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Editorial

The first edition of the Bosnian Journal of Basic Medical Sciences in the year 2004 is a great opportunity for the Editorial Board to authenticate its complete successfulness in realisation of the intentions to progressively achieve the major aim: to issue one edition every three months or four editions per a year.

In addition, there is an increasing number of scientists being interested to publish the results of their investigations in our journal. We also need to emphasise, that an augmented number of authors from the foreign countries are attracted by the scope of the journal.

In this edition, we issue the rest of scientific papers about cloning, which were presented during the Symposium in Sarajevo.

The Association of Basic Medical Sciences has performed necessary additions in its Presidency, Editorial Board and Advisory board.

We are proud to mention that we have daily contacts with younger native and foreign scientists interested in collaboration within our journal.

We take in consideration all suggestions with purpose to improve the quality of the journal. In addition, we are grateful for all of your opinions, advices and recommendations that we constantly try to accept and implement.

Sarajevo, Februar, 2004

Editorial Board
"To those of average curiosity about the wonders of nature, it is likely that two great mysteries have stirred the imagination; and each concerns a birth. Who has not gazed into the heavens on the starlit night and wondered about the birth of the universe? And who has not been stimulated by the sight of the newly born baby to marvel at the unseen events within the mother's uterus that have led to the birth of such a perfect creation?"(1)

These words written by the Professor Sir Graham (Mont) Liggins open Pandora's box of questions, dilemmas, doubts and controversies about human life and its beginning offering everybody lifelong challenge to solve mystery of life.

Entering this field, scientists have been remiss in failing to translate science into the terms that allow mankind to share their excitement of discovering life before birth. Regardless to remarkable scientific development, curiosity, and speculations dating back to Hippocrates, life before birth still remains a big secret. Different kinds of intellectuals involved themselves trying to contribute to the solution of human life puzzle. They are led by the idea that each newborn child will only reach its full potential if its development in uterus is free from any adverse influence, providing the best possible environment for the embryo/foetus.

Considering embryo/foetus, it should be always kept in mind amazing aspect of these parts of human life in which the mother and the embryo/foetus, although locked in the most intimate relationships, are at ALL TIMES two separate people. Accepting embryo/foetus as the person opened new set of questions about its personality and human rights. Today, synthesis between scientific data and hypotheses, philosophical thought, and issues in the humanities, has become pressing necessity in order to deal with ethical, juridical and social problems arising from man's interference in many aspects and stages of life. (2)

**DEFINITION OF THE LIFE**

Proper answer to the question “How to define human life?” is complicated. Nowadays dilemmas consider the respect of human life from the birth to death involving not just biology but other sciences also. Philosophy, theology, psychology, sociology, law and politics evaluate this topic from different point of views. Integration of all would result proper definition.

Some authors say that that life as such does not exist - no one has ever seen it. Szent-Gyorgy says that noun “life” has no significance because there is no such thing as “life”. Le Dantec says that the expression “to live” is too general and that it is better to say a dog “dogs” or a fish “fishes” than a dog or a fish lives. (3)

When defining life it should be considered not just life as it is today but as it might have been in its primordial form and as it will be in the future. All present forms of life appear as something completely new. Life, than, is transferred and not conceived in each new generation. Furthermore, the phenomenon of life has existed on Earth for approximately 3.5 billion years. Consequently, although the genome of a new embryo is unique, the make-up of embryo is not new. If life is observed through the cell than every life (and human also) is considered as a continuum. Human cells and the mankind have been existing on the Earth continuously since the appearance of the first man. However, if definition refers to the single human being or present population, the statement “human life is a continuum” is not acceptable. (4)

Life, in a true sense of word, begins when the chemical matter gives rise, in a specific way to an autonomous, self-regulating, and self-reproducing system. Life is connected with a living being, and it created its own system as an indivisible whole - forms its individuality. One of the most important characteristics of living beings is reproduction. Reproduction is a mean of creating new life by transferring forms of old one into newly formed human being. Therefore, variability, individual development and harmony characterize human beings. Individuality is the most essential characteristic of human being consisting new life but also all human life forms through the evolution, characterized by phenotype, behaviour and the capability to recognize and adapt. Human embryo and foetus gradually develop into these characteristics.

Although we should not forget that in the same way today's research is tomorrow's benefit (1), concerning human life conclusions should not be treated one-sidedly, from one perspective. This reality should be regarded in all its richness: embryo gives biologist, geneticist substance for consideration, but since we are talking about the beginning of a human life, it requires philosophical-
anthropological consideration, theology and social sciences as well. In its protection, we have to include ethics and law. This approach leads to conclusion that it is necessary to reject reductionism as well as integrism and to find “golden middle” in between these two methodologies.

**SCIENTIFIC APPROACH**

Biology characterizes human being by dynamics of the system and its self-control (homeostasis), excitability (response to stimuli of different nature and origins) self-reproducibility, the heredity of the characters, and the evolutionary trend.

For biologists it is important to specify which form of life phenomena, we are referring to: cell, organism population or species. The basic level of organization and the simplest form of life is the cell. Biologically speaking human cellular life never stops - or if it did, the extinction of the human species would result - and is passed on from one generation to another. Human individual organism life is defined within its life cycle, which is temporarily limited; i.e. it has a beginning and the end. (5) It is obvious that life is a highly dynamic phenomenon, which could be described and explained through the careful study of life processes and interactions by interdisciplinary approach. In human spermatozoa and the oocyte are two essential cells involved in creating human life. It is clear that biologists are most qualified to render judgement on this matter. Understanding of beginning of human life and development of the embryo/foetus could provide definitive resolution. However, with a recent possibility of visualizing early human development virtually from conception. Perinatologists should be those who by study, training, practice and research are singularly qualified. (6)

The science enables us data about physical development of the human being but does not provide information about its personality and personal-hood.

**HUMAN EMBRYOGENESIS**

Only proper understanding of the process of human embryogenesis enables answering scientifically the question when the life cycle of human individual starts. Therefore, in the following text the main steps of the human developmental process are going to be briefly described, primarily during the first 15 days after fertilization.

A human being originates from two living cells: the oocyte and the spermatozoon transmitting the torch of life to the next generation. The oocyte is a cell approximately 120 μ in diameter with thick membrane, known as the zona pellucida. The spermatozoon moves, using the flagellum or tail, and the total length of the spermatozoon including the tail is 60 μ. (7)

After singamy, the zygote undergoes mitotic cell division as it moves down the fallopian tube toward the uterus. A series of mitotic divisions then leads to the development of the pre-embryo. The newly divided cells are called blastomeres. From 1 to 3 days after singamy, there is a division into two cells, then four cells. Blastomeres form cellular aggregates of distinct, totipotent, undifferentiated cells that, during several early cell divisions, retain the capacity to develop independently into normal preembryos. As the blastocyst is in the process of attaching to the uterine wall, the cells increase in number and organize into two layers of cells. Implantation progresses as the outer cell-layer of the blastocyst, the trophoderm, invades the uterine wall and erodes blood vessels and glands. Having begun five or more days after fertilization with the attachment of the blastocyst to the endometrium of the uterus, implantation is completed when the blastocyst is fully embedded in the endometrium several days later. Even during these 5-6 days, modern medicine introduces the possibility of making preimplantation genetic diagnosis.

However, at this time, these cells are not yet totally differentiated in terms of their determination to specific cells or organs of the embryo. The term preembryo, then, includes the developmental stages from the first cell division of the zygote through the morula and the blastocyst. By approximately the 14th day after the end of the process of fertilization, all cells, depending on their position, will have become parts of the placenta and membranes or the embryo. The “embryo” stage, therefore, begins approximately 16 days after the beginning of the fertilization process and continues until the end of 8 weeks after fertilization, when organogenesis is complete. (8)

Pre-embryo is the structure that exists from the end of the process of fertilization until the appearance of a single primitive streak. Until the completion of implantation pre-embryo is capable of dividing into multiple entities, but does not contain enough genetic information to develop into an embryo: it lacks of genetic material from maternal mitochondria and of maternal and parental genetic messages in the form of messenger RNA or proteins. So, during the preembryonic period has not yet been determined with certainty that a biological individual will result or would it be one or more (identical twins forming), 50 that the assignment of full rights of a human person is inconsistent with biological reality.

A conclusion is that the pre-embryo requires the estab-
Personality

Defining personality is very complex. There is still no clear definition of personality. One dictionary offers, “what constitutes an individual as distinct person”, but does not define what the “what” is. Another dictionary asserts “the state of existing as a thinking intelligent being”. This definition might lead to the inference that personality increases pro rata with intelligence, or that some people may not have a personality at all if we followed Bertrand Russell’s dictum that “most people would rather die than think and many, in fact, do!” Ken Stallworthy’s Manual of Psychiatry is more help with the ontological point of view, as an individual.

