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ON THE EVE OF THE GREAT JUBILEE – 50 YEARS OF THE MACEDONIAN ACADEMY OF SCIENCES AND ARTS 1967 – 2017

Taki Fiti
President of the Macedonian Academy of Sciences and Arts

This year the Macedonian Academy of Sciences and Arts (MASA) marks and celebrates a great jubilee – 50 years of existence and work of our highest institution in the field of sciences and arts. Although on 22 February 2017 the 50th anniversary of the enactment of MASA in the Assembly of the Socialistic Republic of Macedonia was marked, and on October 10 it will be 50 years since the solemn establishment of MASA, we proudly emphasize that our roots, the roots of the Macedonian and Slavic cultural and spiritual continuity, are far back, in a time dimension which is measured in centuries. Because the mission of the Ss. Cyril and Methodius, the historical events that made Ohrid, with the famous Ohrid Literary School, already in the IX century to become the center of the Slavic educational and enlightening activity, which then spread throughout all Slavic countries, have fundamentally changed our contribution to the treasury of the European culture and civilization. And furthermore, centuries later, in the middle of the XIX century the Macedonian revival began, with a pleiad of our cultural and national activists. These processes at the beginning of the XX century resulted in the establishment of the Macedonian Scientific and Literary Fellowship in Saint Petersburg, led by Dimitrija Chupovski and Krste Petkov Misirkov, whose rich scientific, literary and cultural activities were a significant reflection of our spiritual continuity and identity, and an event that has marked the dawn of the Macedonian Academy of Sciences and Arts. This continuity will remain in the period between the two world wars, with a pleiad of artists in literature, art, music, philological, economic, legal and technical sciences. A few years after World War II, in 1949, in free Republic of Macedonia, the first state University of "Ss. Cyril and Methodius" was established, within which, in less than two decades, solid personnel resources were created which allowed rapid development of the higher education and scientific activity in our country. It was an event of great importance for the establishment of MASA as the highest institution in the field of sciences and arts.

This millennium pace and continuity in the development of art and scientific thought in our region is an indication and evidence that we are not a nation without its own roots, without its own history, without its own culture, and that the attempts to deny our identity, language, name, no matter where they come from, are residual of the Balkan anachronisms, and essentially speaking, they are absurd and retrograde.

Immediately after the establishment of MASA followed a period of rapid development, diversification and enrichment of its scientific and research activities and artistic work. Almost two decades after the establishment MASA entered the phase of its maturity and has grown and has affirmed as the fundament of the Macedonian science, language, culture and history and as one of the pillars and symbols of the statehood of the Republic of Macedonia.

Today, MASA, according to its integral concept, structure and function, has all the necessary attributes of a modern national academy of European type, and of course, performs the three basic functions typical of the European national academies: creating communication space for confrontation of different views and opinions on important issues in the field of sciences and arts, scientific and research work and advisory role.

The scientific and research activities and artistic work, in fact, constitute the core of the activity of MASA. The number of completed scientific and research projects and projects in the field of arts within MASA is impressive – more than 1,000 projects in the past 50 years. Some of these projects are long-term and are mainly related to the strategic issues of specific national interest, and significant is the number of fundamental and applied research in all fields of science and art represented in the Academy. MASA members in their scientific research increasingly incorporate the international dimension in the work – in the recent years more than 60% of the scientific papers have been published in international journals, most of which have been published in journals with impact factor; 50% of

the papers that have been published in proceedings of scientific and professional meetings are related to meetings held abroad, etc. In addition, the works of our renowned writers and poets, members of MASA, are translated into foreign languages, and their work has found its place in world anthologies. Our prominent painters and sculptors of the older and the younger generation have created and create masterworks that are regularly exhibited at home and abroad. It should be particularly noted that our two research centers – Research Center for Energy and Sustainable Development and the Research Centre for Genetic Engineering and Biotechnology "Georgi D. Efremov", that have gained high reputation in the region and beyond, continue to successfully maintain the attained position. The work of the other research centers also enhances, including the newly established ones, which have begun to work on significant international scientific and research projects.

In its half-century of existence and work MASA developed a rich publishing activity. Since its establishment until today around 700 titles have been published – monographs, results of scientific projects, proceedings from scientific meetings, music releases, facsimile and jubilee publications, joint publications with other academies and scientific institutions, publications of solemn meetings, special issues of the departments of MASA etc. A special contribution to the publishing activities of MASA provides the "Trifun Kostovski" Foundation that has been existing and working for 18 years.

MASA proactively follows the changes and the new trends in the scope of the advisory function of the modern European national economies, and in that context the obligations arising from the project SAPEA - Science Advice for Policy by European Academies, initiated by the European Commission in order to intensify the cooperation of the European academies within their advisory role. Through the publication of the results of our scientific and research work, their presentation to the wider scientific and professional public in the country, to the government officials, etc., MASA participates in the policy-making in the field of sciences and arts and in the overall development of the country. The maintenance of the independence of MASA in carrying out the advisory role is our highest priority and principle.

In the recent years MASA has developed extensive international cooperation that contributes to the affirmation of the Macedonian scientific and artistic work and to the increasing of the reputation of MASA and of the Republic of Macedonia in international scale. Today, our Academy cooperates with more than 30 foreign academies and scientific societies and is a member of 7 international associations of academies. In the recent years the cooperation with the academies from the neighboring countries has been intensified, as well as with the Leibniz Society of Sciences from Berlin, and also, within the so-called Berlin process (Joint Science Conference of Western Balkans Process / Berlin Process) the cooperation with the German National Academy of Sciences – Leopoldina, with the French Academy of Sciences, the academies of Southeast Europe and others.

Due to the results achieved in its work, MASA and its members have won a number of high national and international awards. In the past 50 years, MASA has won around 90 awards and recognitions – charters, plaques, certificates of appreciation, medals and decorations from national and international scientific, educational, artistic and other institutions. Particularly, it should be noted that MASA has been awarded with the high decoration Order of the Republic of Macedonia for the contribution to the development of the scientific and research activity and artistic creativity of importance to the development and affirmation of the Macedonian science and state, which is awarded by the President of the Republic of Macedonia, as well as the prestigious Samuel Mitja Rapoport award of the Leibniz Society of Sciences from Berlin, which, for the first time, has been awarded to MASA. Today, 22 members of MASA have the status of foreign, corresponding and honorary members, as well as holders of honorary PhDs at around 60 foreign academies, scientific societies and universities.

• • •

The developmental trajectory of MASA unambiguously confirms that the Academy, in its 50 years of existence and work, faced with periods of heights, but also periods of descents and turbulences that are most directly

linked to the situation in the Macedonian society, i.e. with crisis periods of different nature – the dissolution of the former common state (SFR Yugoslavia), problems with the recognition of the international status of the country after its independence, the embargoes and the blockades of the country in the early transition years, the internal conflict in 2001 and the political crisis in the last two-three years. In such crises and tense periods the criticism for the Academy grew – that MASA is an institution closed in itself, that MASA stays away from the current issues and developments in the country, and so on. On the one hand, it is a result of the insufficient understanding of the social role of the Academy – MASA is the highest scientific institution, where hasty reactions of columnist 'type', with daily political features are not characteristic. On the contrary, MASA uses facts and arguments. The basic activity of MASA, the results achieved in the scientific research and the artistic work is our identification within the national and international professional and scientific community, and beyond, within our society. On the other hand, this criticism and perception of MASA has a real basis in the fact that MASA, as opposed to the huge opus of implemented scientific and research and artistic projects still insufficiently affirms the results of its scientific and artistic production to the public. It is our weakness that we must overcome in the future. Of course, we cannot and must not "turn a blind eye" to the other weaknesses and omissions which, at least from time to time, we have faced with over the past 50 years and which we will face with in the future – insufficient scientific criticism of the events in the field of sciences and arts, insufficient resistance to political influence etc. On the contrary, in the future, we will have to clearly identify the weaknesses and the oversights in our work and to find out the right approaches to overcome them.

Today we live in a world of great science. The strong development of sciences, the new technological model based on information and communication technologies, the new wave of entrepreneurial restructuring of economies and societies, the globalization of the world economic activity, opened new perspectives to the economic growth and the development of individual countries and of the world economy as a whole. However, these processes, by their nature, are contradictory. The latest global financial and economic crisis of 2007-2009 revealed the contradictions of the globalization and the discontent of the people from it – the uneven distribution of wealth and power among individual countries, destruction of the resources and the environment worldwide, exhaustion of power of the existing technology and development models. These processes resulted in other problems – refugee and migration crises, strengthening of the regional and national protectionism despite the efforts to liberalize the international trade, fencing of the countries with walls at the beginning of the new millennium, changes in the economic and technological power and of the geo-strategic position and importance of entire regions and continents, etc. Nevertheless, one thing is a fact – societies that aspire to grow into societies and knowledge-based economies more easily deal with all the above mentioned problems, challenges and risks of the modern world. Of course, moving towards a development knowledge-based model assumes large investments of resources in education, science, research and development and in culture, simultaneously accompanied by well-conceived and devised strategies on development of these crucial areas of the human spirit and civilizational endurance. Hence, this fact, undoubtedly, emphasizes the special significance of the national academies of sciences and arts in achieving this objective.

In the recent years the Republic of Macedonia has been facing with the most difficult political and social crisis in the period after its independence. We are facing a crisis of the institutions, breach of the principles of the rule of law, the phenomenon of "captured state", a decline in the process of democratization of the society and falling behind on the road to the Euro-Atlantic integration processes. The problems that are now in the focus of our reality will require major reforms, much knowledge, energy and political will to overcome them. In this sense, and in this context, the role of MASA and of the overall scientific potential of the country in overcoming the crisis is also particularly important.

The above summarized evaluations and considerations about the development of MASA in the past 50 years, about the achievements in the realization of its basic activity, about the problems it faced and faces with, about the major challenges arising from the new age and which are determined with the changes in the international and national environment, they alone define the main priorities of our Academy in the forthcoming period:

- Our long-term goals are contained in the mission and vision of MASA as the highest institution in the field of sciences and arts. The mission of MASA is through the development of the basic functions that are characteristic for all modern national academies of European type, to give its full contribution to the inclusion of the Macedonian science and art in the modern European and world trends, and our vision is the Republic of Macedonia to become an advanced society based on science and knowledge;

- In the forthcoming years the focus of the scientific and research activity and artistic work of MASA, in cooperation with the other scientific and research institutions in the country and with government experts, will be particularly focused on the elaboration of issues and topics that are most directly related to the sources of the current political and social crisis in the country in order to offer possible solutions, approaches and policies to overcome it;

- The issues related to the Euro-Atlantic integration processes of the Republic of Macedonia, their continuous and persistent scientific monitoring and elaboration and active participation of MASA members in the preparation for the accession negotiations with the EU will remain a high priority on the agenda of MASA. Our ultimate goal is the Republic of Macedonia to become a democratic, economically prosperous and multicultural European country.

- The increasing incorporation of the international dimension in the scientific and artistic work of MASA, through the cooperation with foreign academies, scientific societies and other scientific institutions, through application and work on scientific projects financed by the European funds and the funds of other international financial institutions, also remains our important priority.

Let us congratulate ourselves on the great jubilee – 50 years of the Macedonian Academy of Sciences and Arts.

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INVITED PAPER

ON OUR SCIENCE AND SCIENTIFIC WORKERS

Gjorgji Filipovski[1]

Macedonian Academy of Sciences and Arts

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The idea to write this text comes from the scientific conference held in the Macedonian Academy of Sciences and Arts (MASA), held on October 10, 2016 devoted to the situation and perspectives of the higher education (HE) and the scientific and research activities (SRA). During the preparations for the conference, a study by Acad. Vlado Kambovski *Dispute on science and the higher education in the Republic Macedonia in the 21st century* was published in MASA. Many statistics and official reports on the situation were presented therein.

I would like to give an overview of the development of science over the seven decades since the liberation to present days. I would like to discuss the pressing problems of our science that are awaiting a solution, in addition about the concern of the state for the development of science, the ups and downs of this development, the status of the researchers in the various stages of that development. Undisputed is the fact that a tremendous progress has been done to reach the present state with large capacities in buildings, equipment, personnel, etc. planned for science and higher education.

On these issues I will talk less using statistical figures, and I will talk more from the standpoint of a scientist, the way he has experienced it, as a living witness and a direct participant in the SRA and the HE for 70 years. It seems I am the only living witness.

I was fortunate and privileged to follow that developments from the first day after the liberation. In December 1944 I started my scientific career at the Institute of Agriculture. In the autumn of 1947 I was selected in the first Teaching Council of the Faculty of Agriculture and Forestry as the youngest lecturer. I was also a member of the first academic community when the University was established in 1949. I continued the SRA and the HE activities at the Faculty of Agriculture until 1983, i.e. until the retirement (three years of activity at the Institute and 36 years of higher education activity). Even after the retirement to present days I have continued that activity at the Faculty of Agriculture and at the MASA. In that long period science passed through many stages, and also changed the state's concern for science. The status of the scientific workers changed as well.

Memorable are the first 10-15 years after the liberation with a maximal concern of the state for the development of science, higher education and culture, a concern that even until today has not been repeated. This concern pulled the country out of a terrible backwardness.

In those days, for a short period of time things have happened, for which in other Balkan countries it took decades, and in the old and developed countries it took even centuries. For e.g., at the beginning of 1945 the alphabet was adopted and the literary language was codified. In 1946 the Faculty of Philosophy was established, where for the first time a word in the Macedonian language was heard at a higher education institution. In 1949 the Skopje University was established. Only 4 years passed from alphabet to university. It is unknown in the history of the European nations.

[1] Acad. Gjogji Filipovski was the first Scientific Secretary of the Macedonian Academy of Sciences and Arts elected in 1967

After the liberation we were a total of slightly more than ten scientific workers, of whom only a few were doctors of science, and only three were former lecturers at the Belgrade University. I was only 25 years old and I had an experience of three years as an assistant at the Sofia University. We all became teachers – members of the first academic community at the first university in 1949. At that time the University had less than 100 professors. That was the nucleus from which developed all that we have today in science and higher education. We, the professors, had a privileged profession in the society in those days. We had a higher salary than the others, and we had especially good supply when others barely survived with coupons. Also, with the election for teachers we received approvals for individual apartments, i.e. a big premium for that time when the majority of the citizens lived in shared apartments. I am not mentioning the privileges because I wanted them, but only as an illustration of the great concern of the state for the science and the scientists. Many universities and scientific institutes were established and built in that period.

Of course it could not have lasted forever. A period of stable development of the science from budget funding came, and I would say satisfactory for the possibilities of that period. Later the Council for Scientific Work and the Fund for Funding of Scientific Work were established. For some time I was the head of those bodies, so I know how big was the role of the scientific workers in the creation of science policy and funding of the scientific activities. The budgetary funding was replaced with a fund one, which was more successful. Unfortunately, then came the period when the self-management was introduced, i.e. the establishment of basic organizations of associated labor. This concept was taken from the economy, but applied in the higher education and in science it proved unsuccessful and caused regress in these activities. The scientific workers in their faculties and institutes lost much of their meaning and influence.

A few years before the independence the troubles began. With the independence they intensified. The Council and the Fund were abolished. The scientific workers lost their influence. The funding deteriorated and a period of stagnation and even regression in the SRA has come. The situation worsened further in the last decade when the problems have intensified, which will be discussed later. Science and scientific workers were on the margins of society, sometimes even ignored and discredited. This will also be discussed. Some things that have improved science will not be forgotten.

This article reflects my personal opinion and the responsibility is mine.

Let's start with the problems in our science.

On some pressing problems of our science.

During the 7 decades of free life in the development of science in our country many problems occurred. I chose to write about 5 of the most important, which in the last decade have arisen in particularly enhanced form. Those are:

1. Low percentage of gross domestic product (GDP) allocated for science.
2. Lack of assets for funding scientific and research projects.
3. Blocked election of young scientific workers.
4. Small number of researchers in comparison with the European standards.
5. Non-participation of scientific workers in the creation of the science policy.

According to the World Bank report, the percentage of the GDP in the Republic Macedonia allocated for science is the lowest in the region and in Europe. Usually this percentage shows the concern for science in individual countries. For the whole period since the independence to present days this percentage has ranged from 0.17% to 0.24% with a mean value of around 0.22%. In some other countries in the region this is significantly more (Serbia - 0.8%, Croatia - 1.1%). The European countries on average allocate about 3%, and some European countries even more. Some Asian countries with a rapid development allocate even twice as much as Europe. The EU recommendation for the European countries is 3%, i.e. more than 10 times than us. According to the program of the Ministry of Education and Science (MES) it is planned this percentage in the Republic of Macedonia in 2016 to be 1%, and 1.8% by 2020, but it has not been realized. 6.5 euros are allocated per capita in the Republic of Macedonia and nearly 500 euros in Europe. This percentage was increased in the Republic of Macedonia in 2012 (0.33%) and in 2013 (0.44%). However, it has not improved the projects financing, because this increase was due to the procurement of laboratories and translation of literature. The newly procured equipment will improve the working conditions in the scientific institutions, but without the funds for projects the new equipment will remain underused. Concerning the funds for translation, many feel that they would be more usefully and more effectively used if they were given to the libraries of the scientific institutions for the procurement of foreign literature and journals. Many of the translated books have been earlier used in the original by the researchers.

Nowadays, in the information society, which is based on knowledge and scientific achievements,

the investments in science accelerate the overall development of a country. It is proven by the most developed countries and by the developing countries with very rapid development.

The insufficient projects funds is the constant problem of our science. The funds allocated for science are used for salaries, material costs and projects. The Projects funds are constantly reduced and we have reached the present situation when these funds are rarely granted, and possibly for bilateral projects, i.e. for joint projects with other countries, in a modest amount.

The Vice Rector for science of the University of *Ss. Cyril and Methodius* (UKIM) recently said that in the last 10 years UKIM has not received funds for scientific and research projects. This fact has several negative consequences: the enormous resources invested in science in these seven decades of free life (for buildings, equipment, personnel, etc.), remain unused or underused. In order to make these investments "operable" only a small percentage of those assets is required. Moreover, the lack of funds for projects reduces the scientific production and represents a major problem for the advancement of the scientific workers. Papers are arguably required from them, but no funds have been provided for the papers. In addition, the lack of these funds makes it difficult to prepare the young generation for science because it requires participation in the projects implementation. The lack of funds for science is one of the reasons why UKIM is not among the top 500 universities in the world, unlike, for example, Belgrade and Zagreb University.

This lack of funds for scientific and research projects was experienced by me and my associates. For more than 8 years we have been searching, in vain, means to complete the project for preparation of 63 soil maps and 11 studies on our soils. All the necessary scientific materials have been collected over many decades. The project was successfully completed thanks to the understanding of FAO (UN agency), which has granted us 340,000 dollars from its fund for science. We were embarrassed in front of their experts who helped us because they have shown greater interest in our soils than our country.

The funds for projects and for election of young scientific personnel have been reduced or cancelled in addition to the realization of the concept of combining the lowest GDP per capita with the highest number of universities per million inhabitants in Europe. This concept has been applied only in the Republic of Macedonia. New universities are also established. The concept does not meet our financial and personnel capabilities and needs. It is based on the illusion that quantity replaces quality. A good expert cannot be replaced with 5 trained persons with insufficient knowledge. Practice has shown that this concept has not reflected positively on the level of the higher education and science. I would like to note that in this region there are usually about 3 to 4 universities per million inhabitants, and in some rich countries (California, France) below 2. In the Republic of Macedonia there are 12. Maybe it should be considered to review this concept.

The third and perhaps most painful problem is blocking the election of a scientific offspring. Full professors retire each year, and there is no mechanism to train young and proficient scientists. For this purpose there used to exist funds for assistants that, by engaging in teaching, through completing masters and doctoral studies, which lasted about 8 years in total, were well trained for election into Docent title. By abolishing the assistantship positions the funds for scientific offspring were abolished. I know that there are other ways of training. But they cannot be based on a voluntary basis. The Faculty of Agriculture Sciences and Food, where I was a Professor for 36 years now has only one assistant. In my time, only for my subject there were 2 assistants, i.e. more than at the entire Faculty today. The state of the scientific offspring, unfortunately is similar at the other faculties, too. Without young scientific personnel science has no future. In our country, fortunately, there is plenty of capable personnel who has graduated with high marks and to whom the doors of the universities are closed. They are knocking on other doors abroad.

In the last decade on budget funds tens of thousands of young people with completed studies have been employed. The media reported that some of them stayed at home and received salaries, including those at the Ministry of Education and Science. Only a small percentage of that large number (1-2%) would be sufficient for UKIM to obtain, for example, a thousand young talented and promising people, for each faculty of about 50 that will secure its own future. There are no justified reasons why this has not been done or why it is not being done.

The consequence of the small funds given to science in our country is the small percentage of researchers, especially young people, on 1,000 employees compared with the European countries, where we are striving. According to the World Bank data, in 2007 in our country there were 1.6 researchers per 1,000 employees, 3.4 in Bulgaria, 3.6 in Croatia, and 6.6 in the EU. Numbers speak for themselves.

Finally something about the participation of researchers in the creation of science policy. Today

there are, at national level, bodies in which participate scientific workers, too, within which are proposed strategy, program and funding of the SRA aligned with the overall development of the country. The opinion of the researchers is less important than the opinion of some employees of the MES, which have never dealt with science. It was not always like that. After the independence the Council for scientific work and the Fund for SRA were abolished. With the abolition of the Fund, the fund financing was replaced with a budget financing where scientific workers do not participate and which has far fewer resources. This situation has continued until present days. With the Law on SRA an attempt has been made after two decades to renew the work of these bodies. Relevant legal provisions have been adopted, but concerning the participation of the scientific workers, those legal provision for several years have not been implemented in practice. I do not think that anyone can benefit from it.

I can remember the following matters that have improved science over the past seven decades:

1. The period of the first 10 to 15 years after the liberation when science had a high priority, and in addition, the researchers had a privileged status.
2. I remember in that period the quite unexpected decision to vacate the largest newly built building in Skopje, which belonged to the Ministry of Interior in order to accommodate therein several faculties for which new buildings have not been built. It was unexpected to us, because we knew what an important role that Ministry had in the society in those days. Unfortunately, the earthquake destroyed this building. It was opposite to the Holiday Inn hotel, which is now a parking lot.
3. The financially unlimited support of the University for mitigating the damages of the Skopje earthquake. In a short period of time the university buildings, dormitories, professors' apartments were reconstructed and new buildings for several faculties and dormitories were built. I remember that period well because as a Rector I had the major task to restore and build the University.
4. Great incentive for the promotion of science and art was the investment of large funds for the construction of the new building of MASA and later, after the independence, its annex, too.
5. I remember, as a good one, the decision to establish the Council for Scientific Work and the Fund for Funding of Scientific Work because scientific workers have become a factor in the creation of science policy and funding of scientific activities.
6. I also remember, as a good one, the expert Government and the first parliament that were willing to do everything for science in the situation of the weak opportunities in that period.
7. The group of positive decisions includes the Decision from some years ago to invest in the procurement of new laboratories.

Since the independence the development of science has been stagnant or has been going down. The funds for SRA are reduced, there are not new investments in the university buildings, the scientific facilities and equipment. The funds for scientific and research projects and for the selection of young scientific workers have been abolished. This is especially true for the last decade.

Here are some other items that confirm the marginalization of science and scientific workers, and in some cases their ignoring and discrediting:

Several years ago the Ministry of Science was abolished and was merged with the Ministry of Education. In the joint Ministry the funds for science are reduced and flow into education.

In the joint Ministry of Education and Science, science has a subordinate role not only with the loss of the funds allocated for it. We do not know whether this Ministry has an assistant minister for science because we have not heard from him nor we have seen him. The Ministry's main concern is the education, of which they constantly talk about. We have not heard a word about science. We do not have information about the activities of that Ministry in proposing measures for promoting science. We have not heard that analyses of the situation in science are performed. There is not much desire to hear the opinion of the scientific institutions and scientific workers.

The scientific community has not been informed whether the Government and Parliament periodically consider the state of the SRA and whether they propose measures for its improvement.

The provisions of the Law on SRA relating to the participation of scientific workers in the creation of science policy are not implemented. For example, the National Council for Higher Education and SRA and Technical Development has not been constituted. According to the Law it should be composed of representatives of the Government, the academic community and the business community. The Council for SRA has not been constituted, which is imagined as an expert advisory body to the Minister of Education and Science. The Board of Ethics has not been constituted. All this says that there is no desire to hear the opinion of the scientific workers. One gets the impression that the opinion of

some Ministry employees, who have not worked in science, is more important than the opinion of those who know better what science is.

Since the independence the Parliament has not reached a Long-term strategy for development of HE and the SRA, although laws have provided for that.

In the last several years the Parliament has not adopted a program for the development of the SRA.

Not even the fund on financing the SRA has been constituted, although such funds exist in all countries. The Council for Scientific Work and the Fund for Funding the SRA have been canceled with the independence.

SRA is still treated as an expense rather than as one of the most important pillars on which rests the rapid, stable and sustainable social development.

Lately, some things occurred that clearly illustrate the marginalization and ignorance of science and scientists:

I will begin with the "11 October" award of some time ago. The law provides these prizes for lifetime achievement to be awarded for achievements in science, culture, economy and other social activities. This year, four prizes were awarded and all just in the cultural area. As far as I know, this is the first time that a prize in the field of science has not been awarded. Isn't there anybody who has deserved it? Do they who award prizes know that among scientists there are acknowledged one, not only in the country, that there are those who have also received international acclaims and those who have been visiting professors at prominent universities? Is this the way to treat science and scientific workers?

I will continue with other examples.

This spring, the highest prize "Goce Delchev" was awarded for achievements in the field science in the previous year. I was one of the winners and I was asked to express appreciation on their behalf. When I looked in the audience with the intention to greet the officials, I realized that there wasn't anyone of the top figures of the country. There wasn't any minister. Not even the Minister of Science was present. There wasn't a single representative of the Assembly. Only the deputy minister of science and education was present. There were no television cameras. Among the papers only "Vest" announced the awards and the names of the winners. Only silence from the other newspapers. It is good that is not the case when awarding prizes in other areas. We, the awardees, asked ourselves – why have we deserved this treatment of ignoring the scientific workers. It was not always like that. When I was awarded the "11 October" award twice (before and after the independence) fortunately it was not like that.

However, it is not only this. The winners of the highest state awards and only in the field of culture are awarded pension supplement to a certain amount. It is also received by singers – performers of folk songs. It is a welcomed gesture. Many scientific workers have expressed me dissatisfaction why it does not apply to them. Is it a coincidence or they are forgotten or something third? That third is called discrimination.

I will describe another case of ignoring the science and the scientists. On October 10, on a Solemn meeting MASA marked the 49th anniversary of its foundation. At the same time, MASA declared two prominent foreign scientists for holders of the international recognition "Blaze Koneski" for the study and promotion of our science and culture. Then, a scientific conference was held with 13 papers focusing on the state and prospects of higher education and the scientific and research activities. Again, there wasn't any official, not even one person from the Ministry of Education and Sciences to hear the real situation in those areas and the proposals for its improvement. Maybe that's why they did not come, so as not to spoil their idealized image for those areas, far from the true one. If they were not willing to hear, at least they could have listened.

Now, a few words about the attitude of the media towards science and scientists. In several newspapers every day, one to two pages are devoted to culture, which is to be welcomed. However, about our science, the various scientific events, the achievements of scientific institutions and the scientific workers, about the received awards you will find in the newspapers from time to time only short articles. Again – ignoring. Unfortunately, this situation is not without the fault of the researchers themselves.

I was thinking how to finish this article about the unsatisfactory situation in the area of our science. Reading for the second time the book "On Macedonian Matters" by our great personality Misirkov, I noticed a passage about the importance of science and culture. That paragraph in the original is the following: *Science and literature are the most important factor in the development of a nation to become a nation. The degree of development of science and literature in a nation is measured with its culture and that is how nations are divided: those with cultural and those without culture; those with culture rule, and those without culture are slaves*. It was written 100 years ago by a man with a true vision for comprehensive development of his people when it will be liberated. Do those that today reach decisions about science have the vision of Misirkov from 100 years ago?

Can the current situation in science be improved? Of course it can. It requires a turnabout.

First, in the understanding, as a prerequisite for all other changes. This means that science cannot be treated as an expenditure, but as a very important factor without which there cannot be a quick, overall social progress in the country. Science is treated like that in all developed countries and in the countries with rapid development. All minds that know and can suggest a good strategy for a long-term development of the SRA need to be mobilized, and, also to suggest a program for the implementation of the strategy on shorter timelines. That proposal should be considered by the authorities (Government and Parliament) and a strategy and program to be adopted. It will be the easy part of the job. The implementation is more difficult. This requires a lot of political will, a lot of energy, a lot of money \ deep reforms, consistent with the country's needs and the recommendations of the EU, towards which we strive. Scientific workers are not hopeless. They expect the turnabout to begin next year with the formation of a stable government, regardless of who will form it. They are deeply convinced that countries with such situation in science, such as ours, cannot have a bright future.

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TYPE 2 DIABETES IN PEOPLE FROM CULTURALLY AND LINGUISTICALLY DIVERSE BACKGROUNDS: PERSPECTIVES FOR TRAINING AND PRACTICE FROM NUTRITIONAL THERAPY AND DIETICIAN PROFESSIONS

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ABSTRACT

Objective: To explore the perspectives of nutritional therapy and dietician practitioners, undergraduate students and academics working with people with type 2 diabetes and who are from culturally and linguistically diverse (CALD) backgrounds. **Methods:** A qualitative study design of in-depth semi-structured one-on-one interviews with a total of 24 participants (8 practitioners, 8 students and 8 academics) in the fields of nutritional therapy and dietetics. Open-ended questions focused on the perspectives and experiences (learning, practice and teaching) of working with people of CALD backgrounds who have type 2 diabetes. All interviews were recorded for thematic and textual analysis. **Results:** Inter-related themes which were confirmed with investigator triangulation were the understanding of (i) the concepts of culture and diversity, (ii) the concepts and influences of health, diabetes and food across cultures, (iii) influences within and across cultures and (iv) systems and resourcing. Overarching perspectives across these themes suggested frustration in having sufficient capacity to assess comprehensively, to deliver effective, comprehensive and high quality management plans, and to achieve required health behavioural changes with people from different CALD backgrounds. **Conclusions:** There's a need for improvements in the undergraduate education and training and in professional development programs; training and resourcing of interpreters in delivery of health-related information and working with health professionals; for focus on culturally appropriate management plans that involve consultation with key decision makers in families and communities; and, reviews of the systems for supporting and resourcing nutritional therapists and dieticians in professional development from undergraduate to practice levels.

Keywords: Diabetes service, Culturally and linguistically diverse, CALD, dieticians, nutritional therapy.

INTRODUCTION

Australia is experiencing high rates and costly complications of type 2 diabetes amongst numerous countries worldwide. With a population of just over 20 million, 1.7 million had type 2 diabetes of those that were registered in 2005. It is estimated that by 2031 it will have doubled to 3.3 million [1].

In Australia, accredited 'Nutritionists' and 'Dieticians' are recognized for practice through one or more members of the Federation of Nutrition Organisations, such as Food Standards Australia and New Zealand, the Nutrition Society of Australia, or the Dieticians Association of Australia [2]. The distinctions between a nutritionist and a dietician lies in the number of years to qualify, being around 3 years for

nutritionists and 4-5 years for dieticians, hence, their roles and responsibilities are different. Nutritionists help people achieve their health goals by providing information and advice regarding health and food choices. They also have expertise in public health nutrition and community health [3]. Dieticians however, are qualified to work in private clinical practice, hospitals and in medical nutrition industries. A dietician can prescribe a dietary treatment to people who have medical and physical conditions, such as, food allergies, cancers, overweight, type 2 diabetes. A dietician can also work in public health and community health [3]. In both professions, for type 2 diabetes for example, the key role is to optimize food and nutritional intake for health, often also to achieve weight loss, behavioural change and to work as a contributor / collaborator to a multidisciplinary health service team [4].

There is little empirical research to guide health care professionals regarding the most appropriate approach for working with people of culturally and linguistically diverse (CALD) backgrounds, with type 2 diabetes [5]. There is also little to guide undergraduate teaching, ongoing professional development, policy and resourcing to ensure depth, quality and effectiveness [6]. The purpose of this study was to gather information by interviewing upcoming and existing members of the fields of dietetics and nutritional therapy in an effort to identify descriptive categories that could be used to inform practice and the associated education, policy and resourcing systems.

MATERIALS AND METHODS

Design

The qualitative approach adopted for this study was based on 'interpretative structures, emotionality and power relations that permeate' the health professional and client meetings [7]. Knowledge capture was of interpretations from practitioners, academics, and students in the nutritional therapy and dietetics professions of their interactions in working with CALD background clients who have type 2 diabetes.

Study Setting

Semi-structured in-depth interviews were conducted. An interview guide was used to prompt discussion regarding the cultural diversity and the perceptions/experiences of each interviewee in working with migrant and refugee clients; and to explore strategies for change. This guide was developed by the research team, experienced in mixed methods, which comprised a biomedical scientist, a public and allied health specialist and researcher, and a student of nutritional therapy. Interviews were undertaken by a consultant with expertise in qualitative research, in working with multicultural communities and in anthropology. No payment was provided for participation; the interviewer travelling to the site was nominated by each participant.

A total of 24 in-depth interviews of durations ranging from 1-3 hours were conducted. These were with 16 professionals who each had recognized qualifications in the fields of nutritional therapy or dietetics and who each had been practicing as a health professional (n=8) or teaching and researching at a University (n=8) for at least 3 years. Eight students who were enrolled in undergraduate courses from Universities across Melbourne were also interviewed.

Participants

Participants were recruited using a non-probabilistic maximum variation sampling strategy so that the collected data would show sufficient variation of perception and experience. A general information pack about the study was forwarded to public and private health service organizations that employed nutritional therapists and dieticians, to individual professionals who worked in these fields, and to the academic departments of universities around Melbourne which provide undergraduate education and training and employ academics and researchers in these disciplines. Those interested in participating in the study contacted the research team directly, and were provided with more detailed information sheets about the research prior to signing the consent to participate. All participants gave informed consent to participate in this study, which was approved by the Human Research Ethics Committee of Victoria University, Australia.

Data Collection

Questions were asked sequentially in 3 sections, each with agreed upon questions, statements and probes to elicit information. With exhaustion of responses to each section, the interviewer moved the discussion to the next section. The number of interviewees in each category (practitioner, n=8; academic, n=8; student, n=8) was sufficient to gain confidence that no new themes were emerging. The first section was introductory and to explore experiences and perspectives on the topic of 'cultural diversity'. The second section allowed for an in-depth account of what transpires in meetings between a health professional and the client of CALD background with diabetes. The third section was to foster ideas and innovation for practice, resourcing and systems.

Analytical Procedure

Interviews were recorded with the consent of the participant. Each recording was transcribed verbatim and entered for qualitative analysis in the workspace of the NVivo 9 program (©QSR International Pty Ltd). Cluster analysis was performed to analyse word similarity across transcriptions, and Pearson correlation coefficients were determined for each pair of transcriptions. One researcher (KB) first coded the data according to content analysis to gain 168 codes parsed into 1073 coding references. Through a reiterative process involving group consensus and a focus on straight subjective description, codes were grouped into themes with sub-themes. The derivation of themes was considered complete when all members of the research team concurred that the generated collection of themes captured all of the participants' responses to the interview questions. The descriptive-qualitative approach enabled the researchers to "stay close" to the data and understand the subjective experiences of the participants; this is considered the method of choice when a straight description of the data is desired.

RESULTS

Suggestive of both theme saturation and a suitable sampling technique, cluster analysis revealed a moderate to high degree of word similarity across the 24 transcripts, with Pearson correlation coefficients between transcript pairs ranging from 0.61-0.97.
The results are presented as a summary of the main themes identified in the analysis of the data, accompanied by representative quotations from the participants (practitioner, n=8; academic, n=8; student, n=8). Data analysis provided rich descriptions across different themes suggesting frustration in having sufficient capacity to assess comprehensively, to deliver effective, comprehensive and high quality management plans, and to achieve required health behavioural changes with people from different CALD backgrounds. From the analysis it was evident that there were inter-related themes with investigator triangulation, however, 3 primary theme outcomes were evident.

Understanding the concepts of culture and diversity by health professionals

Culture and diversity is the quality and variety of different cultures, as opposed to a homogenous culture. It is important to understand the conceptualizations of culture and diversity amongst different individuals. We analysed this by comparing the understandings of health professionals, academics and students. It was apparent that across the professions there was great variation in their interpretation of culture and diversity. A group of respondents perspective on cultural diversity was based on language and food:

Practitioner 5: By culture I mean people's language, food ideas, eating patterns, food rituals and beliefs

Student 3: It's not just about cultural diversity but about diversity, right, because we have religious, political, philosophical and linguistic diversity

Student 4: It is about nutrition and it is about cultures too

Student 6: About the different cultures and what they eat I guess...

Student 7: It is people from all different cultures all coming together..

Interestingly, for some respondents, the definition of culture and diversity was related to the individual and experiential level:

Academic 4: It [culture and diversity] is not something that I can define and say that is it. It is broad and it has so many meanings for so many people that it is not something that I feel comfortable defining I guess…

Student 1: I understand cultural diverse when people from different countries. Um, it is hard, difficult and people not understand you…

With the gathering experience of working with people of different cultural backgrounds, came acknowledgement of not knowing what is not known and the difficulties of influencing clinical practice positively with this knowledge:

Academic 4: I think my definition of culture has become more confused over the years…

Student 5: I guess cultural diversity for me was very complicated and brought out more problems than solutions for the patients.

The changing acknowledgement of culture and diversity for practice was noted:

Academic 2: Like all new and 'in' concepts, ideas and philosophies everyone is doing it, thinking it, practicing it and engaging in it. My concerns are that a lot of people are paying lip service to it.

Academic 4: What is culturally right today may change tomorrow…

Even though some respondents, in particular academics, indicated an interest in learning more about cultural diversity and the need for health professions to do so, others, in particular students, were

less interested in advancing the learning of culture and diversity for practice:

Academic 1: We often see people from different cultures and we have very little understanding about who they are; what they think and feel about our ideas around nutrition, cooking practices and health.

Academic 6: I do love cultural diversity. It keeps us all on our toes. It is vibrant and emotionally charged. The cultures have changed and will continue to change and we have really no idea of what, how and in what way we should be working with them…

Student 4: Like if they come into our country isn't it about our rules? Isn't it about what we know? So why do we not just tell them, look this is how it is?

Student 6: And I think that when I go into private practice I am going to be seeing Anglo Saxons mainly.

and perhaps not as cognisant, as the following respondent, of the risks of not learning:

Academic 4: I know that I have to be so careful and aware of the different cultural nuances

Concepts and influences of health, diabetes and food across cultures

Metabolic syndrome (diabetes, cardiovascular disease, cholesterol) is caused by a combination of lifestyle (physical inactivity, lack of sleep, diet, stress, obesity), immunological complexity and genetic factors [8-10]. An understanding of the link between food and diabetes and overall different food groups and its effects on health, varies amongst different cultures and varies according to their educational status. Respondents did not have a clear understanding regarding the different ways in which health, ill-health and their relationship to food was understood by clients from CALD backgrounds:

Academic 4: I think that it is also interesting that people have different ideas about what health is.

Academic 3: Well yeah I have even thought that, what do people think about illness? health? healthy eating?.

One particular responded explored this understanding, to reveal broadness in the concept of health and its relationship to ill-health, and based this on what they were involved or influenced by in the past:

Academic 4: We ran some focus groups with the women and they said that healthy is just having a happy family really.... and, some women said I am healthy when I take my blood pressure tablet and my blood pressure doesn't go up.... and I'am healthy when I can get up in the morning and I can still take 10 tablets not 20 like others in this group.....
because of having starved and survived refugee camps and having long periods of not having food, a chubby child or person is a healthy person or child.

The incidence of type-2 diabetes has increased exponentially in the last 50 years, which is in parallel to obesity and inadequate diet. In 1985 30 million were registered as being diabetic, compared to 285 million in 2010 and 350 million today worldwide. It is recognized as a global epidemic by the World Health Organization [11]. Interestingly, respondents commented on the increasing rates of type 2 diabetes however, this exponential prevalence was viewed differently amongst various communities, ranging from denial to normalization of the condition:

Practitioner 3: They don't like to hear about diabetes because in some cases diabetes is too far removed from their everyday reality. It is what happens but not what you live day in day out.

Student 5: My dad is a diabetic and so is my mother but I have never seen them change their diet or stop drinking so if it was serious they would probably do that. We never speak about it so I assume that it isn't a problem for them. I guess it might sound weird to you but it is normal in my family…

Furthermore, there are major misconceptions and mistaken beliefs relating to what diabetes is and how one should deal with it.

Practitioner 2: Aside from all these factors we have the issues about illness constructions, beliefs, values and misconceptions.

Practitioner 5: I think she did believe that one 'catches diabetes.

Academic 5: They believe that diabetes is caused by sugar ... that in Australia, food is not healthy ... it is injected with hormones that cause so many problems with their health ... that stress levels and family disharmony really cause ... illnesses like diabetes ... if they stop worrying then everything will be okay ... if they could just get their family issues under control then everything will settle with their sugar.

Practitioner 1: I noted that there were language, cultural and health barriers in the hospital. When I say health barriers I mean health beliefs ... For me this is the lived experience of culture; culture's impact on health and illness.

Diabetes is a complex condition, which can affect the entire body [12]. Understanding of what diabetes is, is important, however, patients tend to place insufficient importance on diabetes and on its management:

Academic 5: Diabetes is a funny condition … it is a condition that is ... not seen as important. They can't really see an effect or feel pain therefore it is not a condition that they need to worry about … they tend to disregard information about diabetes and the various warnings.

