are at a higher risk of a more complicated disease outcome rather than nonpregnant women. Comorbidity factors for a complex COVID-19 disease in pregnant women are age, high body mass index, preexistent diabetes or hypertension. Although SARS-CoV-2 is described as a respiratory virus, it has been shown to have multiorgan involvement as well [1]. Around 1 out of 3 hospitalized COVID-19 patients presented with signs of acute kidney insufficiency without having any previous kidney pathology. In general, based on kidney biopsies the damage includes acute glomerulonephritis and acute tubular injury. Based on a retrospective cohort study, there is a high incidence of AKI in patients with COVID-19 that was associated with a 3-fold higher odds of death than COVID-19 without AKI and a 4-fold higher odds of death than AKI due to other causes. These data indicate that patients with COVID-19 should be monitored for the development of AKI and measures taken to prevent this [2]. We would like to report a case of a pregnant woman with COVID-19 who developed macrohematuria.

Case report

A 32-year-old patient, (gravida 4, para 2), presented at 34+2 gestational weeks with a 3-days-history of nausea,

Correspondence to: Rina Purrini, University Clinic for Obstetrics and Gynecology, "Mother Tereza", 1000 Skopje, R. N. Macedonia; E-mail: purrini.r@gmail.com

emesis and diarrhea. On admission she had stable vital parameters. Her temperature was 36.8*C, her blood pressure 130/80 mmHg, her pulse 78 beats per minute, respiratory rate 22 breaths per minute and her oxygen saturation 96%. The patient had her first successful pregnancy, with twins, after three successive in vitro fertilization procedures. During her prenatal history she had a cerclage placement at 13+0 gestational weeks, and she developed pregnancy induced hypertension, controlled with antihypertensive therapy (Tbl. Methyldopa 2x250 mg) as well as anticoagulant therapy (Amp. Enoxaparin 40mg/1x1) starting from 23rd gestational week. Because of her symptoms she had COVID19 test done at our clinic prior to admission. The nasopharyngeal swab returned positive for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. Despite the normal fetal heart rate of both twins, without any uterine activity and an ultrasound imaging with no abnormal findings, the patient was admitted to our hospital for further investigation. On the first day, her blood results showed high acidum uricum levels-692 mmol/L, creatinine 120 μmol/L, urea 12mmol/,L and normal Hgb, Wbc, Hct, CRP, total proteins and albumin levels. Her urine results showed presence of ketones (+) and no presence of proteins. After consulting a nephrologist, a Foley catheter was placed, draining yellow transparent urine. Two hours later, there was 300 ml reddish transparent urine diuresis. On the second day, blood tests showed a further elevation of acidum uricum to 737 mmol/L, as well as a reduction of the total proteins to 52 g/L and albumins to 24 g/L and d-dimer levels of 8168 ngr/mL. The patient was administered 220ml of universal blood plasma, 20% 50mL albumin solution, anticoagulant therapy (Amp. Enoxaparin 40mg/1x1 (patient weight-95kg)), antibiotic Amp. Klimicin 600mg/8h, MgSO4 1gr/h and continued with the antihypertensive therapy with tbl.Methyldopa (2x250mg). The same day, in the evening, her urine presented with massive macrohematuria, which was an indication for an urgent cesarean section, on maternal indication. She delivered twins. The first twin had a weight/height index of 2900gr/52cm, Apgar score of 8/8 and a pH of the umbilical artery of 7.36, while the second twin had a weight/height index of 2420gr/50cm, Apgar score of 8/8 and a pH of the umbilical artery of 7.35. The operation was performed in general anesthesia, but the patient was then transferred to the University Clinic for Infectious Diseases for further evaluation because of hyposaturation, with a decrease of oxygen saturation levels down to 82%, blood pressure of 130/90 mmHg, pulse 95 beats per minute. The patient was hospitalized in the Clinic for Infectious Diseases for 10 days. In the post-partum period, on the first three postoperative days she was put on an oxygen mask support due to hyposaturation below 82%. In the second postoperative day, her urine became yellow and transparent again. She was administered broad spectrum antibiotics, corticosteroids and anticoagulant therapy (Amp. Enoxaparin a 60mg/2x1). She was continuously afebrile and had stable vital parameters. Her blood results on the 2nd postoperative day showed Hgb 100g/L, WBC 14x10*9/L, CRP 112mg/L, acidum uricum 600μmol/L, creatinine 100μmol/L, urea 9mmol/L. She fully recovered on the 10th postpartum day.

The newborns were hospitalized in the neonatology unit, and they tested negative for SARS-CoV-2 infection. They were further investigated and hospitalized for 6 days. Both newborns had stable vital parameters during their hospitalization. The first newborn had body temperature 36,8*C, pulse 114 beats per minute, oxygen saturation 96%, blood pressure 66/42mmHg, respiretion rate 49 breaths per minute, regular diuresis and stool. The second newborn had body temperature 36,9*C, pulse 142 beats per minute, oxygen saturation 99%, blood pressure 57/31 mmHg, respiration rate 40 breaths per minute, regular diuresis and stool. They were released on the 6th day after birth, with stable health conditions.

Discussion

Patients infected with COVID19 are showing signs of kidney damage, even those who have had no prior underlying kidney pathology. Comorbidities commonly associated with renal susceptibility to injury, such as diabetes mellitus, hypertension, cardiovascular disease and advanced age are evident risk factors in the development of COVID-19-associated acute kidney injury [3]. Our case, presented with massive macrohematuria, as a result of endothelial damage of the glomeruli due to her underlying preeclampsia or due to transient glomerular damage because of her COVID19 infection.

The impact of COVID19 in kidneys is not yet clear, however data so far suggests that kidney damage is most likely the result of several processes occurring either in isolation or in tandem. Some pathophysiological mechanisms explain that kidney disease may be caused by SARS-CoV-2 binding to the ACE2 receptor on kidney cells that allow the virus to enter. Detection of coronavirus during the autopsy of kidneys and in the urine of patients infected with SARS-CoV-2 supports the theory that the virus can directly damage the kidneys [4]. Acute tubular injury is a predominant finding in histopathological examination of postmortem tissues, which could also explain the hematoproteinuria observed in many patients. Another mechanism of kidney damage is COVID-19-associated coagulopathy. It is characterized by high D-dimer levels and microvascular damage. COVID-19 tends to have high rates of severe thromboembolic complications and endotheliitis despite of anticoagulation. Based on data, COVID-19-associated organ dysfunction is caused by the “cytokine storm syndrome”. The release of excessive and uncontrolled pro-inflammatory cytokines attacks the lungs, heart and kidneys and thus brings to multiorgan

failure. All of these mechanisms contribute to acute kidney injury, which complicates the course of the COVID-19 disease in infected patients.

Our case, presented with a patient who was COVID19 positive and had controlled pregnancy induced hypertension. Until the COVID19 infection, her pregnancy was uneventful. After the infection, she showed elevation of acidum uricum, creatinine, urea and d-dimers, and no proteinuria. If proteinuria had been present, we would have linked this phenomenon with an aggravation of her pregnancy induced hypertension to preeclampsia. In their recent publication, Dap et al, presented a case of a COVID19 pregnant woman, in which the diagnosis of preeclampsia was wrongly assumed because of proteinuria, due to preeclampsia-like syndrome induced by the severe infection [5]. Similar to this case, our patient who had a controlled prenatal pregnancy induced hypertension, a postpartum macroscopically normal placenta, with no previous kidney pathology, our hypothesis would be that the presence of macrohematuria seems to be the result of the endothelial damage of the glomeruli due to COVID19 infection. Because of the fast deterioration of the patients health condition and the need to terminate the pregnancy on maternal indication, further investigations such as kidney ultrasounds and more detailed urine analysis that would support this case, could not be undergone

Conclusion

There is limited data on long-term effects following the development of acute kidney injury in patients with COVID-19. This disease causes multiorgan damage and it specifically affects the lungs and the kidneys. The presence of comorbidities such as high blood pressure and high body mass index increases the incidence of acute kidney injury in pregnant patients with COVID-19. However, all pregnant patients infected with SARS-CoV-2 should be closely monitored for the development of kidney injury and appearance of macrohematuria. Measures should be taken to prevent this, and the best clinical approach is yet to be found. Therefore these patients should be given multidisciplinary approach in order to make decisions regarding recommended treatments for the best patient outcome.