Genetic uniqueness and singleness coincide only after implantation and restriction have completed, which is about 3 weeks after fertilization. Until that period, the zygote and its sequela are in a fluid process, are not physical individual, and therefore cannot be a person.

It is well known that high percentages of oocytes, which have been penetrated never, proceed on to further development, and that many oocytes which do, are thwarted 50 early in their development that their presence is not even recognized. It is suggested that 30% of conceptions detected by positive reactions to human chorionic gonadotropin (HCG) tests abort spontaneously before these pregnancies are clinically verified.

The newly conceived presents itself as a biologically defined reality. However, the status of the pre-embryo as an individual remains a great mystery. In the present scientific scene especially with the progress of ultrasound technologies, prenatal psychology and therapeutics opened a window into prenatal-life of embryo and foetus confirming the evidence that the embryo/foetus is a true subject itself.

Bioethical Aspects

The idea of embryo/foetus as the miniaturized infant or adult is true in extent that the embryonic i foetal physiologist must be able to apply knowledge of every system obtained in born, yet quite untrue in failing to recognize
the many ways in which life before birth differs fundamentally from life after birth. The newly conceived form presents itself as the biologically defined reality: it is an individual that is completely human in development that autonomously, moment by moment without any discontinuity, actualizes its proper form in order to realize through intrinsic activity, a design present in its own genome. Embryo as a patient is best understood as the subset of the concept of the foetus as the patient. These two concepts opened whole set of questions regarding ethical problems. The embryo as the patient is indivisible from its mother. However, balance is needed in protection interests of embryo/foetus and the mother. One prominent approach to understanding the concept of the embryo/foetus as a patient has involved attempts to show whether the embryo/foetus has independent moral status or personalhood. Independent moral status for the foetus would mean that one or more of characteristics possessed either in, or of the embryo/foetus as a patient has involved attempts to show whether the embryo/foetus has independent moral status or personalhood. (11), (12) Independent moral status for the foetus would mean that one or more of characteristics possessed either in, or of the embryo/foetus itself and, therefore, independently of the pregnant woman or any other factor, generate and therefore ground obligations to the embryo/foetus on the part of the pregnant woman and her physician.

A wide range of intrinsic characteristics has been considered for this role, e.g., moment of conception, implantation, central nervous system development, quickening, and the moment of birth. (13) Given the variability of proposed characteristics, there are many views about when the embryo/foetus does or does not acquire independent moral status. Some take the view that the embryo/foetus possesses independent moral status from the moment of conception or implantation. Others believe that the embryo/foetus acquires independent moral status in degrees, thus resulting in “graded” moral status. Still others hold, at least implicitly, that the embryo/foetus never has independent moral status 50 long as it is in utero. (14) Being a patient does not require that one possesses independent moral status. (15) Being a patient means that one can benefit from the application of the clinical skills of the physician. (16) Put more precisely, a human being without independent moral status is properly regarded as a patient when the following conditions are met: that a human being is presented to the physician for the purpose of applying clinical interventions that are reliably expected to be efficacious, in that they are reliably expected to result in a greater balance of goods over harms in the future of the human being in question. In other words, an individual is considered a patient when a physician has beneficence-based ethical obligations to that individual.

To clarify the concept the embryo/foetus as the patient, beneficence-based obligation is necessary to be provided. Beneficence-based obligations to the foetus and embryo exist when the foetus can later achieve independent moral status. This leads to conclusion that ethical significance of unborn child is in direct link with the child to be born - the child it can become.

LEGAL STATUS OF THE EMBRYO

When discussing low, it should be always kept in mind that medicine is international, but low is not.

The status of the human embryo is not juridical defined and relies on the political, social, and religious influences in each country. It is hard to answer the question when human life should be legally protected. At the time of conception? At the time of implantation? At the time of birth? In all countries (except Ireland and Liechtenstein) juridical considerations are based on roman law. Roman civil law says that the foetus has right when it is born or if it is born-nasciterus.

Few countries agree with definition of beginning of human personality the time of conception. The majority does not grant legal status to the human embryo in vitro (i.e., during the 14 days after fertilization). Thus, even in the absence of legal rights, there is no denying that the embryo constitutes the beginning of human life, a member of the human family. Therefore, whatever the attitude, every country has to examine which practices are compatible with the respect of that dignity and the security of human genetic material.

ARGUMENTS FOR BEGINNING OF HUMAN LIFE AND HUMAN PERSON AT FERTILIZATION

The fundamental approaches of biomedical and social practice must begin with the understanding that the subject before birth is a person and that “personhood” is conferred by successful fertilization of the egg. To hide from this in silence or ignorance should be unacceptable to all as stressed by Scarpelli. (6)

View that human life begins when sperm and eggs fuse to give rise to a single cell human zygote whose genetic individuality and uniqueness remain unchanged during normal development is widely supported. Because the zygote has the capacity to become an adult human individual, it is thought it must be one already. The same zygote organizes itself into an embryo, a foetus, a child and an adult. By this account, the zygote is an actual human individual and not simple a potential one in much the same way as an infant is on actual human person with potential to develop to maturity and not just a potential person. As Scarpelli pointed out recently outside the realm of religious dogma, there has been no one, whose existence can be traced back to any entity other than the
fertilized egg. The biological line of existence of each individual, without exception begins precisely when fertilization of the egg is successful.

The process of fertilization actually begins with conditioning of the spermatozoon in the male and female reproductive tracts. Thereafter, fertilization involves not only the egg itself but also the various investments, which surround the egg at the time it is released from the ovary follicle. Fertilization, therefore, is not an event, but a complex biochemical process requiring a minimum of 24 hours to complete singamy, that is the formation of a diploid set of chromosomes. During this process, there is no commingling of maternal and paternal chromosomes within a single nuclear membrane (pre-zygote); after this process the parental chromosomes material is commingled (zygote).

Among the many other activities of this new cell, most important is the recognition of the new genome, which represents the principal information centre for the development of the new human being and for all its further activities. For the better understanding of the very nature of the zygote, two main features are to be at least mentioned here. The first feature is that the zygote exists and operates from singly on as a being, ontologically one, and with a precise identity. The second feature is that the zygote is intrinsically oriented and determined to a definite development. Both identity and orientation are due essentially to the genetic information with which it is endowed. That is why many do believe that this cell represents the exact point in time and space where a new human individual organism initiates its own life cycle.

ARGUMENTS AGAINST BEGINNING OF HUMAN LIFE AT FERTILIZATION

Today, one largely accepted opinion is that until the 14th day from fertilization or at least, until implantation - the human embryo may not be considered, from the ontological point of view, as an individual. There are at least five main reasons in favour of this opinion:

1. Before formation of embryonic disk embryo is “a mass of cells genetically human”, “a cluster of distinct individual cells” which are each one “distinct ontological entities in simple contact with the others” (18)

2. Until approximately the 14th day after fertilization, all that happens is simply a preparation of the protective and nutritional systems required for the future needs of the embryo. Only when entity called embryonic disc is formed can develop into a foetus and thence into a foetus. (19)

3. Monozygotic twins phenomenon or chimeras can occur. In fact, this seems to be the strongest reason why the embryo is denied the quality of individuality and as a proof that the zygote cannot be an ontologically human being. In approximately one third of cases the embryo divides at about the two cells stage and in the other two thirds the inner cell mass divides within the blastocyst from day 38. Occasionally, the division takes place from day 8-12 but usually it is not complete thereby forming conjoined identical twins on two-headed individuals.

4. Co-existence of the embryo with its mother is a necessary condition for an embryo belonging to the human species and this condition can be obtained only at implantation (13). However, there is evidence that development of human embryo in vitro can continue well beyond the stage of implantation, and that mouse embryos implanted under the male renal capsule can reach the foetal stage. It is also argued, or at least implied, that 50 many human embryos die before or after implantation that is would be lacking in realism to accept that the human individual begins before implantation.

It is well known that high percentages of oocytes, which have been penetrated never, proceed on to further development, and that many oocytes which do, are thwarted 50 early in their development that their presence is not even recognized. Up to 50% of ovulated eggs and zygotes recovered after operations were found 50 grossly, abnormal that it would be very unlikely that they would result in viable pregnancies. It is also suggested that 30% of conceptions detected by positive reactions to human chorionic gonadotropin (HCG) tests abort spontaneously before these pregnancies are clinically verified. The scientific literature is not unanimous on the incidence of natural wastage prior to, and during, implantation in humans, varying from 15% to as much as 50%. The vast majority of these losses are due to chromosomal defects caused during gametogenesis and fertilization. (20)

Genetic uniqueness and singleness coincide only after implantation and restriction have completed, which is about 3 weeks after fertilization. Until that period, the zygote and its sequela are in a fluid process and are not physical individual and therefore cannot be a person.