Many people have a general idea of what diabetes is, but misconceptions about the causes may lead to negative health and management outcomes. In fact these misconceptions have negative impacts on the uptake by the patient of advice. It is interesting that such beliefs exist, and is considered as fate, a curse, a result from past sins, etc.:

Practitioner 6: There's a lot of fatalism that is out of their hands, what's the point in doing that, it's about a curse they put on them in the village…

Academic 6: Someone at church gave me 'mati' (evil eye). I got diabetes because I've been bad.

Practitioner 1: Bad spirits cause disease, medications may not be able to cure you of the bad spirits.

Academic 6: Fruit increases blood sugar, so they don't eat fruit and when you try to explain that it is not all fruit they don't want to hear it. They have this resistance to taking insulin. They believe that insulin means or equals death…

Understandings and influences within and across cultures

The next theme that was extracted from the analysis was participants' perceptions of food and cooking amongst migrant patients with type 2 diabetes. It was the general understanding amongst academics, practitioners and students that certain food practices and cooking methods were unhealthy. Their understanding was that as perceptions of food amongst certain migrant groups were not the same as it is in the western society, this would as a result make it a challenging task to educate them about new and healthier food and cooking methods in the country of settlement. One participant reported,

Student 1: Unfamiliarity with our food or unfamiliarity with our cooking practices here that they don't have that concept of what is healthy eating. A lot of the African refugees don't have... they don't have that concept of what is healthy eating? They don't link…food to health like we do.

This lack of understanding and familiarity of what constitutes "healthy" food and cooking practices that exist in the host society coupled with unhealthy misconceptions of weight and body image can be perceived as a dangerous combination for the management of diabetes. This was evident by,

Student 2: Being overweight is considered healthy. Oh and oh my God, I have seen some of them put so much salt on their food that I nearly died.

Moreover some academics clearly pointed out the importance of adopting their cultural cooking methods and foods into healthy diets.

Academics: How can you integrate foods that are culturally relevant to you into a healthy diet here and I think that is what one of the things that you have the best health outcomes when you can integrate some of their…um… yeah, their cultural diet into a well-balanced Australian diet.

Even though this suggestion about the adaptation of ethnic cooking methods according to a more healthier version of Australian cooking methods is put forward with a well-meaning intention it could lead to assumptions that could negatively impact the relationship between the health care professional and the CALD client. However it was evident that there were also health professionals and academics that appeared to have a more impartial view about cultural and ethnic cooking methods and food consumption patterns of CALD communities. As one pointed out,

The concept of food, as part of the social and religious fabric, is not just about body nourishment. A focus on the world of food and culture is needed in your areas of work.

This quotation however, implies that there is a negative perception amongst some participants in that all ethnic cooking methods and diets were unhealthy and should be adapted to the Australian way, if the diabetic CALD patients want to lead a healthy lifestyle. This lack of understanding would lead to wrong perceptions of cultural / ethnic ways of cooking and therefore, would even lead to a larger gap between the health professional and the CALD client. This signifies either the need to train more health professionals from CALD background or the need to provide more cultural knowledge to students of health disciplines. The lack of knowledge about ethnic food and cooking methods and food practices could lead to dangerous assumptions of ethnic cooking and food practices that would result in widening the gap of communication between the CALD client and the health professional which could consequent-

ly result in the ineffective diabetes education among CALD groups.

Furthermore, the recognition of the importance of gender roles in different cultures is essential when recommending diet regimes to patients with diabetes. The theme of capacity of clients to implement dietary and other lifestyle changes in their homes and communities and the impact of gender roles in some cultures on this capacity was mentioned particularly by many practitioners but also by one academic. In some cultures, for example, the gender roles of 'women doing the cooking', and 'men doing the decision making and talking' are very defined; in others, these roles are less distinct.

Practitioner 7: I ask about gender roles within that culture. For example, what are the roles of a man and the roles of a women and who takes charge of a person's health. How gender roles may impact on that may need to be made.

Practitioner 3: A situation arose when a gentleman didn't like his wife's cooking so therefore he ate 8 bananas instead of the meal his wife had made. He said it is not his place to question his wife's cooking.

Another participant spoke of a situation where she was regarded as insufficiently qualified to give advice or treat a male client who preferred to see a male practitioner. Dependence of females on the males to attend consultation sessions was also identified.

Women of certain cultures don't drive and may not have access to facilities, appointments and education forums. Some women rely heavily on their husbands for lifts to their appointments and the men may not be interested or see a need for the woman to go to their appointment ... There are also issues using public transport in some cultures, for all sorts of reasons.

Interestingly, some participants also expressed their concern over how certain types of body image among males and females could have an impact on the onset of diabetes.

Dietician 6: South African women believed that it was good to be overweight because that was an indication that you had money and were not hungry. This was very important for each person's self-esteem and for the community.

Moreover, the theme that was identified by the researchers, was the hardships that the health professionals went through when working with interpreters. Some interviewees spoke about the importance of working with interpreters, but mentioned also such challenges as much more appeared to be translated by the interpreter than was asked by the practitioner; or translations that did not appear to reflect exactly what was being stated by the practitioner.

Practitioner 6: I will try and have a professional consultation but the interpreter can actually sabotage that and make the whole consultation very unprofessional; having a conversation when I am trying to explain something, interrupting me while I am talking, giving advice.

Academic 4: You need to be aware and check that the translation is correct, that the translators understand the cultural nuances of that and that is within the context of what the client was saying and not taken out of context.

To elaborate on the quote given by Academic 4, points out the notion of cultural nuances and differences and nuances of languages that have been commented on in the final report of the Survey of Asian people and health professionals in the North and West Auckland, 2001 could be brought into light. For example the report that the Asian Health Support Service produced states that an advice such as "you have to take this medicine until finished" could be very well misunderstood by the Asian patient. He/she could understand it as that he/she has to take the medicine until he/she dies (Survey of Asian people and health professionals in the North and West Auckland) [13].

For example, I will try and have a professional consultation but the interpreter can actually sabotage that and make the whole consultation very unprofessional; having conversations when I am trying to explain something; interrupting me while I am talking; giving advice; looking at people's food chart and then proceeding to point to particular foods as if they are running the consultation.

The above quotation from a dietician's interview shows that clearly there was no collaboration between the health professional and the interpreter. It also demonstrates that there is a lack of mutual understanding between the 2 parties. This clearly points out the importance of prior establishment of a professional relationship between the health professional and the interpreter to the actual clinical presentation of the client.

The most common theme that was identified was related to the education, particularly at undergraduate level being the limited learning of cultural diversity offered at tertiary education levels. The processes

of dealing with and taking cultural elements into account of CALD patients were not theoretically taught to undergraduate and postgraduate students in their training. Many participants pointed out the lack of education they received at tertiary education regarding communicating and dealing with patients from CALD backgrounds.

Under the 'theme' of not having learnt enough regarding issues dealing with cultural diversity at the tertiary level or during training, the majority of the participants (18/24) spoke about the minimal amount of cultural diversity learning during their university degree and the need to place greater emphasis on cultural issues within the university's education system.

Academic 1: I don't think the curriculum teaches a lot about cultural diversity.

Practitioner 3: I can't remember having learnt a lot about cultural diversity. In fact it was extremely limited. My experience of cultural diversity happened outside of my course, from friends. I learnt about cultural diversity when I did my work experience.

Many participants believed that education of cultural diversity could not be delivered theoretically in classes.

I would have to say that I don't think one can be taught theoretically about cultural diversity. I think that the only way to learn is to practice.

Rather it should include aspects related to:

- all cultures, not just the cultures that are 'in vogue' at the time
- diversity in terms of religion, philosophy, politics and language
- beliefs, practices, traditions, customs and interpretations
- practical examples using real life scenarios (e.g., working with an interpreter; working with a client who cannot cook certain foods because of home/community influences

DISCUSSION

The successful interaction between cultures and the effective implementation of holistic care is dependent upon health care providers having a degree of cultural awareness and sensitivity to the needs of their migrant and refugee clients. Such 'competency' is theorized as facilitating and promoting the professional's ability to collaborate effectively with the client [14-16] and therefore to provide an effective service. It includes improving practitioner/patient communication and thereby, relationships, understanding of diverse beliefs and perceptions about health and wellbeing [15]. From a social justice perspective, effective cultural health communication is equally important in ensuring fairness in healthcare access by CALD communities.

In addition, they contribute to blame allocation and labelling between health service providers and their clients. Hence, a client may be labelled as 'non-compliant' or 'difficult' for not following a professional's advice; a health service professional may be labelled as culturally incompetent or inappropriate in recommending food and lifestyle changes that are not feasible in faith-based and CALD environments or not practical for the every-day experiences of migrants and refugees as they adjust to the new country and its systems [17].

There has long been a call for healthcare providers to improve their level of knowledge about cultural differences and the different interpretations of health and illness by diverse communities [14, 18]. Effective health communication that is culturally suitable, and that is combined with diabetes prevention education, health promotion and management approaches that are tailored for migrant and refugee communities, is regarded as essential [19, 20]. Cultural diversity is said to be learnt through experience, education and training [21]. Providing training on diverse settings which cater to people from diverse cultures during the years of education to these health professionals would be beneficial and effective in attaining cultural competence. Provision of such training in diverse cultural settings during the years of training could put a good foundation to the future exposure to patients with diverse cultures by these health professionals.

Understanding or having a general idea of the culturally ascribed roles of men and women of different communities would be an important task for the health professional to facilitate optimal diabetes self-management and lifestyle change. Barriers to diabetes self-management included women not being allowed or licensed to drive, not being able to take public transport through fear or a cultural requirements, and not being given permission to talk or make eye contact with practitioners or interpreters during consultations. In some cultures, same sex practitioners were also a requirement. In others, there is a key person who cooks and shops – this person may not be the client. Such cultural differences suggested the need for practitioners to adopt a flexible approach and be sensitive to their ways and mannerisms. It requires the incorporation of searching for gender role and inclusion solutions in conjunction with the client, and/or 'researching'

particular cultural considerations that might impact on the success of the service.

Furthermore, another major challenge that these health professionals faced was their interaction with the interpreters. Viewed collectively, interpreters seemed to be deemed as a 'necessary evil'. Accurate communication was identified as being clearly important for the treatment of clients with diabetes - treatment relying largely on client participation and self-management [22]. However, these client-centred interactions are difficult when there are considerable language barriers between patients and their health care professionals [22]. This was clearly seen as challenges for the interpreters by the health professional participants. For clients with diabetes, skilled interpretation is fundamental. It provides the foundations that underpin entry to, and effectiveness of, the treatment and advice provided by a practitioner. However, finding skilled interpretation is difficult as many are not trained to translate difficult health-related concepts (e.g., prevention of complications; self-management) and the words that are associated with diabetes (the word 'diabetes' itself), its pathophysiology and treatment, and the complications that may result if a client does not self-manage appropriately. These terms are difficult to 'translate' from one English speaker to another, and many are not easily shown or demonstrated with the use of current resources such as flyers in the client's language or generic posters [22]. Adding to the complexity of accurate translation, is the need to present in proper cultural contexts.

The results from this study and those from previous studies suggest that interpreter skills for diabetes care can be improved by focussed training on how to translate specific information that ensures diabetic clients with limited English, an understanding of how to care for themselves and, how to achieve optimal clinical outcomes [22].. Similarly, it is recommended that focus be placed on the understanding that many clients have language barriers that incorporate both verbal and written language; as well as illiteracy and innumeracy in some individuals in their own tongues [23-26]. Such illiteracy and innumeracy at very basic levels makes it difficult for some clients to read and understand prescriptions, their glucometers, nutritional percentages on food packaging, etc. Therefore, interpreter's training should also include how to manage such situations of clients in diabetes education. It is clear that there has to be a prior establishment of a professional relationship and a mutual understanding between the health professional and the interpreter/ translator. This would allow flexibility for both parties when treating the client. Temple and Edwards [27] stated the importance of the complexity of the task of the interpreter in the research process. It is mentioned that the interpretation of an interview involves a set of negotiations in relation to knowledge, language and identity. The involvement of interpreters in the health the care setting was argued and was concluded that the involvement of interpreters in analysing what the client says to the health professional should be taken into consideration if the gap between health professionals and interpreters/translators is to be resolved. For the interpreters' involvement in the analysis of the client's account and their diabetes management, it is important that a relationship needs to be established amongst the 2 professionals prior to their encounter with the client.

A number of project-specific challenges limit the interpretation of the results for policy and practice. Some aspects of this project had not been undertaken at the time of analysing these transcripts. These included the collection of materials used by practitioners and academics to work with, and teach about working with, migrants and refugees; and videos of actual consultations between practitioners and migrant/refugee clients.

CONCLUSION

What is surprising from this study relating to CALD is the very limited documentation or rather a gap in literature regarding diabetes prevalence, complications, health promotion and health practitioners within the CALD communities. There is a distinct lack of cultural education and understanding from health professionals, particularly within Australia and possibly worldwide. If we are to succeed in reducing the incidence of diabetes in the CALD background communities we first need to overcome the barrier created by the lack of cultural understanding of the culturally and linguistically diverse clientele of the health professionals. To achieve this it is important that health practitioners and students engage in an education or training programs to help address and overcome the issues that are confronted when dealing with CALD background communities. Only once this education issue has been resolved, major advances will result in the reduction of diabetes in the CALD communities.

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Резиме

ДИЈАБЕТЕС ТИП 2 КАЈ ЛУЃЕ СО РАЗЛИЧНО КУЛТУРНО И ЈАЗИЧНО ПОТЕКЛО: ПЕРСПЕКТИВИ ЗА ОБУКА И ПРАКТИКА ОД ПРОФЕСИОНАЛЦИ ЗА НУТРИЦИСКА ТЕРАПИЈА И ЗА ИСХРАНА

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Апстракт

Цел: Да се истражат перспективите на нутриционистите и на специјалистите за исхрана, студентите и професорите што работат со лица со дијабетес тип 2, кои се од различно културно и јазично потекло.

Методи: Дизајн на квалитативна студија на детални полуструктурирани поединечни интервјуа со вкупно 24 учесници (8 лекари, 8 студенти и 8 професори) во областа на нутрициската терапија и диететиката. Отворените прашања беа фокусирани на перспективите и искуствата (учење, практика и настава) од работата со луѓе од различно културно и јазично потекло, кои имаат дијабетес тип 2. Сите интервјуа беа снимени за тематска и текстуална анализа.

Резултати: Меѓусебно поврзани теми, кои беа потврдени со истражувачка триангулација беа разбирање на: (i) концептите на култура и разновидност; (ii) концептите и влијанијата на здравјето, дијабетесот и храната во културите; (iii) влијанијата во и меѓу културите; и (iv) системи и ресурси. Перспективите во овие теми сугерираат фрустрација во однос на имањето доволен капацитет за целосна проценка, за изработка на ефективни, сеопфатни и високо квалитетни планови за управување и за постигнување на потребните здравствени промени во однесувањето на луѓето од различно културно и јазично потекло.

Заклучоци: Постои потреба за подобрување на додипломското образование и обука и во програмите за професионален развој; обуката и ресурсите на преведувачите во испораката на информации поврзани со здравјето и работата со здравствените работници; за фокус на културно соодветните планови за управување, кои вклучуваат консултации со клучните донесувачи на одлуки во семејствата и во заедниците; и, ревизија на системите за поддршка и ресурсите за нутрициски терапевти и диететичари во професионален развој од додипломски студии до ниво на практика.

Клучни зборови: служба за дијабетес, културна и јазична различност, различно културно и јазично потекло, диететичари, нутриционистичка терапија

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FACTORS THAT INFLUENCE THE VIROLOGICAL RESPONSE IN PATIENTS WITH CHRONIC HEPATITIS C TREATED WITH PEGYLATED INTERFERON AND RIBAVIRIN

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ABSTRACT

Introduction: The success of the antiviral treatment in patients with chronic hepatitis C depends on the factors related to the virus and the host. The aim of the study is the analysis of the antiviral therapy which is a combination of pegylated interferon and ribavirin, considering various factors that will identify the predictors of the sustained virological response. Material and Methods: This retrospective study included 226 patients, divided in two groups. Patients with sustained virological response and patients without sustained virological response were compared in terms of the following factors: genotype, viral load, gender, age, inflammatory and fibrotic changes in the liver, metabolic abnormalities, obesity and fatty liver. Results: The rate of the sustained virological response is 83.6%, more frequently in patients with genotype 3, with evidenced statistical significance (90.54%). The factors that significantly contribute to sustained virological response are related to the age ($p = 0.0001$), genotype ($p = 0.002$), mode of transmission ($p = 0.005$), inflammatory changes in the liver ($p = 0.028$), body mass index ($p = 0.022$) and insulin resistance ($p = 0.039$). The high rate of sustained virological response is related to the younger age of the patients which indirectly means short Hepatitis C Virus infection duration, absence of advanced liver disease and lack of significant co-morbid conditions. Single confirmed independent predictors of sustained virological response are the age (OR 0.928, $p = 0.0001$) and genotype (OR 3.134, $p = 0.005$). Conclusions: Factors that are related to the virological response are the age, genotype, mode of transmission, inflammatory changes in the liver, body mass index and insulin resistance, but still, independent predictors of sustained virologic response are the age and the genotype.

Keywords: Chronic viral hepatitis C, pegylated interferon and ribavirin, sustained virological response, predictors of virological response.

INTRODUCTION

Chronic hepatitis C is a chronic viral infection that persists for more than six months in more than 75% of the patients, due to the absence of spontaneous clearance of the virus after an acute infection [1]. This chronic inflammation is responsible for the development of more advanced forms of liver deterioration, resulting in cirrhosis which requires liver transplantation [2]. There is an increased occurrence rate of hepatocellular carcinoma (HCC) associated with this type of infection [3]. The prevalence of the chronic hepatitis C virus (HCV) infection worldwide is around 2.8% of the population, which means approximately 185 million people in the world are infected with this disease [4]. There are many ways of transmission of

the virus, but in Republic of Macedonia, the most common way of HCV transmission is intravenous drug abuse (62.3% of the patients), during hemodialysis treatment (32% of the patients) and the rest are related to other ways of transmission (5.7% of the patients) [5]. The severity of liver damage, as well as the success of the antiviral therapy depend on factors related to the virus (genotype and viremia) and factors related to the host [6]. The most common factors related to the host are the age, gender, genetic variations, alcohol consumption, impact of other toxins, immune status, coinfection with hepatitis B virus (HBV) or human immunodeficiency virus (HIV) infection, metabolic disorders such as increased insulin resistance (IR), obesity, abnormalities in lipid metabolism, fatty liver (steatosis), metabolic syndrome (MS), and others [7-9]. According to the data from the literature, IR is independent of visceral fat tissue, hepatic steatosis and genotype of the virus [10]. There is a negative correlation between the sustained virological response (SVR) and IR, the actually increased IR resulted into lower SVR rate, and vice versa, the higher SVR rate is directly responsible for the reduction of the IR [11]. Hepatitis C virus uses the host lipid metabolism for its own lifecycle [12]. Impaired lipid metabolism in patients with chronic hepatitis C infection is determined by the reduction of the total serum cholesterol, LDL, apolipoprotein B and increased steatosis [13]. Obesity, especially trunk thickness is directly related to the increased production of proinflammatory adipocytokine that increases the oxidative stress and weakens the biological response to the treatment regimens based on the interferon therapy [14]. The aim of this retrospective study was to analyze the success of the antiviral therapy in patients with chronic hepatitis C which comprises a combination treatment with pegylated interferon alpha (peg-IFN alpha-2a or peg-IFN alpha-2b) and ribavirin related to various factors (genotype, viral load, the degree of necroinflammatory and fibrotic changes in the liver, gender, age, presence of metabolic abnormalities such as IR, changes in the lipid and glucose profile, presence of obesity and fatty liver) and determination of the SVR predictors.

MATERIAL AND METHODS

The patients included in this retrospective study, in total 226, were patients in age over 18, with verified hepatitis virus C infection [seropositive patients for HCV, as well hepatitis C virus ribonucleic acid (HCV RNA) positivity confirmed with the polymerase chain reaction (PCR) method]. The patients were hospitalized at the University Clinic of Gastroenterohepatology in Skopje for liver biopsy (one of only two institutions in the country where patients are hospitalized for performing liver biopsy before antiviral treatment) in the period from 2009 to 2015. Most of the data was retrieved from the electronic database of the clinic, and the data related to the genotype and HCV RNA titer, were derived from the Research Centre for Genetic Engineering and Biotechnology "Georgi D. Efremov", Macedonian Academy of Sciences and Arts. The exclusion criteria for this study were: active intravenous drug addicts, positive results for other viruses (HBV or HIV), patients with end-stage renal disease, other etiologies of liver disease (autoimmune hepatitis, diagnosed Wilson's disease, hemochromatosis, patients with primary biliary cirrhosis, primary sclerosant cholangitis, α1 antitrypsin deficiency, patients with decompensate liver disease, previous liver transplantation, alcohol abuse (> 20 g/day) and hepatocellular carcinoma. The period of patients treatment depends on the virus genotype: patients with genotype 1 and 4 were treated for period of 48 weeks with pegylated interferon alpha once weekly in subcutaneous dose of 180 μg of peg-IFN α2a, and the dose of 1.5 μg / kg for peg-IFN α2b, and for period of 24 weeks for genotype 2 and 3. In addition patients were treated with ribavirinin daily dose of 1,200 mg per os, for genotype 1 and 4, and daily dose of 800 mg per os, for genotypes 2 and 3. The effectiveness of the treatment was confirmed by SVR, indicating undetectable levels of HCV RNA levels 24 weeks after the completion of the antiviral therapy. The patients who have not achieved SVR are considered as Non Virus Responders (NVR). The study was approved by the local Ethics Committee.

The treated 226 patients were analyzed for the following parameters: gender; age; genotype; mode of transmission of the virus (the patients were divided into two groups: group 1 - patients previous intravenous drugs abusers, group 2 - patients infected by the virus via other way of transmission); level of viremia; histological changes in the liver biopsy (i. Knodell scale to determine the degree of inflammation-HAI-histological activity index, graduated from 1 to 18 and ii. fibrosis- the patients are divided into three groups: group 1- no fibrosis, group 2 - fibrosis, and group 3 - liver cirrhosis); steatosis (patients were divided into three groups: group 0 - no steatosis, 1 - mild steatosis and group 2 - severe steatosis); body weight expressed as body mass index - BMI (calculated according to the formula: weight in kg/height^2 in meters); several laboratory parameters such as: transaminase (aspartate transaminase - AST, alanine aminotransferase - ALT), lipid status (Triglyceride - TG, Total Cholesterol, High-Density Lipoprotein Cholesterol - HDL-C, Low-Density Lipoprotein Cholester-

ol - LDL-C), wherein for dyslipidemia the following cut off ranges referring to the National Cholesterol Education Program Adult Treatment Panel III were determined: TG ≥150 mg/dL or ≥ 1.7 mmol/L; total cholesterol ≥ 200 mg/dL or ≥ 5.17 mmol/L, LDL-C ≥ 130 mg/dL or ≥ 3.36 mmol/L and HDL-C <40 mg/dL or <1.03 mmol/L [15], fasting blood glucose and fasting blood insulin, where Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) was calculated according to the formula: fasting insulin (μU / mL) x plasma glucose (mmol / L) / 22.5.), where the insulin resistance is confirmed when the value is ≥2. In accordance with the virological response the patients were divided into two groups: a group of patients with sustained virological response - SVR and the group of patients Non virus responders - NVR, actually patients who did not have adequate virological response. The above mentioned parameters were compared for those two groups of patients.

Statistical analysis: All data were processed using a statistical computer program SPSS 17 for Windows, where the following statistical tests were used: descriptive statistics (arithmetic mean, standard deviation, standard error, median and inter-quarter interval) for description of the numerical variables, frequencies and percentages for description of the categorical variables. For testing the difference between the numerical variables of the two groups, the Student's T test and Mann-Whitney test were used. For the statistical analysis of the categorical variables the Chi-square test was used. In order to identify the predictors of sustained virological response binary logistic regression analysis was used, by determining the value of the odds ratio (OR) and the 95% Confidence interval. For all analyzes the p value < 0.05 was considered statistically significant, and p < 0.01 was highly significant.

RESULTS

The results of our study showed domination of the male subjects (75%), aged between 18 and 66 years (mean age 33.80 ± 8.65 years). The most common way of transmission of the virus was the intravenous drug abuse found in 63% of the subjects who were former drug abusers (table 1). In our patients the dominant genotype was 3 (present in 67.3%), followed by genotype 1 (at 31.4%), while genotype 2 and 4 were very rare (at 0.9% and 0.4% respectively and due to the small number were not suitable for statistical processing). SVR was achieved in 83.6% of treated patients, and all other baseline characteristics can be seen in Table 1. When the difference of all determined parameters was tested among the group of patients with sustained virological response and the group of Non responders, the following results shown in Table 2 were obtained: SVR was achieved in 85.88% of the male population and 75.51% of female population, with no significant difference between the groups in terms of gender (p = 0.0826). The patients who did not achieve adequate virological response were older (40.2 ± 11.3) compared with the group of patients with SVR where the age was 32.5 ± 7.4, with evident significance of p=0.0001.

Table 1. Baseline Characteristics of Patients With Chronic Hepatitis C Infection

Variable	Patients N=226
Sex, % Male female	 75 25
Age, years, mean ± SD	33.80 ± 8.65
Drug abuse, % Yes No	 63 37
Genotype % Subtype 1 Subtype 2 Subtype 3 Subtype 4	 31.4 0.9 67.3 0.4
HCV viral load, mean ± SD	1804828 ± 5031173
Knodell Histology Activity Index-HAI, mean ± SD	3 ± 2
Presence of fibrosis,%: No fibrosis Fibrosis present Cirrhosis	 73.6 21.3 5.1
Steatosis, %: No steatosis Mild Severe	 55 40 5
BMI, mean ± SD	24.65 ± 4.21
SVR, % Yes No	 83.6 16.4
AST (10-34 U/L), mean ± SD	63 ± 58
ALT (10-45 U/L), mean ± SD	97 ± 86
Triglyceride (0.0-2.0 mmol/L), mean ± SD	1.26 ± 0.75
Total Cholesterol (0.0-5.5 mmol/L), mean ± SD	4.23 ± 1.14
HDL-C (0.9-2.0 mmol/L), mean ± SD	1.16 ± 0.36
LDL-C (2.2-3.7 mmol/L), mean ± SD	2.53 ± 0.97
Fasting glucose (3.6-6.5 mmol/L), mean ± SD	5.34 ± 0.96
Fasting insulin (2-17 μIU/ml), mean ± SD	12.74 ± 16.83
HOMA IR, mean ± SD	2.78 ± 3.55

Abbreviations: HCV: hepatitis C virus; BMI: body mass index; SVR: sustained virologic response; ALT: alanine aminotransferase; AST: aspartate transaminase; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; HOMA-IR: Homeostasis Model Assessment of Insulin Resistance.

Table 2. Factors Associated With Virological Response in Patients With Chronic Hepatitis C Infection

	Response to antiviral therapy		
Variable	**SVR (N=189)**	**NVRs (N=37)**	**P value**
Sex, No (%) Male female	 152 (85.88) 37 (75.51)	 25 (14.12) 12 (24.49)	0.0826 NS[1]
Age, years, mean ± SD	32.5 ±7.4	40.2 ±11.3	0.0001 S[2]
Drug abuse, No (%) Yes No	 123 (90.44) 58 (72.50)	 13 (9.56) 22 (27.50)	0.005 S[1]
Genotype No (%) Subtype 1 Subtype 2 Subtype 3 Subtype 4	 49 (71.01) 1 (50) 134 (90.54) 1 (100)	 20 (28.99) 1 (50) 14 (9.46) 0 (0.00)	0.002 S[1]
HCV viral load, mean ± SD	1894795 ± 5422907	1342136 ± 2057632	0.725 NS[3]
Liver biopsy Knodell Histology Activity Index-HAI, mean ± SD	3.176±2.272	4.258±2.828	0.028, S[3]
Presence of fibrosis, No (%): No fibrosis Fibrosis present Cirrhosis	 137 (86.16) 37 (80.43) 8 (72.73)	 22 (13.84) 9 (19.57) 3 (27.27)	0.359 NS[1]
Steatosis, No (%): No steatosis Mild Severe	 75 (82.42) 53 (81.54) 8 (88.89)	 16 (17.58) 12 (18.46) 1 (11.11)	0.863 NS[1]
BMI, mean ± SD	24.3 ±3.9	26.6 ±5.2	0.022, S[2]
AST (10-34 U/L), mean ± SD	64.9 ± 62.9	56.0 ± 24.6	0.708 NS[3]
ALT (10-45 U/L), mean ± SD	100.2 ± 91.4	81.4 ± 51.6	0.395 NS[3]
Triglyceride (0.0-2.0 mmol/L), mean ± SD	1.2 ±0.7	1.4 ±1.03	0.717 NS[3]
Total Cholesterol (0.0-5.5 mmol/L), mean ± SD	4.3 ± 1.2	4.1 ± 0.9	0.655 NS[3]
HDL-C (0.9-2.0 mmol/L), mean ± SD	1.2 ± 0.4	1.1 ± 0.3	0.711 NS[3]
LDL-C (2.2-3.7 mmol/L), mean ± SD	2.6 ± 1.0	2.4 ± 0.8	0.699 NS[3]
Fasting glucose (3.6-6.5 mmol/L), mean ± SD	5.2 ± 0.8	5.9 ± 1.5	0.02, S[3]
Fasting insulin (2-17 µIU/ml), mean ± SD	12.6 ± 17.8	13.3 ± 9.7	0.037 S[3]
HOMA-IR, mean ± SD	2.7 ± 3.7	3.1 ± 2.9	0.039 S[3]

Abbreviations: SVR: Sustained Virological Response; NVRs: Non Virus Responders; NS: not statistically significant; S: statistically significant; HCV: hepatitis C virus; BMI: body mass index; AST: aspartate transaminase; ALT: alanine aminotransferase; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; HOMA-IR: Homeostasis Model Assessment of Insulin Resistance.
[1] Pearson Chi-square
[2] T test for independent samples
[3] Mann-Whitney U Test

This evident significance was obtained in relation to the mode of virus transmission, i.e. 90.44% of patients who were former intravenous drug abusers have achieved SVR, but only 72.5% of patients who were infected via other virus transmission mode have obtained SVR, or 27.5 % were NVR (p=0.005). Patients with genotype 3 who have achieved sustained virological response (90.54%) were significantly more compared to the patients with genotype 1 (71.01%), with significant difference of p = 0.002. There was no significant difference between the two groups in terms of viral load, where the group with SVR had mean viral load ± SD = 1894795± 5422907, min-max (101 - 59159152), median (IQR) = 781164,0 (168000 - 874000) and for the group NVR, mean viral load ± SD was 1342136 ± 2057632, min-max (103-7717021), median (IQR) = 730000,0 (103460 -1032037). There was also no significant difference in the presence of fibrosis and steatosis among the groups, but the patients who have not achieved sustained virological response had higher Knodell score-HAI, with a mean value of 4.258 ± 2.828, compared to those with SVR whose mean value was 3.176 ± 2.272 (p = 0.028). The greater body weight or higher BMI is an important factor for inadequate virological response so the patients

Table 3. Binary Logistic Regression Analysis to factors contributing to an SVR in Patients With Chronic Hepatitis C Infection

factors	p - value	OR	95% CI for OR
Age	0.0001 S	0.928	0.890 – 0.967
drug users	0.087 NS	0.478	0.205-1,113
Genotype	0.005 S	3.134	1.416-6.932
Knodell HAI	0.053 NS	0.864	0.745-1.002
BMI	0.093 NS	0.909	0.813-1.016
Glicemia	0.065 NS	0.705	0.486-1.022
Insulinemia	0.974 NS	1	0.972-1.028
HOMA-IR	0.863 NS	0.989	0.873-1.121

Abbreviations: SVR: Sustained Virological Response; OR: Odds ratio; 95% CL: 95% confidence interval; S: statistically significant; NS: not statistically significant; Knodell HAI: Knodell Histology Activity Index; BMI: body mass index; HOMA-IR: Homeostasis Model Assessment of Insulin Resistance.

with SVR had a mean BMI of 24.3 ± 3.9, while the patients NVR had a BMI of 26.6 ± 5.2 or evidenced significance was for p = 0.022. Mean value of AST and ALT in the SVR group was 64.9 ± 62.9 U/L and 100.2 ± 91.4 U/L, respectively, while in the NVR group was 56.0 ± 24.6 U/L and 81.4 ± 51.6 U/L, respectively, with no statistical significance. Dyslipidemia was found in 53.8% of patients, but there was no statistical significance in relation to the dyslipidemia between the groups with SVR and those who do not achieve an adequate response. The baseline total cholesterol and its fractions HDL-C and LDL-C in SVR group was 4.3 ± 1.2 mmol/L, 1.2 ± 0.4 mmol/L and 2.6 ± 1.0 mmol/L respectively, while in the NVR group was slightly lower, 4.1 ± 0.9 mmol/L, 1.1 ± 0.3 mmol/L and 2.4 ± 0.8 mmol/L respectively, with no significant difference between groups. The triglycerides were a bit higher in the NVR group 1.4 ± 1.03 mmol / L, unlike in the SVR group were 1.2 ± 0.7 mmol / L, but here there was no statistical significance. Unlike lipids, in the glucose status there was a statistical significance of fasting blood glucose, the fasting insulin and the calculated HOMA IR were significantly higher in the group without adequate virological response (5.9 ± 1.5, 13.3 ± 9.7 and 3.1 ± 2.9 respectively), than in the group with SVR (5.2 ± 0.8, 12.6 ± 17.8, 2.7 ± 3.7 respectively) and the statistical significance was for p = 0.02, 0.037 and 0.039 accordingly. As an information, the insulin resistance was present in 43.6% of the patients. Using multivariate logistic regression analysis, as independent predictors of sustained virological response, were confirmed only the age of the patients [OR 0.928, 95% CI (0.890 - 0.967), p = 0.0001], and the genotype [OR 3.134, 95% CI (1.416 - 6.932), p = 0.005] – table 3. With every additional year of the patient's age, the chances of achieving sustained virological response is reduced by 7.2%. Patients with genotype 3 have 3.134 times (95% CI (1.416 - 6.932)) better chances to achieve sustained virological response than patients with genotype 1.

DISCUSSION

In Republic of Macedonia, as a country that belongs to the group of developing countries, still the first line treatment of patients with chronic hepatitis C is combination therapy of pegylated interferon and ribavirin. Our study showed that several factors influence the achievement of sustained virological response, such as: age, mode of virus transmission, genotype, severity of inflammatory changes in the liver, BMI and evidence of glucose abnormalities, actually insulin resistance. There are a number of studies that have made the analysis of a factors associated with SVR, highlighting the predictors of sustained virological response [16,17].

The age is mentioned in several studies as a predictive factor for achieving SVR, whereas older patients have lower rates of SVR versus younger, as confirmed in our study [18]. This factor is highlighted as an independent predictor using multivariate logistic regression analysis in our study that showed less chance for obtaining SVR for 7.2% after each patient year of age. Despite these, there are studies where age is not a negative predictive factor for sustained virological response, for example the study of Frei et al, (Swiss Hepatitis C Cohort Study Group, 2014), as well as the study of Nishikawa et al. (2012) indicating that interferon combination therapy with ribavirin does not mean inferiority in the elderly and can be safely used in patients with no severe comorbidities [19, 20].

Another factor that is highlighted in our study as important for SVR is the way of virus transmission. According to the study of Kiprijanovska et al.

(2013), in Republic of Macedonia intravenous mode of transmission of HCV through contaminated needles among drug addicts is dominating, found in 62.3% of patients with hepatitis C, where 64% of these patients are carriers of genotype 3 [5]. It is important to point out in our study that this group of patients have a very high rate of SVR, even 90.44%, principally due to a number of factors such as: age (it comes to younger patients), genotype (in this group dominates easy to treat genotype 3) and evidently less frequent metabolic abnormalities such as disorders in the glucose status which are more common in elderly population. Similar results for former drug addicts who showed a high rate of SVR is evidenced in several other studies including the study of Curelac et al. (2011) [21-24].

Our results showed a high rate of SVR, in the most represented genotypes in our country, genotype 3 and genotype 1. In 71.01% of the treated patients with difficult to treat genotype 1 SVR was obtained, and in patients with genotype 3 SVR is evidenced in 90.54%. These values are higher than in other studies where the rate of SVR for genotype 1 is between 40% and 55% and for genotype 3 about 80% [25,26]. Using multivariate logistic regression analysis, the genotype was evidenced as independent predictor of sustained virological response ($p = 0.005$), i.e. patients with genotype 3 have 3.134 times better chance to obtain sustained virological response than patients with genotype 1. This gives us the right to continue the use of this combination therapy of pegylated interferon and ribavirin, especially in patients with genotype 3, comparing the high cost of the new group of direct-acting antivirals.

Factors that affect the virological response are necroinflammatory, fibrotic and steatotic deterioration of the liver which in our study have evident significance only for inflammatory activity [27,28]. The fibrotic changes and steatosis were evidenced in more severe form in the group with inadequate virological response, but with no statistical significance.

The occurrence of obesity in recent years is a worrying phenomenon worldwide, both in developed and developing countries and it takes epidemic size as in the USA and Mexico [29]. The overweight expressed through BMI, is determined as important factor which affects the response to antiviral therapy, as confirmed in our study [30].

Lipid metabolism disorder occurs in all patients with chronic hepatitis C considering the needs of hepatitis C virus in their development cycle to use the lipids of the host [31, 12, 13, 32]. The results of our subjects showed higher values of total cholesterol, including its fractions HDL-C and LDL-C in SVR group, but no significant difference compared with the NVR group, while the level of TG was increased in the NVR group, but also with no evidenced significance in terms of the SVR group, which is also confirmed in other studies [33]. As indicated by some authors, such as Angelico et al. (2009), the higher values of total cholesterol and LDL-C before treatment, favor good therapeutic response, as confirmed in our study [34]. In contrary, the low serum cholesterol may indicate a greater liver damage, progression to fibrosis and adequately poorer response to antiviral therapy.

Changes in glucose metabolism are also often present in patients with chronic hepatitis C, most citing the insulin resistance that eventually could lead to the emergence of diabetes mellitus. Insulin resistance is mentioned in several studies as a factor associated with virological response [35-38]. In the study of Jung et al. (2014), the lower value of HOMA IR was associated with higher SVR rate, but in the group with IR where SVR was obtained, decreasing in HOMA IR after treatment was evidenced [32]. In the study of Romero-Gomez et al. (2005), the insulin resistance, severe fibrosis and genotype 1 are independent predictors of poor virological response [11]. According to the Meta-analysis of Laurito and Parise (2013) where 13 studies with 2238 participating patients were analyzed, it is indicated that IR is associated with a poor virological response, regardless of the genotype [39]. These findings of insulin resistance are confirmed in our study too. Insulin resistance is evidenced in high percentage, i.e. 43.6% of our patients with hepatitis C, but there are studies that showed a slightly higher value, as in the study of Kiran et al. (2013), where even in 51% of the analyzed patients with HCV, IR was evidenced [40]. Although, the group of NVR has a statistically higher value of HOMA IR compared with the SVR group, the multivariate analysis showed that IR cannot be considered as predictive factor for SVR.

All these factors noted as important for achieving sustained virological response will support us in future to have an individualized approach to patients with chronic hepatitis C. Particularly important group are the patients with genotype 3 who usually belong to the group of former intravenous addicted to drugs who are also at a younger age, which in our study have evidence of a high percentage of obtained SVR (something above 90%), so we can treat them with current dual antiviral therapy, a combination of pegylated interferon and ribavirin in the future. For those groups that are difficult to treat (patients with genotype 1, older patients and those who have more pronounced metabolic abnormalities) the last

generation of drugs should be taken into account, which in a country like Macedonia are extremely expensive and therefore, not affordable drugs.

CONCLUSION

Factors that influenced the virological response in our study were the age, the mode of virus transmission, genotype, inflammatory changes in the liver, body mass index and insulin resistance. The rate of sustained virological response is 83.6%, and is statistically significantly more frequent in patients with genotype 3 (90.54%). This high rate of SVR achieved with combined therapy of pegilated interferon and ribavirin is related to the younger age of the patients which indirectly means short duration of HCV infection, absence of advanced liver disease and lack of significant co-morbid conditions. Independent predictors of SVR in our study are the age and the genotype.

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Резиме

ФАКТОРИ ШТО ВЛИЈААТ НА ВИРУСОЛОШКИОТ ОДГОВОР КАЈ ПАЦИЕНТИТЕ СО ХРОНИЧЕН ХЕПАТИТИС Ц

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Вовед: Успехот на антивирусната терапија кај пациентите со хроничен хепатитис Ц зависи од фактори поврзани со вирусот и со домаќинот. Целта на студијата е анализа на успехот на антивирусната терапија со пегилиран интерферон и рибавирин во оваа група пациенти, споредено со различни фактори, кои ќе ги идентификува предикторите на стабилен вирусолошки одговор. *Материјал и методи*: Во оваа ретроспективна студија се вклучени 226 пациенти, поделени во две групи: група со и група без стабилен вирусолошки одговор, кои се споредувани во однос на следниве фактори: генотип, виремија, пол, возраст, инфламаторни и фиброзни промени на црниот дроб, метаболни нарушувања, обезитас и стеатоза на црниот дроб. *Резултати:* Стапката на стабилен вирусолошки одговор изнесува 83,6%, со сигнфикантно повисока стапка од 90,5% кај пациентите со генотип 3. Факторите што придонесуваат значително за постигнување стабилен вирусолошки одговор се поврзани со возраста (p = 0,0001), генотипот (p = 0,002), начинот на трансмисија на вирусот (p = 0,005), воспалителните промени во црниот дроб (p = 0,028), телесната маса (p = 0,022) и инсулинската резистенција (p = 0,039). Високата стапка на стабилен вирусолошки одговор се должи на помладата возраст на пациентите, што индиректно значи пократко времетраење на вирусната инфекција, отсуство на напредната црнодробна болест и недостиг на сигнификантни коморбидни состојби. Како независни предиктори на стабилен вирусолошки одговор се потврдија само возраста (OR 0,928, p = 0,0001) и генотипот (OR 3,134, p = 0,005). *Заклучок:* Фактори што влијаат на виросолошкиот одговор се возраста, генотипот, начин на трансмисија на вирусот, воспалителните промени во црниот дроб, телесната маса и инсулинската резистенција, а како независни предиктори на стабилен вирусолошки одговор се возраста и генотипот.