Conflict of interest statement. None declared.

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ММР

Case report

COMPLETE KAWASAKI DISEASE IN A CHILD WITH TRANSIENT HYPOGAMMAGLOBULINEMIA OF INFANCY - CASE REPORT

КОМПЛЕТНА ФОРМА НА БОЛЕСТА НА КАВАСАКИ КАЈ ДЕТЕ СО ТРАНЗИТОРНА ХИПОГАМАГЛОБУЛИНЕМИЈА НА РАНОТО ДЕТСТВО-ПРИКАЗ НА СЛУЧАЈ

Lidija Kareva, Kristina Mironska, Katarina Stavrik and Arjeta Hasani

Department of Immunology, University Pediatric Clinic, Ss. Cyril and Methodius University in Skopje, Republic North Macedonia

Abstract

Kawasaki disease is an acute febrile illness of early childhood characterized by vasculitis of the arteries. The diagnosis of complete Kawasaki disease should be made in a child who has a fever lasting for 5 days or more and has at least 4 of 5 the clinical criteria: rash, conjunctival injection, oropharyngeal erythema, swelling and erythema of the extremities, and unilateral cervical lymphadenopathy. Incomplete form of the disease is diagnosed when a patient presents with fever for 5 days or longer, 2 or 3 of the principal clinical features, and laboratory findings suggestive of the disease or echocardiographic abnormalities. Kawasaki disease has been described as a complication of various primary and secondary immunodeficiency disorders, hence supporting an infectious etiology of this disease. Immunodeficiencies may result in an incomplete clinical presentation of Kawasaki disease and end up with delay in diagnosis and therefore treatment, which may lead to development of coronary artery aneurysm. We present the case of a 2.5-year-old girl with transient hypogammaglobulinemia of infancy who has a complete form of the disease without coronary artery aneurysm development, to emphasize the occurrence of Kawasaki disease in immune deficiency situations.

Keywords: Kawasaki disease, transient hypogammaglobulinemia of infancy, immunodeficiency

Апстракт

Болеста на Кавасаки е акутна фебрилна болест која се јавува во раното детство и се карактеризира со артериски васкулитис. Дијагнозата на комплетната форма на болеста на Кавасаки може да се постави кај дете кое има покачена температура која трае 5 дена или повеќе, и има најмалку 4 од 5 главни клинички критериуми: осип по кожата, црвенило на коњуктивите, црвенило на орофарингсот, оток и црвенило на екстремитетите, и унилатерална лимфаденопатија. Инкоплетната форма на болеста се дијагностицира кај пациенти кои имаат покачена температура 5 дена или подолго, 2 или 3 од главните клинички критериуми и лабараториски или ехокардиографски наоди во прилог на болеста. Болеста на Кавасаки е опишана како компликација на разни примарни и секундарни имунодефицити што оди во прилог на инфективна етиологија. Имунодефицитните болести често можат да имаат инкомплетна клиничка презентација на болеста што може да резутира со покасна дијагноза и закаснето започнување на лекувањето, кое пак од своја страна доведува до формирање на аневризми на коронарните артерии. Ние презентираме 2,5 годишно девојче со Транзиторна хипогамаглобулинемија на раното детство, кое имаше комплетна форма на болеста, без развој на аневризма на коронарните артерии, со цел да укажеме на можноста за појава на болеста на Каваски кај децата со имундефицит.

Клучни зборови: болест на Каваски, хипогамаглобулинемија на раното детство, имунодефицит

Introduction

Kawasaki disease (KD) is an acute febrile illness of early childhood characterized by vasculitis of the arteries. KD was described in Japan by Tomisaku Kawasaki in 1967 [1]. The diagnosis of complete KD should be made in a child who has a fever lasting for 5 days or more and has at least 4 of 5 the clinical criteria: rash, conjunctival injection, oropharyngeal erythema, swelling and erythema of the extremities, and unilateral cervical lymphadenopathy. Incomplete KD is diagnosed when a patient presents with fever for 5 days or longer, 2 or 3 of the main clinical features, and laboratory findings suggestive of the disease or echocardiographic abnormalities. A diagnosis of atypical Kawasaki syndrome can be made with less than four criteria if coronary artery aneurysms (CAA) are present. KD has predilec-

Correspondence to: Lidija Kareva, University Pediatric Clinic, 1000 Skopje, R. N. Macedonia; E-mail: kvlidija@yahoo.com

tion for the coronary arteries, coronary artery aneurysms can develop in around 25% of untreated cases while early treatment decreases this risk to 3-5% [2]. KD is the leading cause of acquired heart disease in developed nations [3]. Suggestive laboratory findings include elevated erythrocyte sedimentation rate (ESR), elevated C-reactive protein (CRP), hypoalbuminemia, anemia, elevated alanine aminotransferase (ALT), thrombocytosis, leukocytosis, and piuria. The etiology and pathogenesis of KD remains unclear. There has been a suggestion that the etiology of KD is infectious, and that infection triggers hyperactivation and dysfunction of the immune system in genetically predisposed individuals. Six genetic loci were linked to KD through genome studies [4]. The presence of KD in patients with primary and secondary immunodeficiency disorders supports the infectious theory. Transient hypogammaglobulinemia of infancy (THI) is a primary immunodeficiency caused by a transitory drop of the levels of immunoglobulin G (IgG) in an infant beginning between 5 and 24 months of age, while immunoglobulin A (IgA) and immunoglobulin M (IgM) may or may not present as decreased. Levels typically return to reference range at ages 2 to 6 years. THI may be characterized by recurrent infections [5]. In THI, IgG is at least two standard deviations below expected controls [6,7]. We present the case of a two and a half years old girl with THI who developed complete KD without coronary artery aneurism. The aim of the presented case is to emphasize the importance of early diagnosis of KD in immunodeficiency situation like THI in order to protect against coronary artery aneurysm.

Case report

We present a two and a half years old girl with a history of recurrent respiratory infections since the age of 1 year, and 5 days of fever, with pharyngitis and rash. She had received three days course of beta lactam antibiotic for the pharyngitis prior to the hospitalization, but despite antibiotic therapy, elevated temperature continued up to the 40°C, and she started to show swelling of the lymph node on the left side of her neck as well as erythema of the skin with swelling of the hands and feet (Figure 1).

The condition was suspected of allergic reaction to antibiotic and the child was admitted to the hospital. Initial physical examination demonstrated an irritable child with a red cracked lips, strawberry tongue and pharyngeal injection with no exudate. The patient had bilateral hyperemic conjunctivas, and enlarged 2x2 cm tender cervical lymph node on the left side of her neck. She had maculopapular skin rash on the trunk and upper and lower extremities and edema of hands and feet. She was tachycardic with pulse 150 /min. Her immunoglobulin G (IgG) level was 2 g/l which is below normal range for the age; IgA and IgM levels were normal.