5. The product of fertilization may be a tumour, an hydatidiform mole or chorioepithelioma. Though the mole is alive and of human origin, it is definitely not a human individual or human being. It lacks a true human nature from the start and has no natural potential to begin human development.

A teratoma is another clear instance of cells developing abnormally that results from the product of fertilization, but which could not be considered to be a true human
individual with a human nature. It has no potential to develop into an entire foetus or infant. Clearly, the foetus with the teratoma would be a human individual, but not the attached teratoma itself. Obviously, not all the living cells that develop from the concept, the early embryo or the foetus form an integral part of a developing human individual.

DIFFERENT RELIGIOUS TEACHINGS AND HISTORICAL ASPECTS

Catholic Church’s teachings are clearly described in the Introduction Donum Vitae: “A human creature is to be respected and treated as a person from conception and therefore from that same time his (her) rights as a person must be recognized, among which in the first place is the invaluable right to life of each innocent human creature”.

In 1997, the third Assembly of the pontifical Academy for life was held in Vatican City. It has been concluded that “at the fusion of two gametes, a new real human individual initiates its own existence, or life cycle, during which -given all the necessary and sufficient conditions - it will autonomously realize all the potentialities with which he is intrinsically endowed. The embryo, therefore, from the time of gametes fuse, is a real human individual, not a potential human individual. It was even added that recent findings of human biological science recognize that in zygote resulting from fertilization the biological identity of a new human individual is already constituted. (21),(22)

In Western Europe and in the North and South America these opinions are mostly based on Judeo-Christian theology. In Arabian Countries, in Africa and in Asia prevail the influences of the Islamic and Budish religions. Although their approach to the beginning of human life is impressively similar, each of these religions has different attitudes to the problem of embryo research, infertility and its therapy. In a fact, while the Jewish attitude towards infertility is expressed in the Talmud sayings and in the Bible (synthesized in the first commandment of the Talmud), the Christian point of view establishes no absolute right to parenthood. According to the Islamic views, attempts to cure infertility are not only permissible, but also a duty. Buddhism has imposed strict ethics on priests, but it has relatively lenient attitudes toward lay people, so if medical treatment for infertility is available, people should make use of it.

For about two thousand years the opinions of Aristotle, the great Greek philosopher and naturalist, on the beginning of the human being were commonly held. He argued that the male semen had a special power residing in it, pneuma, to transform the menstrual blood, first into a living being with a vegetative soul after seven days and subsequently into one with a sensitive soul 40 days after contact with the male semen. (23)

Aquinas adopted Aristotle’s theory but specified that rational enrolment took place through the creative act of God to transform the living creature into a human being once it had acquired a sensitive soul. The first conception took place over seven days while the second conception or complete formation of the living individual with a complete human nature lasted 40 days. (24) Hippocrates believed that entrance of the soul into the male embryo occurred on the thirtieth day of intrauterine life. It entered into the female embryo on the fortieth day. Actually, this idea was a considerable improvement on the scheme found in the Book of Leviticus, where it is suggested that the soul does not enter the female until forty days after the conception. (25) In short, the rational soul enables the matter to become a human being, an animated body, an embodied soul, a human person. Harvey’s experiments with deer in 1633 proved Aristotle’s theory of human reproduction wrong, without himself finding a satisfactory explanation of human conception. After modern scientists discovered the process of fertilization most people took for granted that human being, complete with a rational soul, began once fertilization had taken place.

It is clear that the answer to the question “When has the human being actually come to life?” could only be given by combining the cognition of different religions, philosophy and various biological scientific disciplines. There is a very fine line between the competence of science and the one of metaphysics, and it greatly depends on the individual’s philosophical principles. Those two, more or less autonomous intellectual disciplines have very often tried dominating one another, or ignoring each other. It is only recently that the majority of scientists and some theologians have come to realize that the separate meanings of scientific and religious “truths” complement themselves thus representing methodologically independent entities. Current science is not interested in what Nature is, but in the facts that could be started regarding it, thus trying to explain the term, rather then inventing it. The main difference between science and religion can be seen in the fact that scientific “truths”, unlike religious postulates, can and must be experimentally verified and the methods of scientific cognition can be easily explained and learnt. While religion favours irrationality, science prefers an entirely rational approach to matters of importance. Intellectual cognition when scientifically expressed usually is in a form of mathematical formulas and presented quantitatively. On the contrary, religion tends to keep its truths in a form of metaphoric expres-
sions, preferring qualitative. Today, there is a tendency, on a higher level, to reopen the dialogue between the science and religion, which was present at the very beginning of our culture. Religion had existed long before science came to life, but science is not to be thought of as a continuation of the religion. Each discipline should preserve its principles, its separate interpretations and its own conclusions. In the end, both of them represent different components of the one and indivisible culture of mankind.

**VISUALIZATION OF EARLY HUMAN DEVELOPMENT**

Significant advances have been made in recent years in visualizing and analyzing the earliest human development. Most of them have been done by introduction of three-dimensional colour Doppler sonography.

Many new parameters about early human development are now studied by Doppler ultrasound. Considerable number of biochemical, morphological and vascular changes occur within the follicle during the process of ovulation and luteinization and most of them can be studied by transvaginal ultrasound with colour Doppler and 3D facilities. (26) If the oocyte is fertilized the embryo is transported into the uterus where under a favourable hormonal and environmental conditions, it will implant and develop into a new and unique individual. The introduction of transvaginal colour Doppler improved the recognition of blood vessels enabling detailed examination of small vessels such as arteries supplying preovulation follicle, corpus luteum and endometrium.

Perifollicular vascularization can help in identification of follicles containing high quality oocytes, with a high probability of recuperating, fertilizing, cleaving and implanting, while 3D ultrasound enables accurate morphological inspection and detection of cumulus oophorus. Follicles without visualization of the cumulus by morphological inspection and detection of cumulus oophorus are now studied by Doppler ultrasound. Considerable number of biochemical, morphological and vascular changes occur within the follicle during the process of ovulation and luteinization and most of them can be studied by transvaginal ultrasound with colour Doppler and 3D facilities. (26) If the oocyte is fertilized the embryo is transported into the uterus where under a favourable hormonal and environmental conditions, it will implant and develop into a new and unique individual. The introduction of transvaginal colour Doppler improved the recognition of blood vessels enabling detailed examination of small vessels such as arteries supplying preovulation follicle, corpus luteum and endometrium.

Perifollicular vascularization can help in identification of follicles containing high quality oocytes, with a high probability of recuperating, fertilizing, cleaving and implanting, while 3D ultrasound enables accurate morphological inspection and detection of cumulus oophorus. Follicles without visualization of the cumulus by morphological inspection and detection of cumulus oophorus are not likely to contain fertilizable oocytes. This information is especially useful in patients undergoing ovulation induction.

Following ovulation, the corpus luteum is formed as the result of many structural, functional and vascular changes in the former follicular wall. Colour Doppler studies of the luteal blood flow velocities enable evaluation of the corpus luteum function in second phase of menstrual cycle and early pregnancy. When the placenta takes over the role of production of progesterone, the corpus luteum starts regressing. After ovulation there is a short period during which the endometrial receptivity is maximal. During these few days a blastocyst can attach the endometrium and provoke increased vascular permeability and vasodilatation at the implantation site. Trophoblast produced proteolytic enzymes cause the penetration of the uterine mucosa and erode adjacent maternal capillaries. This results in formation of the intercommunicating lacunar network - the intervillous space of the placenta. A small intradecidual gestational sac can be visualized by transvaginal sonography between 32 and 34 days. (27)

The secondary yolk sac is the earliest extraembryonic structure normally seen within the gestational sac in the beginning of the 5th gestational week. The yolk sac volume was found to increase from 5 to 10 weeks of gestation. When the yolk sac reaches its maximum volume at around 10 weeks it has already started to degenerate, which can be indirectly proved by a significant reduction in visualization rates of the yolk sac vascularity. Therefore, a combination of functional and volumetric studies by 3D power Doppler helps to identify some of the most important moments in early human development.

The embryonic heart begins beating on about day 22-23, accepting blood components from the yolk sac and pushing blood into the circulation. The embryonic blood begins circulating at the end of the 4th week of development.