Клучни зборови: хроничен вирусен хепатитис Ц, пегилиран интерферон и рибавирин, стабилен вирусолошки одговор, предиктори на вирусолошки одговор

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TRIGONOCEPHALY – OUR EXPERIENCE AND TREATMENT IN THE REPUBLIC OF MACEDONIA

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ABSTRACT

Introduction: Prematurely fused metopic suture results in developmental anomaly named trigonocephaly. The treatment of trigonocephaly is a surgical reconstruction, starting from the simple suturectomy toward the complicated cranial vault reconstructions with aim to obtain enough endocranial space for normal development of the brain and aesthetic correction as well.
The aim: The aim of our paper is to present our experience on this pathology in the Republic of Macedonia, stressing the trigonocephaly as one of the rare forms of craniosynostosis.
Our material: During a period of 20 years (from 1996 to 2015) at the Pediatric department of the Clinic for Neurosurgery in Skopje, we observed 18 babies with trigonocephaly, including one with Carpenter syndrome and trigonocephaly, 14 males and 4 females. All children had simple trigonocephaly, one had syndromic trigonocephaly (Carpenter's syndrome). According to Oi and Matsumoto classification done in 1986[5] severe trigonocephaly is observed in 11 cases and, moderate trigonocephaly in 7 cases.
Our method: Our treatment consisted of slightly modified Di Rocco's[3] surgical procedure named "shell" operation, adding transposition of the "bone flap".
Results: The postoperative period was uneventful except for the expected forehead swelling. The babies were discharged from the hospital on average at the 8th postoperative day. At the three months control after the surgery, the head had excellent aesthetic appearance, with regular psychomotor development according to the age of the patient (Fig 3a and 3b). We had no serious complications except the expected postoperative swelling of the forehead. All operated children had excellent "long term" aesthetic effect and normal psychomotor development.
Conclusion: The early recognition of these anomalies including all craniosynostoses, the deformities of the newborn and infant's head and the preventive operative reconstruction would prevent abnormal disturbance of the psychomotor development during the child's growth. The multidisciplinary approach can prevent new disabled individuals in the society. Our technique allows shortening the entire surgical procedure, especially in the departments where blood saving devices are not available.

Key words: Craniosinostoses, trigonocephaly, our experience, operative reconstruction.

INTRODUCTION

Craniosynostoses represent developmental anomalies of the craniofacial growth in humans that is a premature adhesion of the sutures of the calvaria, which leads to craniostenosis, obstructing the normal psychomotor development of the infants. The consequences of the untreated craniosynostosis can be simple aesthetic disfigurations of the normal shape of the head, but also can lead to mental disruptions,

difficulties in gaining new skills, disturbed behavior, epilepsy, hydrocephalus, headaches, damaging of the cranial nerves (I, II, V, VI, VII), endocrinopathies.[6] The causes for the craniosynostoses are generally unknown, there are a lot of theories and possibilities: the teratogenic effect of the valproic acid, aminopterin, hydantoin, retinoic acid, oxymetazoline, diseases such as hyperthyroidism, rickettsiosis, thalassemia, sickle cell anemia, thyroid diseases in pregnant woman, shunt-induced after treatment of hydrocephalus, amniotic bands, mucopolysaccharidoses, genetic damages, especially of the genes FGFR1–3, NELL1, MSX2, TWIST and GLI3.[1,3,6]

The principle of formation of the craniosynostoses has been modified in dependence of the thoughts and observations of the authorities. Virchow (1851) suspected that the craniosynostosis was a primary malformation while the deformity of the cranial base is secondary; Moss (1959) concluded that the malformation of the cranial base is the essence for appearance of premature fusion of the cranial sutures on the calvaria; and Park & Powers (1920), suggested much more acceptable theory that the primary defect was located in the mesenchymal blast tissue that leaded to anomalies in the cranial vault and the cranial base.[6]

The incidence of craniosynostoses estimates approximately 0.1-1 (0.6) from 1,000 live babies.[4,6] The classification of craniosynostoses distinguishes two groups: nonsyndromic (primary, simple) craniosynostoses and syndromic craniosynostoses (conjoined with other developmental anomalies, usually on the extremities).[3,4,6] The nonsyndromic craniosynostoses are divided depending on the suture that prematurely is closed, respectively, premature of the sagittal suture creates the dolichocephaly (scaphocephaly – head with shape of a boat, the most common 56%), prematurely fused coronal suture - brachycephaly (anterior unilateral – anterior plagiocephaly, 24%, bilateral – acrocephaly, turricephaly, head in a shape of tower), prematurely fused metopic suture – trigonocephaly (wedge-shaped head, 4%), prematurely closed lambdoid suture – posterior plagiocephaly, and premature closure of all sutures gives the form of oxycephaly.[4,6] The syndromic craniosynostoses include the following syndromes: Crouzon, Apert, Pfeiffer, Saethre-Chotzen and Carpenter syndroms.[1,2,4,6]

Diagnosis of the craniosynostoses is made with physical examination of the child (inspection – characteristic shape of the cranial vault, palpation – a prominent thicken prematurely fused suture, volumetric measurements, cranial index, cranial perimeter), x-ray of the skull, EEG, computed tomography with 3D reconstructions, magnetic resonance of the brain (for possible associated anomalies of the brain).[1,2,4]

The treatment of the craniosynostoses is surgical reconstruction, starting with the simple suturectomies going further to the complex cranial vault reconstructions with aim to create enough space for normal development of the brain and the aesthetic correction of the shape of the head as well.

The timing of the surgical procedure for the best result would be the age of 3 to 7 months of the infant. If the intervention is done before the 3 months of age, there is a high rate of recurrence of the craniosynostosis with a need for additional intervention. [7]

THE AIM

The aim of this paper is presenting our experience about this pathology stressing over the trigonocephaly as one of the rare forms of craniosynostosis.

CLINICAL MATERIAL

During the period of 20 years (from 1996 to 2015) in the Pediatric department of the Clinic for Neurosurgery in Skopje, Republic of Macedonia, we observed and treated 18 infant patients with trigonocephaly, including one with Carpenter syndrome and trigonocephaly,14 males and 4 females. All children have simple trigonocephaly, one has syndromic trigonocephaly (Carpenter's syndrome with resistant lower respiratory infection, hypertonia and polydactyly with two thumbs on the right hand). The age of our patients ranged from 7 up to 14 month, an average of 9.5 months with median of 9 months. All children had pronounced trigonocephaly with cranial perimeter lower than 2 standard deviations, hypertonia has been observed in 8 cases and epileptic seizures have been observed in 3 cases. Psychomotor retardation has not been observed. The diagnosis has been confirmed with computed tomography with bone window and in some cases (mostly the latest) with computed tomography with 3D reconstructions of the skull. The measurements of the frontal angle, or of the angle between the two lines drawn through Pterion (bilaterally) and Nasion, described by Oi and Matsumoto in 1986[5], done on the axial CT slices showed a severe trigonocephaly in 11 cases and, moderate trigonocephaly in 7 cases.

SURGICAL TREATMENT

The procedure was done under general endotracheal anesthesia with the infant placed in supine position. The procedure began with bifrontal skin incision and creating of the frontal scalp flap. After

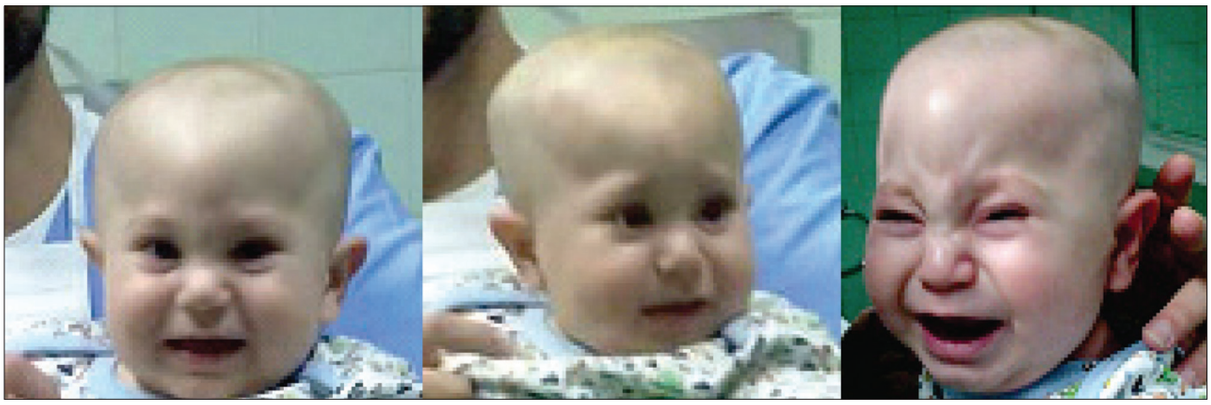

Figure 1. Preoperative trigonocephaly

the elevation of the periosteum, epidural dissection of the free edge of the frontal bone at the great

fontanel is performed, followed by the bifrontal craniotomy with one-piece free bony flap. The upper edge of the bony flap contained the coronal suture, spreading laterally downwards to the both temporal fossas. The lower edge of the bony flap has been made just over the supraorbital rims, after creating a "burr-hole" using diamond drill over the frontonasal suture where the biggest thickening of the metopic suture is observed. The bony flap has been diminished for 1 cm and rotated for 180 degrees.

The most prominent and thick part of the bone was excised, the midline of the bony flap was fractured in fashion of "green-stick" fracture and radial osteotomies were done for complete remodeling of the forehead. The bony flap was fixed forward and distal to the most front part of the cranial base through small bone holes on the free edge of the base with interrupted 2-0 silk sutures. Scalp flap closure has been done with interrupted Blair-Donatti 4-0 polypropylene sutures, without using epicranial drainage.

RESULTS

The postoperative period was uneventful except for the expected forehead swelling. The babies were discharged from the hospital in average on the 8th postoperative day. At the three months control after the surgery, the head had excellent aesthetic appearance, with regular psychomotor development according the age of the patient. (Fig. 4a and 4b). The muscular tonus was better after the reconstruction and correction of the craniostenosis; no further resistant respiratory infections and opisthotonus have been observed. We did not have serious complications except the expected postoperative swelling of the forehead. All operated children had excellent "long term" aesthetic effect and normal psychomotor development. The patient with trigonocephaly and polydactyly in the case of the Carpenter syndrome underwent a second plastic and reconstructive surgery six months after the first surgery in order to reduce the number of fingers on his right hand.

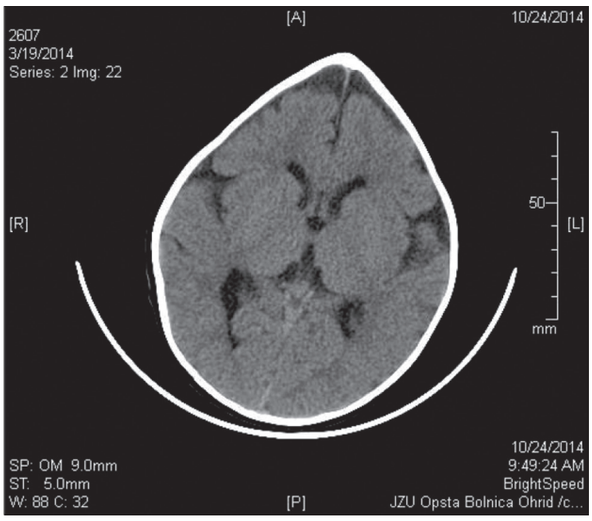

Figure 2. CT scan of the head in case with trigonocephaly

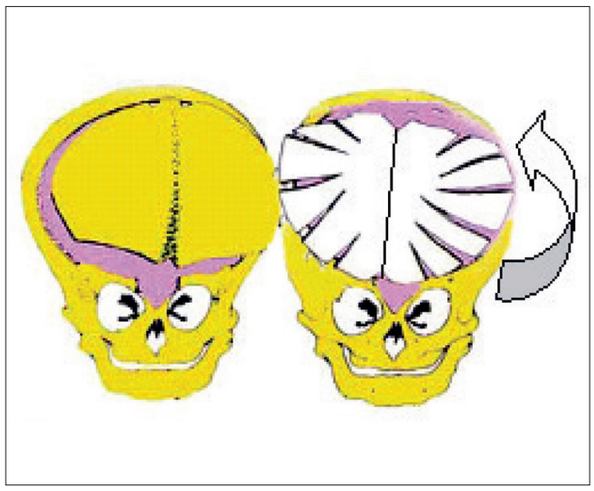

Figure 3. Reconstructed scheme of our treatment, modified Di Rocco "shell" procedure[3]

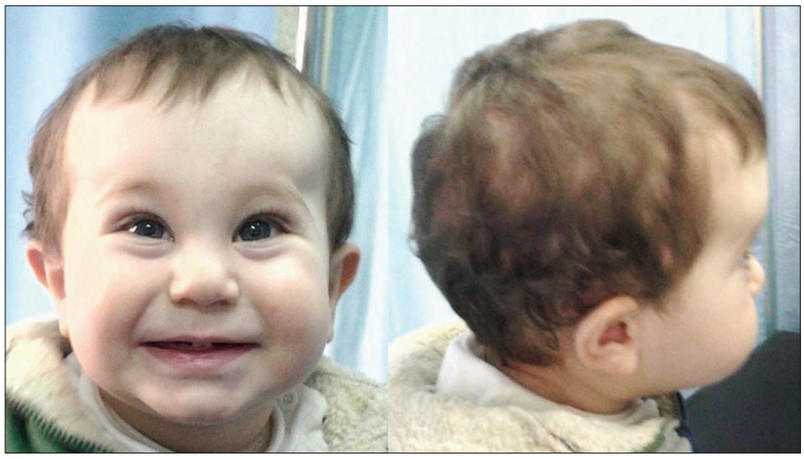

Figure 4a. The patient on Figure 1, *two months after surgery*

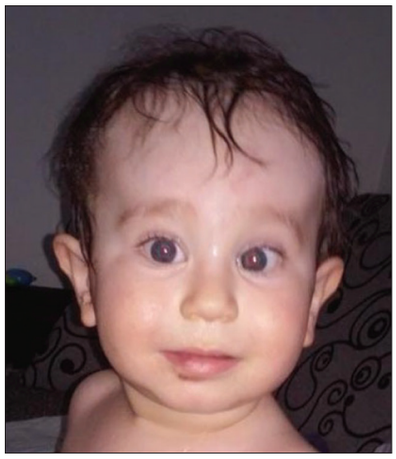

Figure 5b. The same patient *five months after surgery*

We have not observed other complications. The seizures were well controlled in 3 babies with preoperative phenobarbital treatment. The muscular tonus was better after the reconstruction and the correction of the craniostenosis, no further resistant respiratory infections and opisthotonus have been observed. We did not have serious complications except the expected postoperative swelling of the forehead. All operated children had excellent "long term" aesthetic effect and normal psychomotor development.

DISCUSSION

The treatment of the craniosynostoses is surgical reconstruction, starting with the simple suturectomies going further to the complex cranial vault reconstructions including the endoscopic minimal-invasive procedures. The alternative methods like the "conservative remodeling" with helmets are not complete reliable method for correction of these developmental diseases[1]. The eventual postoperative usage of the helmets can increase the effectiveness of the remodeling of the skull. There are a lot of surgical techniques of reconstructions or suturectomies, depending on the type and the severity of the craniosynostosis, the child's age, the association with other anomalies of the cranial base (hypotelorism, hypertelorism, midface malformations…).[1,3]

The trigonocephaly (premature fusion of the metopic suture) takes 4% of all simple non-syndromic craniosynostoses.[6] The clinical appearance is typical, with wedge-shape, triangular forehead, flattened supraorbital rims, thickened metopic suture and cranial index with normal value. The computed tomography of the brain is also typical, with flattened bilateral frontal lobes and small anterior cranial fossa. The only treatment for trigonocephaly is the surgical correction of the deformity. There are various numbers of operative interventions in which the common principle is reconstruction of the whole frontal bone, even including complex reconstructions with corrective osteotomies of the roof of the orbits and the lateral ends of the supraorbital rims for advancement and enlargement of the anterior cranial fossa.

Braid and Proctor[1] suggest that the operative correction is done between the age of 6 and 12 months of the infant for open reconstruction of the anterior part of the vault because of the associated bigger blood lost, the durability of the intervention and the high rate of recurrence if the intervention is done before the age of 6 months of the infant.

Raimondi's[7] opinion about the timing of 7 months of age with follow up do his 13 months of age, having excellent aesthetic effect and no signs of recurrence of the deformity, with normal psychomotor development. The age of our patients at the time of the intervention ranged from 7 to 14 months, an average of 13 months, with median of 9 months.

Apart from the endoscopic treatment, all open surgical procedures include bifrontal craniotomy, creation of free bony flap in one or two pieces, excision of the nasal extensions of the frontal bone and frontal extensions of the nasal bones, lateral advancement of the superior orbital ridges by pivoting on their sectioned or green-stick fractured medial edges, replacement of the frontal bony flaps after modifying their edges, curvature and orientation. The variations include the creation of a free orbital bar and its replacement after opportune remodeling, the insertion of a bone graft in the midline gap resulting from the removal of the upper part of the nasal bones to correct hypotelorism.[3]

Di Rocco's[3] personal surgical technique reaches all the mentioned goals through a procedure named

"shell" operation because of the characteristic form of the frontal bony flap. Essentially the procedure consists of a frontal craniotomy allowing to remove the deformed frontal bone and part of the parietal bones from a line 2cm above the orbital ridges to the anterior fontanel. The flap is remodeled with the drilling of the thick ridge of the metopic suture and anterior displacement of its lateral aspects. Radial osteotomies converging downwards and towards the midline (so mimicking the lines of the shell) diminish the resistance of the bone and allow to modify its curvature. The nasal processes of the frontal bone and the upper part of the nasal bones are removed. The roof and the lateral walls of the orbits are sectioned and the lateral borders of the superior orbital ridges pushed forward in order to compensate for the hypoplastic orbital cavity. The pushing maneuver is made using the medial borders of the superior orbital ridges, cracked only partially, as pivots. The advancement is maintained by replacing the remodeled frontal bone between the advanced superior orbital rims and the anterior border of the parietal bones.

In our cases, there is a modification of the classic intervention with rotation of two free bony flaps without intervention over the superior orbital ridges. The created free frontal bone flap in one piece is osteotomized anteriorly and distally just above the superior orbital ridges with drilling of the most prominent part of the metopic suture and after that it's rotated for 180 degrees with excision of the most prominent wedge part of the bone flaps. The radial converging linear osteotomies are made on the bone flap with separate green-stick fractures for further enlargement of the intracranial space. The frontal bone flap is repositioned and fixed over the superior orbital ridges with one interrupted 2-0 silk suture on both sides of the forehead. The created reconstruction makes an excellent aesthetic and functional effect especially with the enlargement of the anterior and lateral aspects of the frontal lobes of the cerebrum.

The possible side effects of the intervention are bleeding, infection of the wound, with overall incidence under 1%, possible recurrence with further need for additional surgical correction depending on the age of the patient and the type of the craniosynostosis. Our long term results show no complications, neither recurrences. Comparing our results with the results in the literature we observed no difference in the results with the classic surgical procedure for trigonocephaly. However our procedure is shorter and accompanied with less blood loss.

CONCLUSION

The early recognition of these anomalies including all craniosynostoses, the deformities of the newborn and infant's head and the preventive operative reconstruction would prevent abnormal disturbance of the psychomotor development during the child's growth. The multidisciplinary approach can prevent new disabled individuals in the society. Our technique allows shortening of the entire surgical procedure, especially in departments where blood saving devices are not available.

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Резиме

ТРИГОНОЦЕФАЛИЈА - НАШИ ИСКУСТВА И ТРЕТМАН ВО РЕПУБЛИКА МАКЕДОНИЈА

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Апстракт

Вовед: Прерано сраснатата метопична сутура резултира со развојна аномалија наречена тригоноцефалија. Третманот на тригоноцефалијата е хируршка реконструкција, почнувајќи од едноставни сутуректомии кон комплицирани реконструкции на кранијалниот свод, со цел да се добие доволно ендокранијален простор за нормален развој на мозокот и естетска корекција.

Цел: Целта на трудот е презентирање на нашите искуства во врска со оваа патологија во Република Македонија, нагласувајќи ја тригоноцефалијата како една од ретките форми на краниосиностоза.

Нашиот материјал: Во текот на 20 години (од 1996 до 2015 година) во Детскиот оддел на Клиниката за неврохирургија во Скопје опсервиравме 18 бебиња со тригоноцефалија, вклучувајќи и едно со синдром Карпентер и тригоноцефалија, 14 машки и 4 женски. Сите деца имаа едноставна тригоноцефалија, едно имаше синдромна краниосиностоза (синдром Карпентер). Според Класификацијата на Ои и Мацумото, направена во 1986 година[5], тешка trigonocephaly е забележана во 11 случаи, а умерена тригоноцефалија во 7 случаи.

Нашиот метод: Нашиот третман се состои од малку изменета Дирокова[3] хируршка процедура, наречена операција „школка“, додавајќи транспонирање на коскен резен.

Резултати: Постоперативниот период беше мирен, освен очекуваниот оток на челото. Бебињата се отпуштени од болница во просек на осмиот постоперативен ден. Три месеци по операцијата за контрола, главата имаше одличен естетски изглед, психомоторниот развој беше според возраста на пациентите(слика 3а и 3б). Немавме сериозни компликации, освен очекуваниот постоперативна оток на челото. Сите оперирани деца имаа одличен „долгорочен“ естетски ефект и нормален психомоторен развој. Оваа постапка овозможува скратување на оперативното време, посебно во институции каде што нема апарати за рециклажа на крв.

Заклучок: Со раното препознавање на овие аномалии, деформитетите на новороденчето и главата на бебето и превентивните оперативни реконструкции, ќе се спречи абнормално нарушување на психомоторниот развој за време на растот на детето. Мултидисциплинарниот приод може да ја спречи појава на нови лица со посебни потреби во општеството. Нашата техника овозможи скратување на целата хируршка процедура, особено во одделенијата каде што нема уреди за рециклажа на крв.

Клучни зборови: краниосиностоза, тригоноцефалија, наше искуство, оперативна реконструкција

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CIRCONSCRIPT SUBCUTANEOUS ARTERIOVENOUS MALFORMATION OF THE HEAD

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SUMMARY

The aim of this study is to show the various possibilities to treat this rare malformation, accentuating the results of the early surgical treatment before complications.
Material: The authors present 8 cases of patients with subcutaneous arteriovenous malformations, 5 females and 3 males (age of 7, 13, 19, 23, 27, 52 and 58 years) treated in the period of 1999 until 2015 at the Clinic for Neurosurgery and the Clinic for Plastic, Aesthetic and Reconstructive Surgery in Skopje, Republic of Macedonia. This malformation has been observed by the parents in the childhood, around the age of 3 years in all cases. Local red circonscripted nodule, soft, with manually discharging tendency and varicose dilated veins have been observed in all cases, deaf on both sides in one case, while in the older case, a cavernous sinus thrombosis caused unilateral exophthalmia, hyaline indurated ophthalmic vein, vertigo, arrhythmia, heart failure and bradypsychia have been observed. The size of the malformation has been from 2.5 to 7 cm. The diagnostics was done using CT, CT-angiography and digital angiography including external carotid angiography. Endocranial arterials peduncle was present in all cases.
Results: Six cases underwent surgery, while two cases were treated with several treatments of endovascular embolization. The follow up has been ranged from 2 to 15 years. All surgically treated patients improved without recurrence, the exophthalmia, bradypsychia and the heart problems regressed, while in patients treated with endovascular non-complete occlusion the AVM decreased, but still remained.
In conclusion: The Surgical treatment remains a first option if it is possible, and as earlier as possible, while embolization is a useful tool in cases where a complete excision is not possible.

Kew words: Subcutaneous AVM, surgery

INTRODUCTION

Subcutaneous arteriovenous malformations are known as angioma arteriovenosum cirsoides or aneurisma arteriovenosum cirsoides[1] in the literature[1]. They often appear in the subcutaneous tissue of the head and the face, rarely elsewhere on the human body. Although there are some indications in the literature for the traumatic cause whether, at birth or soon after birth, an error in the development of the blood vessels in the normal embryonic arteriovenous seals of the primitive bloodstream is rather adopted.

Therefore at that place appears a constant entry of arterial blood in the veins, which makes them dilated, soar and tortuous due to the increased pressure. The increased blood pressure in the veins causes reactive flourishing of the tissue in the walls of the venous junctions and therefore, they become increased and thicker.

CLINICAL MATERIAL

In the period of 15 year from 1999 to 2015 at the Clinic for Neurosurgery and the Clinic for Plastic, Cosmetic and Reconstructive Surgery in Skopje, Republic of Macedonia we observed and treated eight patients with subcutaneous arteriovenous malformations, five females and 3 males with different ages (...7, 13, 19, 23, 27, 52 and 58 years…) average 28.5 years, median 25 years.

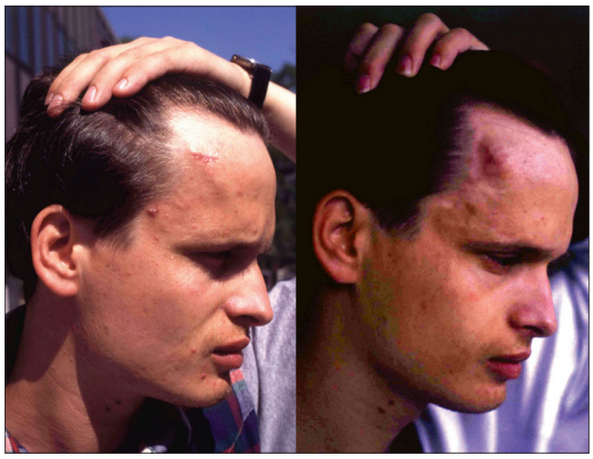

Figure 1. Subcutaneous arterio-venous malformation in right frontal region; before and eight days after the surgical excision.

Figure 2. Subcutaneous arteriovenous malformation in right frontal region;before and one month after the surgical treatment

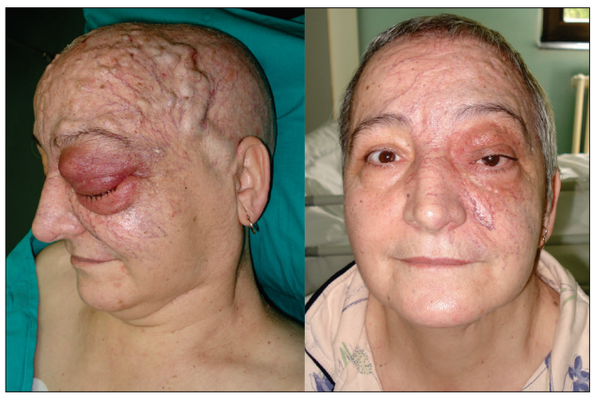

Figure 3. Subcutaneous arteriovenous malformation in the left temporoparietal region complicated with exophthalmia due to sinus thrombosis in the oldest patient; before and two months after the surgery.

The malformation is noticed in the parents in the childhood (about the 3rd year after birth), but due to insufficient knowledge of the pathology and the insufficient diagnostics the medical treatment has been delayed.

The clinical picture in all patients manifested local red circonscript island on the skin of the frontal, parietal or temporal area of the skin of the skull, soft on palpation with the tendency to diminish the volume under pressure, dilated varicose vein of the scalp and systolic noise on auscultation over the swelling. The two youngest patients have a pulsatile swelling of one temporal region, one with loss of audition because of extension to the pyramidal bone. The oldest patient has exophthalmia due to cavernous sinus thrombosis and consecutive purulent conjunctivitis, ptose, hyaline changed dilated varicose veins, arrhythmias, cardiac problems arising from chronic cardiomyopathy and bradypsyhia. The size of the arteriovenous malformation appears from 2.5 to 7 cm in diameter, all with endocranial feeders.

DIAGNOSIS

The diagnosis is set up with clinical examination, computed tomography, magnetic resonance imaging of the brain, CT-angiography and digital pan angiography, showing external carotid arteries of both sides.

TREATMENT

The arteriovenous malformation has been excised in 6 patients, while two patients have been partially treated in the Acibadem hospital with partial endovascular embolization. The oldest patient with exophthalmia has been firstly treated with adequate antibiotic therapy after antibiogram and after the remission of the infection the malformation has been excised.

RESULTS

The revascularization of the malformation in surgically treated patients is not observed. An excellent aesthetic results and complete disappearance of the varicose veins is observed in five cases. Varicose veins regressed in the oldest patient treated surgically, the exophthalmia was withdrawn, the mesh completely open, and remained only a small soft tissue in

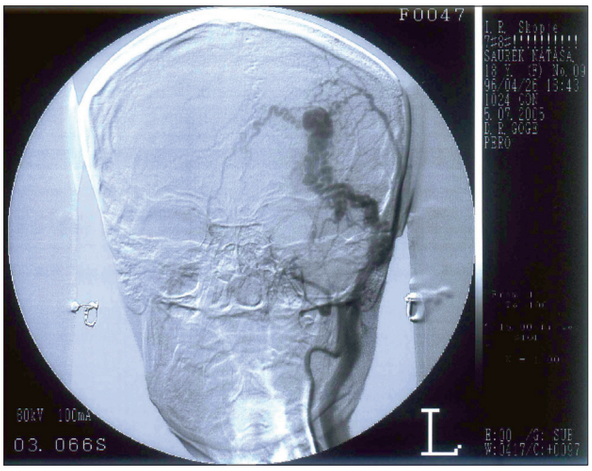

Figure 4. Digital angiography of the patient with arteriovenous malformation in the left frontal region.

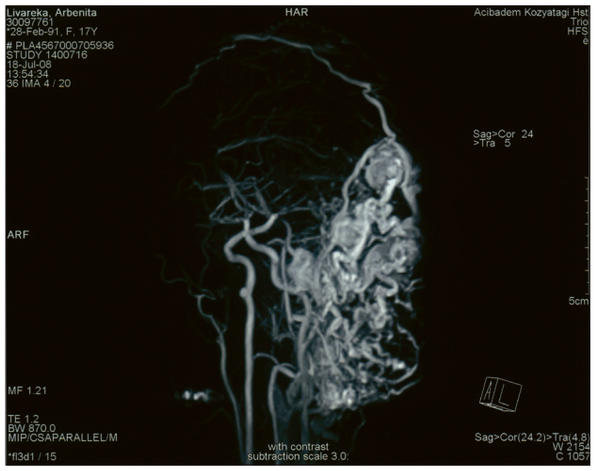

Figure 5. Digital angiography of the patient with arteriovenous malformation in the left temporal region.

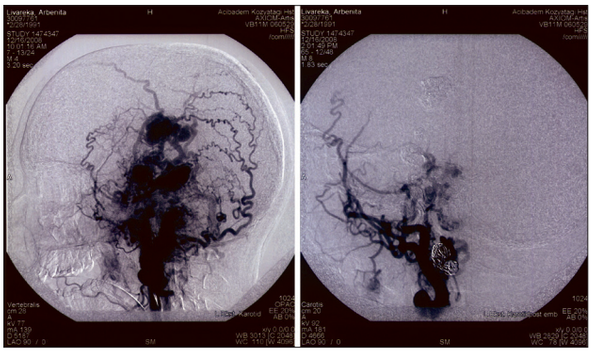

Figure 6. Digital angiography in the patient with subcutaneous arteriovenous malformations in the left temporal region before and after a partial endovascular treatment.

the upper level of the term eyelid which corresponds to the changed hyaline varicose ophthalmic vein. Furthermore, the patient changed her behavior, from depressive and slow she became dynamic, quick in reactions (according to the statement of the children "...completely different personality..."), the cardiac problems disappeared. There is amelioration in the two cases treated with partial embolization, but the malformation still persists on auscultation and angiography.

DISCUSSION

The malformation is not easy to identify because it is not a frequent one[2,6,7,8,9,10,11,12,15,16,19,20]; sometimes it could remain unknown to the specialists in plastic, aesthetic and reconstructive surgery. In the literature there are few published papers on treatment of the subcutaneous arteriovenous malformations of the sculp. According to the published papers, the surgical excision remains radical manner for treatment of these malformations. It is usually feasible when the nidus of the malformation is small and easily accessible for excision. In older age when the varicose developed veins of the scalp may cover the malformation, or when other associated complications may appear changing the surrounding skin, the modern medicine employs the methods of endovascular embolization. However, the endovascular treatment is rarely complete and may lead to incomplete occlusion of the malformation because of the many anastomoses of the blood vessels of the skin[3,18,23]. Apart from that, the endovascular treatment may lead the hair disorder in a certain part of the head causing an unpleasant aesthetic effect. Therefore, a combined surgical treatment with surgical excision of the nidus or ligature of certain arteries or branches of the artery that feed the malformation may be employed.

CONCLUSION

The early recognition and treatment of circonscript subcutaneous arteriovenous malformations of the scalp is of great importance because of the excellent results. A complete angiographic study of the head is mandatory before the treatment. The surgical treatment represents the first option in the medical treatment of these malformations, if possible, and should be realized as earlier as possible. The endovascular treatment may be applied as an additional method in the medical treatment, independent or combined with the operational treatment.

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Резиме

ЦИРКОНСКРИПТНИ ПОТКОЖНИ АРТЕРИОВЕНСКИ МАЛФОРМАЦИИ НА ГЛАВАТА

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Апстракт

Целта на овој труд е да се прикажат различните можности за третман на овие ретки малформации, ставајќи акцент на раниот третман, додека не настанат големи компликации.

Материјал: Авторите прикажуваат 8 случаи на поткожни артериовенски малформации, 5 женски и 3 машки (возраст 7, 13, 18, 19. 23, 27, 52 и 58 години) лекувани во периодот од 1999 до 2015 година на Клиниката за неврохирургија и на Клиниката за пластична, естетска и реконструктивна хирургија во Скопје, Република Македонија. Малформацијата кај пациентите била забележана од родителите во детска возраст (од три години). Болеста се манифестирала со локален црвен цирконскриптен оток на кожата на поглавината, мек на палпација, со тенденција на празнење на притисок до варикозно дилатирани вени на поглавината кај сите четири случаи, глувост на двете уши кај една пациентка, додека кај возрасната личност и со егзофталмус поради додатна тромбоза на sinus cavernosus, хијалино променети дилатирани варикозни вени, аритмии, срцеви проблеми што произлегуваат од хроничното преоптоварување на срцето и брадипсихија. Дијаметарот на малформацијата од 2,5 до 7 cm е неправилен. Дијагнозата е поставена со КТ-скен, КТ-ангиографија и дигитална панангиографија, вклучувајќи и a. carotis externa. Сите случаи имале ендокранијални артериски педункули, кои ја хранеле малформацијата.

Резултат: Третманот кај 6 случаи е изведен оперативно, со ексцизија на малформацијата, додека кај двајца пациенти таа е делумно емболизирана. Постоперативното следење на пациентите изнесува од 3 до 15 години, кај оперативно лекуваните пациенти нема рецидив, додека кај пациентката лекувана со емболизација сè уште постои васкуларна малформација.

Заклучок: Хируршкиот третман претставува прва опција во лекувањето на овие малформации, доколку е можен и што е можно порано, додека емболизацијата може да се примени како дополнителна метода во лекувањето.

Kew words: поткожен AVM, операција

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CASE REPORT

TRANSPOSITION OF SUBCLAVIAN ARTERY – IS IT THE APPROPRIATE CHOICE?

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ABSTRACT

Introduction: To present patients with symptomatic subclavian artery disease and treatment possibility.
Case report: A 53-year-old female was admitted with vertigo and left arm claudication. Duplex-scan and MSCT arteriography verified subclavian artery occlusion. After the endovascular treatment failure, the patient was switched to surgical treatment – subclavian artery transposition. After the successful surgical treatment, the patient was discharged on the second postoperative day. If the patient is a candidate for surgery, the literature review shows good initial and long-term results after the subclavian artery transposition, and emphasizes this technique as superior.
Conclusion: Subclavian carotid transposition is a safe and effective method of treatment in patients after endovascular treatment failure or other indication. Also, the surgical treatment is technically demanding because of the difficult access to the vessel origin, and it requires experienced surgeons.

Key words: subclavian artery, surgery, atherosclerosis

INTRODUCTION

Atherosclerotic steno-occlusive disease of the proximal part of the subclavian artery (SA) is an important cause of posterior circulation ischemia, upper limb ischemia, hand claudication, digital embolization, and angina in patients with a left internal thoracic artery (LITA) graft [1–8]. Also, an additional indication for treatment includes increased inflow for scheduled coronary artery bypass surgery – LITA (left internal thoracic artery) graft [9] and before the thoracic endovascular aortic repair (TEVAR) because the devices are typically placed over the left subclavian artery (LSA) and can result in spinal cord or arm ischemia.[10,11]

There are two modalities of treatment of the steno-occlusive subclavian artery (SA) disease: endovascular treatment (EVT) and open surgery. During the last four decades, a number of surgical techniques have been developed for SA reconstruction including: SA transposition, carotid-subclavian bypass and other extra-anatomic reconstruction including axillo-axillary bypass.

Even EVT have several advantages and seems superior [12], open surgery procedures are indicated in patients with long occlusions (≥4 cm) combined with severe calcification, in patients with occlusions close to the vertebral artery ostium or after EVT failure. [12]

In addition, surgical treatment is indicated in patients with arteritis, aneurysm, before scheduled TEVAR procedure to provide adequate proximal landing zone or in case of arterial injuries. In this report we present a case of a successful SA transposition after EVT failure with literature overview of the possibilities and results of SA steno-occlusive disease treatment.

CASE REPORT AND SURGICAL TECHNIQUE

A 53-year-old female was admitted with vertigo and left arm claudication. Her risk factors were smoking (≥ 20 years) and hypertension. Beside the antihypertensive therapy, levothyroxine was prescribed for several months because of myxedema. On admission, the full laboratory tests including thyroid hormones were in the normal range, with obstructive type reduction of the lung function (FEV 1 – 65%). The preoperative evaluation included extracranial carotid arteries duplex ultrasound scanning as well as subclavian and vertebral arteries and segmental pressure measurement of both upper limb arteries. A duplex scan (GE Vivid 7, GE Healthcare, Wauwatosa, WI, U.S.A.) verified a left SA occlusion with retrograde in the left vertebral artery with no significant stenosis on the carotid arteries. Multislice CT angiography (MSCT; GE Light Speed VCT64, GE Healthcare, Milwaukee, WI, USA.) showed LSA proximal occlusion (length of 20mm) (Figure 1).

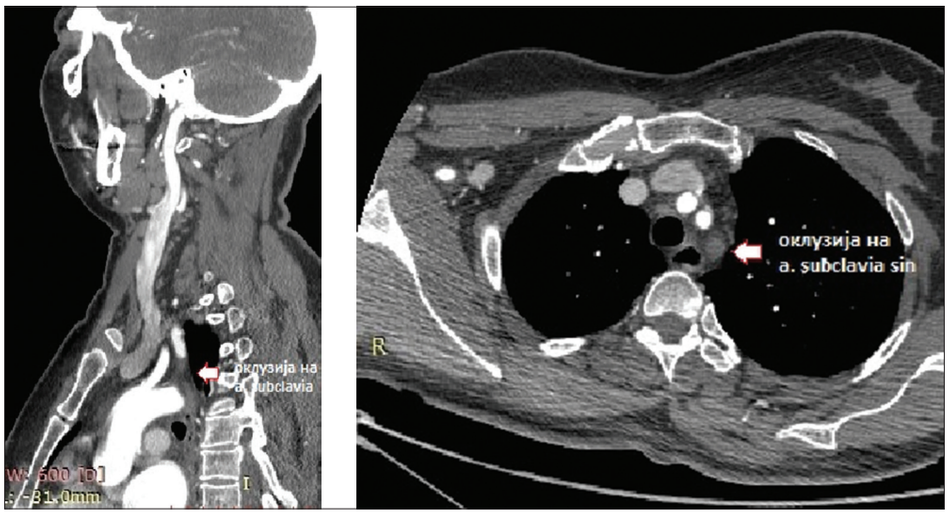

Figure 1. Occlusion of the ostial part of the left subclavian artery – white arrow;
a) sagittal projection; b) axial projection.

The indication for EVT was made by a vascular surgeon and interventional radiologist.

However, percutaneous recanalization failed in this patient using both (femoral and arm) approach due to the inability to cross the lesion with 0.014-in. and 0.035-in. guide wire and the patient was switched to subclavian transposition surgery.

Surgical technique. Via a longitudinal incision over the anterior edge of the left sternocleidomastoid muscle, which begins from incisura jugularis and ends over the anterior edge of the muscle at the level of the thyroid cartilage, we approached the deeper structures. Platysma was cut and medial to the internal jugular vein, the omohyoid muscle was cut first and the common carotid artery (CCA) was isolated for the whole of its length. At the most proximal part of CCA, the medial of the medial head of the sternocleidomastoid muscle in direction towards back, ligating thoracic duct and vertebral vein, the approach for the proximal segment of SA was made. The artery was carefully isolated especially the proximal origin segment and distally to the origin of LIMA. The vertebral artery was separately isolated. The procedure was performed under a systemic anticoagulation (heparin in doses of 100 units/kg) to have the activated clothing time between 250 and 300 seconds. After clamping and cutting of the proximal part of the SA, the blind end of the artery was suture ligated with non resorbable suture, and the distal part was positioned for the anastomosis with CCA under the angle of 90°. With a 4mm puncher, the hole at the side of the CCA was made which was extended afterwards and anastomosed with continuous suture Prolene 6-0. (USP). Flushing was made at the end of the anastomosis and normal pulses were obtained at the subclavian, vertebral, radial and ulnar artery. After the surgery, the patient had no neurological deficits and was extubated in the operating room. The patient was discharged on the second postoperative day with standard therapy (Acetil-salicid acid 100 mg/d) and normal arm/hand pulses. Two weeks later a control color duplex was made and revealed normal flow direction.