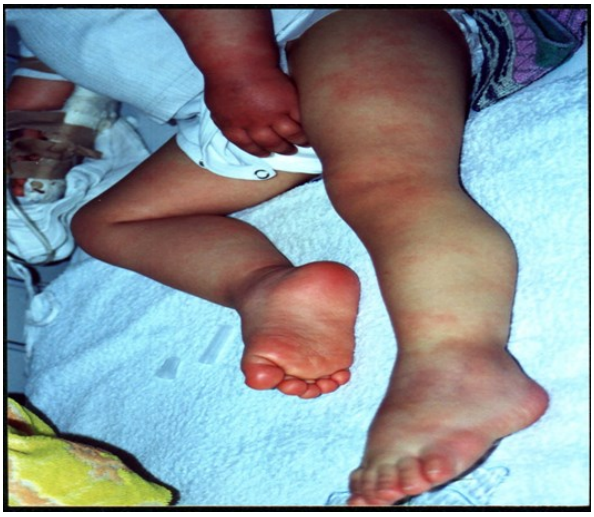

Fig. 1. Kawasaki disease: skin rash and swelling of the hand and feet

The white blood count was 14,000/mm^3, platelet count was 300,000/mm^3, and C-reactive protein (CRP) was 75 mg/L. During the next 3 days the child received fluids, antibiotics and antihistamines, but the erythema became more prominent as well as the swelling of the hands and feet. Temperature continued to be high and reached above 39.5°C. Platelet count rose and reached 550,000/mm^3 and there was elevation of CRP up to 145 mg/L. Cardiac ultrasonography did not identify aneurysm in the coronary artery. Kawasaki syndrome was diagnosed, and the patient was started on high doses of intravenous gamma globulins (IVIG), 2 g/kg divided in equal doses for 4 consecutive days, and aspirin 80 mg/kg/day in four equal doses. Twenty-four hours later, she was afebrile while swelling of the lymph node, palms and feet as well as skin rash disappeared

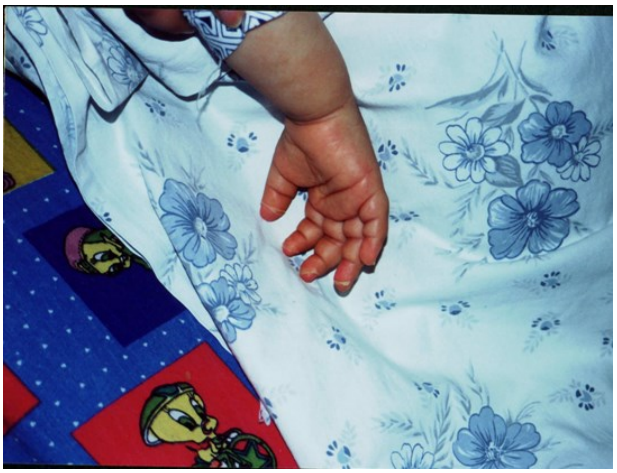

Fig. 2. Kawasaki disease: desquamation of the fingers

in the following week. After 10 days, aspirin was reduced to 5 mg/kg/day and was given for the next 2 months. Two weeks after beginning of the disease desquamation of the palms and feet started (Figure 2). On the 20th hospital day, she was discharged. Regular

laboratory controls were performed every month for the next 6 months together with cardiac ultrasonography which showed no signs of coronary aneurysm. During the follow-up period she did not have infections and at the final control after 1 year her immunoglobulin level was within normal range for the age.

Discussion

A 2.5-year-old girl with THI who had complete KD without coronary artery aneurysm development is presented to emphasize the occurrence of KD in immune deficiency situations. Previously incomplete KD was reported in a 4-year-old boy with THI (8). He was diagnosed with THI at the age of 12 months and his clinical presentation was for incomplete KD with fever more than 5 days and 3 of 5 criteria fulfilled, without developing CAA. Authors discuss incomplete KD in THI as a possible result of the incomplete immune response due to hypogammaglobulinemia, which may result in less antibody response involved in pathogennesis of KD and end up with delay in diagnosis and therefore treatment. Our patient developed complete form of KD, and we assume that more patients with the same condition of the disease should be described in order to draw conclusions about the pathophysiological mechanisms in KD and THI. In our case, the child ceased to have recurrent respiratory infections during 6 months follow-up as a result of high IVIG doses. Her illness was without cardiovascular complications due to early complete presentation of KD and early administration of IVIG. The presence of KD in other patients with primary and secondary immunodeficiency where also described. Majority of the cases with KD and primary immunodeficiency were those with chronic granulomatous disease (CGD). The case of a 2-year-old boy with CGD who developed incomplete KD associated with CAA was described [9]. Further, the case of a 1-year-old boy with CGD who developed several of the characteristic clinical features of Kawasaki Disease, with a second echocardiogram showed dilatation of the left main coronary artery and the right coronary artery in the coronary ostium was presented [10]. Also, a 10 month-old male patient with CGD who had presentation of incomplete KD without CAA was reported [11]. A 10-year-old male with CGD and KD developed incomplete KD, with substantial cardiac dysfunctions but without CAA was also described [12]. Majority of patients with CGD were with incomplete KD and were also associated with the development of CAA, suggesting that the diagnosis of KD in patients with CGD was difficult to establish and vascular damage may progress before onset of the treatment. The most important factor to protect from complications is early diagnosis and early initiation of IVIG treatment within 10 days of symptom onset. In the patient with selective IgA deficiency diagnosis of complete KD had been established on the 5th day and was treated with aspirin, urinastatin and steroid pulse therapy instead of IVIG. No coronary artery aneurysm developed [13]. The second case reported with selective IgA deficiency was a 5-year-old with complete KD without CAA development treated with cyclosporine instead of IVIG [14]. The case of Wiscott-Aldrich syndrome was diagnosed as complete KD at 6 months of age with transient normalization of platelet count during disease course. This patient had been treated with IVIG, with no complications. During the acute phase of KD, the patient's platelet count increased. The investigators suggested that an increase in platelet count may be a result of an increased production of interleukin-6, a known thrombopoietic factor [15]. Reports of KD in X-linked agammaglobulinemia (XLA) patients argue against the presence of autoantibodies in the pathogenesis of KD. So far 4 patients with XLA complicated with KD have been described. Although autoimmunity phenotype is surprisingly common in patients with different types of primary antibody deficiency, it is much less frequent in XLA. There is a report on a 15-month-old boy with XLA who also suffered from Kawasaki disease, as the first report of an association between Kawasaki disease and XLA [16]. There is also a report of a 12-year-old boy on IVIG therapy, who subsequently developed Kawasaki disease [17]. XLA could be considered as a special opportunity to understand autoimmunity in the near absence of immunoglobulins [18]. An 8-month-old male with XLA, sepsis and prolonged fever with development of CAA diagnosed as incomplete KD was described [19], suggesting that infants with XLA and prolonged fever should be monitored for KD and early diagnosis and initiation of IVIG treatment. Four patients with Hyper-IgE syndrome (HIES) and KD have been reported [20]. However, a number of HIES patients with coronary artery aneurysms have not been documented. Patients with CAA and other vascular abnormalities have been reported in the literature as a feature of HIES, or patients who may have also had previous KD (21). Vast majority of patients with primary immunodeficiency described KD were males (87.5%) [19] due to the fact that three primary immunodeficiency disorders associated with KD are X-linked. Incomplete KD was present in 54% of patients compared to 10% described in the literature [19].

The exact classification of KD has long been debated, as the disease has been classified as an infectious, autoimmune, or autoinflammatory disorder. There is evidence supporting all three, and they are not mutually exclusive, as the disease can be considered an infectious driven disease with an aberrant inflammatory response against self, predominantly to the arteries [22]. The basis of autoimmunity and hypersensitivity in some patients with primary immunodeficiency is believed to involve the inability of the host to eradicate microbial pathogens and their antigens completely through the

immune pathways, resulting in an exaggerated and perpetuating inflammatory response.

The most convincing evidence that immunodeficiency predispose to the development of KD comes from the study of adults with the disease. KD is rare in adults and it has been reported that about one-third of adult KD are associated with HIV infection [23-24]. More than 20 cases of HIV patients with KD have been reported. The association of KD with malignancy has also been described. An 11-year-old boy who was diagnosed with acute monocytic leukemia who presented KD complicated with pericardial effusion and left coronary dilation 1 week after chemotherapy [25]. A 3-year-old child with acute myeloid leukemia with complete KD and development of CAA, as well as a 2-year-old boy with Down syndrome and acute myeloid leukemia with incomplete KD without CAA [26] were also reported.

Conclusion

In summary, several immunodeficiency disorders are associated with the development of KD, thus supporting an infectious etiology of this disease which involves the inability of the host to eradicate microbial pathogens completely through the immune pathways, resulting in an inflammatory response. In children with transient hypogammaglobulinemia of infancy, Kawasaki disease should be included in the differential diagnosis of a high-grade prolonged fever, as diagnosis of KD prompts immediate IVIG treatment in order to prevent coronary artery disease.

Conflict of interest statement. None declared.