The start of the embryo-chorionic circulation changes the source of nourishment to all intraembryonic tissues. The survival and further development of the embryo become dependent on the circulation of embryonic/foetal blood. If the embryochorionic circulation does not develop, or fails, the concept is aborted. The embryo cannot survive without the chorion (placenta) and the chorion will not survive without the embryo. A vascular degenerated chorionic villi constitute the hydatidiform mole.

Within the embryo, there are three distinct blood circulatory systems:

1. **Vitelline circulation** (from yolk sac to embryo)
2. **Intraembryonic circulation**
3. **Two umbilical arteries** (from embryo to placenta - foetoplacental circulation)

It is possible to visualize and assess them virtually from conception. (28),(29),(30),(31),(32)

At five weeks from the maternal side of placenta, it is possible to obtain simultaneously three-dimensional imaging of the developing intervillous circulation during the first trimester of pregnancy. Three-dimensional power Doppler reveals intensive vascular activity surrounding the chorionic shell starting from the first sonographic evidence of the developing pregnancy during the 5th week of gestation.
At seven weeks, three-dimensional power Doppler images depict aortic and umbilical blood flow. Initial branches of umbilical vessels are visible at the placental umbilical insertion.

During the 8th and 9th week, developing intestine is being herniated into the proximal umbilical cord.

At nine to ten weeks, herniation of the mid-gut is present. The arms with elbow and legs with knee are clearly visible, while feet can be seen approaching the midline.

At eleven weeks, three-dimensional power Doppler imaging allows visualization of the entire foetal and placental circulation.

During the 11th-12th week of pregnancy development of the head and neck continues. Facial details as nose, orbits, maxilla and mandibles are often visible. Herniated mid-gut returns into the abdominal cavity.

NEW POSSIBILITIES FOR STUDYING EMBRYONIC MOVEMENTS AND BEHAVIOUR

The latest development of 3D and 4D sonography enables precise study of embryonic and foetal activity and behaviour. (33) With four-dimensional ultrasound movements of head, body and all four limbs and extremities can be seen simultaneously in three dimensions. (34) Therefore, the earliest phases of the human anatomical and motor development can be visualized and studied simultaneously. It is clear that neurological development -early foetal motor activity and behaviour needs to be re-evaluated by this new technique. (35),(36) Recently, our group studied the development of the complexity of spontaneous embryonic and foetal movements. (37) With advancing of the gestational age the movements become more and more complex. The increase in the number of axodendritic and axosomatic synapses between 8 and 10 and again between 12 and 15 weeks (38) correlates with the periods of foetal movement differentiation and of the onset of general movements and complex activity patterns such as swallowing, stretching and yawning, seen easily by 4D technique. Seven to eight weeks of pregnancy gross body movements appear. They consist of changing the position of head towards the body. Nine to ten weeks of pregnancy limb movements appear. They consist of changes in position of extremities towards the body without the extension or flexion in elbow and knee. Ten to twelve weeks of pregnancy, complex limb movements appears. They consist of changes in position of limb segments towards each other, such as extension and flexion in elbow and knee. Twelve to fifteen weeks of pregnancy, swallowing, stretching, and yawning activities appear. In addition to these activities, it is now feasible to study by 4D ultrasound a full range of facial expression including smiling, crying and eyelid movement.

It is hoped that new 4D technique will help us for better understanding of both somatic and motor development of early embryo. It will also enable reliable study of foetal and even parental behaviour.

CONCLUSION

“Self-awareness is, one of the fundamental possibility, the most fundamental characteristic of the human species. This characteristic is an evaluation novelty; the biological species form which mankind has descended had only rudiments of self- awareness has however, brought in its train somber companions- fear, anxiety and death awareness” T. Dobzhansky

The question when a human life begins and how to define it could be answered only through the inner-connecting pathways of history, philosophy and medical science. It has not been easy to determine where to draw the fine line between the competence of science and metaphysics in this delicate philosophical field. To a large extent, the drawing of this line depends on one’s fundamental philosophical outlook.
REFERENCES


(9) Declaration of Professors from Five Faculties of Medicine and Surgery of the universities of Rome, organizers of the Conference: The Embryo as a Patient


ARGUMENTS FOR HUMAN THERAPEUTIC CLONING

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INTRODUCTION

For over 30 years, many Western governments have regulated scientific research involving human subjects. According to Knoppers (1) implementation of regulation followed a long and checkered history of research abuse. The regulations evolved largely in response to ethical violations. The Nuremberg Codex exemplifies the progression. It was adopted in 1947. At the conclusion of Nazi Doctors Trial.

Spectacular technical and conceptual advances in modern biology and molecular medicine have solved many problems in a short time. Genetic diagnostics extended well beyond simple inheritance testing, and is now moving into all areas of pathology. Gene therapy, although in a phase of consolidation after an exuberant youth, holds real promise. Understanding of the molecular basis of tissue differentiation, perhaps with the use of nuclear transfer techniques, may allow creation of histocompatible tissue for transplantation purposes (2).

Scientific work will have propounds long-term consequences for medicine, leading to the elucidation of the underlying molecular mechanisms of disease and thereby facilitating the design of rational diagnostics and therapeutics targeted at those mechanisms.

All molecular medicine must operate within a social and ethical context. Prominence of ethical controversy (i.e. presymptomatic genetic testing, or human therapeutically cloning) will very likely diminish with time, as the products of molecular medicine range further away from establishing pure diagnostic and into therapy.

One of the major issues of today’s modern medicine is therapeutically cloning. The main practical purpose of cloning is to generate genetically modified animals to serve as bioreactors. The cloning of mammals is fascinating biological problem, although it is difficult to perform and attempts are rarely successful. The reproductive cloning of humans is likely to cause more individual concern than real social effects, as it is unlikely to become a widespread method of reproduction even if possible and safe.

HUMAN THERAPEUTIC CLONING

Human therapeutic cloning is potentially limitless source of cells for tissue engineering and transplantation medicine. What is human therapeutic cloning? It involves the transfer of a patient’s somatic cell nuclei into enucleated oocytes, development of embryo to the early stage – morula or blastocyste, and isolation of stem cells that can differentiate into immunologically matched tissues. For example, cardiomyocytes could be used to treat patients with heart disease, pancreatic islet cells, for patients with diabetes, or hepatocytes, in a tissue-engineered liver. The main purpose of embryonic stem cell cloning techniques would be to create tissue that would not be subject to graft rejection. This procedure has a great potential, in producing specialized, replacement cells to treat a variety of diseases and conditions including parkinsonism, spinal cord injury, stroke, burns, heart disease, diabetes, osteoarthritis, rheumatoid arthritis (2.).

The cloning of mammals from adult cells has been achieved in several species in the past few years. The first mammal to be successfully cloned from a differentiated animal cell was the sheep (3), despite the fact that there had been previous cloning successes using embryonic cells. The sheep “Dolly” was cloned from an adult somatic cell by the somatic cell nuclear transfer method. Authors who cloned the sheep proved that the differentiation of adult cells (in this case derived from the mammary epithelium) does not involve the irreversible modification of genetic material that is required for the development of the animal to term. Despite this success, somatic cell nuclear transfer cloning is still insufficient, because we still do not know the main factors that distinguish successfully developed clones from clones that do not develop normally (4). Low cloning efficiency (1% of nuclear transfer embryos develop to adulthood) is not really an impediment for agricultural use of cloning because breeding from a single cloned genetically modified individual should be sufficient (5).

Because we do not know precise mechanisms that are involved in the abortions, neonatal deaths and postnatal disease associated with cloning, the human cloning is still dangerous and ethically unacceptable. In a future, we have to give much more emphasis to the development of the nuclear-transfer technology itself and to the genetic and epigenetic mechanisms that are involved in clone failure. Although human reproductive cloning is unacceptable today, production of cells from cloned embryos could offer many potential benefits. Therapeutic cloning according to Davor Solter may also not be affected by low cloning efficiency because this technique does not require a nuclear transfer embryo to develop to adulthood but only to the blastocyst stage, which has a higher success rate (close to 50% on average) (5).
What is present legal status of cloning? Human cloning for any purpose – reproductive or therapeutic – is illegal in Japan. In the United Kingdom, a government-appointed panel recently recommended that scientists should be permitted to create cloned embryos by nuclear transfer for research purposes only, and that these embryos cannot be maintained for longer than 14 days. There are many other countries without any laws whatsoever regarding human cloning, where cloners could move and set up laboratory (6).