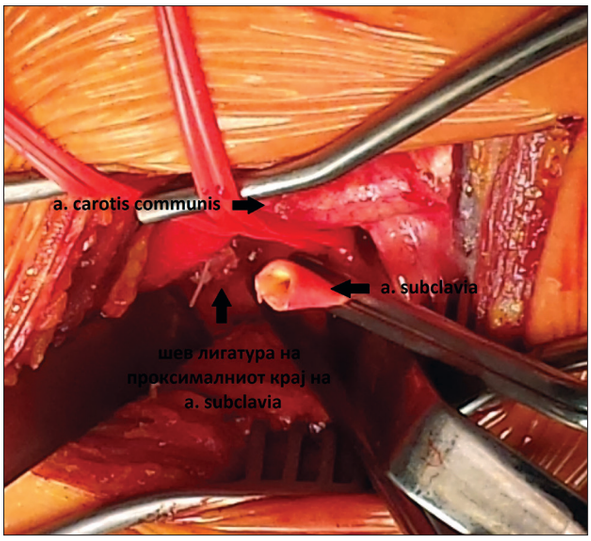

Figure 2. Operative field with longitudinal incision parallel to the medial border of the left sternocleidomastoid muscle. 1. Common carotid artery – upper arrow; 2. Resected left subclavian artery – right lower arrow; 3. Suture ligation of the proximal part of the left subclavian artery – left lower arrow.

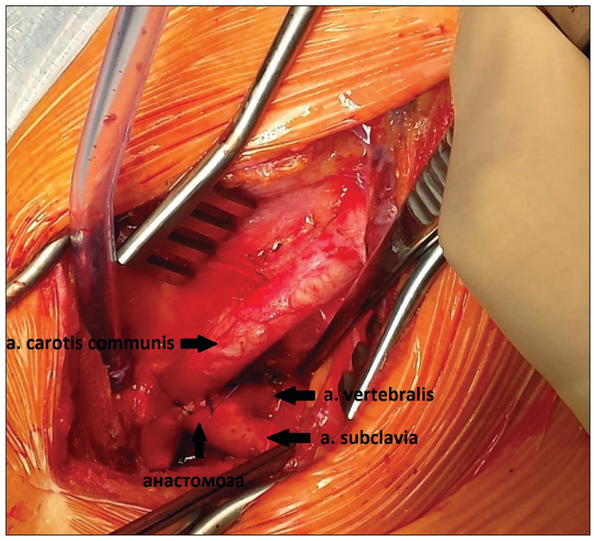

Figure 3. Transposition of the left subclavian artery to the left common carotid artery; 1) Common carotid artery – left upper arrow; 2) Left vertebral artery – right upper arrow; 3) Left subclavian artery – right lower arrow; 4) Anastomosis between the left subclavian artery and the left common carotid artery in termino-lateral fashion.

DISCUSSION

Numerous previous published studies have shown the results of surgical treatment of SA steno-occlusive disease. [13,14,15,16]

There are several surgical techniques for the treatment of SA steno-occlusive disease (including anatomical, extra-anatomical and bypass procedure); but in clinical practice, surgical treatment is technically demanding because of the difficult access to the vessel origin, and it requires experienced surgeons. In addition, the mortality rates after surgery range from 0% to 2.2%, and the risk of cranial neuropathies (such as Horner syndrome) and non-neurologic complications (lymphocele, wound infection, and pneumothorax) is 0%-12%. [13,16,17] On the other hand, the long-term results after surgical treatment vary. Extra-anatomic reconstruction including axillo-axillary bypass grafting is associated with poor long-term results. Superior surgical technique is subclavian-carotid transposition (SCT) with excellent long-term results. [16] Also, Cinà et al. [18] in their review concluded that SCT is safe and effective for reconstruction of the first segment of the SA and patency rates and clinical symptoms freedom are higher with SCT than with carotid subclavian bypass.

However, advances in endovascular techniques provide similar success rates as surgical treatment. [12] But, as it was mentioned above, there are several limitations for EVT. One on the most important is ensuring adequate proximal landing zone for TEVAR procedure to prevent posterior cerebral circulation, prevention of the arm circulation, preservation of LIMA in patients with LIMA – coronary bypass and preservation of C4 collaterals in prevention of perfusion of the spinal cord.

Apart from the advances that the transposition offers as a procedure: easier, faster, excellent patency rate, without prosthetic material, elimination of the type 2 endoleak, we think that the approach has its own role in avoidance of possible complications. This collar incision offers better and easier approach to carotid bifurcation if there is a need for carotid endarterectomy. Also, it offers comfort during performing the procedure because of the avoidance of working in the narrow and limited field (between the two heads of the sternocleidomastoid muscle). Avoidance of possible complications is another advantage because the preservation of the anterior scalene muscle where the anatomic/topographic position of the phrenic nerve is preserves the nerve from possible injury.

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Резиме

ТРАНСПОНИРАЊЕ НА СУБКЛАВИЈАЛНА АРТЕРИЈА – ДАЛИ Е ТОА СООДВЕТЕН ИЗБОР?

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Апстракт

Вовед: Да се претстават пациенти со симптоматска болест на супклавијалната артерија и можноста за третман.

Приказ на случај: 53-годишна жена беше примена со вртоглавица и со клаудикација на левата рака. Дуплекс-скенирањето и МСКТ-артериографијата ја потврдија оклузијата на супклавијалната артерија. По неуспехот на ендоваскуларниот третман, пациентот беше префрлен на хируршки третман – супклавијална артериска транспозиција. По успешниот хируршки третман, пациентот е отпуштен на вториот постоперативен ден. Ако е пациентот кандидат за операција, прегледот на литературата покажува добри почетни и долгорочни резултати на супклавијалната артерија и ја истакнува оваа техника како супериорна.

Заклучок: Транспозицијата на супклавијалната каротида е безбеден и ефикасен метод на лекување кај пациентите по неуспехот на ендоваскуларниот третманот или друга индикација. Исто така, хируршкиот третман е технички напорен, поради тешкиот пристап до потеклото на садот, и тоа бара искусни хирурзи.

Клучни зборови: супклавијална артерија, хирургија, атеросклероза

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CONGENITAL ANOMALIES OF THE KIDNEY AND URINARY TRACT IN CHILDREN BORN SMALL FOR GESTATIONAL AGE

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ABSTRACT

Introduction: Congenital anomalies of the kidney and urinary tract (CAKUT) represent several types of malformations with occurrence of 1 in about 500 live births.

Objective: Small for gestation age (SGA) may influence in prevalence of CAKUT and progression of chronic kidney disease (CKD) in children. The aim of this study was to elaborate our experiences with detected CAKUT in a cohort of SGA born children in Macedonia.

Methods: Our cohort consisted of 100 SGA born children investigated for associated congenital anomalies of urinary tract. We analyzed anthropometric and clinical birth data in children with diagnosed CAKUT and estimated the stage and time of onset of CKD by biochemical and imaging technics.

Results: We revealed 7 (7.0%) SGA born children with congenital anomalies of the urinary tract. Their mean birth weight was very low 1855 gr (-3.93 SDS) and the birth length 45.57cm (-2.17 SDS), as well. A significant growth failure with reduced weight and BMI were noticed at the time of diagnosis. A diagnosis of CAKUT in 4/7 was established in the first few months of life, but in others 3 later in early childhood. Three children revealed with unilateral kidney agenesis, 2 had hypo-dysplastic kidneys and in 2 children was found vesicoureteral reflux. Normal glomerular filtration rate was estimated in 2 children with CAKUT. Stage 2 CKD with GFR 60-90 ml/minx1.73m^2 had 3 children, 1 patient was graded in stage 3 and one child needed kidney transplantation, stage 5 CKD.

Conclusions: We presented 7 SGA born children with CAKUT. An early recognition, assessment and treatment of these anomalies might improve their quality of life.

Key words: born small for gestational age, Congenital anomalies of the kidney and urinary tract (CAKUT), birth weight, birth length, glomerular filtration rate

INTRODUCTION

An entity/acronym - Congenital anomalies of the kidney and urinary tract (CAKUT) covers wide range of renal and urinary tract anomalies with various degree of severity. These structural anomalies may be presented as complete renal agenesis, as the most severe, to renal hypodysplasia, multicystic kidney dysplasia, duplex renal collecting system, ureteropelvic junction obstruction (UPJO), megaureter, posterior urethral valves (PUV), and vesicoureteral reflux (VUR).

CAKUT might be detected in 1 of 500 live newborns. The most severe of these anomalies are fatal for 1 of 2,000 infants during the first month of life. Their incidence is higher in boys, although girls might be also affected. Predominantly they are asymptomatic and not associated with another anom-

aly. There is an evidence of their association with other malformations in syndromic disorders, as in papillorenal syndrome, Meckel–Gruber syndrome, trisomy 18, asplenia, polysplenia etc. [1, 2].

In the majority of patients with CAKUT the inheritance is autosomal dominant with incomplete penetrance, but not all of the family members have the same phenotype expression although they carry the same genetic alteration. Renal anomalies are detected in close relatives of up to 10% of CAKUT patients. Monogenic forms of CAKUT are caused by mutations in *HNF1B* and *PAX2* genes. The Mutations in *HNF1B* and *PAX2* genes that cause syndromic disorders are detected in only 5 to 10% of cases. In 7 affected Sardinian family members with CAKUT by Sanna-Cherchi et al. a heterozygous splice mutation of dual serine/threonine and tyrosine protein kinase gene (DSTYK, MIM 612666.0001) was found [3-7].

Short stature is the most common expected complication in children born SGA. But, they are also at risk for developing obesity, hypertension, insulin resistance, diabetes and cardiovascular diseases in adulthood. These children are with increased risk of renal failure and electrolyte imbalances, as well [8]. Therefore, it is more interesting to investigate the influence of SGA in children with CAKUT if both may contribute to developing the same complications.

SGA is a potential risk factor which may have an influence in increasing the prevalence of the congenital kidney and urinary tract anomalies in children and the progression of CKD, as well. The aim of this study was to elaborate our experiences with detected CAKUT in a relatively small cohort of SGA born children in Macedonia. We followed the established criteria for diagnose of CAKUT in children and their further follow up.

METHODS

The analyzed data are from a cohort of 100 children born small for gestational age. SGA is defined as birth weight and/or length or at least 2 standard deviations (SD) below the mean for gestational age (GA) [9] born at term, whether they remained short after 4 year old or achieved catch up growth. The main interest in this study are patients with diagnosed congenital disorders of urinary tract.

We investigated several variables taken from their first visit in the study. The clinical birth data was evaluated by birth length (BL), birth weight (BW), BL standard deviation score (SDS) and BW SDS. The investigated anthropometrical data were: chronological age, gender, height (centimeters), weight (kilograms) and body mass index (BMI in kg/m²). The diagnose of CAKUT was established by biochemical and imaging technics.

Glomerular filtration rate (GFR) is the most adequate measurement for estimation of the level of kidney function and the stage of kidney disease. GFR in children is stated normal if it is above 90 ml/min/1.73m^2, although with an evidence of functional, morphological or other kidney abnormality. If GFR is 60-89 ml/min/1.73 m^2 it resembles the CKD stage 2. GFR 30-59 ml/min/1.73 m^2 is estimated as CKD stage 3 and GFR 15-29 ml/min/1.73 m^2 as stage 4 and if GFR is less than 15 ml/min/1.73 m^2 that is stage 5 CKD.

CAKUT is very difficult to notice in early childhood until the clinical manifestations do not become apparent. Zhang et al., 2011 [10] recommended ultrasonography as the most preferred screening method for detecting CAKUT in children. There are several other imaging technics that might be useful for detection of these anomalies as radionuclide scanning, voiding cystourethrography (VCUG), X-ray com-

Table 1. Analyzed parameters of SGA born children with CAKUT

No	BW	BW	BL	BL	CA	Gender	H	H	W	W	GFR	PRD	BMI	BMI
	g^r	SDS	cm	SDS	y	-	cm	SDS	kg	SDS	ml/min x 1,73 m^2	-	kg/m^2	SDS
1	1300	-6,4	47	-2	12	M	123,50	-3,30	23,00	-3,51	97,2	agenesio renis	15,08	-1,41
2	1350	-6	40	-5,6	0,1	F	41,00	-6,71	1,85	-7,85	9	agenesio	11,00	-3,76
3	2370	-2,7	47	-1,6	5,2	F	113,00	0,94	25,00	1,98	101,2	agenesio	19,60	2,18
4	1590	-3,7	45	-1,7	4,3	M	92,00	-3,10	12,00	-3,38	88	hypodysplasio	14,81	-0,66
5	2300	-2,5	47	-1,3	0,3	F	59,00	-0,48	4,60	-2,75	61	multicistic dysplastic	13,21	-3,15
6	2130	-3,2	46	-2,1	0,2	M	56,00	-0,90	4,56	-1,57	81,1	VURgr2	14,54	-0,90
7	1950	-3	47	-0,9	0	F	47,00	-3,00	2,00	-4,00	47,2	VUR3	9,05	-4,33
Average	1.855,71	-3,93	45,57	-2,17	3,18		75,93	-2,36	10,43	-3,01	69,24	-	13,90	-1,72

BW-birth weight, BL-birth length, CA-chronological age, H-height, W-weight, GFR-glomerular filtration rate,
PRD-primary renal disease, BMI-body mass index, SDS-standard deviation score

puted tomography, CT scan and magnetic resonance urography.

RESULTS

An analysis performed in a cohort of 100 patients born small for gestational age revealed 7 (7.0%) children with inborn anomalies of urinary tract. Of these 7 children, 4 were girls and 3 were boys.

An estimated clinical birth data showed that the mean birth weight of all children born with primary renal anomalies was low, 1,855 gr or (-3.93 SDS), whilst their birth length was reduced as well, 45.57 cm or (-2.17 SDS), but less than weight (Table 1).

The anthropometrical parameters of these 7 SGA born children during their first visit and diagnosis establishment showed an evidence of growth failure. The average height was reduced in the whole cohort (-2.36 SDS). The short stature was most evident in children born with weight less than 2,000 grams and in children with very low birth weight (VLBW), less than 1,500 grams. The mean weight in all 7 children was even more reduced than height, -3.01 SDS at the time of diagnosis. Their BMI of 13.9 kg/m² (-1.72 SDS) was very low, also.

The diagnosis of CKD in more than half of them, 4/7 children, was established in the first few months of life, and in the other 3 later in the early childhood.

Two children with primary renal disease were diagnosed with normal GFR. Stage 2 CKD had 3 children, one patient was graded in stage 3, one child needed kidney transplantation with GFR 9 ml/min/1.73 m^2, resembling stage 5 CKD.

The performed imaging diagnostic technics revealed three patients with agenesis of one kidney, 2 children had dysplastic kidneys and 2 children were found to have vesicoureteral reflux.

DISCUSSION

Nephrogenesis finishes around the 36 week of gestation. But, if a child is born very premature or with intrauterine growth retardation (IUGR) it is more likely to develop a renal disease due to the reduced nephron number. It was explained, according to Brenner's hyper filtration theory that the reduced nephron number leads to decreased glomerular filtration surface area and, consecutively, to glomerular and systemic hypertension. Hypertension has major influence on the glomerular damage and its sclerosis and on the ending with decreased renal function [11]. Microalbuminuria is the first warning sign for forthcoming impairment of GFR and serious renal disease. Keijser-Veen MG et al., in 2005 presented a study in young adults older than 20 years, born IUGR/SGA, with mainly normal renal function. They found in these patients more often episodes of hypertension and/or microalbuminuria and lower GFR than in patients born with an appropriate gestation age (AGA). These authors suggested that SGA leads to renal failure in adulthood [12].

There are several studies which implicate the influence of the urinary tract anomalies on the birth weight and length and the growth spurt during childhood. Karlberg et al. found that all detected patients with CKD were born with reduced BW and BL SDS by 1 SDS [13]. Franke et al. in their study showed significantly elevated rate of SGA and prematurity in children with congenital CKD [14]. Zivicnjak et al. followed up the growth pattern in boys with CKD. Their growth failure was most evident in the early childhood especially during the first year of life. They did not achieve catch up growth, but it speeded up before puberty, slightly slowed down during puberty, and finally, the late speed up was found only until early adulthood [15].

In our cohort of 100 SGA born children we found 7 patients with congenital anomalies of the urinary tract. The estimated BW of these 7 children was low (-3.93 SDS) or 1,855gr with less reduced BL (-2.17 SDS), which correlates with data from other studies. It is of note that the growth was seriously affected in our children with CAKUT especially in those with very low BW, less than 1,500 gr. It implicates further investigations and treatment. Growth hormone (GH) treatment is useful in short children with CKD. It improves their growth velocity and the final height [16]. We started with GH treatment in 2 short boys older than 4 years and we will follow their growth achievements.

Approximately 30% of all prenatally detected congenital anomalies are diagnosed as CAKUT [17]. The most often noticed anomalies are renal hypoplasia, dysplasia, obstructive uropathy and other kidney congenital impairments in patients with chronic kidney disease [18]. It is still unclear why these children are born IUGR or SGA. Mutations, in particular developmental genes were thought to play a major role in the growth reduction and the structural anomalies of the kidneys, other organs and skeleton, as well [19, 20]. Genetic alterations have been suspects for SGA born children, also. It is of interest to investigate in future, if the same factors responsible for the growth of organs and skeleton influenced the fetal kidney development [21].

The diagnose of CAKUT was established in the majority of our patients (4 out of 7) very early

in childhood, in the first 6 months of life. In other 2 children at the age of 5 and 12 years, respectively. The absence of one kidney is the most common detected congenital malformation in our cohort of SGA born patients. It was noticed in three children. Unilateral renal hypo-dysplasia with or without cystic formations was found in 2 patients as vesicoureteral reflux as well. VUR in one patient was staged as grade 2, but in other as stage 3. An estimated normal GFR was detected in 2 children with unilateral kidney agenesis. Stage 2 of CKD had 2 children with hypo-dysplastic kidneys and a boy with VUR. CKD staged third grade had a girl with VUR. Data from other studies revealed more than one third of patients with CAKUT need surgical treatment. Only one patient of our study 1/7 underwent kidney transplantation due to renal failure.

Our further goal is to investigate if any of our patients with CAKUT have known genetic alteration regarding that or being born small for gestational age.

CONCLUSION

Children born small for gestational age have an increased risk of morbidity and mortality in adulthood due to the development of several different diseases. Their earlier recognition and treatment lead to better life quality and longevity.

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Резиме

ВРОДЕНИ АНОМАЛИИ НА БУБРЕЗИТЕ И НА УРИНАРНИОТ ТРАКТ КАЈ ДЕЦАТА РОДЕНИ МАЛИ ЗА ВОЗРАСТА

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Апстракт

Вовед: Вродените аномалии на бубрезите и уринарниот тракт (CAKUT) претставуваат неколку видови аномалии што се јавуваат кај 1 од околу 500 живородени.

Цел на трудот и хипотеза: Раѓањето мал за возраста може да влијае на преваленцата на CAKUT-от и прогресијата на хроничната бубрежна болест кај децата. Целта на оваа студија е да ги елаборира нашите искуства во откривањето на CAKUT-от кај децата родени мали за возраста.

Методи: Нашата кохорта се состои од 100 SGA родени деца, кои беа испитувани за асоцирани вродени аномалии на урогениталниот тракт. Ние ги анализиравме антропометриските и клиничките податоци на SGA-родените деца кај кои е поставена дијагнозата CAKUT од породилните картони и го проценивме стадиумот и времето на настанок на хроничната бубрежна болест (ХББ) со помош на биохемиски анализи и имиџинг-техники.

Резултати: Откривме 7 (7%) SGA-родени деца со вродени аномалии на уринарниот тракт. Нивната средна породилна тежина беше многу ниска – 1855 гр (-3,93 СДС), како и породилната должина – 45,5 цм (-2,17 СДС). Во моментот на поставувањето на дијагнозата забележавме значаен неуспех во растот, на дофат со намалена телесна тежина и индексот на телесната маса. Дијагнозата CAKUT кај 4/7 од децата беше поставена во првите неколку месеци на животот, а кај другите три подоцна во текот на раното детство. Кај три деца беше најдена агенезија на едниот бубрег, две имаа хиподиспластични бубрези, а кај две деца беше откриен везикоуретрален рефлукс. Кај два деца со CAKUT беше проценета нормална гломеруларна филтрација. Стадиум 2 ХББ со ГФР 60-90 ml/min/1,73 m^2 имаа три деца, еден пациент беше класифициран во трет стадиум и на едно дете му беше потребна трансплантација на бубрег, стадиум 5 ХББ.

Заклучоци: Прикажани се 7 SGA-родени деца со CAKUT. Раното препознавање, проценката и лечењето на овие аномалии може да го подобри нивниот квалитет на живот.

Клучни зборови: родени мали за возраста, вродени мани на бубрезите и на уринарниот тракт, породилна тежина, породилна должина, стапка на гломеруларна филтрација

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CONGENITAL ANOMALIES OF THE KIDNEY AND URINARY TRACT (CAKUT)

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ABSTRACT

Congenital anomalies of the kidneys and urinary tract (CAKUT) are found in 3-6 out of 1.000 of the newborns, or according to some statistics they are represented in 0.5% of all pregnancies. Congenital abnormalities of the kidneys and urinary tract present a family of diseases of various anatomic spectrum, including renal anomalies, and anomalies of the bladder and urethra. The study was retrospective-prospective which means that it included newly diagnosed patients suffering from CAKUT, as well as those patients with already diagnosed and well defined CAKUT on the basis of imaging studies which have been processed according to the protocol for this study.

Key words: Congenital anomalies, kidney, urinary tract, familial screening, outcome.

INTRODUCTION

This paper is motivated by the awareness that so far, in Macedonia there has not been major or serious studies prepared in relation to congenital kidney and urinary tract anomalies. The standard techniques used to examine the kidneys and the urinary tract are: echosonography examination, intravenous pyelography, micturition cystography, diuretic scintigraphy, cortical scintigraphy (Tc-99 DMSA scan), direct radionuclear cystography, and in selected cases computerized tomography and nuclear magnetic resonance.

Factors for development of CAKUT. The first factor is the obstruction of the lower urinary tract in the fetal kidney. If the obstruction is present in the early stadium of the renal morphogenesis at a high degree, the kidney will be dysplastic or aplastic. All these structural modifications might be result of genetic modifications, yet, little is known of the molecular pathogenesis of these anomalies (1).

The second group of factors are the abnormalities caused by genes in the kidney developing (2,3).

The third factor are the substances such as medicines or environmental factors, which are transmitted from the mother to the fetus through the placenta and affect the development of the fetal kidney. Most common substances are those having inhibitory effect of renin-angiotensin- aldosterone system (4,5).

The aim of this study was to analyze the clinical, genetic and prognostic aspects of the congenital anomaly of the kidneys and the urinary tract (CAKUT) in a series of 749 pediatric patients diagnosed at the Pediatric Clinic in the period from 2010 until 2015.

METHODS

Echosonography is widely used for diagnostics of certain organs and organ systems. Ultrasonography is used for early detection of renal agenesis, renal hypodysplasia, multicystic kidneys, diagnosis of the urinary tract obstruction. It is possible to evaluate the size of the kidney, position, anatomy, anomalies, volume and function of the bladder.

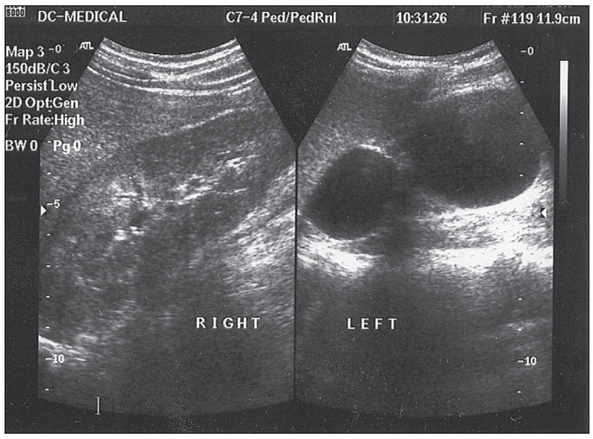


Figure 1. Left cystic dysplastic kidney

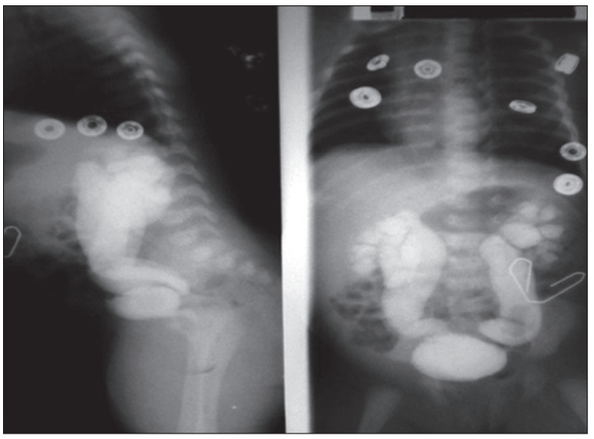


Figure 2. Bilateral severe vesicoureteral reflux

Echosonography examination is necessary in all newborns in which a bilateral serious fetal hydronephrosis is prenatally detected (6,7).

Radiological investigations: Voiding cystourethrography is an examination method by which after retrograde introducing of a contrast agent into the bladder, screening is done during micturition. Voiding cystourethrography is most often done in children suffering from urinary infections in order to confirm or exclude the existence of a vesicoureteral reflux (8).

Radioisotope investigations

^{99m}Tc - Dimercaptosuccinic acid (DMSA) renal scintigraphy

DMSA is an investigation which shows the size, shape and position of a functional renal parenchyma. The lack of a radioisotope in the normal renal position means a diagnosis of a multicystic renal dysplasia or renal agenesis, or in certain cases a unilateral renal hypoplasia.

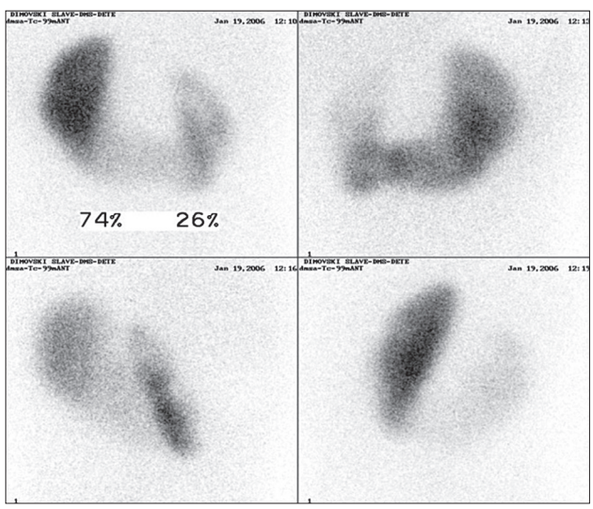


Figure 3. Tc^{99}m DMSA scan shows arcuate kidney

^{99m}Tc - Diethylenetriamine pentaacetic acid (DTPA) renoscintigraphy

DTPA is a noninvasive method for differentiation between a hydronephrosis and hydroureteronephrosis of obstructive type where a surgical correction is needed.

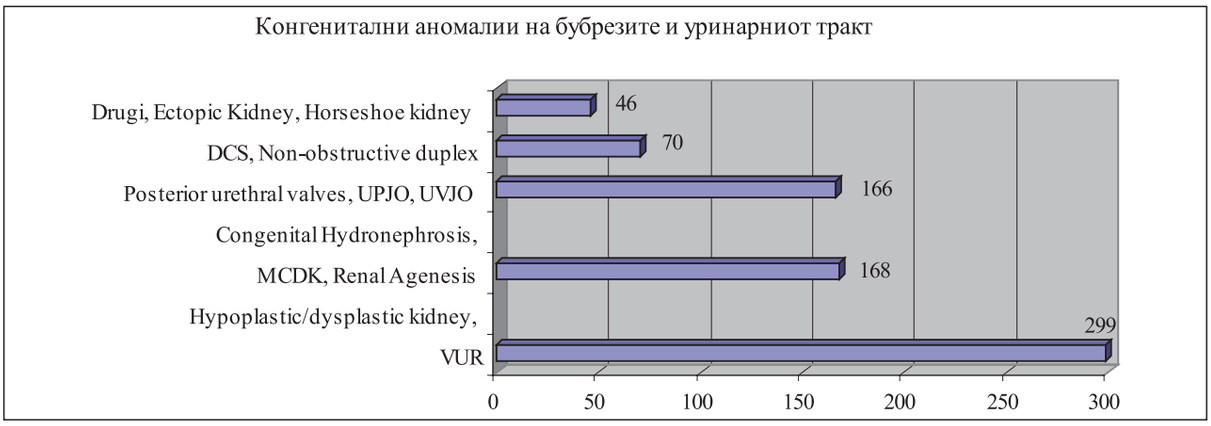
MR Urography (magnetic resonance urography). MR urography is performed with application over gadopentetate dimeglumine – DTPA. MRU is effective in investigation of pediatric uropathological conditions and in the investigating of the congenital anomalies. It provides morphological and functional information (9).

RESULTS

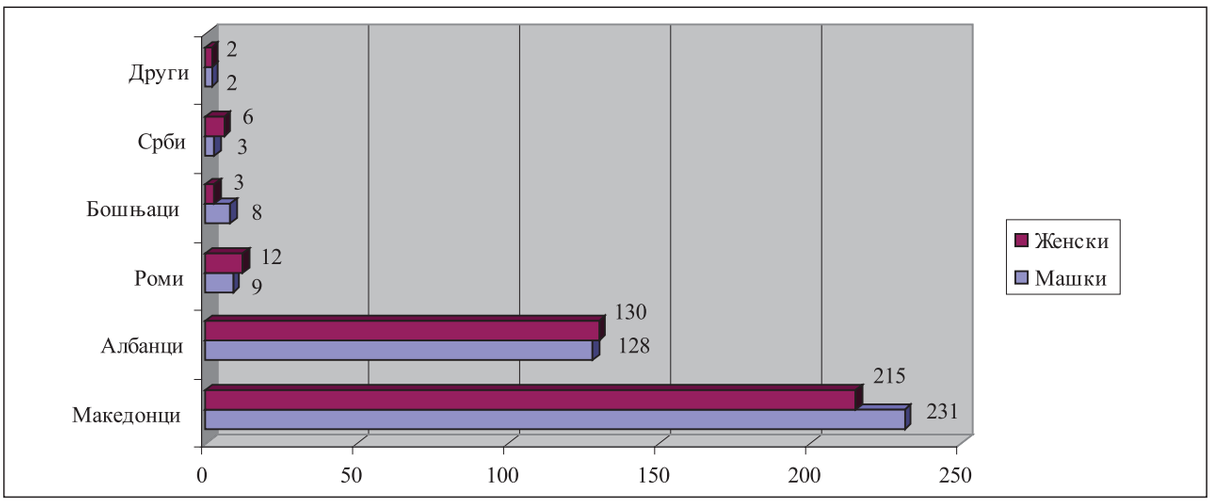
In our series 25% of CAKUT has been detected by prenatal ultrasound screening.

A positive familial history has been found in 12.42% of the patients where no significant statistical difference has been identified in relation to the ethnicity and the type of the malformation. The most common malformation in our series is vesicoureteral reflux (VUR – 39.92%).

- in the whole series of patients, 1/4 of the children had extra renal malformations (in other organs),
- they were least present in children with VUR (16.7%),
- they were most present in children with ectopic kidney and fusion (43.4%),
- ultrasound familial screening was performed in 620 index patients, where a total of 1,614 relatives were tested,
- totally detected families with CAKUT make 10.9%,



(Congenital anomalies of the kidneys and the urinary tract)



(Other–Serbs–Bosniaks–Roma–Albanians–Macedonians) (Male – Female)

- the follow-up of the patients of our series lasted 5-6 years, where the terminal uremia was achieved in 2.1% of the patients.

CONCLUSION

In general, we can conclude that the prognosis of the pediatric CAKUT in Macedonia is excellent. Adverse prognosis is associated with the existence of obstructive anomalies (valvula of the posterior urethra), and bilateral affection (hypo dysplasia, VUR). The favorable results obtained from this study are due to the high non-selectiveness of our series.

With this study we created a database of patients with syndromic and non-syndromic CAKUT, we identified an unambiguous existence of the genetic factor through the familial ultrasound screening and the existence of extra renal abnormalities, thus enabling participation in future multicentric studies.

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Резиме

ВРОДЕНИ АНОМАЛИИ НА БУБРЕЗИТЕ И НА УРИНАРНИОТ ТРАКТ (CAKUT)

Надица Ристоска-Бојковска

Клиничка болница Ацибадем Систина Скопје, Македонија

Апстракт

Конгениталните аномалии на бубрезите и на уринарниот тракт (CAKUT) се присутни кај 3–6 од 1000 новородени или, според некои статистики, присутни се кај 0,5% од сите бремености. Конгениталните абнормалности на бубрезите и на уринарниот тракт претставуваат фамилија заболувања со различен анатомски спектрум, вклучувајќи ги аномалиите на бубрегот, аномалиите на бешиката и на уретрата. Студијата беше ретроспективно-проспективна, што значи дека беа вклучени новодијагностицирани пациенти со CAKUT, како и оние пациенти кај кои е веќе дијагностициран и добро дефиниран CAKUT врз база на имиџинг-студии и тие се обработени по протоколот за оваа студија.

Клучни зборови: конгенитални аномалии, бубрези, уринарен тракт, фамилијарен скрининг, исход

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CORRELATION BETWEEN MICROVESSEL DENSITY AND MORPHOLOGICAL FEATURES IN SKIN SQUAMOUS CELL CARCINOMA

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ABSTRACT

Introduction. Abnormal angiogenesis is described in tumor growth and it facilitates its metastatic spread. Tumors with high angiogenic activity belong to the category of aggressive tumors with poor prognosis for patients.
The aim of this study was to determine the blood vessels density (BVD), i.e. neovascularization at the tumor invasive front in skin squamous cell carcinoma (SCC) in order to determine its possible role in the tumor progression, and to correlate it to the blood vessels density of healthy skin and with the prognostic parameters of the TNM classification: T status, depth of tumor invasion (DI) and tumor histological grade (G), which were also correlated between each other.
Material and Methods. The material consisted of surgical specimens obtained from 30 patients with skin SCC, who underwent surgery.
Tissue samples were routinely processed by standard paraffin technique stained by Hematoxilin-Eosin and immunohistochemically with antibodies against smooth muscle actin (SMA) and CD34. The BVD in the invasive front of the neoplasms was correlated to the healthy skin, tumor status (pT), depth of invasion and grade of histological differentiation (pG).
Results. The histological analysis has shown a high statistical difference in the density of blood vessels in SCC compared to the healthy skin and statistical difference in BVD in neoplasms with different depth of invasion and different grade of differentiation. The density of neovascularzation increased with the deeper invasion and the worse differentiation.
Conclusion. The increased vascularization at the invasive front of SCC with deeper invasion and worse differentiation has pointed out to its possible role in neoplasm progression.

Key words: squamous cell carcinoma, immunohistochemistry, CD34, density of neovascularization, invasive front, histological differentiation

INTRODUCTION

Angiogenesis is a process of formation of new blood vessels from the preexisting normal capillaries in the tissue and occurs in physiological and pathological conditions. Abnormal angiogenesis is described in malignant neoplasms and it is considered that this angiogenesis sustains tumor growth and facilitates metastasis. When the metabolic needs of the tumor increase, the tumor gets an angiogenetic phenotype and begins to recruit blood vessels

from the surrounding stroma. Neovascularization is crucial for the tumor growth and maintenance and removal of the degrading products of the metabolism. It is thought that tumors with a larger quantity of blood vessels are angiogenetic. Tumors with high angiogenic activity belong to the category of very aggressive tumors with poor prognosis for the patients [1,2,3,4].

The first mechanism of tumor vascularization was named vascularization by sprouting [5,6], a process that includes endothelial cells proliferation and migration from the existing blood vessels as well as organizing in tubular vascular structures. Later, some other mechanisms of tumor vascularization were discovered and named as: angioblast recruiting, co-opting vessels, vasculogenic mimicry and mosaic vessels [7, 8].

The new contemporary data of vascular biology identify some key factors that control the vascular growth and are included in the hypothesis suggesting that there is vascular inactivity in the normal tissues due to the dominant influence of endogenous angiogenic inhibitors despite the angiogenic stimulators.

In situations of disturbed balance when the secretion of angiogenic stimulators is increased and the regulation of endogenous angiogenic inhibitors is decreased, preconditions for tumor angiongenesis are developed [6,7,9].

Numerous studies suggest a relationship between angiogenesis, or a vascular network density of the neoplasm and its progression [4,10].

Preventing tumor growth by preventing tumor angiogenesis in the treatment of vascular and solid tumors of the skin such as SCC is a process with great potential in the treatment of neoplasms [11].

There are very few studies on angiogenesis in cutaneous SCC. From the literature focused on this field, we have found data pointing to an increased microvascular density during tumor progression [12], increased peritumoral microvascular density during neoplasm progression [13] or a significantly increased microvasculature in invasive SCC compared to the superficially invasive SCC and solar keratosis [12].

The purpose of this study is to show neovascularization in the invasive front of planocelular carcinoma of the skin to determine its density and to correlate it with the density of the blood vessels in healthy skin, the T status of the neoplasm, depth of invasion and the degree of histological differentiation of the tumor, and to determine the correlation of the individual parameters among themselves in order to determine the possible role of the microvascular density in the progression of SCC.

The aim of our study was to visualize the neovascularity and to determine the density of blood vessels at the invasive front of tumor stroma in skin SCC in relation to the healthy surrounding skin. Whether the change in the density of the neovascularization depends on the degree of histological differentiation of a neoplasm, whether there is influence of tumor stage and what kind of correlation there is between the blood vessels density and the depth of carcinoma invasion are questions we are going to try to give an answer to.

MATERIALS AND METHODS

This retrospective study included surgical specimens obtained from 30 patients with skin SCC, operated at the University Clinic of Plastic and Reconstructive Surgery and at the University Clinic of Maxillofacial Surgery in Skopje. The histological analysis of the surgical specimens was done at the Institute of Pathology, Medical Faculty in Skopje and at the Institute of Histology and Embryology, Faculty of Medicine, Skopje.

Archival materials were used in this study including: paraffin blocks, histopathological specimens and histopathological reports. Patients were grouped by sex, age and location of the neoplasm. Paraffin blocks were additionally used for making new histopathological sections from the carcinoma segment as well as from the resection margins of the surgical material, which were used as control groups for each patient separately. The sections taken for histopathological analysis were routinely examined and stained with hematoxylin-eosin. In addition, there was a special immunohistochemical staining using specific primary monoclonal antibodies against smooth muscle actin: (smooth muscle actin; αSMA (Dako /Clone 1A4/ Code M0851, dilution 1:100) и CD 34 (Dako /Clone QBEnd-10/Code M7165, dilution 1:50) using Avidin-Biotin Immunoperoxydase Complex technique. For the visualization of the antigen-antibody reaction, LSAB and En-Vision kits from DAKO were used.

In the slides stained with antibody against CD34 at a low magnification (10 x 4) certain areas of the greatest vascular density (hot spots) in the invasive front of the neoplasm were identified, while at a high magnification of x400 (Fig. 4) the well-formed vascular channels were counted, with a clearly differentiated lumen, along the invasive front, in a set of 10 visual fields. Immunostaining with smooth muscle actin (SMA) was used in order to prove the presence of non-capillary blood vessels that contain smooth muscle cells in their wall as well as to visu-

alize blood vessels in the normal dermis serving as a control for staining with CD34. The procedure for density determination was identical with that applied in staining with CD34 (Fig. 1, 2, 3).

The depth of stromal invasion in each case is measured by software for histomorphometry LUCA M on the microscope Olimpus BX-41. The distance from the basement membrane of the epidermis to the deepest invasive beach front is accounted for and measured. The resulting values are absolute numbers expressed in micro meters (μm).

The grade of histological diferentiation (G) and tumor local growth/status (pT) were taken from pre-existing histopathological reports. [14], (Fig. 4, 5, 6).