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Case report

UNEXPECTED FINDING OF LARYNGEALHEMANGIOMA - DIAGNOSIS AND MEDICAL TREATMENT

НЕОЧЕКУВАН НАОД НА ЛАРИНГОХЕМАНГИОМ - ДИЈАГНОЗА И МЕДИЦИНСКИ ТРЕТМАН

Hristina Mandzukovska[1], Aspazija Sofijanova[1], Silvana Naunova-Timovska[1], Nikola Nikolovski[2], Goran Micevski[2], Tamara Voinovska[1], Mica Kimovska-Hristov[1], Spasija Neskova-Jankovic[1] and Olivera Jordanovska[1]

[1]University Clinic for Children's Diseases, [2]University Clinic for Ear, Nose, Throat - Faculty of Medicine, Ss. Cyril and Methodius University, Skopje, Republic of North Macedonia

Abstract

Introduction. Laryngeal hemangioma is a rare and slowly progressing vascular tumor causing stridor or obstructive respiratory symptoms.
Case report. We present an unexpected finding of laryngeal hemangioma in a 10-year-old boy with a severe general condition. Upon admission at our Department, he was immediately intubated. The next day, after extubation, his condition again deteriorated. Fiberbronchoscopy examination was performed; we confirmed a laryngeal hemangioma. Inadequate investigations and diagnosis of laryngeal hemangioma in early childhood cause a life-threatening condition.
Conclusion. Regular and appropriate medical examinations are necessary for an adequate diagnosis. Early medical treatment is necessary to avoid more severe conditions and complications.

Keywords: laryngeal hemangioma, larynx, fiberbronchoscopy

Апстракт

Вовед. Ларингеалниот хемангиом е редок и бавно прогресивен васкуларен тумор, кој предизвикува стридор и респираторни симптоми.
Приказ на случај. Во овој приказ на случај презентираме неочекуван наод на ларингеален хемангиом кај 10 годишно дете со тешка клиничка слика. На нашиот оддел беше веднаш интубирано. Следниот ден, по екстубација неговата состојба повторно се влоши. Беше направена фибер-бронхоскопија; се потврди присуство на ларингеален хемангиом. Ненавремени, несоодветни испитувања и дијагноза на ларингеалниот хемангиом во раното детство, може да предизвикаат состојба опасна по живот.
Заклучок. Редовни и соодветни медицински прегледи се неопходни за правилна дијагноза. Раниот медицински третман е неопходен за да се избегнат потешки состојбии компликации.

Клучни зборови: ларингеален хемангиом, ларинкс, фибер-бронхоскопија

Introduction

Laryngeal hemangiomas are a rare condition in pediatric population. They occur in different anatomic locations of the larynx and manifest with different clinical symptoms such as obstruction, dyspnea, dysphagia, hoarseness, etc. [1,2]. Although in the studies of Dickison P and Hoornweg MJ the incidence of hemangiomas in children was 2.6% to 9.9%, due to the rarity of laryngeal hemangiomas, the exact incidence of the tumor remains unreliable [3,4].
In 2014 the American Academy of Pediatrics (AAP) made recommendations for treatment of infantile hemangioma while calling for additional research of the treatment of hemangioma in the airways of young children [5]. Several recommendations of medical treatment have been proposed in the management of hemangiomas. Laryngeal hemangiomas often require urgent treatment. According to the general situation, if there is a life-threatening airway obstruction, surgical resection with endoscopic laser excision or open excision is necessary [6]. Tracheotomy can help bypass the presence of hemangiomas. Interferon treatment, systemic corticosteroids, chemotherapeutic therapy and β-adrenergic blockade for small laryngeal hemangiomas are effective for appropriate therapy [7,8].
In the present study, the unexpected diagnosis of laryngeal hemangioma and medical treatment of a child is described.

Correspondence to: Hristina Mandzukovska, University Clinic for Children's Diseases, 1000 Skopje, R. N. Macedonia; E-mail: hbicevska@yahoo.com

Case report

On March 23, 2016, a 10-year-old boy from Switzerland was transferred from the Institute for Lung Diseases in Children "Kozle" to our Pediatric Intensive Care Unit (PICU) at the University Children`s Hospital. The boy 's parents reported a history of asthma (over the past two years with inspirational stridor), treated with β-adrenergic blockade and corticosteroids.

On admission the child was in a severe general condition, tachycardic (187/min), with biphasic stridor, irregular breathing and respiratory insufficiency. The initial gas blood analysis showed a severe respiratory acidosis (pH-7.11 pCO_2-103mmHg, pO_2-28mmHg, O_2-60%). The boy was immediately intubated (module SIMV) and after one hour the parameters of gas blood improved (pH- 7.35 pCO_2 -53, pO_2-62 mmHg, O-95%), as well as the general condition. The biochemical, hematological and microbiological analyses (Table 1) were within border references for his age.

Table 1. Hematological analyses

Analyses	Initial value	First control	Second control
hematological	Hb-141	Hb-133	Hb-130 g/l
	Er-5.9	Er-5.59	Er-4.87 x10^{12}/l
	Le-3.7	Le-4.5	Le-6.3 x10^9/l
	Tr-75	Tr-82	Tr-88 x10^9/l
	Hct-43.2	Hct-40.1	Hct-38.4 %
gas blood	pH 7.11	pH-7.35	pH-7.5
	pCO_2-103	pCO_2-53	pCO_2-41 mmHg
	pO_2-28	pO_2-62	pO_2-68 mmHg
	sO_2-60	sO_2-95	sO_2-94 %
	BE-1.7	BE-0.6	BE-1.7 mmol/l

Table 2. Biochemical and microbiological analyses

Biochemical and microbiological analyses	Values
electrolytes	Na-131mmol/l
	K-4.2 mmol/l
	Ca-1.95 mmol/l
	P-1.78mmol/l
hepatogram	AST-97 U/L
	ALT-27 U/L
	LDH-423 U/L
creatinine	60 umol/L
blood urea BUN	4.0 mmol/l
total protein	65 g/L
albumin	41 g/L
glycemia	15.64….5.4 mmol/l
CRP	0.2….. 2.0 mg/l
hemostasis	PT-13 s
	aPTT-29 s.
	TT-25 s
	D-dimer 1014 ng/ml
microbiological	negative

The chest X-ray in the projection of lungs demonstrated stripblotchy shadows in the lung parenchyma on both sides.

The initial therapy started with intravenous administration of cefotaxime, aminoglycoside, and corticosteroids. After application of nasogastric tube (NG), gastrointestinal bleeding in the NG tube was manifested. In addition, he was given intravenous proton pump inhibitors. During the night shift, the child's condition improved (conscious with stable vital signs, afebrile).

On his second day at ICU, after the routine assessment (clinical evaluation, gas blood) the general condition of the child was still normal; with spontaneous breathing and conscious. Our decision was to extubate him, but unfortunately after extubationhis condition immediately deteriorated (hard wheezing, irregular breathing, unconscious, bradycardic 30/min). During the second intubation, a massive bleeding in the endotracheal tube caused an additional complication, a life-threatening airway obstruction.

The intubated child was transferred to the University Clinic for ENT where fiber-bronchoscopy examination was performed. The results of the fiber-bronchoscopy was an unexpected lesion that looked like a large vascular tumor-hemangioma located on plica venticularis lateris dextri, which obstructed the lumen of the border between the larynx and the trachea to a maximum diameter of under 2 cm. The child was posted for extirpation under general anesthesia. The minimal bleeding that occurred was treated with medications. The laryngeal hemangioma was extirpated and sent for histopathological examination (blood vessel hyperplasia and hemangiectasis squamous mucosa were observed beneath the squamous mucosa; in the cavernous vessels erythrocytes were found and lymphocyte infiltration was observed around the vessels). The histopathology findings verified the lesionas cavernous hemangioma. The first control fiber-bronchoscopy examination after two weeks showed no complications.The child had a normal voice after 6 weeks. At 6 months follow-up, there was no evidence or clinical signs of laryngeal hemangioma.The contol fiber-bronchoscopy and child`s follow-up were realized in the abroad (we got the informations from the parents).

An informed consent was obtained from the parents. The study protocol was approved by the Ethics Co-

mmittee of the University Children`s Hospital and the University Clinic of Otorhinolaryngology.

Discussion

Laryngeal hemangiomas are a very rare condition and have been reported in different locations (vocal chamber, arytenoid cartilage and aryepiglottic fold), and rarely occur in the larynx. Symptoms include hoarseness, dyspnea, dysphagia or a pharyngeal foreign body sensation. Without proper diagnosis and treatment, a high mortality rate must be expected [9,10].