**EMBRYONIC AND ADULT STEM CELLS**

Stem cells are clonogenic self-renewing progenitor cells that can generate one or more specialized cell types. Stem cells can be divided (in vertebrates) in two groups: embryonic and organ or tissue specific stem cells. Embryonic stem cells are pluripotent stem cells derived from the inner cell mass of the blastocyst, capable of generating all differentiated cell types in the body. Embryonic cells generate second group – organ/tissue specific stem cells. Such multipotent stem cells generate the cell types comprising a particular tissue in embryos or in some cases in adults. More research is needed to solve some current problems and questions: how to reprogram the nucleus of the adult cells without the need for an enucleated egg; how to put the cells together to create or recreate functional structures, how to modify the genome of the patient’s cells before the nuclear transfer procedure etc. It is our hope that by understanding how the cytoplasmic component direct development, we may eventually be able to reprogram the nucleus of adult cells without the need for an enucleated egg. Furthermore, it may be possible to modify the genome of the patient’s cells (through targeted gene alterations or engineered chromosomes) before the nuclear transfer procedure, so that after “reprogrammation”, the clones develop only into groups of specialized cells and tissues, rather than into a whole organism (7).

There is still the task of putting the cells together to create or recreate functional structures. For relatively simple tissues, such as skin and blood vessels substitutes, this may involve seeding cells onto masses or sheets of polymeric scaffold. Creating vital organs will be much greater challenge, and will require assembling different cell types and materials with great combinatorial and architectural complexity.

The creation of embryos for the purpose of research has been ethically and politically contentious. The term human embryo is defined as any organism, not protected as a human subject ... that is derived by fertilization, parthenogenesis, cloning, or any other means from one more human diploid cells. American National Institute of Health (NIH) has concluded that pluripotent stem cells are not themselves “organisms” under the definition. NIH may fund research on such stem cells. It raises the ethical question of where the embryos are obtained and the possibility of complicity in embryo destruction (8).

According to Winston (9) many of ethical objections regarding embryonic stem cells could be resolved by more research. In time destruction of large number of embryos might be avoidable. One possibility is to derive embryonic stem cells from embryonic blastomeres before blastocyst formation, blastomeres can be removed from the embryo without risking damage; it may be possible to collect cells mass at slightly later development stage. Cells could be banked and preserved in prolonged culture. An alternative to cloning should be reviewed. One possibility is to study heterokaryons produced by fusion of an embryonic cell (rather than egg) with somatic-cell nucleus; such hybrids could have potential for targeting Duchenne muscular dystrophy.

Although the destruction of a human embryos is lamentable there is a considerable moral difference between creating and destroying embryos solely to obtain stem cells and destroying unwanted human embryos that will never be used for reproductive purposes, to achieve benefit for those with serious disease and disorders (8).

The question of the definition and status of the human embryo is emerging as one of the most problematic issues for scientists. J.F. Mattei feels that, on the one hand, we cannot deprive ourselves of the therapeutic potential of the embryo solely on the basis of protecting it; on the other hand, Mattei wonders whether these few cells at the bottom of a test tube truly merit the name embryo. Does the embryo results from fertilization? Yes, when fertilization took place in utero. But when the fertilized egg is at bottom of a test tube, its spontaneous process is not to develop into a living being. Therefore, Mattei thinks that is not possible to combine, in the same concept and the same name, the in vitro embryo and the in vivo embryo. All this reinforced by the progress in therapeutic cloning (9).

The cloning of human beings has been officially unlawful in Europe since the Additional Protocol of the Council of Europe Convention on Human Rights and Biomedicine came into force in March 2001. On the other hand, the Council of Europe decided in favor of therapeutic cloning. Some experts think that is the first stage leading to reproductive cloning (6,8,10).

**ARGUMENTS FOR HUMAN THERAPEUTIC CLONING**

Indeed, there are strong arguments for embryonic stem cells research: our legal approach to abortion, our readiness to remove ectopic pregnancies, human preimplanta-
tion have only a limited potential to became humans. Most are lost before menstrual period. Finally, there is general public approval of in vitro fertilization; only around 10% of transferred IVF embryos produce a baby.

The promise of stem cell research for millions of patients may afford an outcome in which the ethical debate can be resolved. We can ask ourselves where be morality in letting millions of people continuing to suffer from chronic life-threatening disease. Human pre-embryos should be treated with respect. But, as Lanza pointed does a blastocyst warrant the same rights and reverence as that accorded a living soul – a parent, a child or a partner – who might die because we failed to move the moral line (11).

It seems increasingly likely that somatic cell nuclear will be developed and tested in humans, not in an attempt to create a child, but in effort to prevent and treat a long list of diseases.

ADULT STEM CELLS

Adult stem cells, the multipotent cells, exist in many adult organs and could serve as potential tool in future therapy. They can be isolated and in some cases expanded ex vivo. And even, they can be transplanted back to adult animals where they can differentiated and function approximately like in the normal organ.

Organ-specific stem cells can overcome their intrinsic restrictions upon exposure to a novel environment perhaps via genomic reprogramming. A dult stem cells from one tissue/organ can be induced to differentiate into cells of other organs (bone marrow-to-brain, bone marrow-to-liver, skin-to-brain, brain-to-heart).

There are some problems with adult stem cells, which could be possibly resolved by future research. Here are some of those problems: it is difficult to expand them and impossible to grow in large numbers, they don't have the same plasticity or broad range of potential as embryonic stem cells, we don't know the impact the aging process would have the same gene defect (this problem is also applied to embryonic stem cells) (2,5,7).

There are some other options. Recent observations on cell cultures from amniotic fluid and on amniotic epithelial cells provide evidence that they may represent new sources for the isolation of cells with the potency to differentiate into different cell type. A wide variety of investigations have provided evidence that cells of all three germ layers (ectoderm, mesoderm and endoderm) depending on the gestational age, fetal pathology, etc. can be detected in human amniotic fluid. Amniotic fluid can serve as a source of cells for fetal tissue engineering (12).

CONCLUSION

What are expectations? Cloning has the potential to contribute to improvements in veterinary and human medicine, with the prospect that non-reproductive human cloning strategies might provide future therapies for severe, incurable disease. Any stem cell can turn into any tissue given the appropriate conditions. More research has to be done before we understand whether there are restrictions on this process, whether it involves reprogramming that can lead to other unpredictable cellular behaviors and finally whether it even occurs at sufficient high frequency to be clinically useful. Until then there are no ethical and moral reasons to forbid stem cell therapeutic cloning. Before we start seriously with human therapeutic cloning, we have to learn more about the basic molecular mechanisms that are involved in nuclear reprogramming.
REFERENCES

(1) Knoppers B.M.: From medical ethics to "genetics". The Lancet Perspectives, 356 Suppl. 10; S38, 2000
ABSTRACT

BACKGROUND AND PURPOSE:
To investigate the histopathologic characteristics of atherosclerotic lesions in diffuse coronary artery disease and to evaluate the possible inflammatory role of chronic infection with Chlamydia pneumoniae (CP).

MATERIALS AND METHODS:
For 10 patients (males, mean age 61 years) who were surgically treated for grave diffuse coronary artery disease, histomorphological analyses of endarterectomized segments of the coronary arteries were performed. Serological analyses for the detection of CP antibodies in peripheral blood were done, preoperatively.

RESULTS AND CONCLUSIONS:
Diffuse and concentric atherosclerotic changes from VI to VIII stage according to the Stary classification were found. Immunohistochemical methods revealed infiltrates of T-lymphocytes (80% of cases), B-lymphocytes (40% of cases) and macrophages (80%). Using the nuclear marker for proliferation activity MIB-1, single MIB-1 positive cells were found in 40% of cases. Features of arteriologenesis and vasculitis of newly formed arterioles (as well as thickening of the wall of newly formed arterioles) were found in the vessel wall of 8 patients, 7 of them had chronic infection with CP (preoperative micro-immunofluorescent test results: 1:32<lgG≤1:512 and lgA≥32), one had passed CP infection (1:32 ≤lgG<1:512, lgA negative). These features were absent in 2 patients, both recovered from CP infection and had not the chronic CP infection at the time of surgery. DNA of Chlamydia pneumoniae was detected using the polymerase chain reaction (PCR) method in the vessel wall of 3 patients who were chosen randomly for this method. This study suggests an inflammatory and proatherogenic role of CP in a high grade atherosclerotic coronary artery wall in diffuse coronary artery disease.