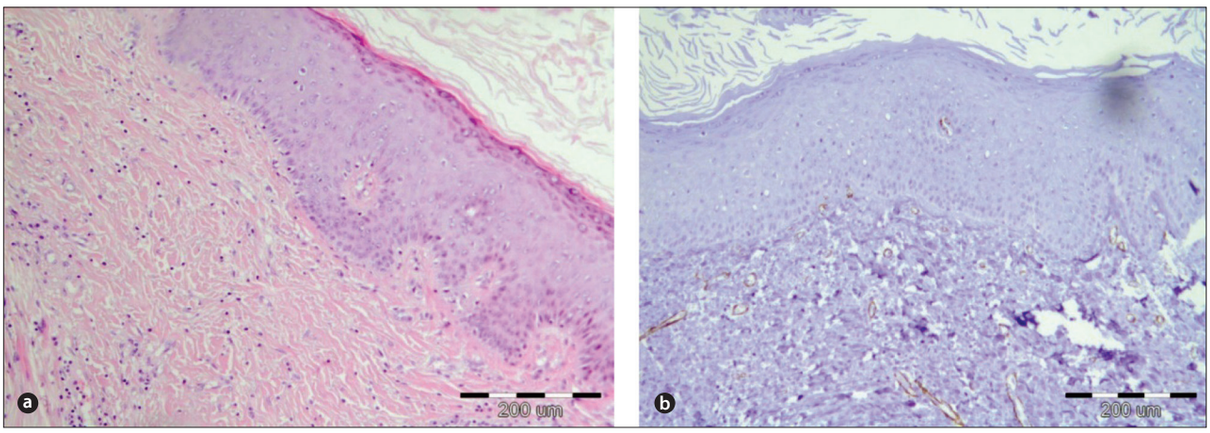


Figure 1. Microphotographs: a) Normal skin H.E. (10 x4) b) Normal skin Immunostaining with CD34 (10x4)

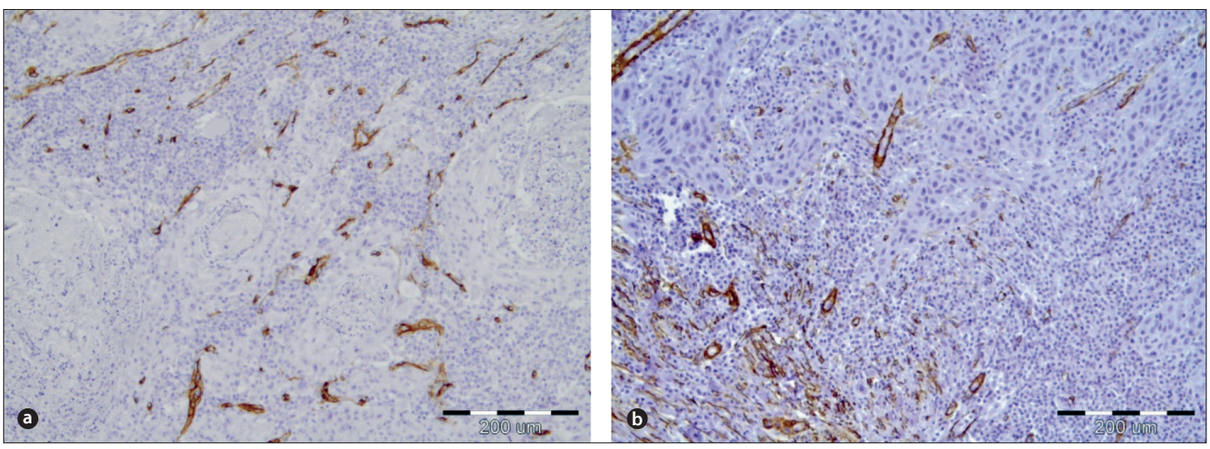


Figure 2. Microphotographs. a) Immunostaining with CD34 (10x10). b) Microvessel density in the invasive front of G2 SCC (CD34 10x10)

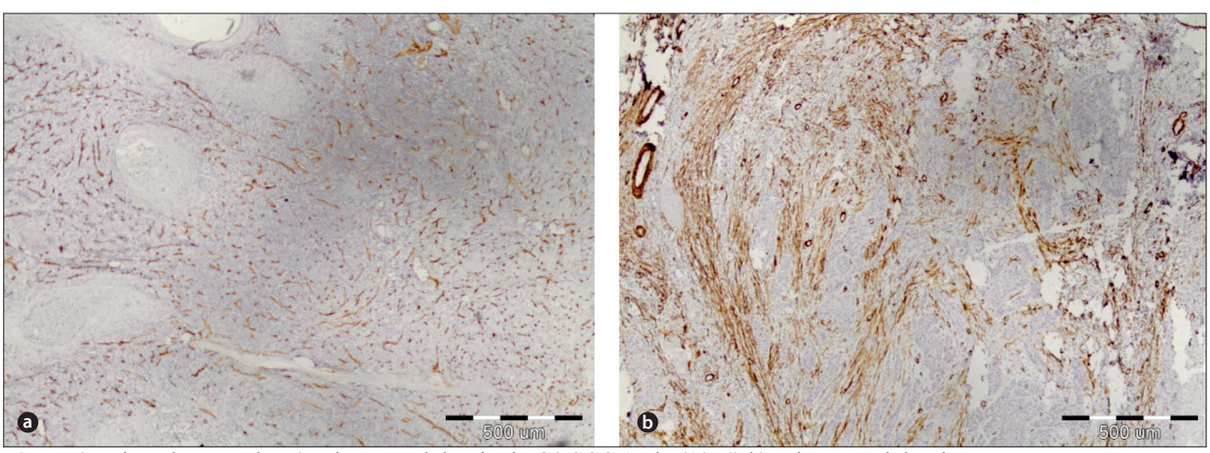


Figure 3. Microphotographs. a) Microvessel density in G3 SCC Actin (10x4) b) Microvessel density, immunostaining with Actin (10x10)

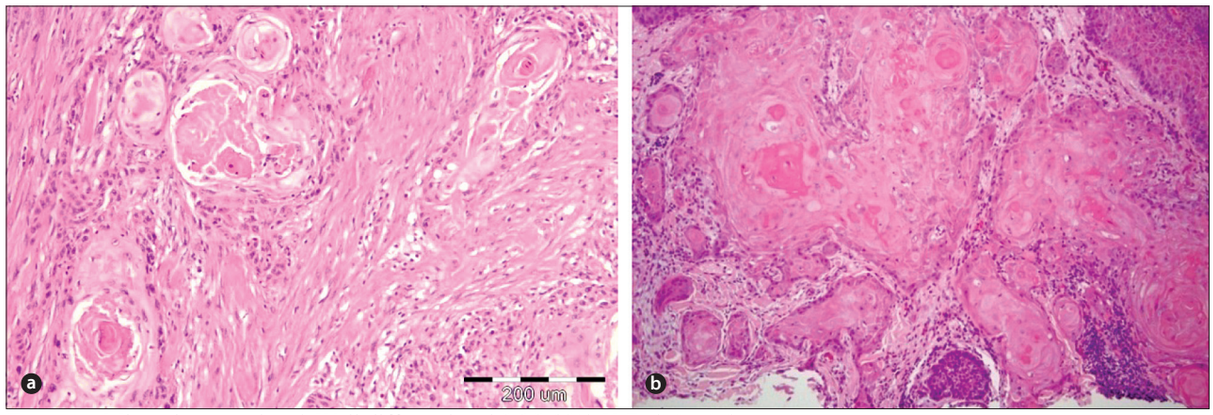


Figure 4. Microphotographs. a) Well differentiated SCC (G1) H.E. (10 x 10)
b) Another case of well differentiated SCC H.E. (10 x 10)

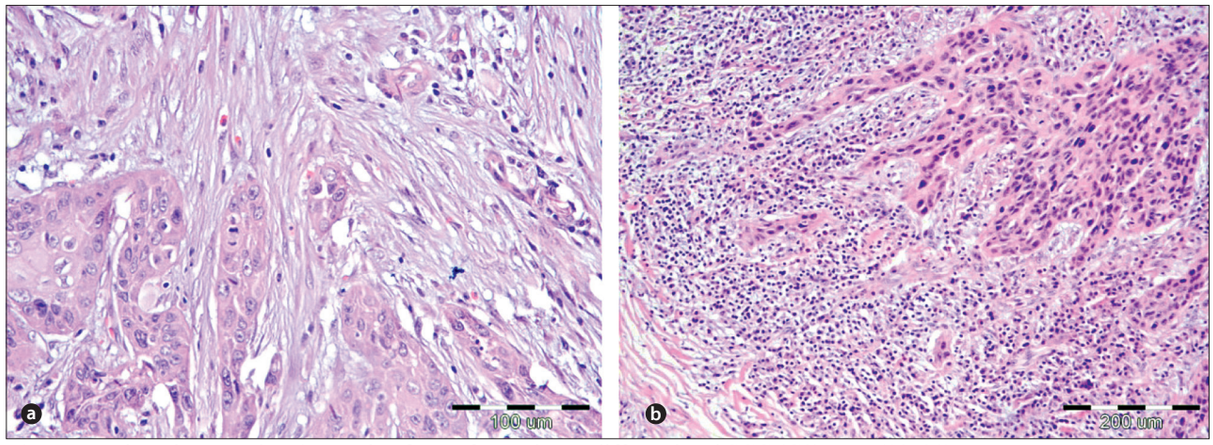


Figure 5. Microphotographs. a) Invasive front of a moderate differentiated SCC (G2) H.E. (10x20)
b) The invasive front of an another case of a moderate differentiated SCC) H.E. (10x10)

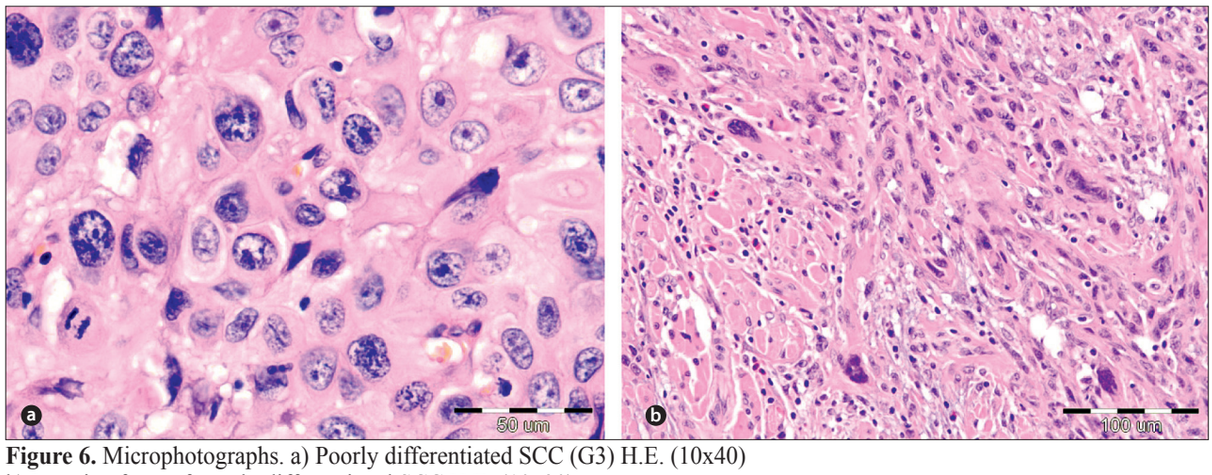


Figure 6. Microphotographs. a) Poorly differentiated SCC (G3) H.E. (10x40)
b) Invasive front of poorly differentiated SCC H.E. (10x20)

The density of the neovascularization in each separate case was determined as a sum of all stained and counted vascular lumina found in the 10 visual fields. Both, the minimum and the maximum count of blood vessels were determined (from all visual fields) in each case and their mean value was calculated. Identical procedure was conducted on the sections from the surrounding healthy skin, involving determination of the vessel density in the dermis.

For the evaluation of the results modern statistical methods of analyses were used by employing the computer software. The statistical package SPSS 11.0 was used for creation of databases. The following statistical tests were applied:

- Kruskal-Wallis test,
- Mann Whitney U test is an equivalent to Student's t-test;
- Spearman's rank correlation

RESULTS

Of the total number of 30 analyzed patients, 10 (33.3%) were females, aged 70-98 years (mean age 85.7±8.4), whereas 20 (66.7%) were males, aged 57-89 years (mean age 74.2 ±10.4) (Table 1).

The most frequently present skin region in the examined group was the face, in 13 cases (43.3%), and the least present were regions of the forehead, neck, breast, eyelid, arm, and leg with one case each (3.3%) (Table1).

There were 21 cases (70.0%) with pT1 tumors and 9 cases (30.0%) with pT2 tumors (Table 1) and there were 12 (40.0%) G1 tumors, 13 (43.3%) G2 tumors and 5 (16.7%) G3 tumors (Table 1).

Values obtained by measuring the depth of invasion ranged from the lowest 1561.2 μm to the highest 13000.1 μm, mean value 4991.71±1741.9 (Table 1).

The depth of the stromal invasion was the smallest in well-differentiated (G1) squamous cell carcinomas (mean 2579.28±697.26), and the largest in the poorly differentiated (G3) squamous cell carcinomas (mean 9219.896±2268.882) (Table 2).

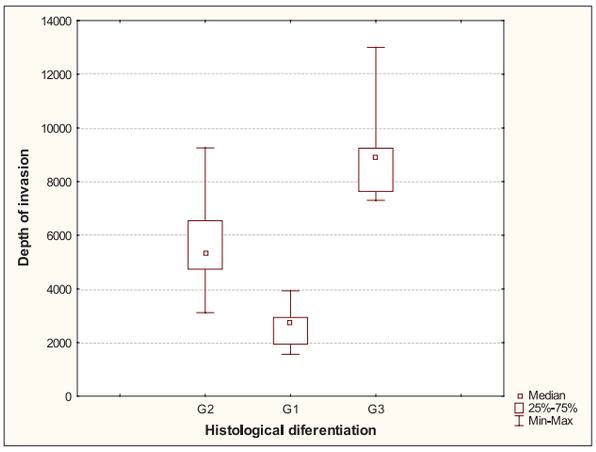
Table 1. Distribution of cases according to: location, tumor status of the neoplasm (pT), grade of differentiation (G) and depth of invasion

Location	Number and %
Skin of face	13 (43.33%)
Skin of nose	3 (10.0%)
Skin of ear auricle	3 (10.0%)
Skin of abdomen	3 (10.0%)
Skin of scalp	2 (6.66%)
Skin of forehead	1 (3.33%
Skin of eyelid	1 (3.33%)
Skin of neck	1 (3.33%)
Skin of breast	1 (3.33%)
Skin of hand	1 (3.33%)
Skin of lower limb	1 (3.33%)
Tumor status (pT)	**Number and %**
pT1	21 (70.0%)
pT2	9 (30.0%)
Grade of differentiation	**Number and %**
G1	12 (40.0%)
G2	13 (43.33%)
G3	5(16.66%)
Depth of invasion	**mean±sd**
	4991.71±2741.9μm
Depth of invasion	**min. / max.**
	1561.2 μm / 13000.1 μm

Table 2. Average depth of stromal invasion in skin SCC expressed in micrometers according to the grade of differentiation (G) of neoplasms

Grade of differentiation	Depth of stromal invasion (μm)
G1 (N = 12)	2579.28±697.26
G2 (N = 13)	5592.33 ±1532.48
G3 (N = 5)	9219.896±2268.882

The difference in depth of the stromal invasion in skin SCC, which was registered in neoplasms with different grade of histological differentiation, was statistically significant (Kruskal-Wallis test: H (2, N= 30) =23.47711, p =0.00008) (Figure 7).



Legend: (G1) – well-differentiated carcinoma, (G2) – moderately differentiated carcinoma, (G3) – poorly differentiated carcinoma

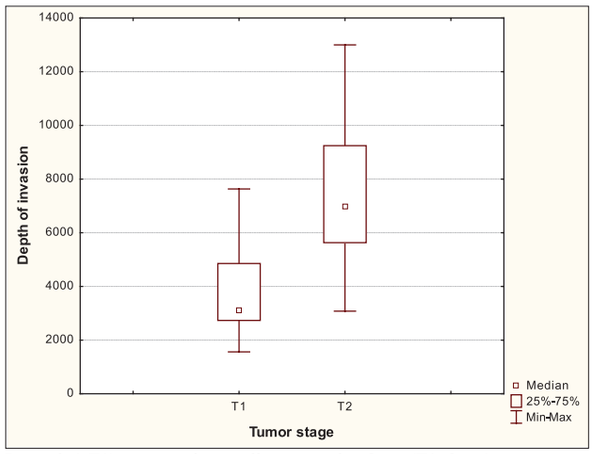
Figure 7. Statistically significant difference between the depth of invasion in skin SCC with different grade of differentiation

The depth of stromal invasion differed in neoplasms with different tumor stage (pT).

Table 3 shows that in pT1 neoplasms smaller or equal to 2 cm the depth was smaller (3957.534±1843.46) than in pT2 neoplasms larger than 2 cm where the depth of the stromal invasion was 7404.770±3066.01 ($p < 0.05$ (Mann-Whitney U test-z = 2.96429 p = 0.003034) (Figure 8)

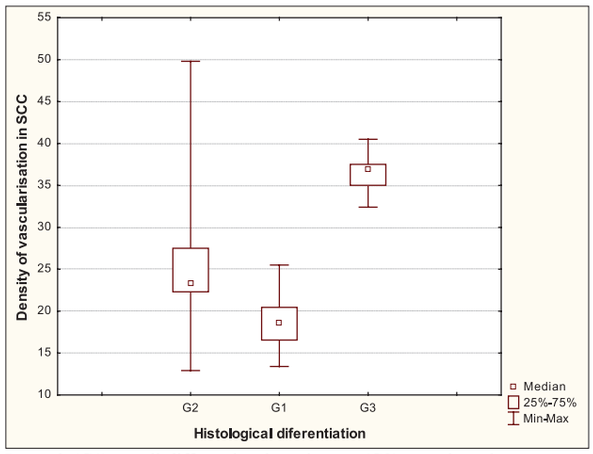
Table 3. Average depth of stromal invasion in skin SCC expressed in micrometers according to the tumor status (pT1 and pT2) of the neoplasm

Tumor status of neoplasm	Depth of stromal invasion (μm)
T1 (N = 21)	3957.534±1843.468
T2 (N =9)	7404.770±3066.009



Legend: (pT1) – tumor size smaller or equal to 2 cm (T1≤2 cm), (pT2) – tumor size larger than 2 cm (T2>2 cm)

Figure 8. Statistically significant difference between depth of invasion in skin SCC of skin in neoplasms with pT1 and pT2 tumor status



Legend: (G1) – well-differentiated carcinoma, (G2) – moderately differentiated carcinoma, (G3) – poorly differentiated carcinoma

Figure 9. Statistically significant difference in the density of neovascularization in skin SCC according to the grade of histological differentiation (G1, G2, G3)

Table 4. Vascular density in the invasive front of skin SCC and the normal surrouding skin and grade of diferentiation of the neoplasms (G)

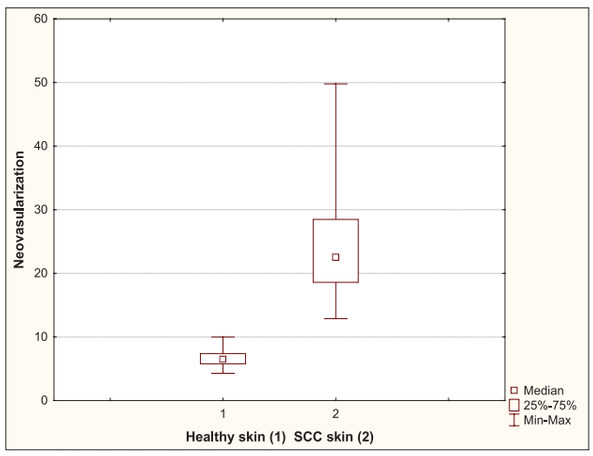
	G1 (N = 12)	G2 (N = 13)	G3 (N = 5)
Total number of blood vessels in visual field	187.92±37.99	256.61±83.34	364.80±30.13
Minimum number of blood vessels in visual field	15.08±3.44	13.07±2.53	17.40±4.16
Maximum number of blood vessels in visual field	24.75±5.59	33.0±9.88	39.0±4.63
Mean number of blood vessels in visual field ± SD	18.79±3.79	25.44±8.43	36.48±3.01
Vascular density in normal surrounding skin			
Total number of blood vessels in visual field	66.33±15.95	68.61±9.13	63.20±8.70
Minimum number of blood vessels in visual field	4.16±1.19	4.92±2.10	3.80±1.78
Maximum number of blood vessels in visual field	12.33±3.20	13.69±3.22	11.60±3.28
Minimum number of blood vessels in visual field ± SD	6.63±1.59	6.86±0.91	6.32±0.87

The lowest value of vascular density in the invasive front of the neoplasm was found in the well-differentiated tumors (G1), and the highest value was found in the poorly differentiated tumors (G3). The mean values of the vascularization were in range of 15.8 to 24.75 blood vessels (Table 4) and they showed statistically significant difference of BVD in the groups of carcinomas with different grade of differentiation (Kruskal-Wallis test: H(2, N=30)=16.02890, p =0.0003) **(**Figure 3)**.**

The difference registered in the mean values of vascular density in tumors compared to that in normal skin was statistically significant (Mann-Whitney U test-z=6.652991, p=0.0000001) (Figure 10).

Neovascular density in the invasive front of the neoplasm in terms of tumor status was different but not statistically significant (Mann-Whitney U test-z = 1,42557, p = 0.153993).

With regard to tumor stage (pT) of neoplasms with SCC, no statistically significant difference in the density of vascularization was observed between the tumors measuring T1≤2 cm and T2 >2 cm (Mann-Whitney U тест-z=1.42557, p=0.153993) (Figure 5).



Legend: 1 – healthy skin, 2 – carcinoma

Figure 10. Statistically significant difference in density of neovascularization in skin SCC compared to the density of vascularization in normal surrounding skin

Table 5. Mean density of vascularization according to tumor status (pT) of the neoplasm

Tumor status of neoplasm	Density of vascularization
pT1 (N = 21)	23.63±9.27
pT2 (N =9)	26.93±6.88

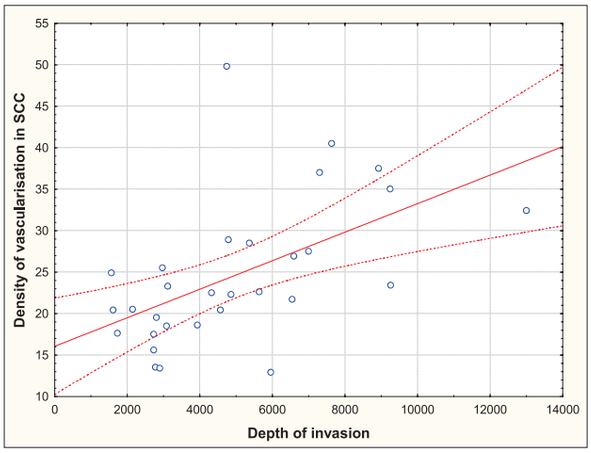


Figure 11. Correlation of the vascular density in the invasive front of the SCC of the skin and the depth of invasion

The density of the neovascularization in SCC of the skin was in positive statistically significant correlation to the neoplastic depth of invasion (Spearman Rank Order Correlations- r=0,5455, p=0,00018 (Figure 11)**.**

The statistical analysis of the examined parameters: tumor status (pT1/pT2), grade of histological differentiation (G1, G2, G3), depth of invasion and density of blood vessels at the invasive front of skin SCC according to the sex and age of the patients as well as according to location of neoplasms showed no statistical significance.

DISCUSSION

The blood vessels in the healthy human skin are usually passive and in general the neovascularization is not noticed except in follicular angiogenesis which is included in the separate phases of follicle cycle. The normal dermal matrix in the area of the base membrane around the microvascular endothelial cells are thought to function as a natural inhibitor of angiogenesis, ensuring the passivity of the blood vessels in healthy skin [15].

Recent data show that thrombospondin-1 (TSP-1) and 2-thrombospondin (TSP-2) are the main physiological inhibitors of dermal angiogenesis. TSP-1 and TSP-2 are members of the matrix glycoprotein family and deposit in the dermal-epidermal basal membrane, which promotes an angiogenetic barrier by separation of the non-vascularized epidermis from the vascularized dermis. Endostatin, angiostatin, vasostatin and interleukin 12 (IL-12) play the same role. They are together identified as inhibitors of tumor angiogenesis and tumor growth *in vivo* [9,15].

Potent pro-angiogenic factors in the skin angiogenesis are the following: vascular endothelial growth factor (VEGF-A), basic fibroblast growth factor (bFGF-2) and interleukin-8 (IL-8). VEGF-A is the main angiogenic growth factor which is bound to two types of tyrosine receptors VEGFR-1 and VEGFR-2 that can be found in vascular endothelial cells. VEGF-A is secreted by tumor cells and influences upon releasing of matrix metalloproteinases (MMPs) from endothelial cells. The released MMP-2 and MMP-9 degrade the extracellular matrix (ECM) tracing invasive pathway of endothelial cells in the neighboring tissue. Additionally MMPs dissolve ECM by which new concentrations of bFGF-2 and VEGF-A are released [9,10,15,16, 17]. In situations of disturbed balance when the secretion of angiogenic stimulators is increased and the regulation of endogenous angiogenic inhibitors is decreased, preconditions for tumor angiongenesis are developed [6,7,9].

In the early 70s Judah Folkman postulated the hypothesis that tumors need vascularization in order to grow and that some diffusible molecules regulate that process. The process of onset of angiogenesis is balanced by pro- and anti-angiogenic factors. At the same time, he believed that perhaps by disturbing and preventing angiogenesis in tumors, the tumor growth would be slowed down, the tumor mass would be decreased and tumor regression would appear [17,18,19].

Inhibition of angiogenesis would include: inhibition of angiogenic growth factor production, increased production of angiogenic inhibitors or inhibition of receptors' activity and signaling of blood vessels [20, 21, 22].

Not all tumors are angiogenic from the beginning of their growth. Clinical and experimental data about formation of tumors influenced by chemical cancerogenic substances, confirm that tumor progression happens by a switch from a prevascular to a vascular phase. The first data about the angiogenic potential of premalignant lesions came from the Folkman's laboratory where the transition from hyperplasia to neoplasia was shown.

Premalignant lesions can be found in all epithelial organs and are characterized by disordered proliferation, loss of cell uniformity and architecturally different organization. Some changes are reversible, but some develop further on towards carcinoma *in situ* and then in invasive carcinoma [23, 24].

The activation of vascularization in premalignant lesions is characterized by proliferation and migration of endothelial cells and by the ingrowth of new blood vessels from the preexisting ones, by which higher density of the new blood vessels is evident, while together with the increased expression of VEGF-A/VEGFR-2 and decline of TSP-1 are indicators for the early angiogenic switch or inclusion of angiogenesis in skin squamous cell carcionoma (SCC). Growth factors from the VEGF family play a fundamental role in the growth and invasion of SCC. The blockage of VEGFR-2 receptors leads to inhibition of angiogenesis and invasion of SCC. The levels of TSP-1 and TSP-2 physiologic inhibitors of skin angiogenesis, located on the basement membrane, are decreased in SCC, which has shown to be an indicator or phase that precedes the invasion. Studies have shown deletion of the chromosome 15, which is the location of the gene TSP-1. In the experimental studies there are proofs that if a copy of the chromosome 15 is added or TSP-1 is given, tumor growth will be suppressed [25,26].

The accumulated scientific-research data on the human body confirm that angiogenic intensity can be a prognostic indicator in numerous malignomas including: head and neck SCC, malignant melanoma, cancers of the lungs, breast, stomach, urinary bladder, prostate, cervix and invasive skin SCC and indicated the relationship between angiogenesis, i.e. density of the vascular net in a neoplasm and its progression which is associated with tumor aggressiveness and poor prognosis. Disabling tumor growth by preventing tumor vascularization i.e. angiogenesis, represents an oncologic therapeutic approach to the treatment of vascular and solids following the consistency of skin tumors such as SCC [27].

Angiogenesis in SCC has been examined in tumors that occur in several different anatomic sites in order to determine its role in progression of tumors or their aggressiveness and consequently the outcome of the disease [28]. Analysis of angiogenesis in solar keratosis, in superficially invasive and invasive SCC of the skin, has shown higher microvessel density compared to the neighboring normal skin, pointing out to the fact that angiogenesis appears early in the developmental stage of cutaneous SCC and that neovascularization is parallel with the tumor progression [29].

The analysis of the microvascular density in SCC and basal cell carcinoma of the skin, using immunostaining with CD34 and determination of the levels of VEGF, showed that the correlation between the high vascular density and high level of VEGF in SCC indicate a possible role of angiogenesis in determining more aggressive types of cancers [30,31]. The vascular density is confirmed to be higher in SCC than in basal cell carcinoma. The association of microvascular density and neck metastasis in oral SCC points on the key role of angiogenesis in oral SCC [32].

However, a variety of data can be found in the literature. One study conducted on head and neck malignant tumors presented correlations between vessel density, bad prognosis and onset of metastases [33,34], while another study found no correlation between angiogenesis and tumor stage and prognosis [35]. It is still unclear whether these discrepancies due to different tumor stages, localization or methods of analysis [36].

The rate of metastasis in skin SCC is low and ranges from 4.5% in tumors of thickness (depth of invasion) between 2-6 mm and 15% in tumors exceeding a thickness of 6 mm [37]. In the absence of generally accepted criteria for evaluation of the early stages of SCC Breuninger's classification has been accepted, which is more relevant than Broder's one and which is based on the degree of differentiation [38]. Based on Breuninger's classification of carcinomas of thickness <2 mm, no increased vessel density was observed. Only in carcinomas exceeding a thickness of 2 mm the vascular density was significantly increased. The location of the tumor did not have influence on the vascular density, but had an impact on the depth of infiltration into the subendothelial stroma [39].

This suggests that in skin SCC the increased vascular density might be a prognostic factor and together with the disease stage might be an indicator of tumor aggressiveness and disease outcome [40]. Discrepancies between data presented in the literature may be due to: origin of the specimens (human or animal), different dynamics of tumor progression, different causes that influence on tumor development, i.e. virally- or ultraviolet-induced carcinogenesis. On the other hand, skin epithelial tumors remain surface tumors for a considerable period and may not require increased angiogenesis since they are completely well-nourished by diffusion similar to the epidermis. Increased angiogenesis would be required if tumor mass increases and consequently hypoxia increases in that area [41].

Taking into consideration that in the skin SCC, determining the T parameter of pTNM classification means measurement of the largest diameter of the neoplasm, which is not always identical to the one that determines the depth of the invasion, many authors have tried to explore the depth of invasion of SCC as a prognostic factor. [42,43].

Tan WJ and others have investigated the prognostic significance of the depth of the invasion in carcinoma of the tongue and found that it is an important prognostic indicator in cancer of the tongue [44].

In another research the prognostic factors in penile SCCs were analyzed. It was found that the depth of the invasion and the vascular invasion were significant predictors of cancer progression [45].

Kristensen GB *et al.* determined the major prognostic factors in early SCC of the uterine cervix and discovered that the clinical tumor size and depth of invasion were the main prognostic factors in patients with early squamous cell cervical carcinoma [46].

CONCLUSION

The analysis of this study showed significant increase of the microvascular areas in an invasive front of SCC compared to the normal skin and showed correlation between the density of the neovascularization, the depth of the invasion and the grade of neoplasm, elements which according to numerous authors represent predictors of tumor progression.

Neovascularization significantly increases in neoplasm with higher grade and the higher depth of invasion. Hence, the increase in vascularity in worse differentiated carcinomas influences the depth of invasion and it plays a role in the progression of the neoplasm.

The significant change in the vascular density of SCC in contrast to the normal skin and its gradual increase in tumor stroma of cancers with worse differentiation and incised depth of invasion suggests that neoangiogenesis is a process that is associated with the needs of the neoplasm, i.e. it facilitates its spread.

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Резиме

КОРЕЛАЦИЈА ПОМЕЃУ ГУСТИНАТА НА КРВНИТЕ САДОВИ И МОРФОЛОШКИТЕ КАРАКТЕРИСТИКИ КАЈ ПЛАНОЦЕЛУЛАРЕН КАЦИНОМ НА КОЖА

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Апстракт

Вовед: Малигните тумори стимулираат ангиогенеза за да растат и да метастазираат. Туморите со висока ангиогенетска активност се вбројуваат во категоријата на многу агресивни тумори, со неповолна прогноза за пациентите.

Цел на трудот е одредување на густината на крвните садови, т.е. неоваскуларизацијата во инвазивниот фронт на туморската строма кај планоцелуларен карцином на кожата во однос на здравата кожа, како можен показател за туморска прогресија. Одредувана е густината на крвните садови кај планоцелуларен карцином на кожа во однос на длабочината на инвазијата, степенот на хистолошката диференцираност и различната туморска големина на неоплазмите.

Материјал и методи: Материјалот за изведување на ова истражување го сочинуваат оперативни материјали од 30 пациенти со планоцелуларен карцином на кожа, кои биле оперирани. Примероците на ткивата се обработени со стандардна парафинска техника, боени со Hematoxylin-Eosin и имунохистохемиски со антитела за мазен мускулен актин (smooth muscle actin, SMA SMA) и CD34. Густината на крвните садови во инвазивниот фронт на неоплазмата беше споредувана со здравата кожа, туморската големина p(T), длабочината на инвазијата и степенот на хистолошката диференцираност на туморот p(G).

Резултати: Хистолошката анализа покажа висока статистичка разлика во густината на крвните садови кај планоцелуларниот карцином на кожа во споредба со здравата кожа и статистички разлики во густината на крвните садови во однос на длабочината на инвазија и степенот на диференцираност на неоплазмата. Слабата диференцираност на неоплазмата и зголемената длабочина на инвазијата покажуваат зголемување на неоваскуларизацијата.

Клучни зборови: планоцелуларен карцином, имунохистохемија, SMA, CD34, густина на неоваскуларизација, инвазивен фронт, хистолошка диференцијација

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CASE REPORT

EXTREMELY LOCALLY ADVANCED OVARIAN MALIGNANT MIXED MULLERIAN TUMOR IN 37-YEARS-OLD FEMALE

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ABSTRACT

Ovarian carcinosarcomas, rare variant of ovarian carcinoma, composed of both carcinomatous and mesenchymal components, solid and/or cystic, fleshy and hemorrhagic, frequently spreading beyond the ovary, are treated with surgery and adjuvant chemotherapy according to the treatment principles of ovarian carcinomas due to the small number of reported cases and lack of randomized studies. We report a case of a 37-year-old woman with clinical signs of extremely locally advanced tumor of ovarian origin, infiltrating the lower left quadrant of the abdominal wall with necrosis of the covering skin. Prior biopsy of the left ovary and omentum confirmed poorly differentiated serous adenocarcinoma. Bulky tumor the size of a child's head, originating from the left ovary and infiltrating into the lower left quadrant abdominal wall was debulked with wide excision of the abdominal wall and creation of wide defect of the lower left part of abdominal wall covered with Dexon mesh. After the recovery, the medial part of the defect with exposed mesh was closed with pedicled tensor fasciae latae fasciomyocutaneous flap, while the lateral part of the defect was covered with split thickness skin graft. Optimal surgical cytoreduction and adjuvant chemotherapy in case of extremely locally advanced ovarian malignant Müllerian tumor provide satisfactory recurrence-free survival period.

Keywords: locally advanced ovarian mixed tumor, carcinosarcoma, surgical debulking, adjuvant chemotherapy

BACKGROUND

Ovarian carcinosarcomas, also called malignant mixed Mülleriantumours (MMMT) are rare variant of ovarian cancer, accounting for less than 1% of all ovarian tumors, with fewer than 400 cases reported in the literature [1,2,3]. Carcinosarcomas pathologically consist of mixture of two malignant components, epithelial and mesenchymal, occurring throughout the female genital tract, most commonly in the uterus. Ovarian carcinosarcomas are three times less prevalent than those of the uterus [4]. According to the WHO, carcinosarcomas are regarded as metaplastic carcinomas and are supposed to be of epithelial monoclonal origin [5].

Histologically, carcinosarcomas are composed of both carcinomatous and mesenchymal components, which may be either homologous (originating from tissue normally present in the ovary) or heterologous (containing osteoid, chondroid or rhabdomyoid cells). Macroscopically the tumors are solid and/or cystic, fleshy and hemorrhagic and frequently spread beyond the ovary and over the peritoneal surfaces.

The stage classification used for carcinosarcoma of the ovary is the same FIGO system that is applied to the other ovarian adenocarcinomas.

The treatment principles of ovarian carcinosarcomas are the same as those for epithelial ovarian cancer, but the evidence for doing so is lacking due

to the small number of cases and lack of randomized studies. This is also valid as far as adjuvant chemotherapy is concerned.

Ovarian carcinosarcoma carry a particularly unfavorable prognosis. No effective chemotherapeutic regimen and radiotherapy exists. Optimal cytoreductive surgical debulking is crucial and the FIGO stage is considered as the only prognostic factor [3].

CASE REPORT

37-year-old woman was admitted at the Clinic for Digestive Surgery in Skopje with clinical signs of extremely locally advanced tumor, of ovarian origin, infiltrating the lower left quadrant of the abdominal wall. Upon clinical examination, large tumor the size of a child's head, with signs of necrosis and hemorrhage, completely infiltrating the full thickness of the lower abdominal wall and necrosis of the covering skin was established. Laboratory examination upon admission revealed RBC $2.58x10^{12}$/L, hemoglobin 67g/L, hematocrit 0.220 L/L, platelets $506x10^{9}$/L, albumin level 25g/L, total protein level 50g/L and CRP 48.9 mg/L.

13 months prior to the admission, the patient underwent surgery because of tumor of the right ovary. During the surgery, the right ovary was removed and biopsy of the left ovary and large omentum was performed. The pathohistological analysis confirmed presence of poorly differentiated serous adenocarcinoma of the right ovary and tube, with tumor implants onto the left ovary and malignant cells in the ascites. The pathological stage was IIC (pTNM=pT2c G3 pNx C4 G3 LV1).

The patient was advised for reoperation and chemotherapy, which were declined by the patient.

7 months prior to the admission, CT of the abdomen and pelvis was performed, verifying large tumorous mass in the lower left quadrant of the abdomen with wide interface with the lower left part of the anterolateral abdominal wall. One month later, second CT of the abdomen and pelvis was performed, verifying several tumorous masses of different sizes, the largest being 9 cm in diameter; compression of the anterior abdominal wall; enlarged pelvic lymph nodes, secondary deposit with diameter of 35mm in the VIII segment of the liver and medium quantity of ascites.

Indication for elective operation was established and median laparotomy was performed. Intra-operative finding was bulky tumor with size of a child's head, originating from the left ovary and infiltrating into the lower left quadrant of the anterolateral abdominal wall (Figure 1).

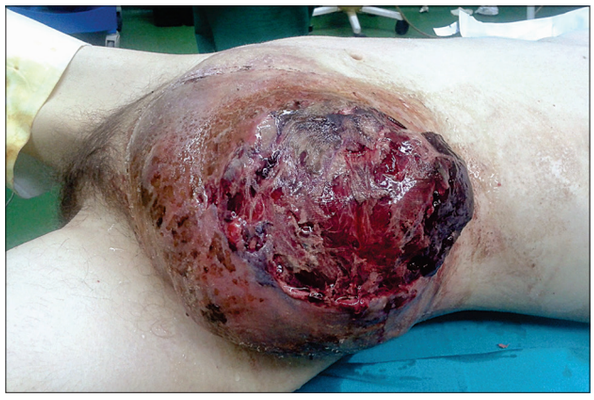

Figure 1. Large tumor, with signs of necrosis and hemorrhage, completely infiltrating the full thickness of the lower abdominal wall and necrosis of the covering skin

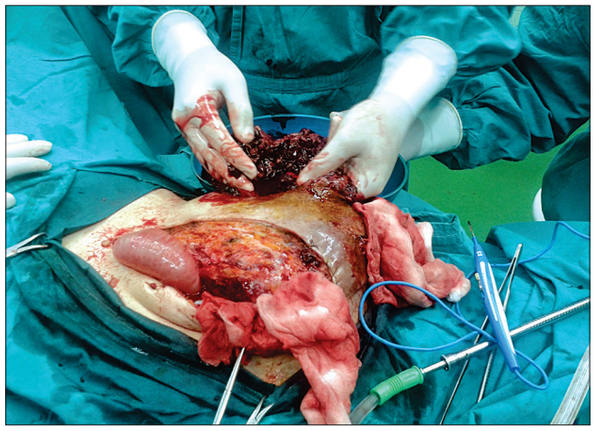

Figure 2. Tumor debulking and wide excision of the abdominal wall

Debulking of the major part of the ovarian tumor was performed, with omentectomy and wide excision of the abdominal wall. The median laparotomy was closed but the wide defect of the lower left part of the anterior abdominal wall was covered with Dexon mesh covered with Vaseline gauze dressing (Figure 2).

The size of the full thickness resected abdominal wall was about 15x10cm. Since a prompt reconstruction was needed, it was decided to close the medial part of the defect with exposed Dexon mesh with a pedicled tensor fasciae latae fasciomyocutaneous flap, while the lateral part of the defect was covered with split thickness skin graft (Figure 3).

The patient received chemotherapy with Paclitaxel 285mg and Carboplatin 490 mg.

Results 6 months after the initial surgery are shown in Figure 4.

13 months after the initial surgery local recurrence was noted on the lateral abdominal wall and CT scan of the abdomen was indicated, revealing local recurrence in the lateral abdominal wall in

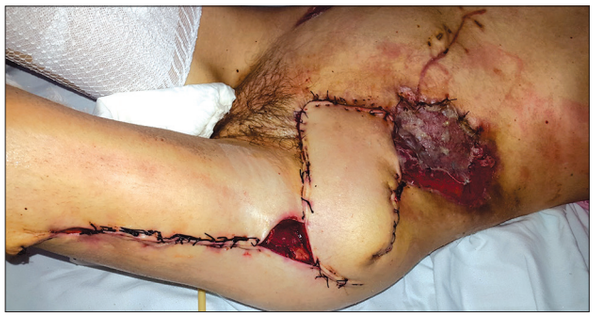

Figure 3. Reconstruction of the defect

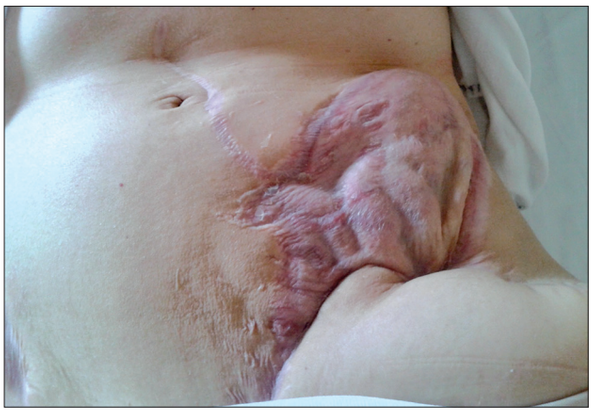

Figure 4. Antero-lateral abdominal wall 6 months after debulking and wide excision of abdominal wall

the vicinity of the previous incision, ascites and mass on the peritoneum with 3 cm in diameter. The lateral abdominal wall mass was excised in general anesthesia creating atypical laparotomy approach for excision of the peritoneal mass. After the operation, the patient received chemotherapy for the second time.