In the present study we diagnosed an unexpected lesion of large vascular tumor-hemangioma, which obstructed the lumen of the border between the larynx and the trachea. The child was 10 years old, with reported history of treated asthma. On admission his general condition was severe; he was immediately intubated, and after few hours the general condition was improved. The fpllowing day he was extubated and after few seconds the condition deteriorated; he was reintubated and transferred for fiber-bronchoscopy examination (unexpected large lesion, laryngeal hemangioma), and was immediately extirpated.

In this case, probably the general practitioner did not conduct the appropriate investigations that should have to be realized earlier when the first symptoms manifested.

Sometimes fiber-bronchoscopy examination cannot confirm a diagnosis at first, but the diagnosis should be kept in mind in the first year of life. Sometimes the correct diagnosis might be missed if laryngoscopy is performed when the child is on steroid therapy or is intubated. To confirm a definitive diagnosis of laryngeal hemangioma an imaging technique, such as ultrasound, CT, or MRI is required [11].

What should we do if we establish a diagnosis of laryngeal hemangioma?

Therapy has been modified during the years. According to the current pediatric recommendations, the larynx hemangioma treatment of choice is propranolol [12].

For small laryngeal hemangiomas, observation and oral application of systemic β-adrenergic blockade is usually sufficient. The first-line therapy includes propranolol which is novel in the pediatric population.Within 6 weeks complete elimination of laryngeal hemangioma was confirmed (endoscopically visible disease) [13].

Larger hemangiomas require treatment, and this can include surgical resection, corticosteroid injections, steroids, ethanol injections, cryosurgery, radium or gold implants, interferon treatment, laser surgery and surgical exitrpation [10,14].

Laryngeal hemangiomas often require urgent treatment for life-threatening airway compromise. Multiple treatment modalities have been proposed in the management of airway hemangiomas. Until nowadays, debate still exists on the best approach. Successful therapy and treatment include intralesional steroids, laser intervention endoscopic laser excision and life-saving surgical resection.

Conflict of interest statement. None declared.

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Case report

BILATERAL X-LINKED JUVENILE MACULAR RETINOSCHISIS - OBSERVATION AND TREATMENT

БИЛАТЕРАЛНА ЈУВЕНИЛНА МАКУЛАРНА РЕТИНОШИЗА: ОБСЕРВАЦИЈА И ТРЕТМАН

Natasha Trpevska Shekerinov, Milena Golubovic and Emilija Gjosevska Dashtevska

[1]University Clinic for Eye Diseases, Faculty of Medicine, University "Ss Cyril and Methodius" Skopje, [2]PZU D-r Bogoev, Skopje, Republic of North Macedonia

Abstract

We present a case report of a 33-year-old man with bilateral juvenile foveomacular retinoschisis. The patient came to the University Clinic for Eye Diseases in Skopje, Department of Retinal Diseases and Vitreous Surgery and complained of progressive vision loss in both eyes over the last few years, but with a significant decline in visual acuity on the left eye in the last year. After the examinations, he was diagnosed with X-linked juvenile retinoschisis (XLJR). The finding of the left eye was more pronounced, with established macular hole, and the patient was surgically treated with pars plana vitrectomy. The condition of the right eye is still being monitored. The aim of this paper was to present the anatomical and functional results of surgical treatment-vitrectomy with peeling of the inner border membrane and gas tamponade in foveal macular retinoschisis, as well as the importance of long-term observation in individuals with XLJR. By presenting this case, we want to draw attention to this rare genetic disease, which leads to serious, significant consequences for visual function.

Keywords: bilateral macular X-linked juvenile retinoschisis (XLJR), genetic disorders, vitrectomy

Абстракт

Приказ на случај, маж на 33 год. возраст со билатерална јувенилна фовеомакуларна ретиношиза. Пациентот се јави на Универзитетската Клиниката за очни болести во Скопје, на одделот за ретина и витреална хирургија и даде податок за прогресивно намалување на видот, обострано, во период од последните неколку години, но со значителен пад во последната година, на левото око.
По направените иследувања беше поставена дијагноза Х-врзана јувенилна ретиношиза (XLJR). Наодот на левото око беше повеќе изразен, со формирање на макуларна дупка, поради што пациентот беше хируршки третиран, бепе направена парс плана витректомија. Десното око се обсервира.
Цел. Да се прикажат анатомските и функционалните резултати од хируршкиот третман витректомија со пилинг на внатрешната гранична мембрана и тампонада со гас кај фовеална макуларна ретиношиза, како и значењето на долгорочната обсервацијата кај индивидуите со XLJR.
Преку презентација на случајот сакаме и да го насочиме вниманието кон ова ретко генетско заболување, кое доведува до сериозни, значителни консеквенци по видната функција.

Клучни зборови: билатерална макуларна Х-врзана јувенилна ретиношиза (XLJR), генетски заболувања, витректомија.

Introduction

X-linked juvenile retinoschisis (XLJR) is an inherited vitreoretinal dystrophy characterized by splitting of the neurosensory retina in the area of the macula and on the periphery of the retina.
The splitting may be in a different layer of the neurosensory retina, in the nerve fiber layer or the inner plexiform layer, the outer plexiform layer, or the outer nuclear layer [1]. XJR is a rare genetic disease with an incidence of 1/5000 to 1/25 000 [2].
It is a mutation in the XLRS1 gene located on the short arm of the X chromosome (Xp22.2-p22.1) [2,3]. The gene responsible for encoding the protein-retinoschisin is crucial for the formation of a junction between cells in the inner plexiform layer and synaptic connections between bipolar cells and photoreceptors [2]. In fact, the dysfunction of this protein due to mutation disrupts the structural integrity of the retina, leading to the formation of cysts and cystic cavities, which can develop in all layers of the retina [4]. Given that the disease is X-linked, men are affected and women are carriers [3]. After all, in mothers who carry the gene, her fema-

Correspondence to: Natasha T Shekerinov, University Eye Clinic-Skopje, R. N. Macedonia; E-mail: n_trpevska@yahoo.com

le children can be 50% carriers, and her male children have a 50% incidence of developing the disease.
Men with a gene mutation can not pass it on to their sons, but their daughters will be carriers [4]. Although rare, however, if both parents are affected, XLJR may also occur in girls [4].
The disease is characterized by a slow course, reduced visual acuity, and progressive scotomas in the visual field, corresponding to areas of the affected retina. XLJR is present at birth and the symptoms progress over time.
The onset of the disease is early, between 5-6 years, and children have difficulty reading due to blurred vision and are usually diagnosed on routine systematic examinations, with a visual acuity between 20/200 and 20/50 [1,2]. Hyperopia and nystagmus are also common [3].
The diagnosis of XLJR is made by fundus examination where splitting of the retinal layers can be seen, and by additional examinations such as optical coherence tomography of the posterior segment of the eye (OCT), ultrasound, computed perimeter, electroretinogram (ERG) and visual evoked potentials (VEP).
The juvenile form of retinoschisis is a more severe form, while the acquired form may remain asymptomatic. Acquired form usually occurs between 50-70 years, but can occur among younger people. It is equally present in both males and females [3].

Case report

A 33-year-old man came to the University Clinic for Eye Diseases, Skopje, in September 2019, to the Department of Retinal Diseases and Vitreous Surgery. He complained of a progressive decrease in vision in both eyes over a period of several years, but with a significant decline in visual acuity in the left eye in the last year. XLJR was initially diagnosed in a private ophthalmology practice, where he had been monitored until then. The best-corrected visual acuity (BCVA), according to Snellen's optotype, was 0.1 in the right eye and 0.05 in the left eye. Intraocular pressure was normal. The patient denied other illnesses, did not use any substances, or took long-term medication. The family history for related eye diseases was negative.
Biomicroscopy of the anterior segment showed a normal finding, while on fundus examination (indirect ophthalmoscopy) macular retinoschisis of both eyes was seen, on the left with an incomplete macular hole, atrophy of the retinal pigment epithelium, and thickening of the inner limiting membrane (Figure 1a and 1b).
OCT of the posterior segment showed cystic spaces in the fovea, with a central macular thickness of 220 μ on the right eye and 674 on the left (Figure 2 and Figure 3).