INTRODUCTION

The modern view on the aethiopathogenesis of atherosclerosis includes the inflammatory process on the vessel wall. This inflammation might at least partly be caused by certain infectious agents, among them Chlamydia pneumoniae is a visible candidate (1, 2). Pathogenetic mechanisms of possible Chlamydial involvement in the progression of atherosclerosis have been already discussed (3). Seroepidemiological, laboratory and pathological studies (4-7) revealed CP as »being there« in atherosclerosis, however, whether CP is an initiator, promoter or an innocent bystander in the atherosclerotic process remains unproven (8). Diffuse coronary atherosclerosis is a special entity of coronary atherosclerosis where long segments of coronary arteries are diffusely atherosclerotically damaged (9). During the surgical treatment with the classic by-pass technique, many times it is necessary to make an endarterectomy of the diffusely involved artery segment. This is the procedure where the surgeon with a special knife has to cut off the damaged intima of the coronary artery wall (10). Endarterectomized sequesters offers the unique opportunity for studying the atherosclerotic vessel wall.

The aim of our research was to study pathohistological changes in the endarterectomized segments of the coronary artery wall in patients with serologically proven chronic CP infection where we were expecting to find distinct pathomorphological features regarding the patients without chronic CP infection.

MATERIALS AND METHODS

We histologically analysed the endarterectomized segments of the coronary arteries for 10 patients who were surgically treated because of diffuse coronary artery disease. All our patients were males, their mean age was 61+-3 years. Eight of them suffered myocardial infarction before the operation, and angina pectoris was present for 3–24 months before the operation. All patients had arterial hypertension, their mean blood pressure was 145/90 mm Hg (receiving therapy!). Also, all were obese.
their mean BMI was 30.0±4.4 kg/m². Nine patients were hyperlipidemic and had been receiving therapy with statin, 4 patients had diabetes. 90% of our patients were smokers, preoperatively. Immediately after the operation, the tissue samples of the endarterectomy were fixed in 10% buffered formalin for 24 hours and embedded in paraffin. Four mm sections were cut, deparaffinized, and stained with hematoxylin-eosin (HE), Masson-trichrome and Weigert. For the immunohistochemical analyses the sections were heated in a microwave oven (15 minutes for CD3, CD79α, CD68, and 25 minutes for the proliferation marker Ki-67). For the detection of the proliferation marker Ki-67, the MIB-1 monoclonal antibody was used. The sections were washed with a phosphate-buffered saline solution (PBS). Then, the primary antibodies were applied: CD3 were incubated overnight in a wet chamber at 4 °C (DAKO, Glostrup, Denmark; dilution 1:40); MIB-1 monoclonal antibody, incubation for 30 minutes at room temperature (DAKO, Glostrup, Denmark; dilution 1:80); CD68 monoclonal antibody, incubation for 30 minutes at room temperature (DAKO, Glostrup, Denmark; dilution 1:40); MIB-1 monoclonal antibody, incubation overnight in a wet chamber at 4 °C (DAKO, Glostrup, Denmark; dilution 1:100). After washing in PBS, streptavidin-biotin complex/horseradish peroxidase was applied for 30 minutes at room temperature. Positive controls for CD3, CD79α, CD68, and MIB-1 tonsils were used.

In the step serial sections of endarterectomized segments, the severity of atherosclerosis was graded according to the Stary classification (11, 9). Cell infiltration, proliferation activity of cells and tissue, capillarogenesis and arteriologenesis in the endarterectomized segments were investigated.

Preoperatively, a venous blood sample was taken for the determination of the antibody levels IgG, IgM and IgA to CP, at least 3 times in each patient over a period of 6 months to prove a stable titer. Serological studies were performed in the Institute of Microbiology of the Medical Faculty, University of Ljubljana by the microimmunofluorescence method (MIF), utilising Chlamydia pneumoniae, Chlamydia psittaci and Chlamydia trachomatis elementary bodies (MRL Diagnostics, USA) as antigens to detect specific IgG, IgM and IgA antibodies. Serological evidence of CPn infection was based on the criteria published by Grayston et al. (12). A fourfold rise in IgG/IgA titer in paired sera or an IgM titer ≥ 1:20 in any serum were considered as presumptive evidence of acute or recent infection with CP. Titers of IgG ≥ 1:32 and < 1:512 were presumed to be due to past infection with CP. Titters with stable IgG and IgA titers ≥ 1:32 were presumed to be chronic infection with CP. A negative result was defined as an IgG/IgA titer < 1:32 and IgM titer < 1:20. The sera tested for IgM or IgA antibodies were pre-treated to remove possible free and complexed IgG antibodies, following the manufacturer’s instructions (MRL Diagnostics, USA).

The study was approved by the National Ethics Committee and an informed consent was obtained by each patient.

RESULTS

Seven of our patients had chronic infection with CP (1:32≤ IgG ≤1:512 and IgA ≥1:32) and 3 patients had past infection with CP (1:32≤ IgG <1:512 and IgA negative). These 3 patients served us as a small control group. In all 10 patients infection with other types of Chlamydia (trachomatis, psittaci) was excluded.

Histopathologic examination of step-serial hematoxylin-eosin sections of endarterectomized sequesters revealed that the arterial intima was diffusely and concentrically atherosclerotic invoved, with narrowed lumen and proliferated intima (Picture 1), sometimes with cholesterol crystals in lipid core diffuse atheromas, but predominantly with proliferative fibrous tissue, somewhere calcified.

The greatest stage of atherosclerosis (according to Stary classification) - type VIII lesions - were found in 6 patients, type VII lesions were found in 2 patients and type VI to VII lesions in 2 patients. Macrophage infiltrations were found in 80% of cases, T-lymphocytes in 80%, B-lymphocytes and plasma cells in 40%. Single MIB-1 positive cells were found in 40% of cases, either in the area of mononuclear infiltrates or outside the area of mononuclear infiltrates, where these cells were confined predominantly to the area of fibrointimal hyperplasia (see Picture 2). Single MIB-1 positive cells were also found among smooth muscle cells in the vessel wall of arterioles.

We found neoangiogenesis in the inner part of atherosclerotic changed intima in 80% of cases. In 70% of cases it was capillarogenesis, in 60% we found newly formed arterioles and small arteries. Both forms, capillarogenesis and arteriologenesis, were found in 50% of cases. No angiogenesis was found in 20% of cases; these were 2 patients without chronic CP infection. Most newly formed arterioles and small arteries had thickened and hyalinized vessel walls. In 60% of cases, arterioles showed features of vasculitis - in their vessel walls and perivascularly the infiltration of polymorphonuclear cells (neutrophils) was found (Picture 3).

We had the opportunity to test 3 (2 with chronic and 1 with past CP infection) out of 10 patients for the presence of CP DNA in the vessel wall with the PCR method. In all 3 cases we obtained positive results - CP DNA was found.
Cross section through the endarterectomized segment of the coronary artery: the intima is diffusely and concentrically atherosclerotic involved. The diffuse mononuclear infiltrate is visible as well as neoangiogenesis in the outer parts of the intima. (x25)

Single MIB-1 positive cells (arrow) in the connective tissue of the atherosclerotic plaque and a single MIB-1 positive smooth muscle cell in the vessel wall of the arteriole/small artery. (x 320)

Vasculitis of newly formed arteriole. In the perivascular infiltrates, neutrophils are found. (x160)

**DISCUSSION**

The patients with chronic CP infection and the patients without chronic CP infection did not differ in the stage of atherosclerosis or the extent and severity of mononuclear infiltration (regarding macrophages and T-lymphocytes), but B-lymphocytes and plasma cells were more commonly found in the atherosclerotically changed coronary artery wall of patients with chronic CP infection. However, arteriologenesis was the unique morphologic feature in chronic CP infection as well as the vasculitis of newly formed arterioles and small arteries. Indeed, in one patient with past CP infection these signs were also present; it is possible that seronegativity was achieved recently, since the CP-DNA was still present in his vessel wall. In the last decade it is well known that classical risk factors such as hypelipidaemia, hypertension, diabetes, obesity and smoking do not account for all the incidence of atherosclerosis and cardiovascular disease. Other risk factors have been investigated and infectious agents and their possible role in the atherosclerotic process became apparent (13). The connection between CP and coronary artery disease was first mentioned in 1988 by a Finnish research group (14) and was supported by many references afterwards (15, 16). However, although possible pathogenetic mechanisms are already known (3), it is still unknown whether CP plays a causal, contributory or bystander role.