DISCUSSION

Basically ovarian carcinosarcoma is a mixture of two different malignancies, epithelial and sarcomatous, which behave in independent manners. This is evident in their pattern of metastasis: transperitoneal spread being almost exclusively accomplished by malignant epithelial deposits and with great difficulty by the sarcomatous component [6].

The few case series present in the literature tend to agree that maximal cytoreduction appears to correlate with better progression free survival and overall survival and the complete cytoreduction should be the goal of surgical treatment [7,8].

Secondary cytoreduction in epithelial ovarian carcinoma is a standard proven to extend survival whenever R0 is achieved; on the other hand, ovarian carcinosarcoma does not seem to follow this principle [9]. On the contrary, there are reports that show statistically significant survival benefit for women who have optimum cytoreduction after surgery [1].

In case of not achieving optimal debulking of the tumor, there are also conflicting reports. The size of the residual disease after tumor debulking did not significantly impact the survival according to Barakatet al. [10], while Muntz et al. [11] state the opposite.

Research in malignant mixed mesodermal tumors of the uterus have suggested that the sarcomatous and carcinomatous components both arise from a single malignant precursor cell which has undergone metaplastic change to a sarcomatous form in some areas of the malignant tissue which contributed to the presence of both histological types [12].

Carcinosarcomas of the ovary are very aggressive tumors that are usually diagnosed at an older age compared to women with epithelial ovarian cancer. Ovarian carcinosarcomas are usually at an advanced stage at the time of the diagnosis, and survival after the diagnosis varies by stage of the disease and histological type [13]. The overall median survival period is 8 months [1].

One of the most perplexing situations ever to confront the surgeon is an open abdomen in the absence of adequate somatic substance to effect secure closure of the abdominal cavity. Major defects in abdominal wall substance pose few basic problems. First of all, there is various depth of tumor infiltration into the abdominal wall. No thought of subsequent closure should ever influence the surgeon to be less thorough or to accept less than adequate tumor resection. Closure of the abdominal wall under excessive tension regularly fails because of subsequent tissue disruption and serious wound infection. In cases where there is too great a gap between the abdominal side walls , insertion of a sheet of synthetic mesh to bridge the defect will maintain visceral position within the abdomen proper. In selection of a fascial substitute, certain fabric characteristics appear to be crucial. The substance should be pliable to preclude erosion into major structures, inert to avoid greater inflammatory response, porous to allow free drainage of the exudates and to have fiber resilience sufficient to maintain mesh integrity offering potential for permanence.

CONCLUSION

Optimal surgical cytoreduction and adjuvant chemotherapy in case of extremely locally advanced ovarian malignant Müllerian tumor provide satisfactory recurrence-free (13 months in our case) and survival period (19 months in our case).

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Резиме

ЕКСТРЕМНО ЛОКАЛНО НАПРЕДНАТ ОВАРИЈАЛЕН МАЛИГЕН МУЛЕРИСКИ ТУМОР КАЈ 37-ГОДИШНА ЖЕНА - ПРИКАЗ НА СЛУЧАЈ

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Апстракт

Оваријалните карциносаркоми, ретка варијанта на оваријалните карциноми, составени и од мезенхимална и карциноматозна компонента, солидни и/или цистични, месести или хеморагични, често проширени и подалеку од овариумот, се лекуваат хируршки и со адјувантна хемотерапија, според принципите за лекување на оваријалните кариноми, како резултат на малиот број на опишани случаи и недостигот на рандомизирани студии. Ова е приказ на случај на 37-годишна жена со клинички знаци на екстремно локално напреднат тумор од оваријално потекло, кој го инфилтрира долниот лев квадрант на абдоминалниот ѕид со некроза на препокривачката кожа. Претходната биопсија на левиот овариум и оментум покажа лошо диференциран серозен аденокарцином. Bulky-тумор со големина на детска глава, со потекло од левиот овариум и со инфилтрација во долниот лев квадрант на абдоминалниот ѕид, беше ослободен со широка ексцизија на абдоминалниот ѕид и создавање широк дефект на долниот лев дел на абдоминалниот ѕид, кој беше покриен со Дексонска мрежа. По заздравувањето медијалниот дел на дефектот со изложената мрежа беше затворен со фасциомиокутан флеп на петелка од тензор на широката фасција, додека латералниот дел од изложената мрежа беше покриен со кожен графт со половична дебелина. Оптималната хируршка циторедукција и адјувантна хемотерапија во случај на екстремно локално напреднат оваријален малиген мулериски тумор обезбедува задоволителен период без рецидив и преживување.

Клучни зборови: локално напреднат мешан тумор на јајниците, карциносаркома, хируршко остранување, адјувантна хемотерапија

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PROGNOSTIC FACTORS ON THE POSITIVITY FOR METASTASES OF THE AXILLARY LYMPH NODES FROM PRIMARY BREAST CANCER

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ABSTRACT

Aim: The aim of the study was to identify the impact of T stage, the presence of estrogen, progesterone, HER2neu receptors and the values of the Ki67 on the positivity for metastases of the axillary lymph nodes, from primary breast cancer.

Material and methods: 290 surgically treated patients for breast cancer were included in the study. All cases have been analyzed by standard histological analysis including microscopic analysis on standard H&E staining. For determining the molecular receptors - HER2neu, ER, PR, p53 and Ki67, immunostaining by PT LINK immunoperoxidase has been done.

Results: Patients age was ranged between 18-90 years, average of 57.6±11.9. The mean size of the primary tumor in the surgically treated patient was 30.27 + 18.3 mm. On dissection from the axillary pits 8 to 39 lymph nodes were taken out, an average of 13.81±5.56. Metastases have been found in 1 to 23 lymph nodes, an average 3.14±4.71. In 59% of the patients there have been found metastases in the axillary lymph nodes. The univariate regression analysis showed that the location, size of tumor, differentiation of the tumor, stage, the value of the Ki67 and presence of lymphovascular invasion influence on the positivity of the axillary lymph nodes. The presence of the estrogen receptors, progesterone receptors and HER2neu receptors showed that they do not have influence on the positivity for metastatic deposits in axillary lymph nodes. The multivariate model and the logistic regression analysis as independent significant factors or predictors of positivity of the axillary lymph nodes are influenced by the tumor size and the positive lymphovascular invasion.

Conclusion: Our study showed that the involving of the axillary lymph nodes is mainly influenced by the size of the tumor and the presence of lymphovascular invasion in the tumor. Ki67 determined proliferative index in the univariate analysis points the important influence of positivity in the axillary lymph nodes, but not in the multivariate regressive analysis.

Key words: breast cancer, axillar status, tumor size, T stage, estrogen receptors, progesterone receptors, HER2neu receptors, Ki67, lymphovascular invasion

INTRODUCTION

Involvement of axillary lymph nodes with metastatic disease from primary breast cancer is the most significant prognostic factor of the disease. Some factors are well known to influence the prognosis of the disease and early appearance of the local and distant relapse.

The axillar status (involvement of lymph nodes in axilla with metastatic diseases from primary breast cancer) together with the size of the primary breast tumor are the main factors that determine the stage of breast cancer, but also predict the prognosis of the breast cancer disease (1). Up to now all information about the axillar status were taken from the examinations of the axillar lymph nodes that we take out

with the axillar lymphadenectomy. This is part of the surgical treatment of patients with breast cancer – radical mastectomy or breast conserving surgery followed with axillar lymphadenectomy.

Introducing the procedure - detection of the sentinel node and biopsy is minimally invasive procedure that determines the first drainage lymph node in the axillar pit (2). The examination of this lymph node at the same surgical intervention gives us information about the status of this lymph node, but also gives us information about other lymph nodes in axilla.

Knowing the status of the axilla is very important, for the planning of the further therapeutic procedure.

AIM

To analyze which factors have an influence on the positivity of the axillar lymph nodes, with point to tumor size, persistence of estrogen, progesterone and Her 2new receptors on tumor cell surface, Ki67, at our patients.

MATERIAL AND METHODS

290 surgically treated patients for breast cancer that have a complete history for all parameters were included in the study. All the cases have been analyzed with standard histological analysis including macroscopic and microscopic analysis on standard H&E staining. For determining the molecular receptors immunostaining with PT LINK immunoperoxidase has been done for HER2neu, ER, PR, p53 and Ki67.

We performed the statistical analyze with the statistical program Statistica 7.

RESULTS

Patient's age ranged between 18-90 years, average of 57.6. The mean size of the primary tumor in the surgically treated patient was 30.27 $\pm$ 18.3 mm. On dissection, 8 to 39 lymph nodes were taken from the axillary pits, an average of 13.81. Metastases have been found in 1 to 23 lymph nodes, an average 3.14. In 59% of the patients metastases in the axillary lymph nodes have been found.

The univariate regression analysis showed that the location, size of tumor, differentiation of the tumor, stage, the value of the Ki67 and presence of lymphovascular invasion influence on the positivity of the axillary lymph nodes. The presence of the estrogen receptors, progesterone receptors and HER2neu receptors showed that they do not have influence on the positivity for metastatic deposits in the axillary lymph nodes. The multivariate model and the logistic regression analysis as independent significant factors or predictors of positivity of the axillary lymph nodes are influenced by the tumor size and the positive lymphovascular invasion. (Table 1.)

DISCUSSION

Axillar lymphadenectomy gives us parameters for axillar status, but at same time it is a therapeutic procedure. On the other hand, axillar lymphadenectomy was followed with many unlike features and complications as sensation in the arm, reduction of the arm mobility and lymphedema (3). Using the thesis of Fisher and Veronesi, that breast cancer is the systemic disease at the moment of the diagnosis, so it needs to be treated as systemic disease with drugs that work in the whole body (chemotherapeutic, antihormonal therapy, immunotherapy) (4,5). So, the axillar status is the first diagnostic tool and in many instances, especially if it is not involved with metastatic disease, which is in 40-70%, it is not necessary to do an axillar lymphadenectomy. This situation will be more reliable with introducing mammographic screening, with detecting much smaller tumors and without involved lymph nodes in the axillar pit (6).

The prediction of the axillar status can be used to predict the whole axillar status, to predict the sentinel node and to predict the non-sentinel node status if the sentinel node is positive. In the last case it is possible to use prediction, and not to do axillar lymphadenectomy in case when the sentinel lymph node is positive, because in 40-50% cases the other lymph nodes is negative (7, 8, 9, 10).

Many authors use some standard methods for prediction of the axillar status, as clinical examination, mammography, ultrasonography, and also introduce new methods like ultrasound guide biopsy, CT, NMRI, Pet-CT, SPETCT, contrast examinations. In many cases they have detected enlarged lymph nodes, but it is impossible to guarantee that all this is metastatic changed (low sensitivity) (11, 12, 13, 14). With the use of these methods it is possible only to lower the rate of false negative results (15).

The introduction of the SLND detection, especially if both types of detection are used, as vital blue due (methylene blue) and the radioisotope Technetium with colloid particles (radiocolloid) at the end of the last century give us very successful tool for the SLND detection, which histological examination gives us a successfully status of SLND, but also the status of the whole axilla. The successful rate

of SLND detection is 98% (74-99%), and the false negative rate is less than 5% (0-19%) (16, 17, 18, 19, 20). The false negative rate can be lower using the extirpated sentinel lymph node in the investigation and not only the histological examination of the frozen sections, but also the use of the immunohistochemical analyses with cytokeratin, or the use of OSNA (analyzing the amplification of the RNA copies of CA19)(16, 17, 21, 22, 23).

In literature there are many investigations for determination of the factors that can predict the positivity of axilla, SLND and NSLND if SLND is positive. Those factors can be divided in few categories:

- Epidemiological (age, race, side, localization)
- Clinical (palpable tumor, palpable axillar lymph nodes, location of the tumor)
- Pathological (histology of tumor, differentiation of cells, neovascularization of the tumor, vascular and lymphovascular invasion, extensive intraductal component, persistence of the receptors on the surface of the cells – estrogen, progesterone, Her-2 new, persistence of p53 proteins, persistence of factor of proliferation Ki67. By knowing these parameters it is possible to determine the subtype of the breast cancer.
- Biochemical (CEA, CA 15-3)
- Genetic (BRCA 1, BRCA2, VEGFC, MIB1, CCR7, CXCR4) (24-60).

Some of these investigations can be provided to the material taken from the tumor before the surgical intervention with “core” biopsy, which is very important for planning further therapeutic steps (42).

As first prediction the axillar status gives us the possibility to introduce SLND biopsy as minimally invasive surgery, especially in the early stages, but also in some cases with well-defined tumors which are in the early stage, it is possible not to do lymphadenectomy. If we know the axillar status before the beginning of the treatment we can:

- plan to perform the SLND investigation at the early stages of breast cancer (T1 or T2 with clinically negative axilla), and if this node is negative not to further conduct the axillar lymphadenectomy. In the literature it is referred that SLND was indicated at 60-70% of patients with breast cancer, and at 60-70% of them will be with negative SLND, and it will be not necessary to perform the lymphadenectomy. This is the reality especially in regions where mammographic screening is done.
- plan not to perform SLND or ALND in rare cases, in different tumors, older patients, with very low chances for metastases in the lymph nodes.
- plan to use other therapeutic opportunities as systematic therapy or radiotherapy.

Many of the factors that were examined as predictors for axillar status are very well known. Also, there are known pathophysiological mechanisms of their action, and it is very well known how is their action to the biology of the tumor and how they work to spared the disease in the body. So, estrogen receptors are on the surface of the cell. The connection of the estrogen and the estrogen receptors activate many processes in the cell and favor the raising and the dividing the cells. So, estrogen favors the rising of the tumor. Giving the drugs that blockade the estrogen receptors or drugs that blockade the synthesis of the estrogen will stop the rise of the tumor. The same situation is with the persistence of Her-2 neu receptors. HER2 is a membrane tyrosine kinase and oncogene that is overexpressed and gene amplified in about 20% of breast cancers. When activated it provides the cell with potent proliferative and anti-apoptosis signals and it is the major driver of the tumor development and the progression of the breast cancer. The over expression will activate many pathways in the cell, so the cells will raise and divide uncontrolled, so the tumor will raise and will not be under control. Giving the target drug – monoclonal antibody - Trastuzumab (Herceptin) will block these receptors, and the tumor will be under control. Moreover, giving chemotherapeutics which interact with all the cells that divide fast; the tumor will be under control. Ki67 is a factor that shows the proliferative activity of the tumor cells. Ki67 is in correlation with the S phase of the cells and mitotic activity. Normal breast cell has a proliferative activity of 3% (3% of the cells are in dividing stage). A bigger activity of 20 % shows the aggressive tumor with bad prognosis and shorter survival (61, 62, 63).

Many investigators analyze many factors, how they enable, or in combination can predict the status of the axillar lymph nodes, the SLND status and in recent time the NSLND status. Postaci, Jiao, Jaime Jans, Ugras, Gangi, Pijnappel, Sawaki, Brenin, Chung, Chadha, Tan, Gajdos, Qiu, Ashturkar, Wu, Tseng, Ko, Li, Ngo, Yoo, Danko, Cabioglu, Capdet, Susini, Wasuthit are part of authors that in the last decade investigated which factors influence the positivity of the axillar lymph node or the positivity of the sentinel node. They investigate all the factors that can be investigated like epidemiological, clinical, histo-

pathological, genetic, and molecular. Mainly, from all those studies the dominant factors that can influence the positivity of the axillar nodes are: the size of the tumor, location, histology, grade of differentiation, lymphovascular invasion. But, also in many investigations other factors that can influence the positivity of the axillar lymph nodes are referred: age, persistence of estrogen, progesterone and Her 2 neu receptors on the surface of the cells, subtype of breast cancer, the values of Ki67, multifocality, EIC and other. In only few studies VEGFC, MIB1, CEA, CA 15-3, CCR7, CXCR4 and others were referred (24-47).

In the studies of Jiao, Pijnappel, Sawaki, Gangi, Qiu one of the essential factors that predict the axillar involvement is the persistence of the hormonal receptors and Her 2 receptors on the tumor cell, moreover, it is well defined that Luminal and Her enriched the tumors lymph nodes are more often were involved in the metastatic disease. On the other hand, triple negative tumors rarely have involvement in the lymph nodes with metastatic disease, however, this type shows early distant metastasis and worse prognosis. But many others studies show that the persistence of the hormone receptors, Her 2 receptors on the surface of the tumor cells has no influence on the involvement of the axillar lymph nodes with metastases. So it is interesting which are the factors that influence the fact that the same factor in one study is the main factor, and in other study it is not an important one (25, 28, 29, 30, 36).

In our study the univariate regression analysis showed that the location, size of tumor, differentiation of the tumor, stage, the value of the Ki67 and the presence of the lymphovascular invasion influence the positivity of the axillary lymph nodes. The presence of the estrogen receptors, progesterone receptors and HER2 neu receptors showed that they do not have influence on the positivity for the metastatic deposits in the axillary lymph nodes. The multivariate model and the logistic regression analysis as independent significant factors or predictors of positivity of the axillary lymph nodes are influenced by the tumor size and the positive lymphovascular invasion.

The predicting of the NSLND positivity is important, and it is very current in the last years, because according to some investigations 30-40% of Z011 in the axillar pit are only sentinel node positive, so in these patients it is not necessary to do axillar lymphadenectomy (48).

For this reasons there were defined many nomograms for predicting status, where different factors from three to nine were incorporated, with various combinations. So now it is actual not to do the axillar lymphadenectomy also in patients with positive 1 or 2 sentinel lymph nodes in which the nomogram assists the prediction of the further progress of the disease in other lymph nodes in the axillar pit. These patients must be treated with systemic therapy and locally radiotherapy (48).

Factors that are included in many of the nomograms are: tumor size, tumor differentiations, lymphovascular invasion, number of positive SLND, number of negative SLND, size of metastasis in SLND, type of SLND detection, type of histological examination of SLND, number of CK19 determined with OSNA, Ki 67 and others (49- 60).

Most popular nomograms are:

- MSKCC that involves: size of tumor, differentiation of tumor-G, number of positive SLND, number of negative SLND, type of detection SLND, LVI, multimodality and positivity for estrogen receptors. This is the most frequently used, and one of the best for prediction.
- Stanford that involves: size of tumor, size of metastases in SLND, and LVI.
- Tenon that involves: size of tumor, ratio between positive and negative SLND, size of metastases in SLND.
- Bolster that involves: size of tumor, LVI, size of SLND metastases.
- Cambridge that involves: differentiation of tumor-G, ratio between SLND+ and SLND, size of SLND metastases.
- MDA that involves: size of SLND metastases, size of tumor, LVI, number of extracted SLND.
- Mayo that involves: age, size of SLND metastases, number of SLND positive, number of SLND negative, size of Tumor.
- Ljubljana that involves: size of metastases in SLND, number of SLND negative, number of SLND positive, size of tumor, LVI, ultrasound findings.

The investigation of the factors that involve NSLD are done by: Metini, Xiang, Miao, Nadem, Van der Hoven, Yao lung Kuo, Cordero, Pepeles, Gur, Gserini, Fredman, Gullen, Van la Para, Wiliams (49-60). Some of them test some nomograms in their patients and suggest which is the best for prediction. But no one can predict with 100% safety, status of axilla, or SLND in all patients, so it is necessary as minimum to do the detection and the biopsy of the sentinel node, which is further histology examined. By detecting the status of the sentinel node we can safely predict the status of other lymph nodes in axilla.

CONCLUSION

Our study showed that the involving of the axillary lymph nodes is mainly influenced from the size of the tumor and the presence of lymphovascular invasion in the tumor. Ki67 determined the proliferative index in the univariate analysis and points out the important influence on the positivity in the axillary lymph nodes but not in the multivariate regressive analysis.

Table1. Characteristics of the primary breast cancer in our patients

Variable	Axilla positive (no=171)	Axilla negative (no=119)	Total (no=290)	p
Tumor size				
Tis	3 (1,75%)	10 (8,40%)	13 (4,48%)	
T1a	22 (12,86%)	15 (12,60%)	37 (12,76%)	
T1b	5 (2,92%)	11 (9,24%)	16 (5,51%)	
T1c	21 (12,28%)	27 (22,68%)	48 (16,55%)	
T2	93 (54,38%)	50 (42,02%)	143 (49,31%)	
T3	13 (7,60%)	2 (1,68%)	15 (5,17%)	
T4	14 (8,19%)	4 (3,36%)	18 (6,19%)	1,0 ns
Location				
Central	39 (22,8%)	22 (18,49%)	61 (21,03%)	
Inner	19 (11,11%)	18 (15,12%)	37 (12,06%)	
Lateral	113 (66,08%)	79 (66,39%)	192 (66,91%)	0,79 ns
Histology				
Ductal	141 (82,46%)	96 (80,67%)	237 (81,44%)	
Lobular	18 (10,53%)	9 (7,56%)	27 (9,31%)	
Other	12 (7,02%)	14 (11,76%)	26 (8,97%)	0,86 ns
Nuclear grade				
1	3 (1,75%)	13 (10,92%)	16 (5,52%)	
2	115 (67,25%)	87 (73,11%)	202 (69,65%)	
3	53 (30,99%)	19 (15,96%)	72 (24,48%)	0,99 ns
Estrogen receptors				
Positive	130 (76,02%)	85 (71,43%)	215 (74,14%)	
Negative	41 (23,98%)	34 (28,57%)	75 (25,86%)	0,53 ns
Progesteron receptors				
Positive	139 (81,29%)	87 (73,11%)	226 (77,93%)	
Negative	32 (18,71%)	32 (18,71%)	64 (22,07%)	0,75 ns
Her 2 new receptors				
Positive	52 (30,41%)	38 (31,93%)	90 (31,03%)	
Negative	119 (69,59%)	81 (68,07%)	200 (68,97%)	0,37 ns
P53				
Positive	88 (51,46%)	43 (36,13%)	131 (45,18%)	
Negative	83 (48,54%)	76 (63,86%)	159 (54,82%)	0,92 ns
LVI				
Positive	99 (57,89%)	18 (15,13%)	117 (40,34%)	
Negative	72 (42,10%)	101 (84,87%)	173 (39,65%)	1,0 ns
Ki67				
< 20	58 (33,92%)	68 (57,14%)	126 (43,20%)	
> 20	113 (66,08%)	51 (42,86%)	164 (56,80%)	0,99 ns
Stage				
0			3 (1,03%)	
IA			43 (14,83%)	
IB			9 (3,10%)	
IIA			83 (28,62%)	
IIB			126 (43,45%)	
IIIA			43 (14,83%)	
IIIB			15 (5,17%)	
IIIC			34 (11,72)	

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Резиме

ПРОГНОСТИЧКИ ФАКТОРИ НА ПОЗИТИВИТЕТОТ ЗА МЕТАСТАЗИ НА АКСИЛАРНИТЕ ЛИМФНИ ЈАЗЛИ КАЈ ПРИМАРЕН КАРЦИНОМ НА ДОЈКА

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Апстракт

Цел: Целта на студијата беше да се одреди влијанието на Т-стадиумот, присуството на естроген, прогестерон, HER2neu рецептори и вредностите на Ki67 на позитивитетот за метастази во аксиларните лимфни жлезди кај примарен карцином на дојка.

Материјал и методи: Во студијата беа вклучени 290 хируршки третирани пациенти поради карцином на дојка. Сите случаи беа анализирани со стандардните хистолошки анализи, вклучувајќи микроскопска анализа со стандардно H&E-боење. За одредување на молекуларните рецептори – HER2neu, ER, PR, p53 и Ki67, беше применета обработка со PT LINK-имунопероксидаза.

Резултати: Пациентите беа на возраст од 18 до 90 години, просечно 57,56±11,9. Средната големина на примарниот тумор беше 30,27±18,3. Кај пациентите од пазувната јама беа извадени од 8 до 39 лимфни

јазли, просечно 13,81±5,56. Зафатени со метастаски депозит беа од 1 до 23 јазли, просечно 3,14±4,71. Позитивност на аксиларни лимфни јазли е детектирана кај 59%. Со универијантна регресиска анализа беа издвоени следниве фактори, кои влијаат на позитивност на аксила: локација, големина на туморот, диференцираност на туморот, стадиум, вредност на Ки67 и лимфоваскуларна инвазија. Присуството на естрогени рецептори, прогестеронски рецептори и HER2neu рецептори покажа дека тие немаат влијание на позитивитетот за метастатските депозити во аксиларните лимфни јазли. Мултиваријантниот модел на логистичка регресиска анализа, како независни сигнификантни фактори, односно предиктори за позитивноста на лимфните јазли во пазувната јама, ги потврди големината на тумурот и позитивната лимфоваскуларна инвазија на тумурот.

Заклучок: Од иследувањата во нашата серија се утврди дека на позитивитетот на аксиларната јама влијание има големината на туморот и позитивната лимфоваскуларна инвазија на туморот. Факторот Ki67, кој ја презентира способноста за делба, биолошката агресивност на туморот, во универијантната анализа укажува на значајно влијание за позитивитетот на аксиларните лимфни јазли.

Клучни зборови: малигном на дојка, аксиларен статус, големина на туморот, Т-стадиум, естрогени рецептори, прогестеронски рецептори, херцептински рецептори, Ки67, лимфоваскуларна инвазија

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SYNERGISTIC EFFECT OF HYPEROXIA AND BIOTRAUMA ON VENTILATOR-INDUCED LUNG INJURY

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ABSTRACT

Patients undergoing mechanical ventilation in intensive care units (ICUs) may develop ventilator-induced lung injury (VILI). Beside the high tidal volume (Vt) and plateau pressure (Pplat), hyperoxia is supposed to precipitate lung injury. Oxygen toxicity is presumed to occur at levels of fraction of inspired oxygen (FiO_2) exceeding 0.40. The exposure time to hyperoxia is certainly very important and patients who spend extended time on mechanical ventilation (MV) are probably more exposed to severe hyperoxic acute lung injury (HALI). Together, hyperoxia and biotrauma (release of cytokines) have a synergistic effect and can induce VILI. In the clinical practice, the reduction of FiO_2 to safe levels through the appropriate use of the positive end expiratory pressure (PEEP) and the alignment of mean airway pressure is an appropriate goal. The strategy for lung protective ventilation must include setting up FiO_2 to a safe level that is accomplished by using PaO_2/FiO_2 ratio with a lower limit of FiO_2 to achieve acceptable levels of PaO_2, which will be safe for the patient without local (lungs) or systemic inflammatory response. The protocol from the ARDS-net study is used for ventilator setup and adjustment. Cytokines (IL-1, IL-6, TNFα and MIP-2) that are involved in the inflammatory response are determined in order to help the therapeutic approach in counteracting HALI. Computed tomography findings reflect the pathological phases of the diffuse alveolar damage. At least preferably the lowest level of FiO_2 should be used in order to provide full lung protection against the damage induced by MV.

Keywords: hyperoxia, ventilator-induced lung injury, cytokines, lung-protective ventilation strategies

INTRODUCTION

Providing effective life-support with minimized risk and optimized comfort in patients with respiratory failure are still the principal objectives of mechanical ventilation (MV) [1]. Despite its life saving effects, MV might have adverse effects: ventilator-associated pneumonia, impaired cardiac performance, and difficulties with sedation and paralysis. The mechanical ventilation may also lead to serious damage in both healthy and diseased lungs; a process called ventilator-induced lung injury (VILI). In the past the inadequate ventilation strategy was considered to be responsible for the onset of VILI, that is, histopatologically identical with the acute lung injury (ALI). However, recently, more detailed factors for development of VILI have emerged: loading of alveolocapillary membrane, duration of exposure to MV, intensity of the exposure (tidal volume; Vt), end-expiratory pulmonary volume, magnitude of the available "baby lung" heterogeneity (which includes atelectasis, consolidation and edema) and hyperoxia. Endotoxin, vascular pressures and fluid/transfusions are additional factors leading to VILI

[2,3]. High Vt and high plateau pressure (Plat) can give excessive distension, or "stretch," of the aerated lungs, thus resulting in volutrauma and barotrauma. Besides the volutrauma and barotrauma, atelectrauma is another important causative factor leading to VILI. Ventilator-induced lung injury arises not only from repeated application of high mechanical forces that tear fragile tissue directly, but also from initiation of signaling that culminates in inflammatory changes [4]. The term "biotrauma" denotes release of cytokines (mediators) secondary to epithelial injury caused by barotrauma or volutrauma [5]. Ventilator-induced lung injury greatly assists patients with the most severe form of lung injury, acute respiratory distress syndrome (ARDS) [6].

A question arises why hyperoxia, especially if it lasts long, is not being considered as a risk factor for the development of VILI along with the other factors. Oxygen therapy has been used in the care of critically ill patients since early years of the last century. However, from the beginnings of the 1970s an increasing understanding emerged that oxygen therapy can cause pulmonary toxicity [7]. Hyperoxia is detrimental for mechanically ventilated patients and even fraction of inspired oxygen (FiO_2) levels of 0.40 and lower can provoke pulmonary toxicity, thus leading to VILI. The exposure time to hyperoxia is certainly very important and patients who spend extended time on MV are probably more exposed to severe hyperoxic acute lung injury (HALI) [8].

Hyperoxia is supposed to precipitate lung injury through the production of reactive oxygen intermediates [9]. Hyperoxia provokes cytokine release, which is involved in the inflammatory response. Endothelial and epithelial cells injury, increased pulmonary capillary permeability and a marked increase in the inflammatory cells are the main manifestations of HALI [10]. Microscopically the prominent findings are: hyaline membrane formation in alveoli, alveolar septal edema and fibrosis, and diffuse hyperplasia of the alveolar lining layer with formation of a cuboidal epithelial lining [7]. The combination of high Vt and hyperoxia causes significantly greater reductions in the lung compliance, increased alveolar-capillary membrane permeability, gives more severe pulmonary surfactant dysfunction [11], and increases expression of pro-inflammatory mediators [12,13]. These notions have been confirmed in a several number of studies, which showed that cyclic opening and collapse of the alveoli even at low inspiratory pressures and low inspiratory volumes increased stretch and shear forces resulting in lung injury and surfactant dysfunction [14,15]. Inflammation and more specifically, cytokines such as tumor necrosis factor - alpha (TNFα) and interleukin (IL)-I are thought to decrease surfactant components either directly [14-17] or indirectly by inducing alveolar leakage of proteins that subsequently inhibit surfactant function [14-18]. Cytokines play the most important role in inflammation. They are low molecular weight soluble proteins that transmit signals between the cells involved in the inflammatory response [19]. Cytokines are produced by bronchial, bronchiolar, and alveolar epithelial cells but also by alveolar macrophages and neutrophils [20]. In almost all studies in-vitro, ex-vivo and in-vivo models, using different species and applying various techniques, hyperoxia induced elevation of cytokines. Furthermore, in almost all studies, cyclic overstretch has increased alveolar levels of IL-8 or its rodent equivalent macrophages inflammatory protein (MIP)-2. MIP-2 is the most potent leukocyte chemoattractant and its role in the pathogenesis of VILI is very important [21,22]. Other proinflammatory cytokines such as IL-1and IL-6 were elevated in a large number of studies.

FIO_2 LEVELS: DIFFERENT ATTITUDES, BELIEFS AND PRACTICES

Considerable variations exist in the attitudes, beliefs, and stated practices relating to the management of oxygen therapy in the ICUs patients. A Canadian questionnaire study has shown that most respondents believed that the levels of FiO_2 up to 0.40 are not harmful and this is the ideal value when partial pressure of oxygen in arterial blood (PaO_2) permits this [23]. Another newer study from the Netherlands investigated the beliefs and actual clinical practice regarding the oxygen therapy in critically ill patients where the majority of ICUs clinicians acknowledged the potential adverse effects of prolonged exposure to hyperoxia and reported a low tolerance for high oxygen levels, in actual clinical practice; a large proportion of their ICUs patients was exposed to higher arterial oxygen levels [24, 25].

However, there is evidence of poor outcomes after hyperoxia in a number of patients mechanically ventilated, but in most cases this did not lead to adjustment of ventilator settings [10]. All the doctors in ICUs have their "own" mode of setting the ventilator, but almost always patients on MV are exposed to greater than normal concentration of oxygen. Additionally, in some patients it is not possible to develop a ventilation strategy that is non-injurious in all lung regions and hence the problem becomes more complex.

Clinical practice shows that whenever we have a low PaO_2, the first step we undertake is to set the FiO_2 on a higher level. It is well-known that this is not the right way to increase PaO_2, but in spite of that, we are used. In this protocol, higher PEEP with lower FiO_2 is used. The higher PEEP levels are set and adjusted according to each patient's arterial-oxygenation response to the PEEP/FiO_2 settings [29]. (Table 1).

Table 1. Higher PEEP levels with Lower FiO_2

FiO_2	03	0.3	0.3	0.3	0.3	0.4	0.4	0.5
PEEP	5	8	10	12	14	14	16	16

always repeat it and moreover, for a longer period of time. FiO_2 setting value is usually about 0.4, but even oxygen toxicity is presumed to occur at levels of FiO_2 exceeding 0.40, there are no studies which have examined the effect of FiO_2 value less than 0.4 on the lungs. It would be of great benefit to perform studies that will apply protective ventilation strategies with different levels of FiO_2, but especially with FiO_2 under 0.4.

RATIONALE STRATEGY FOR LUNG PROTECTIVE VENTILATION

In order to achieve lower FiO_2 level than 0.4, which is safe for the patient, it is necessary to decrease FiO_2 to safe levels through appropriate use of the positive end expiratory pressure (PEEP) and the alignment of the mean airway pressure. An acceptable level of PaO_2/FiO_2 ratio with a lower limit of FiO_2 must be accomplished. This way, adequate tissue oxygenation with FiO_2 levels less than 0.40 will be achieved.

The modes of mechanical ventilation are adjusted according to the protocol of the ARDS-net study (based on open lung concept) [26]. The ARDS network study gives a golden protocol for lung protective ventilation. Ventilation protective strategies are used routinely without fulfilling the criteria for ALI/ARDS [27]. The ARDS network study demonstrated a compelling survival advantages when using low tidal volumes rather than conventional MV with high tidal volumes in patients with ALI or ARDS [28].

According to the protocol, the ventilator set up and adjustment is as follows: MV is adjusted to the body mass, with an initial Vt of 8 ml/kg/bw and reducing Vt by 1 ml/kg/bw at intervals not more than 2 hours, until reaching the Vt of 6 ml/kg/bw, with plateau airway pressure (Pplat) not exceeding 30 cmH_2O. Furthermore, PaO_2 between 55-80 mmHg or saturation of the arterial blood oxygen (SaO_2%) 88-95% and minute ventilation of 6–35 respirations per minute adjusted to achieve arterial pH ≥7.30 if possible and inspiration:expiration time 1:1–1:3 are accepted values. A minimum PEEP of 5 cmH_2O, and incremental PEEP/ FiO_2 combinations

From the National Heart, Lung, and Blood Institute ARDS Clinical Trials Network. N Engl Med 2004; 351:327 [29].

However, it is still unclear which is the optimal PEEP that has to be used to avoid overdistension of the alveoli and de-recruitment, and to minimize VILI [30,31].

Regarding Pplat, to reach Pplat ≤ 30 cm H_2O after each change in PEEP or VT, Pplat (0.5 second inspiratory pause) has to be checked [27].

Recruitment maneuvers are performed to maximize the amount of open lung while avoiding the high tissue stresses that lead to VILI. Extended sigh recruitment maneuver has better improvement of oxygenation in arterial blood than CPAP recruitment maneuver [32,33].

Additionally, bedside the monitoring, the following is necessary: heart rate, electrocardiogram, non-invasive mean arterial pressure, respiratory rate, oxygen saturation, FiO_2, arterial blood gas analysis (monitored half an hour after every MV adjustment), serial chest X-ray and chest/lung computed tomography that reflect the pathologic phases of diffuse alveolar damage [34]. Immunological analyses of cytokines: IL-1, IL-6, TNF**α** and MIP-2 also have to be made.

Precise control of the arterial oxygenation to minimize the possible harms of hypoxemia is an issue that is extremely important, as at present there is no direct evidence to support the implementation of permissive hypoxemia [35].

The effects of open lung ventilation with reduced FiO_2 of 0.4 will prevent not only local, but systemic inflammatory response as well. Furthermore, the effects of setting FiO_2 values at 0.3 will be more reliable. The effects will of course depend on the condition of the lungs (healthy or diseased prior to MV). Evidence for oxygen use in different medical conditions where efficacy and/or safety are uncertain relies on anecdotal experiences, case reports, or small, underpowered studies and require large randomized controlled clinical trials [36].

The rationale of this approach will determine the exact FiO_2 level necessary for safe MV.

CONCLUSION

Patients undergoing MV might develop VILI. Hyperoxia is detrimental for mechanically ventilated patients and may lead to VILI. VILI can appear as a result of too much O_2, large tidal volumes, high inspiratory pressures, cyclic opening/closing of the alveoli and all these lead to release of cytokines. The release of cytokines leads to biochemical injury, which is the concept of biotrauma. The lungs are metabolically active organs composed of epithelium and endothelium that create many substances. They are a door to many pathogens and may be source of systematic inflammation. Alveolar epithelial cells are important for maintaining alveolocapillary barrier and can act as immune effector cells in response to exogenous stimuli (MV). The mechanical ventilation together with hyperoxia are responsible for the cytokine increase and may play a role in initiating a possible systematic inflammatory response. Together, hyperoxia and biotrauma have a synergistic effect and can induce VILI.

The prevention of VILI means also prevention of HALI. Lung-protective ventilation strategies provide avoidance of cyclic opening and closing of alveoli, limitation of inspiratory pressures and volumes, appropriate level of the end-expiratory pressure and together with the appropriate (preferable lowest) level of FiO_2 can give full lung protection against the damage induced by mechanical ventilation.

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Резиме

СИНЕРГЕТСКИ ЕФЕКТ НА ХИПЕРОКСИЈА И БИОТРАУМА НА ВЕНТИЛАТОР-ПРЕДИЗВИКАНА ПОВРЕДА НА БЕЛИТЕ ДРОБОВИ

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Апстракт

Во единиците за интензивна нега (ЕИЛ) пациентите што се поставени на механичка вентилација (МВ) можат да развијат вентилатор-предизвикана повреда на белите дробови (ВИЛИ). Покрај големиот дишен волумен (Vt) и плато-притисок (Pplat), и хипероксијата ја влошува белодробната повреда. Се претпоставува дека кислородна токсичност се јавува при вредности на инспираторен кислород (FiO_2) што се поголеми од 0,40. Времето на изложеност на хипероксијата е, секако, многу важно и пациентите што поминуваат подолго време на МВ се веројатно повеќе изложени на тешка хипероксична акутна белодробна повреда (ХАЛИ). И двете, хипероксијата и биотраумата (ослободување на цитокини) имаат синергистички ефект и можат да предизвикаат ВИЛИ. Во клиничка практика целта е намалување на FiO_2 на безбедни вредности со соодветна примена на позитивен притисок на крајот на експириумот (PEEP) и усогласување на притисокот во дишните патишта. Стратегијата на белодробна протективна вентилација мора да вклучи поставување на FiO_2 на безбедно ниво, кое се постигнува со користење на односот PaO_2/FiO2, со долна граница на FiO2 за да се постигне прифатливо ниво на PaO_2, кое ќе биде безбедно за пациентот без локален (белите дробови) или системски инфламаторен одговор. Протоколот од ARDS-нет студијата се користи за поставување и приспособување на вентилаторот. Се испитуваат цитокините (IL-1, IL-6, TNFα и MIP-2) што се вклучени во инфламаторен одговор, со цел да помогнат во терапевтскиот пристап на ХАЛИ. Наодите од компјутеризираната томографија ги одразуваат патолошките фази на дифузното алвеоларно оштетување. Потребно е да се користи најниско ниво на FiO_2 за да се обезбеди целосна заштита на белите дробови од оштетување што е предизвикано од МВ.

Клучни зборови: хипероксија, вентилатор-индуцирана повреда на белите дробови, цитокини, вентилација стратегии за заштита на белите дробови

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NEONATAL HYPOGLYCEMIA: RISK FACTORS AND OUTCOMES

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ABSTRACT

Background and aims: Severe neonatal hypoglycemia (HG) leads to neurologic damage, mental retardation, epilepsy, personality disorders, impaired cardiac performance and muscle weakness. We aimed to assess the clinical characteristics of children with hypoglycemia in a random population of newborns.
Patients, methods and results: We investigated 84 patients (M:F=35:48) born at the University Clinic for Gynecology and Obstetrics in Skopje (hospitalized in the NICU) who were found to have hypoglycemia. In total 89.25% of the babies were premature. The mean birth weight was 1795.95 +/596.08 grams, the mean birth length was 41.92+/- 4.62 cm, while the mean gestational age was 33.05±3.19 weeks. 32 children (38.08%) were very low birth weight (<1500g), 38 (45.22%) were low birth weight (1500-2500g), while there were 8 children (9.52%) appropriate for age BW and no high BW for age patients (>4000 g).
HG duration was 2.42+/-2.41 hours. In the group as a whole, hypoxic-ischemic encephalopathy (HIE) was found in 3 children (3.57%), infections in 22 (26.18%), respiratory distress syndrome (RDS) in 9 patients (10.62%), intracranial haemorrhage in 2 patients (2.38%). There were no inborn errors of metabolism. There were two deaths (2.38%).
Conclusion: Neonatal HG is a significant factor in the overall neonatal mortality. HG can also cause severe invalidity. We found that infections, LBW and low gestational age were most commonly associated with neonatal HG. However the Spearman test showed weak direct correlation, without statistical significance. Neonatal HG requires complex and team interaction of prenatal and postnatal approaches to reduce the incidence of seizures, their consequences and the overall mortality. Special consideration is to be taken in measures that avoid neonatal infections, HIE, LBW and low gestational age. Further studies on a larger population are needed to fully understand and prevent the phenomenon of HG in newborns.

Keywords: neonatal hypoglycemia, co-morbidities, low birth weight, HIE, mortality.