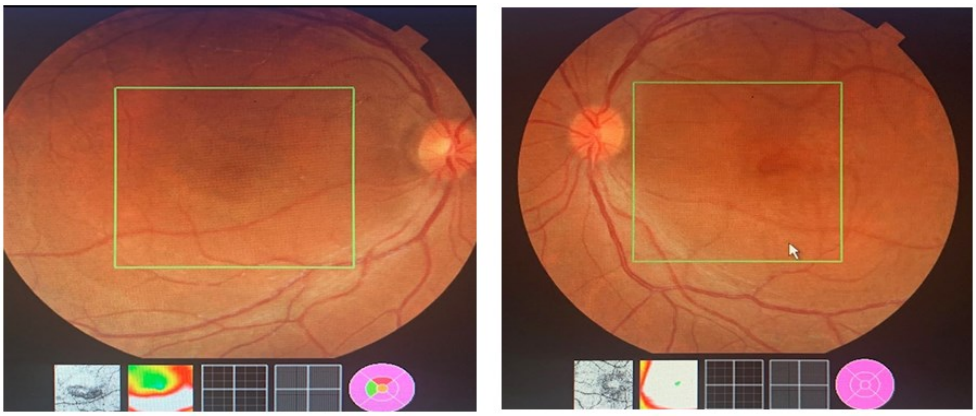

Fig.1a, 1b
Photo fundus view of the right and left eye
Foveolar retinoschisis - radial cystic spaces on both sides, with macular hole formation in the left eye

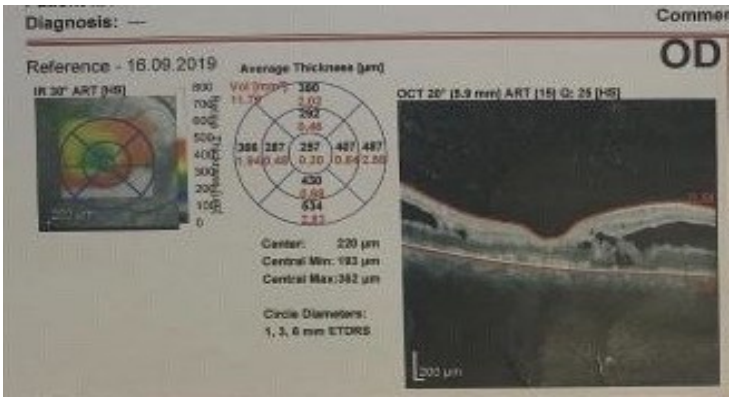

Fig. 2. OCT on the right eye: central thickness (foveal thickness): 220 µm, September 16, 2019

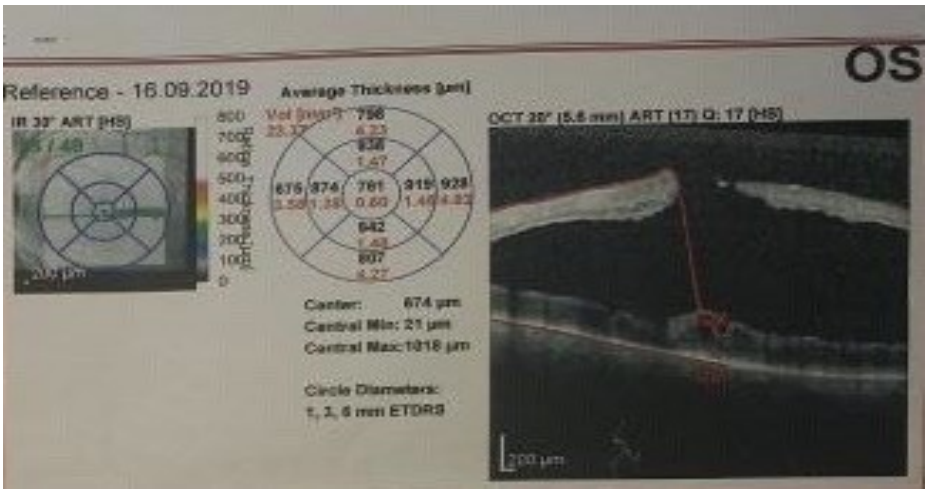

Fig. 3. OCT on the left eye: central thickness (foveal thickness): 674 µ, September 16, 2019

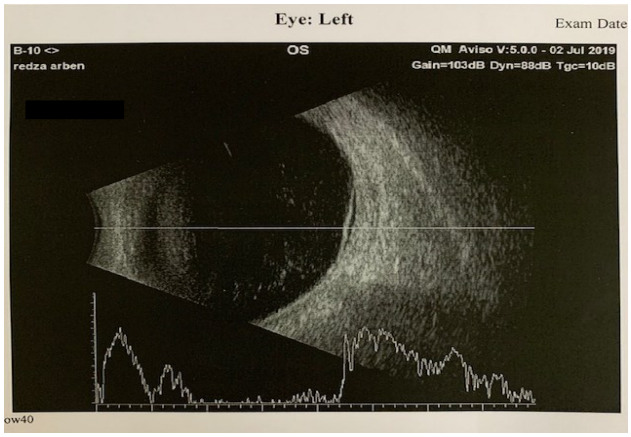

Fig. 4a. Ultrasound finding on the left eye, July 2019

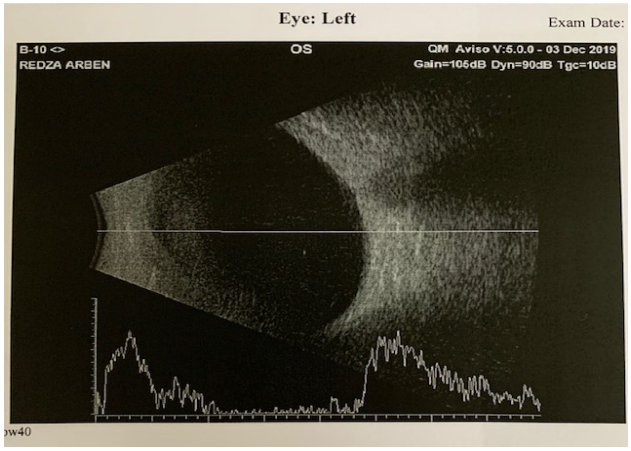

Fig. 4b. Ultrasound finding of the left eye, postoperatively, December 2019

The electroretinogram (ERG) showed abnormal scotoptic and photoptic responses on both sides. In fact, a typical finding for XLJR is the absence of b-wave and subnormal a-wave, i.e. negative ERG.

Taking into account all these diagnostic methods, including ultrasound, the diagnosis of bilateral juvenile foveal retinoschisis with incomplete macular hole of the left eye was made (Figure 4a and 4b).

During the period of the conservative treatment and monitoring, the visual acuity of the left eye progressively decreased to 0.01 with clinical findings of foveal cysts, thickening of the inner limiting membrane, and formation of an incomplete macular hole (Figure 3).

Oral acetazolamide did not improve visual acuity.

As indicated, surgical treatment was performed on the left eye - pars plana vitrectomy (PPV) with peeling of the inner limiting membrane (ILM peeling), and gas tamponade.

We continued to monitor the right eye, where there was a slight progression of the findings - enlargement of the intraretinal cystic spaces and increased macular thickness on OCT (Figure 5).

Postoperative results showed significant regression of the left eye finding, with clinical and anatomical improvement, reduction of foveal thickness, and reduced retinal cysts, as seen on the control OCT (Figure .5).

After surgery, visual acuity in the left eye was slightly improved, to 0.05 without correction. At the last check-up, 10 months postoperatively, visual acuity was stable and was again 0.05 without correction.