Our study contributes to the evidence that CP might play the contributory role in the genesis of atherosclerosis. A special histomorphologic picture has been found in endarterectomized segments of atherosclerotically damaged coronary arteries. These changes speak for the presence of chronic inflammation and immune reaction (B-lymphocytes, plasma cells, neoangiogenesis). Finding newly formed arterioles and small arteries is highly suggestive of the complex pattern of pathogenetic process. Indeed, ischemia in atherosclerotic tissue induces capillarogenesis (17) and this is the expected finding in atherosclerotic tissue. However, ischemia is an unlikely stimulus for arteriologenesis/arteriogenesis, since vascular endothelial growth factor (VEGF), the only growth factor with a clear connection to hypoxia, is not a mitogen for smooth muscle cells that form arteriolar wall (18). Other cytokines (FGF, IGF-1, TGF-beta), released during inflammation from monocytes and lymphocytes, trigger the proliferation of smooth muscle cells in arteriologenesis and arteriogenesis. We believe that growth factors released from monocytes and lymphocytes at inflammation caused by CP, influence the formation and growth of arterioles and small arteries. Interestingly, it was quite common to find MIB-1 positive cells (in 40 % of all cases), mostly in endothelial cells, fibroblasts and also in smooth muscle cells of arterioles, indicating the extensive proliferating activity of these components of the atherosclerotic artery wall.
Moreover, finding vasculitis of the newly formed vessels (in 60% of arterioles and small arteries, vascular and perivascular infiltration with neutrophils was found) shows that the damaging process is continuing, leading to further damage and the early atherosclerosis of newly formed vessels. It is important to emphasise that all these special pathohistological signs were absent in two patients without chronic CP infection (they had only past CP infection). However, in the group of 3 patients with past CP infection, there was one with arteriogenesis and vasculitis; probably, seronegativity was achieved recently, moreover since CP-DNA was still present in his vessel wall.

Finding CP-DNA with the PCR method in all 3 patients who were randomly selected for this method is another proof for CP being present in the artery wall not to rely only on serological criteria for chronic infection. In conclusion, our study speaks for the contributory and continuous role of CP in atherosclerosis in diffuse CAD. Inflammatory and immune responses in the artery wall are triggered in the chronic infection with CP and a special histomorphologic picture in the atherosclerotic tissue of endarterectomized segments of coronary arteries was found.

REFERENCES

ABSTRACT

Introduction: Without sufficient insulin treatment, acceptable level of glycoregulation, avoidance of dislipoproteinaemia and maintenance of body mass is difficult to achieve in patients with type 1 diabetes mellitus (DM). On the other hand, sometimes it is difficult to prevent weight gain, endogenous hyperlipidaemia and iatrogenic insulin resistance.

Aim: To compare metabolic control indicators in patients with type 1 DM in patients treated conventionally to those on intensified insulin regimen.

Material and methods: A sample of 52 persons with type 1 DM, without late complications and long duration of the disease, was selected. Among them 19 (36.5%) persons were treated with insulin in 4 or 5 doses, and 33 (63.5%) conventionally, in 2 doses. All the participants had biochemical indicators of metabolic control determined (glycosylated Hb, fasting and postprandial glycaemia, total cholesterol, triglycerides as well as lipoprotein fractions, HDLC and LDLC), body height (BH) and weight (BW) measured, body mass index calculated (BMI) and blood pressure measured (BP).

Results: In the group treated conventionally we found significantly higher mean values of BMI as compared to those on intensified insulin regimen (23.2 ± 2.0 kg/m², and 21.2 ± 1.2 kg/m² respectively, p<0.01) and proportion of those with overweight was as well significantly higher (27.3% versus 0%, p =0.012). We noted higher mean values of systolic (134.2 ± 17.6 mmHg, versus 123.4 ± 12.7, p<0.05) and diastolic (83.2 ± 10.1, versus 74.0 ± 9.7, p<0.01) BP. Biochemical indicators of glycoregulation were significantly worse with, at the same time, higher total dose of applied insulin (55.9 ± 8.5 IU, versus 46.3 ± 10.0 IU, p <0.01), and insulin units per kg of body weight (0.84 ± 0.11 IU/kg versus 0.77 ± 0.15 IU/kg, p<0.05).

Conclusion: Results indicate that intensified insulin treatment is more favourable variant of treatment, by which the certain level of insulin resistance, which might be present in patients treated with two higher insulin doses, is probably reduced. Therefore it improves metabolic outputs, blood pressure values and body mass index but also may have beneficial impact to economic aspect of insulin treatment as well.

Key words: type 1 diabetes mellitus, insulin regimens, insulin resistance

INTRODUCTION

The management of type 1 diabetic patients remains continuous challenge to clinicians, particularly in regard to optimal insulin dosage which will adequately reduce hyperglycaemia, avoid hypoglycaemia, prevent lipolysis and maintain optimal muscle and adipose tissue mass and eventually prevent late complications of the disease(1, 2). Although subcutaneous application of insulin is per se non physiological because of the first by pass of the liver, for vast majority of patients, it remains the only available option for the time being (3). It can be delivered by so called intensified or conventional insulin regimen (4). The first one includes insulin administration 4 or 5 times daily, in the form of short- or rapid acting type before meals and intermediate or long-acting one at bedtime and if needed during the day. The other type of insulin regimen is standard or conventional one with two dosages of mixed insulin (short or rapid and intermediate acting) before breakfast and dinner. The insulin dosage in insulinopenic patients should be titrated cautiously and individually in order to prevent catabolic but as well hyperanabolic state, characterized by excessive weight gain, endogenous hyperlipidaemia and possibly iatrogenic insulin resistance (5, 6, 7).

The main aim of this cross-sectional study is to compare metabolic control and nutritional indicators in patients with type 1 diabetes mellitus treated conventionally to those on intensified insulin regimen.

MATERIAL AND METHODS

A sample of 52 persons with type 1 diabetes mellitus, with similar age and relatively short duration of the disease, who were treated on outpatient basis at the Department of Diabetes and Endocrinology of Clinical Centre University of Sarajevo, was selected. It did not include patients with other forms of autoimmune endocrine diseases, those with signs of diabetic or other forms of nephropathy and liver diseases. They did not have other kind of diabetic microvascular or macrovascular complications. All the participants were individual-
ly and carefully instructed about self management, with emphasis on dietary advices and physical exercise in the period prior to the beginning of the study. They did not experienced any acute deterioration of the disease, major infection, surgery or severe stress in the period of at least 4 months.

The sample included 19 participants on intensified insulin treatment (IIT), with 4 or 5 insulin applications daily in the form of human, short-acting one before main meals and intermediate acting insulin at bedtime or in the morning. The other group consisted of 33 persons treated conventionally (CIT), by two dosages of mixed insulins (short and intermediate acting) before breakfast and dinner. There was not significant difference between the groups of participants in regard to the age and the duration of the disease (Table 1).

All the participants had biochemical indicators of metabolic control determined: glycosylated Hb (HbA1c), fasting glycaemia (FG), postprandial glycaemia (PG), total cholesterol (TCh), triglycerides (TGL), HDL-C and LDL-C. All analyses were carried out in the same venous blood samples collected from the fasting subjects except for the postprandial blood glucose values and HbA1c. The tests were carried out by Institute of Clinical Biochemistry, Clinical Centre University of Sarajevo.

Glucose levels were measured in venous blood samples using the automated glucose-oxidase reaction. Glycosylated Hb (HbA1c) levels were measured using the BIO-RAD Micro Column Test. Total cholesterol as well as HDL-C and LDL-C and triglyceride levels were measured enzymatically on an automated Abbot-Spectrum using Trace Cholesterol, HDL-C, LDL-C and Triglyceride commercial kits (PEG-6000 Method).

Nutritional status was assessed by measuring body weight (BW) and body height (BH) and calculating the body mass index (BMI) with a BMI of less than 18.5 indicating undernourishment and higher than 25 indicating being overweight. Individuals were weighed and measured using a Secca 770 digital scale and Secca 225 height statometer. All the participants have the blood pressure measured according to the country-wide integrated noncommunicable diseases intervention programme (CINDI) protocol (8) using a calibrated sphygmometer, Reister 600/306, Diplomat.

**STATISTICAL ANALYSIS**

The results were expressed as mean values with standard deviation or proportions expressed in percentages. The significance of the differences between the mean values among the groups was tested using the Student’s unpaired t-test, while the difference between proportions was assessed using $\chi^2$ test. The differences were considered significant at the level of $p < 0.05$. Connection between variables was assessed by Pearson correlation coefficient with confidence limits of 95%.

**RESULTS**

We found significantly better indicators of glycoregulation and majority of lipid control indices in the intensively (IIT) treated group (Table 2 a). Although improvement in HDL-C levels were noted this difference was not statistically significant.

Proportion of those who achieved more satisfactory glycaemic control was also significantly higher in the IIT group (Table 2 b), although the differences in lipid parameters were not significant.