INTRODUCTION

The neonatal period is marked by increased risk of HG and seizures (1-3). Neonatal HG is the most common form of a metabolic disturbance in newborns, while seizures are the most common symptom of neurological dysfunction (2,3). The incidence of neonatal seizure is high: 10-25% in neonatal intensive care units (NICU). Strikingly in NICU 15% of newborns with seizures will die and 35- 40% will have significant neurological defects (2).

Hypoxic-ischemic encephalopathy (HIE) is found in ~50% of the patients with neonatal HG (1). HIE may potentiate the permanent brain damage caused by HG (3). In neonates there are multiple other causative factors for HG: intracranial hemorrhage, infections, metabolic disorders, CNS malformations, birth trauma, and metabolic disorders (2, 3).

Delay in therapy often results in poor neurological outcome (4, 5). It is of note that the worst neurological outcome in large number of children is

observed in neonates and infants with persistent and recurrent severe hypoglycemia (3, 6).

We aimed to assess the incidence, etiology, and outcome of HG in newborns hospitalized at the neonatal ward and NICU of the University Clinic for Gynecology and Obstetrics.

PATIENTS AND METHODS

This study was carried out in the neonatal intensive care unit of University Clinic for Gynecology and Obstetrics in Skopje, Macedonia.

The following classification of neonatal HG was applied: capillary blood glucose (CBG) values were considered normal when ≥ 2.5 mmol/l, or HG was mild (2.2-2.4 mmol/l), moderate (1.6-2.1 mmol/l) or severe HG (<1.6 mmol/l)(7).

The diagnosis of neonatal seizures was based on clinical observation, multiple measurements of CBG (from the first hour after birth, than consecutively the 3rd, 6th hour of life, and/or until the resolution of the HG episode. The etiology of the seizures was assessed using clinical examination, laboratory results, and/or imaging (ultrasound).

The Diagnosis of HIE was determined by analyzing data from the medical history and physical examination, taking into regard the Apgar score, arterial blood gas results, and neuroimaging. The American Academy of Pediatrics and the American College of Obstetricians and Gynecologists defined those features for the diagnosis of birth asphyxia: 1) Profound metabolic or mixed acidemia (pH<7.00) in umbilical arterial blood. 2) Apgar score of 0-3>5 minute after birth. 3) Signs of neonatal encephalopathy (seizures, coma, or hypotonia), 4) Multiple organ involvement (kidney, lungs, liver, heart, intestines). Children were breastfed, rarely formulas were used..

Clinical examination, blood cell count, C reactive protein, and positive blood culture lead to the diagnosis of sepsis. Pulmonary infections were diagnosed clinically and confirmed by microbiological analysis of the deep tracheal aspiration. The existence of intracranial hemorrhage was demonstrated with US or CT scan.

The biochemical analysis was performed using the ARCHITECT plus c4000, Integra 400.

The statistical analysis included parametric and non-parametric statistics using SPSS software.

RESULTS

We investigated 84 patients (M:F=35:48) as a random group born at the University Clinic for Gynecology and Obstetrics and admitted to the NICU in Skopje. The delivery in 33 (39.27%) cases was vaginal, and in 51 (60.69%) caesarean section. Among all the patients 89.25% were preterm.

The mean birth weight was 1795.95 +/596.08 grams, the mean birth length was 41.92+/- 4.62 cm. 32 children (38.08%) were very low birth weight (<1500g), 38 (45.22%) were low birth weight (1500-2500g), while there were 8 children (9.52%) appropriate for age BW and no high BW for age patients (>4000 g) (Fig 1).

The Apgar score was 6.65 +/-1.11 at 0 minutes and 7.17+/-1.19 at 5 minutes.

The blood glucose level at 0 hours was 2.17+/-0.17, at one hour 4.08+/- 1.9. at 2 hours 4.37+/-1.74, at 3 hours 4.17+/-2.002, at 4 hours 4.39+/-1.89 and at 5 hours the glycaemia was 4.28+/-1.86 mmol/l. HG duration was 2.42+/-2.41 hours. In the group as a whole, hypoxic-ischemic encephalopathy (HIE) was found in 3 children (3.57%), infections in 22 (26.18%), respiratory distress syndrome (RDS) in 9 patients (10.62%), intracranial hemorrhage in 2 patients (2.38%) (Fig.2).

There were no inborn errors of metabolism. There were two deaths (2.38%) in children with infections, low birth weight and hypoglycemia.

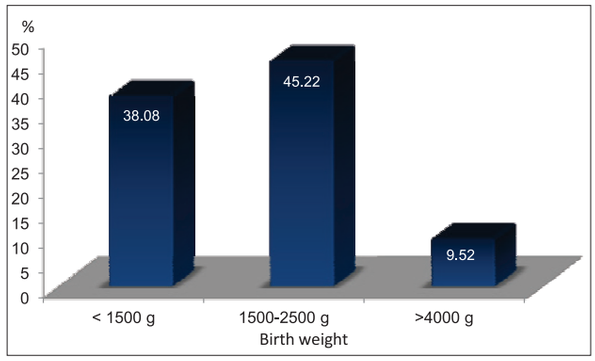

Figure 1: Birth weight distribution

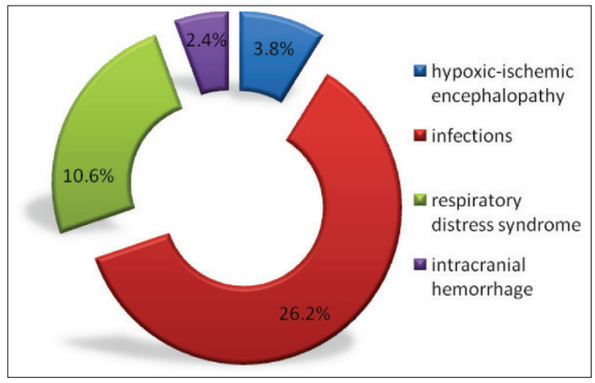

Figure 2: Co-morbidities in children with neonatal hypoglycaemia

Although the Spearman test showed weak direct correlation, there was not statistical significance for the association among HG and infections (multiple R = 0,752101; p=0,526683), gender (multiple R = 0,782301; p=0,577641), LBW (0,114932 weak direct correlation, but no statistical significance p=0,347025)(Fig.1) and low gestational age (-0,112608 weak direct correlation, but no statistical significance p=0,307799) (Fig. 3). HIE and HG were not correlated, probably due to the low number of patients with HIE (3 patients).

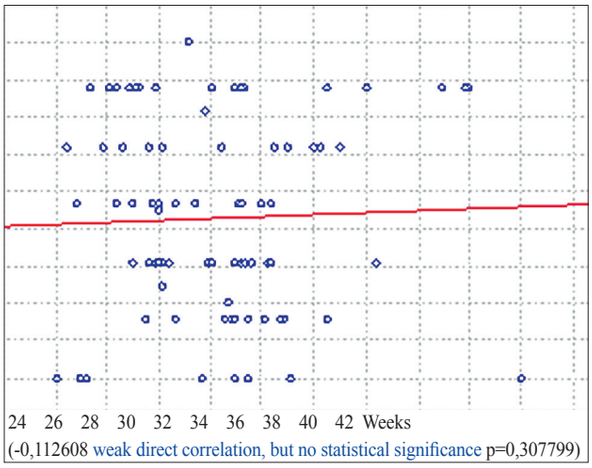

Figure 3: Birth age and hypoglycaemia

DISCUSSION

In neonates long term sequelae can occur within a wide range of low serum glucose values. Even transient moderate HG can result in neurological damage (8). The duration and severity of NH greatly influences the creation of permanent neurological damage, although the nadir glucose concentrations and the duration of HG that can damage newborn brain are not precisely determined (2, 3).

Serum glucose levels in neonates normally decline until age 1-3 hours and spontaneously increase afterwards. In fact, there is no rigorously determined specific blood glucose concentration for a definition of NH for infants (9-15).

The definition of neonatal HG that we used for the study was that of the clinical settings. HG in newborns was defined by a plasma glucose level of less than 30 mg/dL (1.65 mmol/L) in the first 24 hours of life and less than 45 mg/dL (2.5 mmol/L) thereafter. Many experts recommend that values of blood glucose <50 mg/dL in neonates should be vigorously treated (3).

As previous studies have already reported estimating the frequency of neonatal HG many factors can be influenced by many factors: the definition used, the type of glucose assay, the compartment measured (serum, whole blood), the population investigated, the method and timing of feeding.

In the USA NHG is estimated to 1.3-3 per 1000 live births (2), while in Japan, among neonates born at 35-36 weeks' gestation >80% of admissions to the NICU after birth were due to apnea or HG (16). Other authors (17-22) report an incidence between 7-11%. Population-based studies (Harris County, USA) reported an incidence of 1-3.5/1000 live birth (I 3). There is an expected high incidence in NICU: 10-25% (23).

We also observed that all the neonates had HG of early onset (before 72 hours). Mostly neonatal HG was early: 59.6% and 81% of neonates had early onset seizure in reports by different authors (23, 26).

In addition we also found that HIE was not the most frequent factor in NHG seizures (3.57%). HIE was the most frequent factor described by other authors (17, 24, 25). HIE may potentiate the role of HG in causing brain damage. Contrary to our results many authors reported that the HIE is the leading cause of neonatal HG and seizures (27-29).

Infections were the most common finding in children our study: 26.18%. Reported prevalence of seizures among children with infections is between 24.5 and 28.7% (17, 23). This difference might be due to the high number of risk deliveries in our hospital. Exact causes remain to be determined.

Intracranial bleeding in this series of patients was found in 2.38% of newborns. Others have found a higher percentage of intracranial bleeding: 6.9% -9.0% (1, 17, 23). Differences in the observed frequency stem from the different populations of patients: full-term versus pre-term, LBW versus ABW, patients from neonatal wards versus NICU.

The overall mortality remains high, especially in children with co-morbidities. HIE and infections are the most common leading causes of death. The mortality rates ranged between 9 and 14.7% (2, 17). We had a mortality of 2.38%.

The most common risk factors for neonatal HG were asphyxia, birth weight less than 1500 g, sepsis, convulsion, and meningitis (2, 3, 31). EEG is a gold standard for neonatal epilepsy (32). Among many other measures in lowering the neonatal HG, there is no evidence that the universal screening of glucose levels in the first hours should be applied to all newborn infants (33).

In conclusion, neonatal HG is a major factor in neonatal mortality (34). Neonatal HG is also a major factor in permanent neurological consequences. It is of note that major contributing factors for neonatal

HG should be actively searched and preventive measures must be taken in a timely manner. In this study LBW, low gestational age, HIE and high percentage of neonatal infections were the most frequent conditions associated with neonatal HG. It is of note that a larger group of patients is needed for more reliable data. Nevertheless it is obvious that preventive measures should address the LBW, HIE, infections and the low gestational age.

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Резиме

НЕОНАТАЛНА ХИПОГЛИКЕМИЈА: РИЗИК-ФАКТОРИ И ПОСЛЕДИЦИ

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Апстракт

Тешката неонатална хипогликемија (НХ) води до невролошки оштетувања, ментална ретардација, нарушувања на личноста, епилепсија, намалување на срцевата функција и мускулна слабост. Нашата цел беше да ја ги анализираме клиничките карактеристики на група новородени со хипогликемија, избрани по случаен избор.

Во ова ретроспективна студија иследивме 84 пациенти (м : ж=35 : 48), кои беа родени во Универзитетската клиника за гинекологија и акушерство и лекувани на Одделот за неонатална интензивна нега. Средната родилна тежина беше 1795,95+/-596,08 грама, средната родилна должина 41,92+/-4,62 цм, средната гестациска возраст 33,05±3,19 недели. 32 деца (38,08%) имаа многу ниска родилна тежина (>1500g), 38 (4,22%) беа со многу ниска родилна тежина (1500–2500g), родени со соодветна гестациска тежина беа 8 (9,52%), а родени со зголемена телесна тежина немаше.

Траењето на ХГ беше 2,42+/-2,41 часа. 94,4% од децата беа предвремено родени. Во целост, хипооксично исхемична енцефалопатија имаа 3 деца (3,57%), инфекции – 22 деца (26,18%), респираторен дистрес-синдром – 9 деца (10,62%), интракранијална хеморагија имаше кај двајца пациенти (2,38%). Не беа најдени деца со вродени грешки во метаболизмот. Две деца (2,38%) завршија летално.

Неонаталната ХГ може да предизвика тежок инвалидитет и е значителен фактор во вкупната неонатална смртност. Студијата покажува дека инфекциите, ниската родилна тежина, малата гестациска возраст се често асоцирани со ХГ. Неонаталната ХГ бара комплексна и тимска соработка и во пренаталниот и во постнаталниот пристап за да се редуцира фреквенцијата и последиците на неонаталната ХГ. Специјална грижа треба да се посвети на намалувањето на бројот и сериозноста на неонаталните инфекции, хипооксично исхемичната енцефалопатија, ниската родилна тежина и малата гестациска возраст. Понатамошни студии со поголеми примероци се потребни за да се разбере целосно феноменот на неонаталната ХГ и за да се преземат мерки за нејзина превенција.

Клучни зборови: неонатална хипогликемија, коморбидности, ниска родилна тежина, морталитет

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BDI IN THE ASSESSMENT OF DEPRESSION IN DIFFERENT MEDICAL CONDITIONS

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ABSTRACT

As a common disorder, depression must be diagnosed not only in psychiatric but also in different medical settings, especially in patients with chronic diseases. Beck Depression Inventory is valuable and sensitive self-report inventory used worldwide for assessment depressive symptoms. In this research we present obtained scores of BDI in different group of disorders and we showed that BDI scores are related to the clinical condition, as well as with the age.

Key words: depression, self-report, BDI

INTRODUCTION

Depression is a common but very serious mood disorder. It causes severe symptoms that affect practically all daily activities, such as sleeping, eating, or working. To be diagnosed with depression, the symptoms must be present for at least two weeks.

The World Health Organization (WHO) has ranked depression the 4th leading cause of disability worldwide and projects that by 2020, it will be the second leading cause [1].

Direct information on the prevalence of major depression does not exist for most countries, so, there is wide variability in prevalence estimates. Moussavi et al. in Lancet (2007) published data for lifetime prevalence estimates which ranged from 1.0% (Czech Republic) to 16.9% (US), with midpoints at 8.3% (Canada) and 9.0% (Chile). The 12-month prevalence estimates ranged from 0.3% (Czech Republic) to 10% (US), with midpoints at 4.5% (Mexico) and 5.2% (West Germany). However, lifetime prevalence is two-three times higher that of 12-month prevalence suggesting that between one-third and one-half of lifetime cases have recurrent episodes in a given year. The ratio of 12-month to lifetime prevalence estimates in some reports was significantly lower on average in reports concerned to high income (37.7%) than low-middle income (53.3%) countries.

In the report about global World Health in 2008 it was pointed that depression has a greater effect on overall health than angina, arthritis, asthma or diabetes.

The wide variability in the prevalence estimates is presumably due to a combination of substantive, measurement, and study design factors. The WHO World Mental Health (WMH) Survey Initiative in order to study cross-national differences in prevalence, proposed a common protocol and instrument, the WHO Composite International Diagnostic Interview (CIDI) Version 3.0. The 12-month prevalence estimate in 18 WMH countries ranged from 2.2% (Japan) to 10.4% (Brazil) [2, 3].

A number of consistent socio-demographic correlates, as well as the evidence for a wide range of adverse effects of major depression have also been found across countries. Cross-national data document associations of depression with some factors such as low education, high teen child-bearing, marital disruption, unstable employment, low marital quality, low work performance, low earnings, a wide range of secondary disorders etc. [4, 5].

The criteria for depressive disorders have been explained in the Diagnostic and Statistical Manual of

Mental Disorders. There are several DSM editions, the newest one is DSM-V, edited by American Psychiatric Association in 2013. In this manual, unlike in DSM-IV, the chapter "Depressive Disorders" has been separated from the previous chapter "Bipolar and Related Disorders." The common feature of all of these disorders is the presence of sad, empty, or irritable mood, accompanied by somatic and cognitive changes that significantly affect the individual's capacity to function.

Since depression cannot be diagnosed with any laboratory testing the diagnose is mainly based upon symptomatology and medical history following criteria noted in DSM-V. However, because of possible comorbidity, some main laboratory must be done, such as: complete blood count, thyroid function check, creatinine and blood urea nitrogen, liver function check, fasting blood glucose, cholesterol, calcium and magnesium level and folic acid and vitamin B_{12} levels.

Untreated depression increases the chance of other risky behaviors such as drug or alcohol addiction. It also can ruin relationships, cause problems at work, and make it difficult to overcome serious illnesses. Additionally, depression carries a high risk of suicide. It is especially a possible outcome in males, opposite to suicide tentamens in female population.

In this context, depression must be diagnosed at time, and treated corresponding current medical knowledge.

Without proper treatment, including pharmacotherapy (antidepressants) and psychotherapy, untreated depression can last for weeks, months, or years. The other possible modalities for treatment are Transcranial Magnetic Resonance, Biofeedback or Cranial Electrotherapy Stimulation (CES).

Together with clinical symptomatology, the diagnosis is based more frequently on psychological testing but neuroimaging techniques and QEEG recordings are also available.

There are several tests for diagnostic of depression: Hamilton scale (HAM-D), Zung Self-Rating Depression Scale, Montgomery-Asberg Depression Rating Scale, Geriatric Depression Scale, Beck Depression Inventory etc. Except the HAM-D (used by professionals), which was developed as a measure of treatment outcome rather than a screening or diagnostic tool for depression, all others are based on self-reports.

The most frequently used self-report measures are Beck Depression Inventory I and II, Center for Epidemiologic Studies Depression Scale, Geriatric Depression Scale, Hospital Anxiety and Depression Scale, and Patient Health Questionnaire-9. Some of these measures have become integrated into routine clinical practice (as screening tools) in large managed-care organizations

The aim of this article is to present the results obtained with the original BDI for testing depressive symptoms in different groups of patients in the period over 10 years.

METHOD AND SAMPLES

In this research the original BDI was used for testing depressive symptoms in different groups of patients in the period over 10 years.

Beck Depression Inventory follows the criteria for depression listed in the fourth edition of the Diagnostic and Statistical Manual of Mental Disorders. The test consists of twenty-one questions that not only assess the presence of depression, but also the severity of depression as well. There are three versions of the BDI—the original BDI, created by Aaron T. Beck, first published in 1961 and later revised in 1978 as the BDI-1A, and finally the BDI-II, published in 1996. The BDI is widely used as an assessment tool by health care professionals and researchers in a variety of settings. Each question has a set of at least four possible responses, ranging in intensity. When the test is scored, a value of 0 to 3 is assigned for each answer and then the total score is compared to a key to determine the depression's severity. The standard cut-off scores are as follows: 0–9: indicates minimal depression; 10–18: indicates mild depression; 19–29: indicates moderate depression; 30–63: indicates severe depression [6].

For this research BDI is applied in 510 patients, comprising followed groups:

(1) For checking possible postpartum depression BDI was applied in 150 randomly selected women, examined within the first week after delivery. Mean age of women was 29.23 years (SD± 5.11). In the majority there was the first childbirth (mean 1.34).

(2) Patients treated with chronic maintenance dialysis comprised a sample of 230 patients; 110 females (mean age 55.5 ± 13.5 years), and 120 males (mean age 54.5± 14.3 years). The mean duration of maintenance dialysis was 8.3 ± 5.8 years (from 0.5 to 24 years). Patients were selected randomly from three dialysis centers in R. Macedonia.

(3) Patients with different ophthalmological problems (N=100); mean age 41.6 ± 15.9 years, both sexes included. Patients were divided in two groups: serious ophthalmological diagnoses, where we expected psychological problems (N=65) and simplest ones (N=35) as control.

(4) Group of adolescents (N= 20) with obsessive compulsive disorder (OCD); mean age 16, 33±1, 83 years, both genders.

(5) Adolescents with chronic diseases: a) cystic fibrosis (N = 25 mean age = 17.5±2.6 years); b) malignancies (N=20, mean age=19.5±1.3 years) c) juvenile rheumatoid arthritis (N=15, mean age 13.5 ± 0.56 years) and d) diabetes mellitus (N = 25, mean age = 13.5 ± 1.5 years, 18 girls and 7 boys).

(6) Adults females with moderate hypertension (N= 25) mean age 65.2 ± 7.5 years tested as voluntaries for evaluation the effects of cranial electrostimulation (CES) with Alpha Stim.

RESULTS

Table 1 presents some characteristics of all evaluated patients.

Table 1. Some characteristics of evaluated groups

Diagnosis	*Number*		*Mean age (years)*
Postpartum depression (PPD)	150	females	29.23 ± 5.11
Dialyzed patients	230	110 females 120 males	55.5 ± 13.5 54.5 ± 14.3
OCD	20	both sexes	16.33 ± 1.83
Cystic fibrosis (CF)	25	both sexes	17.5 ± 2.6
Malignancies	20	both sexes	19.5 ± 1.3
Juvenile rheumatoid arthritis (JRA)	15	both sexes	13.5 ± 0.56
Diabetes mellitus T1 (T1DM)	25	18 girls 7 boys	13.5 ± 1.5
Adults with moderate hypertension	25	females	65.2 ± 7.5

Postpartum depression (PPD) is a complex combination of physical, emotional, and behavioral changes that happen in a woman after giving birth [7]. According to DSM IV there are three types of mood changes in this period: "baby blues", PPD and postpartum psychosis.

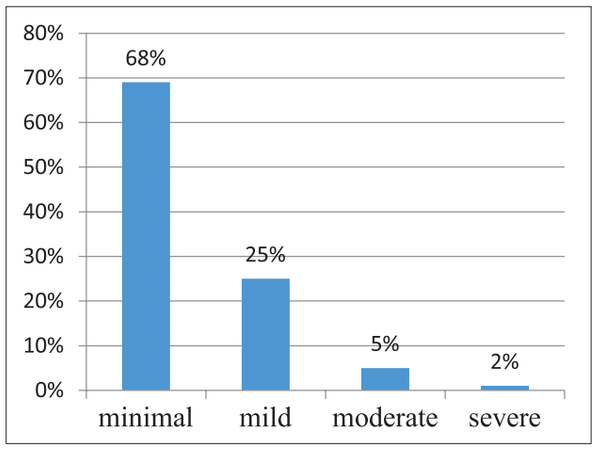


Figure 1: Level of postpartum depression obtained with BDI

The diagnosis of postpartum depression is based not only on the length of time between delivery and onset, but also on the severity of the depression. Generally, the incidence worldwide is supposed to be 10-20% [8]. In Hispanic women the prevalence of significant symptoms of PPD was found to be much higher (54.2%) [9].

A number of factors can increase the risk of postpartum depression, including: a history of depression during or before pregnancy, age at time of pregnancy (the younger the women is, the higher the risk), ambivalence about the pregnancy, number of children, having a history of premenstrual dysphoric disorder, limited social support, living alone, marital conflict, especially domestic violence [10-12].

The level of depression in this group of women obtained with BDI is presented on Figure 1. It is obvious that the most frequent is the state of minimal postpartum depression.

Correlation between the age and depression scores is presented on Figure 2. The calculated Pearson's coefficient was r= - 0.15 which means minimal negative correlation. This result confirms that younger women are more susceptible for depressive

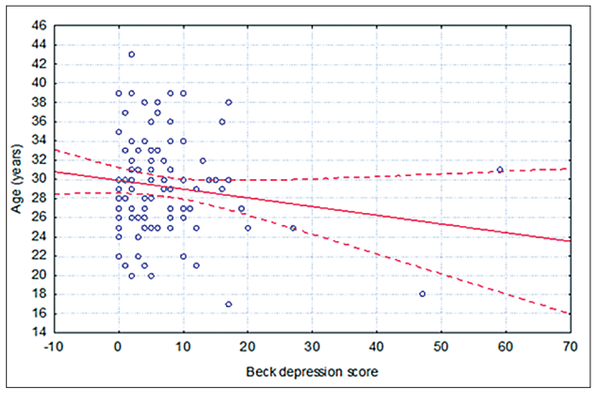


Figure 2: Correlation between age of women and Beck depression scores

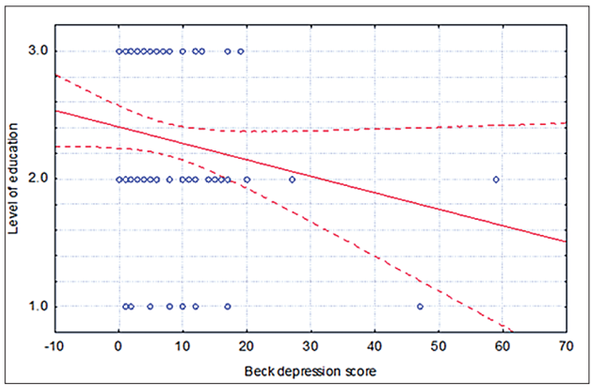


Figure 3: Correlation between level of education (1- primary school; 2- high school; 3- university degree) and Beck depression scores

reaction. Concerning education (Figure 3) minimal negative correlation between depression and education is obtained. It means that higher education influence as beneficial factor for PPD.

The hemodialysis as a treatment of choice for the end-stage renal diseases disrupt a normal lifestyle and require considerable psychological and social adaptation. The mean duration of maintenance dialysis in the evaluated sample was 8.3 ± 5.8 years (from 0.5 to 24 years). It was expected that chronic stress related to the process of dialysis increases the level of depression. Obtained scores for BDI in dialyzed patients is shown on Figure 4.

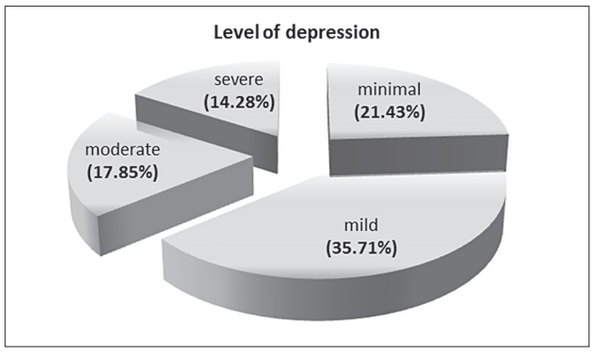
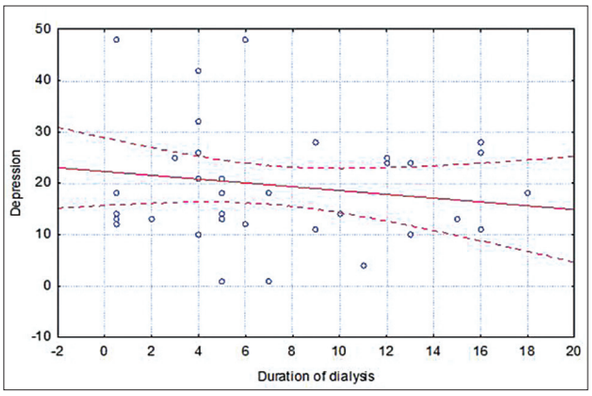


Figure 4: Level of depression in dialyzed patients graduated by scores obtained with BDI

Depression can be described as being composed of two components: an affective (mood) one and a physical (somatic) one (e.g., loss of appetite, fatigue). In participants with concomitant physical illness like chronic renal failure, the BDI's reliance on physical symptoms such as fatigue, may artificially inflate scores due to symptoms of illness, rather than those of depression. For this reason, there are several precautions that must be taken when interpreting the results. However, the BDI has been shown to be valid and reliable with results corresponding to a clinician rating of depression in more than 90% of all cases.

Regarding the duration of dialysis (in years) and depression, minimal negative correlation was obtained (r= -016) which is not statistically significant (Figure 5).



[Correlation r = -0.16; p = 0.32 (p>0.05)]

Figure 5: Correlation between duration of dialysis and depression score.

Eyes, being the organs of vision, detect light and convert it into electro-chemical impulses in neurons. Having this characteristics, vision is the most important sensorial part of the information system. Visual loss leads to reduced ability to perform routine activities of daily living. It is obvious that any kind of visual problems can be the risk for stable mental health. Patients with binocular disease have severe difficulties performing fine visual tests such as reading, and are often faced with serious lifestyle issues such as impending loss of driver's license, work, and independence [13].

It was described in many studies that depressive symptoms and anxiety are two common, practically normal responses to a glaucoma diagnosis [14]. It is similar with other ophthalmological diseases, but in the everyday practice mental health stays not so important. In addition, medical school doesn't usually discuss the soft skill of how to talk to patients. On the patient's part, the Internet will often give inaccurate disease information or take facts out of context. Still, depressed, anxious, or cognitively impaired patients are less likely to adhere to their medication regimen, putting themselves at an even greater risk for complications. On the other side, new researches show that even minimal depression can affect visual function in age-related macular degeneration (AMD) patients [15]. Table 1 presents serious ophthalmological patients (N=65) included in this study.

The rest of 35 patients are diagnosed as chronic conjunctivitis, emmetropia, astigmatisms, and simple hypermetropia, and this group is comprised as control.

Table 2: Patients with serious ophthalmological problems

Diagnosis	*Number of patients*
Diabetic Retinopathy	5
Glaucoma	10
Age-related Macular Degeneration (AMD)	10
Cataract	10
High Myopia (Myopia Alta)	5
Presbyopia	25

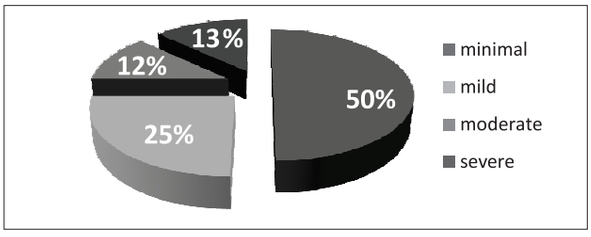


Figure 6: Level of depression in ophthalmological patients

BDI scores obtained in all group are presented on Figure 6.

Results obtained by BDI for all patients show that 50% of evaluated patients manifested minimal, 25% mild, 12% moderate, but even 13% severe depression (see Figure 6). This finding is very important because the moderate and severe depressions were unrecognized and untreated. It also raises the importance for deeper communication and support for all patients with vision's problems.

The highest scores for depression indicating moderate and severe depression are obtained from patients with age-related macular degeneration, proliferative diabetic retinopathy, as well as with glaucoma and cataract. Obtained results for the 35 patients with minimal eyes problems showed practically normal BDI scores (scores 0-9).

The correlation between BDI scores and age in this group of patients is presented on Figure 7. It

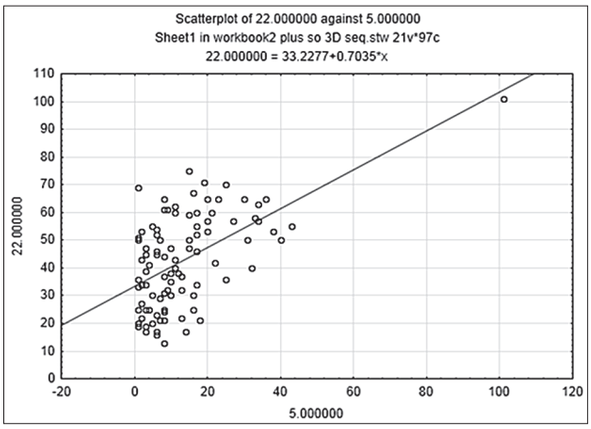


Figure 7: Correlation between obtained scores for depression and age

is very important to point out that all patients with moderate and severe depression are over 50 years old.

Obsessive–compulsive disorder (OCD) is a mental disorder where people feel the need to check things repeatedly, have certain thoughts repeatedly, or feel they need to perform certain routines repeatedly. People with this disorder are unable to control either the thoughts or the activities. Common activities include hand washing, counting of things, and checking to see if a door is locked. Often they take up more than an hour a day. The condition could be associated with tics, anxiety disorder, and an increased risk of suicide. In general, such behavior can disturb the normal functioning of the person. It is estimated that OCD affects 1–3% of the general population. The National Comorbidity Survey Replication found a median age of onset in OCD of 19 years, with 21% of cases having onset by age 10 [16].

The BDI results are showing that among this group of adolescents N=20 mean age 16.33 ± 1, 83 with OCD we did not find scores for clinical depression (mean BDI=7 ± 0.5).

The group of chronic patients comprised adolescents with cystic fibrosis, JRA, T1DM and different forms of malignancies.

The group with Cystic fibrosis comprised 25 adolescents with mean age 17.5 ± 2.6 years. Obtained BDI scores in majority of patients was 8.5 ± 0.8 which correspond to the state of no depression. Only in two patients BDI was 15 and 16 respectively, which means moderate depression. These results corresponded with the clinical presentation of depressive mood, fatigue and sleep problems, especially manifested in a girl, 19 years old who had family problems, but not related to the diagnosis. A few weeks later, she overcame this depressive reaction. The other patient was a boy aged 17.5 years and the depressive reaction was related to the disruption of the emotional relation with his girlfriend.

In patients with T1 diabetes mellitus BDI showed normal scores related to no depressive signs (mean scores 9.02 ± 1.3). Practically the same results are obtained in a group of JRA patients which was unexpected because the frequency of pain sensations in the last group.

The Beck depression inventory (BDI) was also applied in adolescents with malignant diseases. The group's results showed total scores of 14.66, which are under the cutoff for depression. However, in 8 patients the score on BDI was over 19 which corresponds to manifest depression.

In all depressed adolescents the psychological treatment was organized.

For testing the effects of cranial electrostimulation with Alpha Stim^R (CES) we evaluated a group of adult women recruited as voluntaries with moderate hypertension (N= 25), mean age 65.2 ± 7.5 years. The obtained mean BDI scores before the treatment were 25 ± 2.45 (confirming moderate depression) and were reduced to 14 ± 1.33 (mild depression) after the treatment. The obtained statistical significance is on the level $p<0.05$ and confirmed the effectiveness of this therapeutic modality for both, depression and hypertension.

Concerning all obtained BDI results in this study, it was obvious that the depressive mood is higher in older patients with any medical condition. In this context, BDI was higher in dialyzed patients, people with ophthalmological problems as well as women with moderate hypertension.

DISCUSSION

It is known that historically, depression was described in psychodynamic terms as "inverted hostility against the self". By contrast, the BDI was developed in a way by collating patients' descriptions of their symptoms and then using these to structure a scale which could reflect the intensity or severity of a given symptom. In this context, Aron Beck developed a triad of negative cognitions about the world, the future, and the self, which play a major role in depression.

Structured as self-report for 21 statement, scored with answers 0-3, the obtained higher total scores for BDI indicate more severe depressive symptoms.

In this article we showed that the use of BDI in practice is very useful for confirmation of the depression not only in psychiatric patients, but in a larger population. As it can been illustrated, BDI differentiated women in postpartum period in groups with minimal (68%), mild (25%), moderate (5%) and severe depression (2%). This finding is important especially for selection of non-diagnosed patients in obstetric practice [17].

In a group of dialyzed patients, it was expected that depression will be frequent mental problem. BDI showed the presence of minimal depression in 21.43%, mild in 35.71%, moderate in 17.85% and severe in 14.28%. This finding is very important for the medical staff included in the treatment of the end-stage renal diseases for organizing prevention/treatment of psychological comorbid problems in these patients [18].

Unexpectedly, the population treated in ophthalmological setting tested with BDI showed scores for severe depression in 13%, mild in 12%, moderate in 25%, and minimal in 50%. We concluded that depression appeared as an important mental problem in ophthalmological practice. It is usually unrecognized and untreated. Depression could be the risk factor for treatment and prognosis of the eyes diseases. Generally, all more severe eyes problems have a high impact on patients' daily life and causes restrictions of their psychological well-being, autonomy and mobility. For this reason, the role of doctors and nurses involved in the treatment of ophthalmological disorders is very subtle. In this context we proposed some measures for mitigation of psychological problems [19].

We also showed that adult women with moderate hypertension manifested moderate depression as well, which was underdiagnosed. Additionally, we showed that cranial electrostimulation is good non-pharmaceutical modality for treatment of moderate depression [20].

The used BDI was nonsignificant in adolescent patients with CF, T1DM, and JRA as well as in the patients with different forms of malignancies [21, 22, 23].

Our findings suggest that depression is highly correlated with age. In the adolescent period coping mechanisms are very strong and powerful and they help to overcome depressive reactions in majority of patients.

However, non-psychiatric clinicians have difficulty recognizing depression in their patients. One meta-analysis found that non-psychiatric clinicians accurately diagnosed depression in only 36% of depressed patients; another meta-analysis found a rate of 47% [24, 25].

As a general comment regarding any assessment of depression is that users of self-report instruments without psychological background/experience in the management of clinical issues related to depression may need supervision.

Concerning the age related depressive signs, Sharp and Lipsky (2002) reported that psychometric data on the BDI are mixed, so the BDI may not be the best screening measure for elderly patients [26]. Based on the research that used different depression rating scales in the elderly population, it was shown that the GDS is the best validated instrument in various geriatric populations. For example, in cognitively intact elders, the GDS appears to be a well-validated instrument in various treatment settings.

The BDI was revised in 1996 and as result the BDI-II appeared. It is developed in response to the American Psychiatric Association's publication of the DSM-IV, which changed many of the diagnostic criteria for Major Depressive Disorder [27].

Items involving changes in body image, hypochondriasis, and difficulty working were replaced. Also, sleep loss and appetite loss items were revised to assess both increases and decreases in sleep and appetite. All but three of the items were reworded; only the items dealing with feelings of being punished, thoughts about suicide, and interest in sex remained the same. Finally, participants were asked to rate how they have been feeling for the past two weeks, as opposed to the past week as in the original BDI. Since the publication of the BDI-II, a sparse literature exists regarding its reliability, validity, and utility specifically among older adults.

Like the BDI, the BDI-II also contains 21 questions, each answer being scored on a scale value of 0 to 3. Higher total scores indicate more severe depressive symptoms. Steer, Rissmiller, and Beck (2000) reported that the BDI-II had good internal consistency ($\alpha = .90$) and that the total score was not significantly correlated with gender, age, or ethnicity of the patients.

In our country, we mainly used the older BDI for assessment depressive signs, and we are pretty satisfied with obtained results [28].

The results of the study of Sangmee Ahn Jo et al. 2016 showed that the Korean version of the BDI is appropriate for screening for depression and 16 is the optimal cut-off score for the Korean elderly [29].

The BDI has the same problems as other self-report inventories, in manner that scores can be easily exaggerated or minimized by the person completing them. Additionally, the way the instrument is administered can have an effect on the final score. If a patient is asked to fill out the form in front of the examiner in a clinical environment, social expectations have been shown to elicit a different response compared to administration via a postal survey.

However, BDI was used widely in the selection of depressive symptoms in different groups of patients such as: psoriatic patients [30], multiple sclerosis [31, 32], adolescents from dysfunctional families [33], high school adolescents [34], patients with dry eyes disease [35], in medical students [36], patients with coronary disease [37], patients with rheumatoid arthritis [38] etc.

Application of BDI in participants with concomitant physical illness may artificially inflate scores due to symptoms of the illness, rather than of depression. In an effort to deal with this concern Beck and his colleagues developed the "Beck Depression Inventory for Primary Care" (BDI-PC), a short screening scale consisting of seven items from the BDI-II considered to be independent of physical function. Unlike the standard BDI, the BDI-PC produces only a binary outcome of "not depressed" or "depressed" for patients above a cutoff score of 4.

Although designed as a screening device rather than a diagnostic tool, the BDI is sometimes used by health care providers to reach a quick diagnosis.

Some important remarks

- Depression as a commonly occurring disorder worldwide could be successfully assessed with BDI as a self- report inventory
- Lifetime prevalence estimates of major depression vary across countries, with prevalence generally higher in high income versus low-middle income countries
- Depression and it's severity confirmed with scores obtained with BDI is age-related
- Depression is comorbid with many other diseases, especially with chronic ones.

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Резиме

БДИ ВО ПРОЦЕНКАТА НА ДЕПРЕСИЈА ВО РАЗЛИЧНИ МЕДИЦИНСКИ СОСТОЈБИ

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Апстракт

Како многу често заболување, депресијата треба да биде дијагностицирана не само во психијатриските туку и во други медицински установи, особено оние што се занимаваат со хронично болни пациенти. Бековиот инвентор за депресија е валиден и сензитивен саморапортирачки инструмент, кој се користи насекаде во светот за проценка на депресивните симптоми. Во ова истражување се презентирани добиените скорови од БДИ кај разни групи растројства и покажано е дека тие се зависни од клиничката состојба, како и од возраста.

Клучни зборови: депресија, саморапортирање, БДИ

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CLINICAL EVALUATION OF ROOT END RESECTION BEVEL IN PERIAPICAL SURGERY

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ABSTRACT

Root end resections play an important role in the success of periapical surgery. Beveling of the root end resections can vary significantly depending on the surgical technique, the root and canal morphology.
The intention of this article was to clinically assess the root resections bevels and to estimate their relation to applied periapical surgeries.
A prospective clinical study consisted of sixty periapical surgeries performed on teeth with chronic periapical lesions. Thirty periapical surgeries were performed in a conventional manner, while thirty were contemporary ultrasonic surgeries. Following the completion of strictly planned and performed intraoperative procedures, the resection bevels were assessed. To obtain the real bevel angles a compass was used. Root resections were significantly less beveled in all teeth operated with contemporary ultrasonic surgery, with mean values between 2.1° to 7.8°. The number of roots and their dilacerations didn't influence the root resection bevel. For comparison, root resections were significantly beveled in all conventionally operated teeth, with mean values of 46°. Due to the technical limitations of the conventional periapical surgery, mandibular premolars were exclusively operated with ultrasonic periapical surgery, with mean values of resection bevel not exceeding 20.7°. Significantly lesser resection bevel associated with ultrasonic periapical surgery contributes to root preservation and favorable surgical outcome.