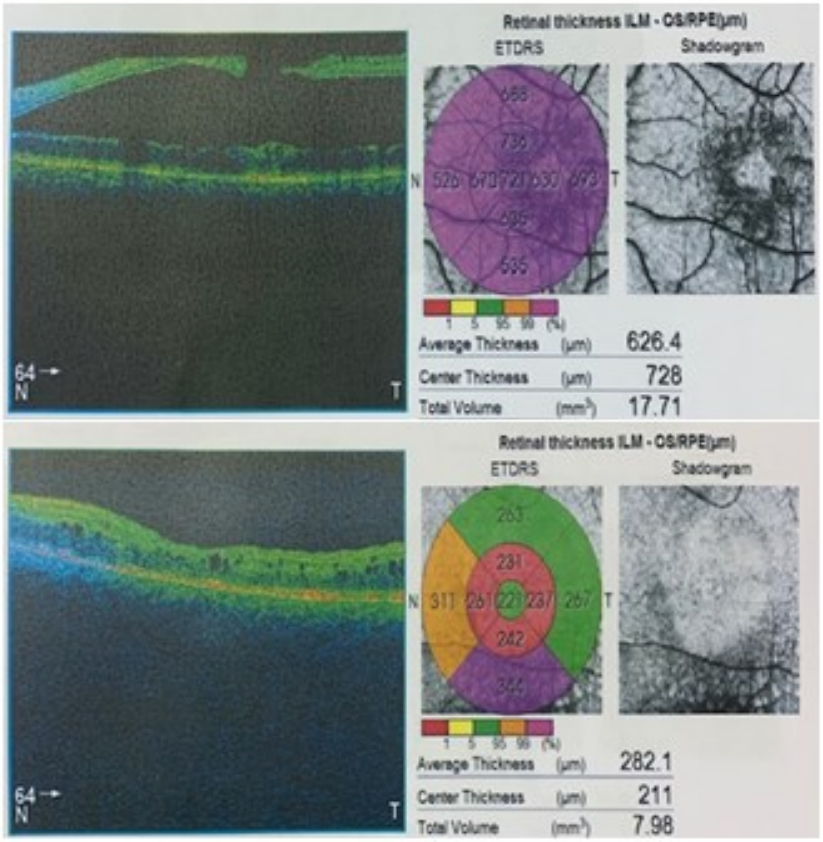

Fig. 5. Comparative OCT finding on the left eye, before and after surgery. AT 626 µm → AT 282 µm

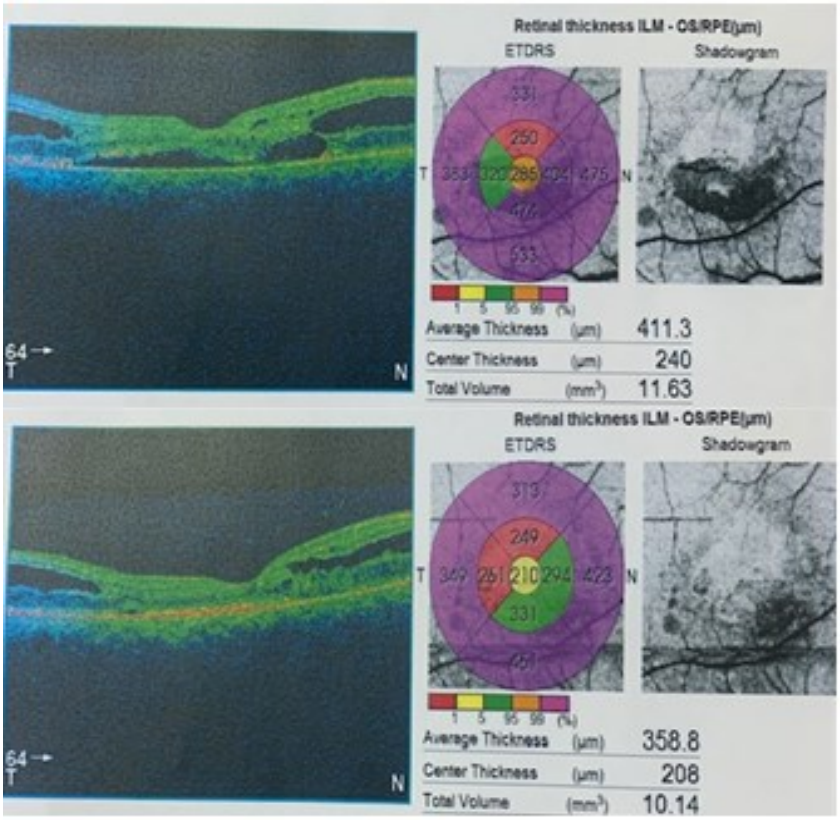

Fig. 6. Comparative OCT finding on the right eye, 6-months follow-up, from July to December 2019 (AT 358µ µ AT 411µ)

Discussion

X-linked retinoschisis (XLRS) is a disease with heterogeneous clinical expression, with broad phenotypic inter- and intrafamilial variability [4].
X-linked retinoschisis (XLRS, OMIM 312700) is one of the causes of juvenile macular degeneration, which occurs in men at an early age. It is characterized by symmetrical, bilateral macular involvement and usually starts to occur in the first decade of life, with cases occurring as early as three months [3,4]. The main subjective symptom is vision loss with a significant decrease in visual acuity from 20/60 to 20/120. Visual acuity often worsens during the first and second decade, but afterwards remains relatively stable until the fifth or the sixth decade [5].
The typical clinical presentation of XLRS is splitting of retinal layers, i.e. cystic spaces ordered in a stellar pattern in the area of the macula, with involvement of the peripheral retina. Isolated macular forms of juvenile retinoschisis as our presented case are quite rare. Approximately half of cases have bilateral peripheral retinoschisis, most commonly in the infotemporal region (Figure 3) [5].
As a result of these changes, strabismus, nystagmus, axial hyperopia, color vision defects (red-green dyschromatopsia), and foveal ectopy may be present [5]. Possible complications include vitreous hemorrhage, retinal detachment, which is actually an indication for surgical treatment-pars plana vitrectomy, or occurrence of neovascular glaucoma [6].
Hass, in 1898, first described two brothers with typical cystic maculopathy and peripheral choroidal atrophy as "retinal and choroidal changes" [7]. At that time, the diagnosis was based only on ophthalmoscopic examination, so the changes were described in the literature under a wide range of names, according to different clinical manifestations and etiologies.
Then in the 1960s, abnormal ERG findings were reported in individuals with XLRS, where the b/a ratio was abnormal in almost all cases (ERG b/a<1) [8]. Since then, ERG has become an important test in this disease, with a typical finding in patients with XLRS, which has been confirmed in our case.
ERG usually shows a selective decrease in dark amplitude adapted b with relative preservation of α-amplitude in affected men [9]. However, recent studies have shown that the ERG response is much more variable than previously thought [9]. Some individuals with X-linked juvenile retinoschisis and confirmed pathogenic variant of RS1 may show a normal ERG in which the b-wave is still present [9].
Today, optical coherence tomography of the posterior segment (OCT) and fundus autofluorescence (FAF) are very important diagnostic methods for the XLJR, with a pathognomonic finding (Figures 3 and 4) [9].
SD OCT as a non-invasive rapid method is an indication for screening and is an important imaging method, which plays a key role in showing the degree of splitting of the neurosensory retina and the size and volume of cystic cavities, especially in the early stages. OCT allows monitoring of macular retinoschisis, even when fundoscopy in children or ERG can not be performed [10].
The authors in a study point out that OCT shows small cysts in the peripoveolar region and larger cystic spaces in the fovea in most school-age individuals with XLRS [11]. Cystic spaces are less obvious after adolescence. On the other hand, OCT findings in elderly patients may be normal, due to the flattening of the cysts with age, [11] or may be in different layers of the retina, in the nerve fibers (NFL), ganglion cells (GCL), inner nuclear (INL), outer plexiform (OPL), outer nuclear (ONL) or the photoreceptor (PL) layer, and over time may form a large central cavity that can progress to nonspecific macular atrophy [11].
Variations in presentation, progression, clinical course, stages, and different findings on SD OCT highlight the variability of this genetic disease among members of the same family, as well as between both eyes of the same patient, as is shown in our presented case (Figures 3 and 4) [12].
The clinical manifestations of foveal lesions vary widely, from radially furrowed (about 3%), microcystic lesions (34%), honeycomb-like cysts (8%), or combinations thereof (31%), to non-cystic changes, including pigmentation, loss of the foveolar reflex (8%), or appearance of atrophic lesions (8%) [13].
Recent studies have shown that the degree of retinoschisis is greater in the inner nuclear and outer nuclear-plexiform layers, in contrast to some previous histopathological studies which claimed that retinoschisis occurs more in superficial retinal layers, such as the inner limiting membrane and nerve fibers layer [13]. Some authors have suggested that this discrepancy may be due to technical limitations of histopathological studies [13].
XLJR can progress to retinal detachment in approximately 5%-22% of affected individuals. Meanwhile, about 4% to 40% of people with X-linked juvenile retinoschisis may have vitreous hemorrhage [13].
Studies show that young boys may experience vitreous hemorrhage and/or retinal detachment (RD), with or without neovascular glaucoma, which in differential diagnosis may suggest Coats disease, while in school-age boys may be manifested with poor vision, amblyopia or strabismus.
In elderly patients the disease may be present with retinal pigmentation or maculopathy, and not all patients show an electronegative ERG finding (b/a<1) [14].
The delayed onset of the b wave seen in a study by Bowles *et al*. performed in 68 men with XLJR suggested that photoreceptor synapses or bipolar cell dysfunction increased with age [14].

Fluorescein angiography, as a diagnostic procedure for diseases of the posterior segment of the eye, has no significant features in XLJR. The finding appears normal, especially in younger people, as in the case shown, and in elderly patients atrophic changes in the retinal pigment epithelium may occur. In contrast, fundus autofluorescence is very important, and in XLJR is manifested by increased autofluorescence in the fovea, which is a characteristic sign [9,10].

In 1997, identification of the RS1 gene (gene ID: 6247; OMIM: 312700) showed that XLRS was caused by a mutation located in the p22 region of the short arm of the X chromosome (localization: Xp22.2-p22.1, GenBank AF014459) [9].

To date, it is the only known gene associated with XLRS, encoding retinoschisin (RS1), which is primarily present in photoreceptors and bipolar cells [5]. It is also involved in cell adhesion and cell interactions on membrane surfaces [15].

Retinoschisin has been shown to have: hidden leading sequence (LS; 23 AA), domain RS1 (Rs1D; 39 AA), highly conserved discoidin domain (major structural feature of RS; 157 AA), and C-terminal segment (5 AA) [5]. The domain of discoidin is essential for normal anatomical and functional integrity of the retina, but its deficiency causes splitting in different retinal layers i.e. retinoschisis [5].

Previous research has shown that RS1 is synthesized and secreted mainly by the inner segment of the photoreceptors, which is identified in a healthy retina, but it has later been shown to be present in other cells in the outer retinal layers. This molecular mechanism explains why retinoschisis can occur in different layers of the retina [5,16].

According to the latest publications, using the OCT method, retinoschisis is mainly detected in the INL (inner nuclear layer, composed of Müller and bipolar cells) and in the PRL (plexiform retinal layer) [16].

The dysfunctional, defective RS1 accumulates in these layers leading to cyst formation and splitting in retinal layers [16]. In cases of nonspecific foveal atrophy, electrophysiological and molecular genetic tests are necessary to confirm the diagnosis [16].

To date, no satisfactory treatment is available to stop the formation and progression of retinoschisis in patients with confirmed XLRS [17].

Studies highlighting the importance of vitrectomy in XLRS present patients with progressive retinoschisis, i.e. with progressive decline in visual acuity, and fovea involvement, or complications, and also emphasize the effect of treatment, excellent anatomical outcomes, and stabilization of visual acuity [17].

In our presented case it was confirmed that after the performed surgical operation, PPV, the visual acuity was stabilized and there was a significant regression of the central thickness of the fovea.

The vitrectomy may be an effective and essential treatment in patients with progressive XLJR retinoschisis in order to prevent further vision loss and other serious complications [18]. Some studies have shown that given the benefits of the operative outcome, there is an indication for treatment of the contralateral eye in order to stabilize the condition [16,18].

Recently, a comparative study was presented comparing vitrectomy as surgery versus observation in patients with a progressive form of XLRS. Patients included in the study had no retinal detachment or vitreous haemorrhage, but had significantly reduced visual acuity from progressive macular retinoschisis, or had peripheral retinoschisis with a high risk of macular involvement. The authors noted that vitrectomy may improve visual acuity and reduce the risk of complications expected in patients with progressive XLRS [18].

Ikeda *et al.* suggested that surgical treatment can improve visual acuity only in the non-progressive form of XLRS [19]. It means that early surgery, before complications occur and before vision is compromised, can result in a successful anatomical and functional outcome [19].

Gene therapy is considered a potential treatment in the near future. In people with confirmed mutations in XLRS, genetic diagnosis may help ensure safe reproduction in *in vitro* fertilization.

While retinoschisis is expected to be found in an individual with a positive family history of XLJR, making a diagnosis in individuals without a known familial genesis may be more difficult.

Differential diagnoses include Goldman-Favre syndrome, acquired retinosis, retinitis pigmentosa, Morbus Stargardt, Stickler syndrome, Wagner disease, cystoid macular edema, macular dystrophies, and exudative retinal detachment [20].

When retinal changes are subtle, amblyopia may be the primary diagnosis. Prevention of amblyopia and observation to prevent secondary complications is of particular importance in order to maintain the visual acuity necessary for social functioning.

It is necessary to evaluate the male relatives and children of the affected individuals in order to identify them as early as possible for taking preventive measures.

If the pathogenic variant RS1 is known in the family, molecular genetic testing can be performed to determine the nature of the inheritance and implications of the genetic disorder for appropriate genetic counseling and to assess the genetic risk and status of family members.

Conclusion

It is of particular importance to increase the awareness of ophthalmologists for early detection of XLJR.

There is a need for regular screening examinations of children under 10 years of age by a pediatric ophthalmologist or retinal specialist, patient education and careful monitoring, which should enable early identifica-

tion and treatment of any complications that may result in a significant vision loss.

Those affected by XLJR need support, observation, genetic counseling, vocational rehabilitation assistance and relevant social services assistance, i.e. to enable these patients to function as optimally as possible in the environment.

Conflict of interest statement. None declared.

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In memoriam

ПРИМАРИУС Д-Р СТЕФЧЕ БОЈАЏИЕВ
(1947-2021)

На 28 Април 2021 година по кратко боледување, во 74 година од животот, почина примариус д-р Стефче Бојаџиев.

Роден е на 26 септември 1947 година во Битола, каде го завршува основното и средното образование. Медицински факултет завршил во Скопје каде е и активен член на Црвениот крст.

Се вработува во Душевната болница во Демир Хисар. Специјализацијата по невропсихијатрија ја завршува во Скопје при што бил автор на многубројни научни и стручни трудови.

Лекарската пракса ја продолжува во невропсихијатриското одделение во Битола. Во 2000 година ја добива титулата примариус и шеф на неврофизиолошкиот кабинет.

Добитник е на 7-Априлската награда по повод „Денот на здравјето“ во 2006 година.

Неколку години по ред држи бесплатни едукативни предавања и трибини во основните и средни училишта во Битола на тема „Во борбата против дрогата“. Еден е од основачите на Центарот за зависници во Битола, помагајќи беспоштедно, на многу млади луѓе во справување со најголемото „Зло на денешницата“.

Автор е на неколку книги од областа на неурологијата и психијатријата: „Паркинсонова болест“, „Психопатија“ и „Психосоматски заболувања“. Ова се први книги во нашата земја од оваа област. Беше долгогодишен, ценет и активен член на Научното друштво во Битола.

Д-р Христо Бојаџиев
Специјалист по Спортска медицина-Битола

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 Индеџ 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 лsмпхобластиц леукемиа ин цхилдхоод. Н Енгл Ј Мед 1991; 325:1330-6.
б) заеднички автор
ГИВИО (Интердисциплинарs гроуп фор цанцер царе евалуатион). Редуцинг диагностиц делаs ин бреаст цанцер. Поссибле тхерапеутиц имплицатионс. *Цанцер* 1986; 58: 1756-61.
в) ***без автор*** **-** анонимно. Бреаст сцреенинг: нењ 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 пхsциологs: 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.

Адресата на Редакцијата
Даме Груев бр. 3
Градски ѕид блок ИИ,
1000 Скопје,
Тел.: ++ 389 02 3162 577

Електронска адреса (Е-маил): млдЖунет.цом.мк

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.