Key words: periapical surgery, root end resection and bevel

INTRODUCTION

Root end resections are an important aspect of the periapical surgery. The root end is resected in order to identify the root canal and provide access to the source of infection (1). A root resection of 2-3 mm eliminates an apical area with the highest incidence of accessory canals (2) and exposes the root surface within reach of visual inspection and further instrumentation. The apex of most teeth contains multiple foramina and by removing the apical 2–3 mm of the root most of these can be removed (3) but care must be taken not to compromise the crown-to-root length ratio.

Historically, resection and beveling was carried out in order to improve access to the root canal for preparation with a round bur (4). The conventional (bur) type of periapical surgery is virtually unfeasible if root resections are not beveled, resulting in steeper root resections (approximately 45°) (5). Beveling exposes dentinal tubules, which can allow the leakage of bacterial byproducts and irritants from the root canal past the root end filling (6,7). Gilheany P et al. (8) in their research confirmed a positive correlation between steeper root resections and increased apical permeability due to the exposed dentinal tubules. The least apical permeability was noted in cases with horizontal root resections (marked at 0°). Increased apical permeability was additionally emphasized in periapical surgeries performed on teeth with failed endodontic treatment. The results of the spectrophotometric analysis of a dye penetration in the resected root surfaces performed by Ichesco WR et al. (9) confirmed an increased apical permeability in the endodontically treated teeth in comparison with those that were not.

Apart from the steeper root resections, conventional retropreparations fail to coincide with an axial root inclination (10).

The current biological evidence and the advances in the surgical techniques suggest reduction of the root resection bevel. The root is therefore resected perpendicular to the root canal to reduce the number of exposed dentinal tubules (11).

Horizontal or near horizontal root resections can be maintained exclusively when ultrasonic periapical surgery is performed. This contemporary surgical alternative was first introduced by Carr GB (12) in 1992. Ultrasonic armamentarium consists of miniature 90° angled surgical retrotips which eliminate the need for steep resections and great osteotomies. When used in conjunction with visual enhancements this surgical technique necessitates an almost perpendicular root resection. Most of the studies with concern to the subject of root resection bevel and periapical surgery were performed in laboratory environment on extracted teeth. Therefore our goal was to clinically assess the root resections bevel and to estimate their relation with different methods of periapical surgery.

MATERIAL AND METHODS

Study design: the prospective clinical study consisted of sixty maxillary and mandibular teeth with periapical inflammatory lesions. Thirty teeth were subjected to periapical surgery with conventional retrograde approach, while the rest were treated with contemporary ultrasonic periapical surgery. After the preliminary examinations, the surgical field in every case was anesthetized with submucosal infiltration of 2% scandonest with adrenaline (mepivacaine hydrochloride, Septodont).

All surgeries were performed by the same surgeon. Various full-thickness mucoperiosteal flaps were used depending on the teeth location and the extension of the periapical pathology. After elevating the flap, the bone was removed from the apical area to gain access to the lesion and root end. The surgical debridement of the cortical and cancellous bone was performed with a bur and sharp spoon excavator. To prevent the bone dehydration, the area was intermittently rinsed with saline solution during the entire surgical procedure. After the apical root exposure, root end resections were executed with a straight handpiece and a fissure bur (Figure 1).

Conventional periapical surgeries were carried out with small reverse conical burs. (Figure 2). Ultrasonic periapical surgeries were executed with diamond coated (dc) retrotips (source EMS - Nyon Switzerland) (Figure 3). The root bevel was determined by the need for access and visualization to complete the root-end preparation and filling. Following the completion of the strictly planned and performed intraoperative procedures, the bevel angles were assessed.

Descriptive statistics (with mean values and percentages) and Mann Whitney U test were used to present the study results.

RESULTS

Fifty four teeth (73.3%) underwent periapical surgery due to unsuccessful endodontic treatment. A preoperative radiographic evaluation of the endodontic treatments was presented in Table 1. A satisfied endodontic treatment was evident only in 6.8% of the cases. The rest of the subjects demonstrated radiological features of unsuccessful endodontic treatment, such as: inconsistent root canal filling (11.4%); unfilled 2mm of the root canal (40.9 %); unfilled 2mm of the root canal with external root resorption (4.5%); unfilled apical portion of the root canal (27.3%); overfilled root canals (2.2%) and perforated root canals in the middle section (via falsa) (6.8%).

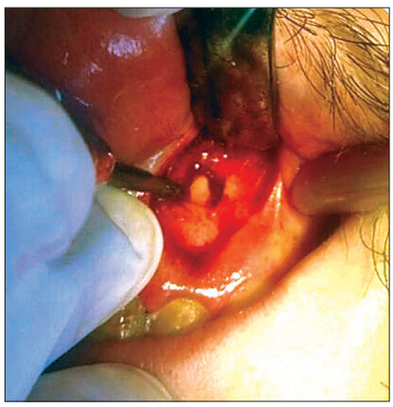

Figure 1.
Root end resection

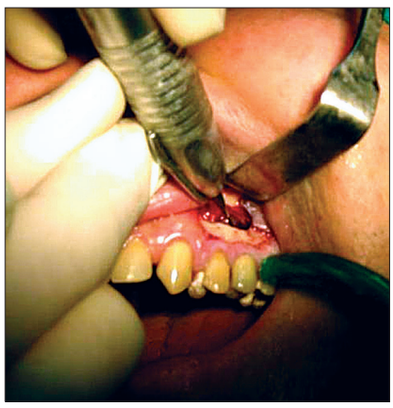

Figure 1.
Conventional periapical surgery

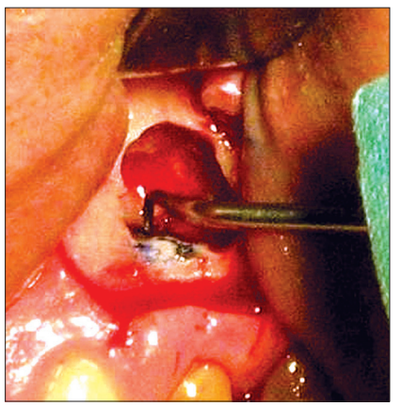

Figure 3.
Ultrasonic periapical surgery

Table 1. Distribution of operated teeth according to preoperative radiographic evaluation of the endodontic treatment (N=44)

Radiographic evaluation	Number of teeth	%
satisfying root canal filling	3	6.8
root canal with inconsistent canal filling	5	11.4
root canal with unfilled apical 2mm	18	40.9
root canal with unfilled apical 2mm and external resorption	2	4.5
root canal with unfilled apical portion	12	27.3
overfilled root canal	1	2.2
root canal perforated in middle section	3	6.8
Total	**44**	**100**

Table 2. Distribution of re-operated teeth according to radiographic evaluation of the surgical treatment (N=16)

Radiographic evaluation	Number of teeth	%
correct periapical surgery	2	12.5
unsatisfying periapical surgery with unsealed apical 2 mm	2	12.5
unsatisfying periapical surgery with unsealed apical 4 mm	3	18.75
unsatisfying periapical surgery with unsealed apical third	3	18.75
unsatisfying periapical surgery with inconsistent canal filling	3	18.75
unsatisfying periapical surgery with uncompleted root resections	3	18.75
Total	**16**	**100**

Sixteen teeth (26.7%) were re-operated. Preoperative radiographic evaluation of primary surgeries are presented on table 2. Periapical surgeries were correct in 12.5%. The rest were unsatisfying with: unsealed apical 2 mm (12.5%); unsealed apical 4 mm (18.75%), unsealed apical third (18.75%); inconsistent canal filling (18.75%) and uncompleted root resection in 12.5% of the cases.

The mean values of root resection in relation to the periapical surgeries assessed for every morphological group of operated teeth are presented in Table 3.

The root resections in ultrasonically performed periapical surgeries were significantly less beveled with average values ranging from 2.1° to 7.8°. The number of roots and their dilacerations didn't increase the root resection bevel.

Quite the opposite the conventional periapical surgeries were performed with significantly higher root bevel, with mean value of 46°. Due to the technical limitations of the conventional surgery mandibular premolars were exclusively operated with ultrasonic technique with mean value of root resections of 20.7°.

The statistical Mann Whitney U Test analysis revealed significant differences between the mean values of root resection bevel for two methods of periapical surgery for all groups of operated teeth (Table 4).

Table 4. Mann Whitney U Test between mean values of root bevel in regard to surgical methods

Operated teeth	Statistical significance
MCI	p = 0.0011
MLI	p = 0.0014
MK	p = 0.0339
MP	p = 0.0022
MdI	p = 0.0021

Table.3 Mean values of root resection bevel in operated teeth according to the morphological classification and surgical technique

Morphological group	Conventional periapical surgery		Ultrasonic periapical surgery	
	root resection bevel (mean value)	sd	root resection bevel (mean value)	sd
MCI	31.0°	4.3°	2.1°	1.1°
MLI	35.0°	4.0°	3.8°	2.7°
MK	36.0°	5.5°	2.3°	1.2°
MP	41.0°	4.4°	7.8°	6.9°
MdI	36.0°	1.4°	19.0°	0.0°
MdP	/	/	20.7°	8.1°

MCI – maxillary central incisors MK- maxillary canines; MLI – maxillary lateral incisors MP – maxillary premolars
MdI- mandibular incisors; MdP- mandibular premolars

DISCUSSION

In cases of endodontic failure the periapical surgery should be restricted to cases in which a non-surgical approach is impossible or has failed (13,14). When an improper or defective root canal filling is the cause of endodontic failure, and the root canal is coronary accessible and negotiable,

surgical treatment is not considered the treatment of choice (15). The majority of teeth included in this study had periapical lesions associated with failed endodontic treatment (Table 1). The periapical radiographs revealed that periapical lesions correlated with unfilled 2mm of the root canal-s in 40.9 %, and unfilled apical portion of the canal in 27.3% of the cases. Endodontic retreatment was considered unfeasible because the teeth in question had either intra canal posts or were covered with porcelain prosthetic restorations. Subsequently periapical surgery with retrograde approach was a treatment of choice. For cases with overfilled root canals (2.2%) and root perforations as a result of procedural errors (6.8%) periapical and periradicular surgeries were the treatment solution.

Periapical re-surgeries were performed in teeth with periapical lesions associated with unsuccessful surgical treatment (26.7%). The criteria for failure include clinical signs or symptoms, and radiographic evidence of uncertain or unsatisfactory healing. The radiological evidence of unsuccessful periapical surgery were continual periapical radiolucencies correlated with unsealed apical 4 mm (18.75%), unsealed apical third (18.75%), inconsistent canal filling (18.75%) and uncompleted root resection in 12.5% of the cases (Table 2).

Periapical surgery can be performed using conventional or modern techniques. Conventional periapical surgery is executed with a small bur that hinders the surgical access to the apical canal system, unless the root resection is steeper.

The introduction of loupes, ultrasonics, and compatible root-end filling materials has made modern periapical surgery more predictable, and the success rates are now high (16,17,18). The current microsurgical techniques permit precise performance of the surgical procedures, thus eliminating the disadvantages of the traditional periapical surgery. Contemporary ultrasonic surgery is performed with miniature 90° angled retrotips that enable perpendicular access on the minimally resected root surface.

Periapical surgery consists of surgical debridement of pathologic periapical tissue, root-end resection, root-end cavity preparation and root-end filling to seal the root canal.

Root end resection is an important step. The correct root resection enables visual and instrumental approach to the entire resected root surface without compromising the root length and the integrity of the vestibular lamina. Kim and Kratchman suggest that at least 3 mm of the root-end must be removed to reduce 98% of apical ramifications and 93% of lateral canals (19). Using a water-cooled fissure bur, an apical resection about 3 mm from the apex is performed, using a limited bevel.

Kratchman SI. (20), Rubinstein R. et al (21); Von Arx T. et al. (22), Gilheany P. et al. (8), Kim S. et al. (5) recommended root resections with a shallow bevel, not more than 10° in order to reveal the entire canal system with simultaneous preservation of the vestibular lamina.

In their investigation performed on extracted teeth, Melhaff et al. recorded significantly higher values for the resection angle in periapical surgeries performed with bur (35.1° mean value) versus ultrasonic surgeries, where the average resection bevel had amounted value to 16.0° (23).

According to Petrovic V. et al. (24) in certain cases root resection can be even steeper (45° and more), but such circumstances increase the risk of incomplete elimination of apical ramifications.

If the root resection is steeper, removal of the apical ramifications will be partial, larger amount of dentinal tubules will be exposed and the surgical outcome will be challenged.

Vertucci RJ. et al. (25) consider exposed dentinal tubules to present a potential vector for apical permeability.

The results from our clinical study displayed significant correlation between the root resection bevel and the applied surgical techniques.

The mean values of root resections in conventional periapical surgeries ranged from 31° for maxillary central incisors (MCI) to 41° for maxillary premolars (MP).

As expected, the mean values of root resections in ultrasonic periapical surgeries were lesser, ranging from 2.1° for maxillary central incisors (MCI) to 7.8° for maxillary premolars (MP). Due to the technical limitations of the conventional periapical surgery, mandibular premolars were exclusively operated with ultrasound technique with mean root resection value of 20°.

Our findings were in accordance with the recommendations of Kim S. (3), Gilheany P. et al. (11), Mehlhaff D. et al. (23), Kratchman SI. (20).

There is no biological justification for a steep bevel angle. It was strictly for the convenience of the surgeons for apex identification and for the subsequent apical preparation (13,14). In fact, beveling causes significant damage to the very tissue structures that the surgery is designed to save, i.e. buccal bone and root. By diagonal resection, the result of steep beveling, the buccal bone is removed along with a large area of the root causing, in effect, a large osteotomy. Furthermore, beveling frequently misses the lingually positioned apex, causes elongation of

the canal and reduction of the root diameter, thereby weakening it (26, 27, 28).

Root resection beveling also influences the depth of the retropreparation. Ninety degree root resection requires apical preparation with minimal depth of 1mm for quality retro-obturation. The increasing of the root resection bevel requires deepening of the apical cavity Gilheani et al. (8), which is difficult to achieve.

CONCLUSION

The results from our clinical study revealed that root end resections bevel correlated with periapical surgical techniques. The significantly lower values for the root resections were associated with ultrasonic periapical surgery. Lesser resection bevel preserves the root length, lessens the apical permeability and creates favorable circumstances for superior surgical outcome.

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Резиме

КЛИНИЧКА ЕВАЛУАЦИЈА НА ЗАКОСУВАЊЕТО НА КОРЕНСКИТЕ РЕСЕКЦИИ ВО ПЕРИАПИКАЛНАТА ХИРУРГИЈА

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Апстракт

Коренските ресекции играат важна улога во периапикалната хирургија. Закосувањето со кое тие се изведуваат може значително да варира во зависност од оперативните техники, коренската и канална морфологија.

Целта на овој труд беше да се направи клиничка евалуација на закосувањето на коренските ресекции и да се утврди нивната корелација со применетите оперативни техники.

Проспективната клиничка студија интегрираше шеесет индицирани периапикални хируршки процедури на заби со хронични периапикални лезии. Триесет периапикални хируршки интервенции беа со конвенционален приод, додека другите беа изведени со современа ултрасонична метода. По целосното финализирање на сите интраоперативни процедури, закосувањето на коренските ресекции беше евидентирано поединечно, за секој случај, со примена на шестар и агломер. Коренски ресекции со значително помало закосување, со средна вредност помеѓу 2,1° и 7,8°, беа евидентирани кај сите заби оперирани со современата ултрасонична хируршка техника. Бројот на корените и нивната дивергентност не влијаеја на закосувањето на коренските ресекции. Спротивно, значително закосени беа коренските ресекции евидентирани кај сите конвенционално оперирани заби, со средна вредност од 46°. Поради лимитираните перформанси на конвенционалната периапикална хирургија, мандибуларните премолари беа оперирани исклучиво со ултрасонична техника, со средни вредности на коренските ресекции не поголеми од 20,7°. Значително помалото закосување на коренските ресекции, евидентирано за ултрасоничната хирургија, придонесува кон презервација на коренскиот супстрат и кон подобар оперативен исход.

Клучни зборови: периапикална хирургија, коренска ресекција и закосување

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MEETING REPORT

5th RARE DISEASE SOUTH EASTERN EUROPE (SEE) MEETING, SKOPJE, MACEDONIA (NOVEMBER 15th, 2016)

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ABSTRACT

The fifth SEE meeting on rare diseases (RDs) was held in Macedonian Academy of Sciences and Arts (MASA) the November 11th, 2016. Several lectures dealt with mucopolysaccharidosis, glycogen storage diseases and the possibilities for their diagnosis and treatment. Enzyme replacement treatment (ERT), its availability, effects (or the lack of it) on the brain, and further prospects of eventual gene treatment were comprehensively exposed and discussed. Special accent was on Gaucher, Morquio IVA, Hunter and the audience was given new knowledge on the complexities of diagnosis and treatment. A block of lectures on rare renal diseases was also impressive. From renal stones, their molecular and genetic mechanisms to different forms of CAKUT the use of NGS and other molecular methods in diagnosis of RDs. Mitochondrial diseases, the novelties and importance of early discovery were comprehensively exposed. Special lecture was given on the complement system. Endocrine disruptors, microprolactinomas were also the topic of the meeting. A rather reach session of posters was also presented.

Key Word: Rare Diseases, rare renal disease, growth disorders, Gaucher..

Momir Polenakovic (MASA, Macedonia)

has given an update on RDs, first defining RDs as those that affects less than 1/2000 (Europe), 1/1250 in the USA, while in Japan RD is the one that affects less than 1/50,000 patients. It is estimated that 6-8% of European population (27-32 million) suffers from 5-8.000 RD. Impressively, the majority of RDs affect children (75%) and, unfortunately 30% of children die before the fifth birthday.

In renal diseases, over 100 conditions meet the epidemiological criteria to be defined rare: polycystic kidney disorders, Fabry, Alport disease but also less known and rarer conditions. Other organs might involve with the kidney disorder. Early diagnosis of renal RD is important in order to take appropriate therapeutic measures, either siple ones (potassium citrate and large hydration in cystinuria) or expensive as it is enzyme replacement therapy in Fabry disease. Both treatment enables long term renal survival and good quality of life. Progresses in genetics, particularly new technologies of next generation sequencing have opened unprecedented opportunities for the identification of faulty gene(s), novet treatments and personalized medicine

In Macedonia we lack comprehensive registries at the national level. The published articles of Macedonian health professionals on renal RD in PubMed journals present only the tip of the iceberg.

Milosevic Danko (Zagreb, Croatia)

presented the atypical hemolytic syndrome (aHUS) in 5 Croatian Children. Two children affected during infancy were treated with PEX/FFP, CVVHD and Eculizumab. The first described patient at the age of 10 months patient was found to be **heterozygous** for a substitution in exon 27 of the *C3* gene (c.3478G>A); **heterozygous** for the ***CFH*** **H3** haplotype and **homozygous** for the **MCPggaac**

haplotype of the *CD46* gene. A **heterozygous** mutation causing amino acid change (**Q950H**) in scr16 of **complement factor H** and heterozygous for *CFH* c.-331C>T polymorphism was also found, as other siblings with MCP S274I mutation and is **homozygous for the MCPggaac** haplotype. Interestingly, his sister had a novel heterozygous mutation (p.**S274I**) in *CD46*. Siblings were treated with methylprednisolone and PEX/FFP. All affected children have anormal life without physical/mental consequences, but for one child who has mild mental retardation.

Adrijan Sarajlija (Belgrade, Serbia)

gave a talk on glycogen storage diseases (GSDs) in one center. He stressed that the strict distinction among various types is impossible since many of these entities affect multiple organs. GSD type 1 with its two subtypes is considered as the most common GSD. Worldwite there is a much higher prevalence of subtype 1a, while in Serbia approximately 80% of patients affected with subtype 1b with . Most common mutation in in *G6PC* gene causing GSD 1a is c.247C>T. Other GSD in Sebia include types 2, 3, 6 and 9. Treatment of GSDs is nutritional treatment with exception of GSD type 2 (Pompe disease) for which enzyme replacement therapy is available. Gene therapy is still in development.

Mitochondrial diseases – Are they rare or common?

Johannes A. Mayr (Salzburg, Austria)

had a fascinating talk on mitochondral diseases., Mitochondria provide the vast majority of energy (ATP) in oxidizing cells. Mutations in the 37 mitochondrial genes and 250 nuclear genes have been associated with human disease.

Impressively, the clinical presentation of these rare disorders is summarized by the slogan: “Any symptom, in any organ or tissue, at any age and with any mode of inheritance”.

The new sequencing techniques allowed for the discovery of several new and unexpected genes.

Furthermore, defects in the mitochondrial energy metabolism were reported as early as more than 90 years ago by Otto Warburg in solid tumors. Furthermore, an increasing number of underlying biochemical and genetic defects were identified in the last decade, in neurodegenerative diseases and in the aging process.

Velibor Tasic (Skopje, Macedonia)

described the super rare disease of Pitt Hopkins through the personal travails and suffering of a child and his family. Pitt Hopkins syndrome (PTHS) is a neurodevelopmental disorder due to haploinsufficiency of the mutated TCF4 gene. Severe mental retardation, wide mouth, and intermittent overbreathing are the signs of the syndrome.

The prevalence of PTHS is estimated between 1 in 34,000 to 1 in 200,000-300,000.

PTHS is considered an Autism Spectrum Disorder. TCF protein is important for development of the nervous system and the brain, and is also found in patients with schizophrenia (overexpression of the TCF4 protein). There may be overlap with clinical syndromes and patients may be misdiagnosed as Angelmann, Mowat Wilson and Rett syndrome.

The current research approach tries agents that are able to enhance/augment the function of TCF4 activity without directly activating the protein.

Gaucher Disease (GD) was a theme of talks by Zoran Gucev (Skopje, Macedonia) Milan Lakocevic (Belgrade, Serbia) and Zlate Stojanovski (Skopje, Macedonia). GD is an autosomal recessive lysosomal storage disorder caused by deficiency in glucocerebrosidase. Partial deficiencies cause Type 1 GD, largely a disorder of macrophages with the pathologic hallmark the Gaucher cells (an enlarged, often multinucleate macrophage with crumpled cytoplasm). Infiltration by Gaucher cells leads to hepatosplenomegaly, hypersplenism, and displacement of haematopoietic bone marrow. Bony involvement encompases recurrent osteonecrosis, osteoporosis and fractures. More severe enzyme deficiency leads to accumulation of endogenously derived substrate in neuronal cells and a brain disorder: slowly progressive neurodegenerative disease with myoclonic epilepsy (both forms of Type 3 disease), to a rapidly fatal infantile form (Type 2 GD). Since 1991 enzyme replacement therapy (ERT) is available with recombinant technology and most recently by gene activation techniques. ERT has dramatic effects on visceral and bone marrow disease, while the effects on bone are slower and sometimes partial. Both Milan Lakocevic and Zoran Gucev kept our attention on ERT, while Zlate Stojkovski presentet a patient with GD an polycythaemia vera rubra.

Zoran Gucev (Skopje, Macedonia)

took us to a journey of rare diseases with the hallmark of a growth disorder as diagnosed in Mac-

edonia. Growth disorders are a rather diverse and frequent occurrence in childhood. A variety of rare endocrine (LHX4, IGHDII, SHOX hormonal deficiencies…), metabolic diseases (Hunter, Morquio IV A and B, Lesch-Nyhan…), disproportionate short stature (spondilocostal dysplasias, overgrowth syndromes…), dysmorphias (Adams Oliver Syndrome, Wiedeman Steiner, Rubinstein-Tayby…) were describee. The unforeseen usefulness of anomalies in the prenatal diagnosis, in uncovering of novel molecular mechanisms and in discovering novel treatments is comprehensively exposed. Several new genes (*PIK3CA, TBX6* …) discovered in the course of investigation of growth disorders are also presented.

Rossella Parini (Monza, Italy)

gave a talk on mucopolysaccharidoses (MPS). Those genetic progressive diseases cause disabilities, reduced quality of life and early death.

Central nervous system (CNS) damage is seen in the most severe forms of MPS I and MPS II, in the totality of MPS III and in some MPS VII patients, while it is almost absent in MPS IV and VI. Only MPS I had a possible curative treatment which was the bone marrow transplantation.

The enzymatic replacement therapies (ERT) at present are available for MPS I, MPS II, MPS VI and MPS IV. In general ERT improves of some clinical signs and stabilisates others.The major disadvantage is that ERT cannot cross the blood-brain-barrier. ERT efficacy is more evident when patients are treated early. Therefore the neonatal screening is strongly supported.

Joachim Seitz (Hamburg, Germany)

presented all the perils of the endocrine disruption as cause of (rare) diseases. Plastics on land and see reach food and drinking water. At the cellular and molecular level some of those organic substances act like hormones and severely interfere with the physiological hormone cascade, which explains the origins of the term "endocrine disrupting chemicals" (EDCs).

Oliver Bartsch (Meinz, Germany)

Genetic causes of developmental delay (DD) and intellectual disability (ID) have been increasingly found with the application of next-generation sequencing (NGS). In >100 patients with DD/ID and normal findings by karyotyping and microarray analysis NGS was performed. A definite diagnosis was achieved in 25% of families.

NGS tremendously increased the diagnostic yield, by 25%. His laboratory is now replacing the MPIMG1-test by whole exome sequencing (WES).

Jan Halbritter (Leipzig, Germany)

gave a lecture on nephrolithiasis (NL). NL is contributing to development of chronic kidney disease (CKD). Ca-based (e.g. Ca-oxalate >> Ca-phosphate) calculi are the most frequent. NGS identified more than 30 monogenic forms of over the last two decades. Distinct pathomechanism are found depending on the physiological function of the defective protein. Modern NL metaphylaxis may include mutation. Zoltan Prohaska (Budapes, Hungary) shed a sharp light on meny dark corners of the complex complement system.

Ljiljana Saranac, Ivana Markovic (Nish, Serbia)

turned the audience's attention on microprolactinoma in childhood and adolescence. The estimated incidence was 0.1 per million children. Galactorrhoea/amenorrhoea syndrome in women and gynecomastia/ hypogonadism in adult men, hyperprolactinaemia in children has different clinical expression influencing growth, puberty and weight gain. The diagnosis is made on the basis of consistently elevated PRL levels (at least 3 measurements) and dynamic tests of all pituitary axes.

A neuroradiological investigation (pituitary MRI) is necessary for adenoma confirmation.

Wheather micro-or macroadenomas, prolactinomas are treated medicaly with dopamine agonists. The medical treatment is able not only to normalize PRL levels, but also to produce a rapid tumor mass shrinkage. Due to senescense phenomenon microprolactinomas never progress to macroprolactinomas.

Stefan Chichevaliev, Vesna Aleksovska (Skopje, Macedonia)

informed about the policies, practice and their impact on the quality of life of patients with RDs.

The investigation on relevant international relevant documents, as well as documents from countries from the region: Bulgaria, Slovenia, Serbia and Croatia following improvements are required:

1) increasing the financial support for procurement of medicines and medical devices (100%); 2) raising awareness among health professionals for RD (81,2%); 3) establishing an international professional collaboration for research, monitoring and promotion of the doctrine and practice for RD (81,2%); and 4) establishing a database and knowledge for RD (90%).

A voluminous sessions with poster presentation followed, given by specialists, residents and students.

- Daniela Chaparoska. NEUROLEPTIC MALIGNANT SYNDRONE: CASE REPORT
- Gordana Kiteva-Trenchevska. REFRACTORY EPILEPSY IN TOUBEROS SCLEROSIS COMPLEX
- S. Nikolovska, V. Grivcheva-Panovska, K. Damevska, D. Chaparoska , N. Baneva. KLIPPEL-TRENAUNAY SYNDROME – CLINICAL EXPERIENCE
- Nada Petrova. TUBEROUS SCLEROSIS COMPLEX - A CASE REPORT
- Irena Rambabova Bushljetik, Gjulsen Selim, Igor Kuzmanovski, Menka Lazarevska, Milan Risteski, Jelka Masin-Spasovska, Olivera Stojceva Taneva, Ladislava Grcevska, Goce Spasovski. USE OF mTOR INHIBITOR – EVEROLIMUS IN PATIENT WITH BILATERAL ANGIOMYOLIPOMA AND SUBEPENDIMAL ASTROCYTOMA ASSOCIATED WITH TUBEROUS SCLEROSIS COMPLEX
- Vesna Dichoska, Ana Stamatova, Emilija Sahpazova, Velibor Tasic, Dorottya Csuka, Zoltán Prohászka. ATYPICAL HEMOLYTIC UREMIC SYNDROME
- Nikolina Zdraveska, Anet Papazovska Cherepnalkovski, Natasa Aluloska, Jana Jovanovska, Katica Piperkova. SYSTEMIC MASTOCYTOSIS - REPORT OF A RARE CONDITION IN NEWBORN
- L. Milenkova, A. Papazovska, N. Aluloska, N. Zdraveska. FAMILIAL OCCURRENCE OF MULTICYSTIC DYSPLASTIC KIDNEY
- Kreshnik Pollozhani, Hana Starova, Kristijan Bundovski, Emilija Sahpazova. ATYPICAL HEMOLYTIC-UREMIC SYNDROME-A CASE REPORT
- Ana Stamatova, Meri Miholova , Velibor Tasic. UROFACIAL OR OCHOA SYNDROME
- A. Janchevska, K. Kuzevska - Maneva, L. Tasevska Rmush, A. Sofijanova, O. Jordanova, A. Ugrinska. UNUSUAL PRESENTATION OF HASHIMOTO'S DISEASE WITH THYROID-ASSOCIATED OPHTALMOPATHY IN A 12.5 YEAR OLD GIRL
- Marija Dimishkovska, Zoran Gucev, Svetlana Kocheva, Vjosa Kotori, Momir Polenakovic, Dijana Plaseska-Karanfilska. NOVEL MUTATION IN FANCA GENE (c.3446_3449dupCCCT) IN TWO UNRELATED PATIENTS FROM MACEDONIA AND KOSOVO
- V. Jovanovska, R. Kacarska, K. Kuzevska Maneva, B. Gjurkova Angelovska , M. Neskovska Shumenkovska, E.Shukarova Angelovska. WILLIAMS-BEUREN SYNDROME WITH SUPRAVALVULAR AORTIC STENOSIS. CASE REPORT
- Salihu S, Tosevska K, Cekovska S. SDS PAG ELECTROPHORESIS IS A RELIABLE SCREENING TOOL IN PATIENTS WITH OCRL MUTATIONS
- Milan Velkov, Aleksandar Petlickovski, Momir Polenakovic, Zoran Gucev. SYNDROME NIJMEGEN BREAKAGE AND A DIFFUSE LARGE B CELL LYMPHOMA
- Katarina Abraseva, Marko Kostovski, Marina Krstevska-Konstantinova. RUBINSTEIN-TAYBI SYNDROME WITHOUT *CREBBR* AND *EP300* GENE ALTERATIONS
- Eva Markovska, Natasa Petrovska, Zoran Gucev. UNBALANCED TRANSLOCATION 8;13 IN A 13 YEAR OLD BOY WITH FACIAL DYSMORPHIA AND MENTAL RETARDATION
- Ana Stamatova, Zoran Bozinovski, Aleksandar Solev, Sanja Ivanovska, Dijana Plasevska Karanfilska, Momir Polenakovic, Zoran Gucev. A VARIANT OF FIBRODYSPLASIA OSSIFICANS PROGRESSIVA CAUSED BY THE *G328E* (c.9836>A) MUTATION OF THE *ACVR1* GENE
- Teodora Stojanovska, Kristijan Bundovski, Gjorgji Bozhinovski, Velibor Tasic. ISOLATED CONGENITAL ANOMALY OF THE KIDNEY AND URINARY TRACT IN A CHILD WITH 22q11.2 MICRODELETION SYNDROME
- V. Ambarkova, N. Stavreva. DENTAL ASPECTS OF YOUNG PATIENTS WITH ECTODERMAL DYSPLASIA SYNDROME
- Kristijan Bundovski, Teodora Stojanovska, Gjorgji Damjanovski, Velibor Tasic. NUTCRACKER PNENOMENON IN A PATIENT WITH RECURRENT GROSS HEMATURIA

- S. Fustik, A.Stamatova,_T. Jakovska, L. Spirevska. EARLY VERSUS LATE DIAGNOSIS OF CYSTIC FIBROSIS: A NEED FOR IMPLEMENTATION OF NEONATAL SCREENING
- Sanja Ivanovska, Snezana Jancevska , Marina Krstevska Konstantinova. WIEDEMANN-STEINER SYNDROME IN A PATIENT WITH NORMAL HEIGHT AND NO ALTERATION IN *KMT2* GENE

Резиме

ПЕТТИ СОСТАНОК ЗА РЕТКИ БОЛЕСТИ НА ЈУГОИСТОЧНА ЕВРОПА, СКОПЈЕ, МАКЕДОНИЈА, 15 НОЕМВРИ 2016 ГОДИНА

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Апстракт

Петти состанок за ретки болести на југоисточна Европа се одржа во МАНУ на 11 ноември 2016 година. Повеќе предавања се занимаваа со мукополисахаридози, болести на гликогенските депоа и можностите за нивна дијагноза и третман. Ензимската заместителна терапија, нејзината достапност, ефектите (или нивниот недостаток) на мозокот), и идните изгледи за евентуална генетска терапија беа сеопфатно изложени и дискутирани. Посебен акцент беше ставен на Гоше, Morquio IVA, Хантер I и публиката стекна нови знаења за комплексноста на дијагнозата и третман. Групата предавања за ретките бубрежни заболувања исто така беше импресивна. Од бубрежни камења, нивните молекуларни и генетски механизми до различните форми на CAKUT употребата на NGS и други молекуларни методи во дијагнозата на RDS. Митохондриските болести, новините и значењето на раното откривање беа сеопфатно изложени. Специјално предавање беше одржано за комплементниот систем. Ендокрините нарушувачи и микропролактиномите исто така беа тема на состанокот. Исто така, беше презентирана прилично богата постер сесија.

Клучни зборови: ретки болести, ретки бубрежни болести, нарушување на растот, Гоше.

In our article,

Stankov A, Belakaposka-Srpanova V, Bitoljanu N, Cakar L, Cakar Z, Rosoklija G:

"VISUALISATION OF MICROGLIA WITH THE USE OF IMMUNOHISTOCHEMICAL DOUBLE STAINING METHOD FOR CD-68 AND IBA-1 OF CEREBRAL TISSUE SAMPLES IN CASES OF BRAIN CONTUSIONS",

Prilozi (Makedon Akad Nauk Umet Odd Med Nauki) 2015, 36:141-145, an important sentence was omitted from the opening of paragraph 2 of "Materials and Methods". The following sentence should be inserted as the first of that paragraph:

The method was an adaptation, from thin paraffin sections to thick, of the microglial staining method published by Schnieder *et al* [23].

The authors apologize for the omission.

In our article,

Marija Cvetanovska, Zvonko Milenkovik, Valerija Kirova Uroshevik, Ilir Demiri, Vlatko Cvetanovski:

"FACTORS ASSOCIATED WITH LETHAL OUTCOME IN PATIENTS WITH SEVERE FORM OF INFLUENZA",

Prilozi (Makedon Akad Nauk Umet Odd Med Nauki) 2016, 37 2-3: 63-72 the following sentence of the abstract: "The patients were divided in two groups: survived (n = 75) and deceased (n = 75)" is to be replaced with the following sentence: "The patients were divided in two groups: survived (n = 75) and deceased (n = 12)". The authors apologize for the error.

УПАТСТВО ЗА АВТОРИТЕ

Списанието „Прилози“ на Одделението за медицински науки на Македонската академија на науките и уметностите излегува трипати годишно и е цитирано во Index Medicus и во Medline и е достапно на www.manu.edu.mk/prilozi. Во него се објавуваат едиторијали, изворни научни трудови, научни соопштенија и прегледни статии (клинички, лабораториски и епидемиолошки искуства, прикази на случаи, куси соопштенија, писма до уредникот, историски записи и др.) од областа на медицинските науки. Трудовите не треба да ги содржат резултатите што авторите веќе ги објавиле во други публикации или списанија.

Трудовите предложени за објавување во „Прилози“ ги рецензираат двајца стручњаци од соодветната научна област, кои за авторите остануваат анонимни.

Трудовите се објавуваат на англиски јазик со резиме на англиски и на македонски јазик.

Трудот треба да биде отчукан со новинарски проред (28–30 реда), на бела хартија од формат А4, со маргини најмалку 3 cm или на компјутер – програма Word for Windows, со приложена дискета или CD.

Обемот на оригинален научен труд, вклучувајќи ги и прилозите (илустрации, графикони, табели) не смее да биде поголем од еден авторски табак (30.000 знаци, односно 16 страници од 28 реда). Обемот на кратките соопштенија не треба да биде поголем од седум страници.

На трудот, покрај името и презимето на авторот/авторите, треба да биде наведена установата или организацијата во која е изработен трудот.

Трудот треба да содржи апстракт од најмногу 250 збора на англиски јазик, како и апстракт на македонски јазик. Апстрактот треба да содржи краток приказ на целта на трудот, методите на работа, битните резултати (со нумерички податоци) и основни заклучоци. Заедно со апстрактот треба да бидат доставени најбитните клучни зборови. Клучните зборови за медицинските науки се наведуваат во согласност со M.E.S.H.

Трудот по правило треба да содржи: вовед, материјал и методи, резултати, дискусија и заклучок. Воведот мора да биде краток, со јасно дефинирана цел и со досегашно познавање на проблемот. Материјалот и методите треба да содржат доволно податоци од кои читателот ќе биде во состојба да ги повтори испитувањата без дополнителна информација. Резултатите треба да бидат напишани кратко и јасно, а дискусијата да ги објасни резултатите.

Мерните единици и другите технички податоци мораат да бидат усогласени со Sl-системот.

Илустрациите се приложуваат посебно. Графиконите и цртежите треба да бидат на паус или на бела хартија, контрастни, а ознаките и бројките во графиконите сразмерни на големината на цртежот, за да останат читливи по редуцирањето на големината на цртежот. По правило не треба да бидат повеќе од четири. Нивното

место во текстот да биде означено. Сите илустрации треба да имаат легенди на англиски јазик.

Табелите можат да бидат приложени посебно, но нивното место во текстот да биде означено. Насловите на табелите треба да бидат напишани на англиски јазик.

За трудовите од областа на медицинските науки, во принцип, важат упатствата објавени во „Brit. Med. Journal“, Vol. 296, 1988, p. 101–105 („Ванкуверските правила“), односно N. Engl. J. Med., Vol. 324, 1991, p. 424–428.

Литературата се цитира во оригинал, и тоа по следниов редослед: презиме и почетна буква од името на авторот, наслов на трудот, назив на списанието, година на објавување на цитираниот труд, годиште и број, страници (од‡до). Доколку се цитира книга или зборник на трудови, се наведува и издавачот и местото на издавање (пред страниците). Ако се цитира труд од повеќе од три/шест автори, по третиот/шестиот се додава „и сор.“, односно *„et al.“*. Во текстот на трудот се наведува првиот автор и годината ставени во загради [ ], односно бројка во загради, доколку библиографијата е нумерирана. При прва употреба на кратенка, во заграда да се даде нејзиниот полн назив.

Кратките соопштенија кои се објавуваат во сп. „Прилози“ (до 7 чукани страници) не мора да ги содржат поглавјата вовед, материјал и методи, дискусија и заклучок, но тие мораат да бидат содржани во текстот.

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Ракописите се доставуваат до Уредувачкиот одбор на сп. „Прилози“ на Одделението за медицински науки на МАНУ во два примерока (оригинал и копија) и електронска верзија на трудот.

Авторите на трудовите треба да ги покријат трошоците за печатење и за интернет-објавување на своите трудови во износ од 250 ЕУР во денарска противвредност.

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The journal *Prilozi, Oddelenie za medicinski nauki (Contributions, Section of Medical Sciences of the Macedonian Academy of Sciences and Arts)* is published three times a year and is cited in Index Medicus and Medline and available on www.manu.edu.mk/prilozi. The journal publishes editorials, original research works, research reports and reviews (clinical, laboratory and epidemiological experiences, case studies, short communications, letters to the editor, historical notes, etc.) from the area of medical sciences. The manuscripts should not represent research results which the authors have already published in other books or journals.

The papers submitted for publication in *Prilozi* are peer-reviewed by two experts from the respective scientific field who remain anonymous to the authors.

The papers are published in English, accompanied by a summary written in Macedonian.

The paper should be typed with double-spacing (28–30 lines), on a white paper in A4 format, with margins of 3 cm, or on a computer using Word for Windows program enclosing the CD, or USB.

The length of the original research paper, including the annexes (illustrations, graphs, tables, etc.) should not exceed a signature or printer's sheet (30,000 signs, that is, 16 pages with 28 lines each). The length of shorter reports should not exceed seven pages.

The submitted manuscript must contain the name/s and surname/s of the author/s, the name and address of the institution and or organization where it was prepared.

The abstract should not exceed 250 words, written in English, and a summary in Macedonian. The abstract should represent briefly the goals, methods, main results (with numerical data) and basic conclusions of the research. The most essential key words must be added to the abstract. The key words for the medical sciences are given according to M.E.S.H.

The manuscript contains an introduction, materials and methods, results, discussion and a conclusion. The introduction must be concise with a clearly defined goal and with previous knowledge of the problem. The materials and methods ought to contain sufficient data to enable the reader to repeat the investigation without seeking additional information.

The results should be presented briefly and clearly, and the discussion should explain the results.

The measuring units and other technical data should be given according to the SI-system.

Illustrations are submitted separately. Graphs and drawings should be prepared on tracing paper or on a white sheet of paper, in contrast, while the markers and figures in the graphs must be proportional to the size of the drawing in order to remain readable after the reduction of the size of the drawing. There should not be more than four

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Tables can be enclosed separately, but their places in the text must be indicated. The titles of the tables should be written in English.

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Short reports published in the journal *Prilozi* (up to 7 typed pages) do not need to have separate sections for introduction, materials and methods, discussion and conclusion, but these elements can be incorporated in the text.

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The authors of the manuscripts need to cover the costs of printing and online publishing for their manuscript in total amount of Macedonian MKD equivalent to 250 EUR.

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