Body weight, body mass index, systolic and diastolic blood pressure were significantly lower in the ITT group as well as proportion of overweight and hypertensive patient (Table 3 a, Table 3 b). There were no undernourished patients in both groups.

We noted significant negative correlation between the number of insulin injections with postprandial glycaemic levels ($r = -0.50$), body mass index ($r = -0.49$) and glycosylated Hb ($r = -0.44$) (Table 4). The higher the number of insulin injections the lower lipid parameters were noted as well (Table 5). Negative correlation was found between the number of insulin injections and total IU, DBP, SBP and IU/kg (Table 6).

**DISCUSSION**

Athough majority of previous studies indicated superiority of intensive insulin regimens in regard to prevention and delay of late microvascular complications (9, 10, 11) there has not been strong evidence that equal glycaemic control is significantly less likely to be achieved with 2

---

**Table 1. Characteristics of participants by the type of insulin regimen**

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Age (y)</th>
<th>Males</th>
<th>Females</th>
<th>Duration (y)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IIT</td>
<td>19</td>
<td>27.5 ± 7.6</td>
<td>8 (42.1%)</td>
<td>11 (57.9%)</td>
<td>5.1 ± 2.4</td>
</tr>
<tr>
<td>CIT</td>
<td>33</td>
<td>31.8 ± 10.2</td>
<td>15 (45.5%)</td>
<td>18 (54.5%)</td>
<td>5.5 ± 3.4</td>
</tr>
</tbody>
</table>

- **Student's t-test**: $t = 1.714$, $p > 0.05$
- **$\chi^2$ test**: $\chi^2 = 3.13$, $p = 0.077$; $\chi^2 = 3.38$, $p = 0.067$

1 - Intensified insulin treatment
2 - Conventional insulin treatment
dosages of appropriate mixtures of short and intermediate acting human insulins (12, 13). In addition, majority of above mentioned studies noted significant weight gain and hypoglycaemic episodes with intensified insulin regimes. In that regard, the question of developing the syndrome of insulin resistance as seen as an underlying disorder in type 2 diabetes related to overweight has been raising a lot of concern recently (14, 15, 16).

Our results indicates that majority of metabolic indicators were improved with intensified insulin regimen. At the same time the total number of applied daily insulin units and moreover insulin units per kg of body weight, were significantly less in this group. This indicates improved insulin sensitivity with IIT, so that the smaller amounts of exogenous insulin was sufficient to achieve satisfactory glyco and lyporegulation (Table 2 a, Table 3 a). The finding of higher proportion of hypertensive patients can also be attributed to higher level of insulin resistance in CIT group (Table 3 b).

It is very likely that 2 higher daily insulin dosages promote to some extent weight gain and compromise patients' adherence to dietary recommendations. In addition to achieving more favourable metabolic outputs, it is interesting that the cost of insulin treatment was decreased, as we noted 16.3% of reduction of total daily insulin requirements from 56 to 47 IU (Table 3 a) and 9.4% in regard to IU/kg (0.85 to 0.77IU/kg). Although investments in self-monitoring can initially increase the costs of IIT, as was shown in DCCT (10, 18, 19), in future we can expect lower rate of micro and macrovascular complications which would contribute to the lower cost in long term period (17, 20).

Although overall metabolic control was closer to optimal in IIT group, it could be even better, which would possibly be achieved with currently available rapid and long acting insulin analogues with superior pharmacokinetic properties (21).

Table 2. Parameters of glyco- and liporegulation by the type of insulin regimen

### a) mean values

<table>
<thead>
<tr>
<th></th>
<th>HbA1c (%)</th>
<th>FG (mmol/l)</th>
<th>PG (mmol/l)</th>
<th>TCh (mmol/l)</th>
<th>LDLC (mmol/l)</th>
<th>HDLC (mmol/l)</th>
<th>TGL (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IIT</td>
<td>6.9 ± 0.8</td>
<td>7.1 ± 1.4</td>
<td>10.1 ± 2.1</td>
<td>4.9 ± 0.4</td>
<td>2.9 ± 0.5</td>
<td>1.20 ± 0.27</td>
<td>1.65 ± 0.79</td>
</tr>
<tr>
<td>CIT</td>
<td>7.9 ± 1.2</td>
<td>8.3 ± 1.1</td>
<td>12.2 ± 1.7</td>
<td>5.4 ± 0.9</td>
<td>3.4 ± 0.9</td>
<td>1.09 ± 0.25</td>
<td>2.12 ± 0.71</td>
</tr>
<tr>
<td>t-test</td>
<td>3.257</td>
<td>3.152</td>
<td>3.954</td>
<td>2.564</td>
<td>2.590</td>
<td>1.430</td>
<td>2.227</td>
</tr>
<tr>
<td>p-value</td>
<td>p&lt;0.01</td>
<td>p&lt;0.01</td>
<td>p&lt;0.05</td>
<td>p&lt;0.05</td>
<td>p&lt;0.05</td>
<td>p&lt;0.05</td>
<td>P&lt;0.05</td>
</tr>
</tbody>
</table>

1 - intensified insulin treatment
2 - conventional insulin treatment
3 - glycylated haemoglobin
4 - fasting glycaemia
5 - postprandial glycaemia
6 - total cholesterol
7 - low density lipoprotein cholesterol
8 - high density lipoprotein cholesterol
9 - triglycerides

### b) proportions

<table>
<thead>
<tr>
<th></th>
<th>HbA1c ≤ 7.0 n (%)</th>
<th>FG ≤ 7.0 n (%)</th>
<th>PG ≤ 10.0 n (%)</th>
<th>TCh ≤ 4.8 n (%)</th>
<th>LDLC ≤ 3.0 n (%)</th>
<th>HDLC ≥ 1.0 n (%)</th>
<th>TGL ≤ 1.7 n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IIT</td>
<td>14 (73.7)</td>
<td>12 (63.2)</td>
<td>12 (63.2)</td>
<td>9 (47.4)</td>
<td>12 (63.2)</td>
<td>16 (84.2)</td>
<td>9 (47.4)</td>
</tr>
<tr>
<td>CIT</td>
<td>6 (18.2)</td>
<td>5 (15.2)</td>
<td>2 (6.1)</td>
<td>10 (30.3)</td>
<td>14 (42.2)</td>
<td>24 (72.7)</td>
<td>8 (24.2)</td>
</tr>
<tr>
<td>X²-test</td>
<td>15.7</td>
<td>12.6</td>
<td>19.9</td>
<td>1.51</td>
<td>2.07</td>
<td>0.9</td>
<td>2.93</td>
</tr>
<tr>
<td>p-value</td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
<td>p=0.218</td>
<td>p=0.149</td>
<td>p=0.343</td>
<td>P=0.087</td>
</tr>
</tbody>
</table>

1 - intensified insulin treatment
2 - conventional insulin treatment
3 - glycylated haemoglobin
4 - fasting glycaemia
5 - postprandial glycaemia
6 - total cholesterol
7 - low density lipoprotein cholesterol
8 - high density lipoprotein cholesterol
9 - triglycerides
Table 3. Nutritional status indicators, blood pressure values, and insulin units by the type of insulin regimen

a) mean values

<table>
<thead>
<tr>
<th></th>
<th>BW (kg)</th>
<th>BMI (kg/m²)</th>
<th>SBP (mmHg)</th>
<th>DBP (mmHg)</th>
<th>IU</th>
<th>IU/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>IIT¹</td>
<td>60.7 ± 7.4</td>
<td>21.2 ± 1.2</td>
<td>123 ± 13</td>
<td>74 ± 10</td>
<td>46.8 ± 10.3</td>
<td>0.77 ± 0.16</td>
</tr>
<tr>
<td>CIT²</td>
<td>67.7 ± 9.8</td>
<td>23.2 ± 2.0</td>
<td>134 ± 18</td>
<td>83 ± 10</td>
<td>55.9 ± 8.5</td>
<td>0.85 ± 0.11</td>
</tr>
<tr>
<td>t-test</td>
<td>2.707</td>
<td>4.000</td>
<td>2.352</td>
<td>3.255</td>
<td>3.473</td>
<td>2.162</td>
</tr>
<tr>
<td>p-value</td>
<td>p&lt;0.01</td>
<td>p&lt;0.01</td>
<td>p&lt;0.05</td>
<td>p&lt;0.01</td>
<td>p&lt;0.01</td>
<td>p&lt;0.05</td>
</tr>
</tbody>
</table>

¹ - intensified insulin treatment
² - conventional insulin treatment
³ - body weight
⁴ - body mass index
⁵ - systolic blood pressure
⁶ - diastolic blood pressure
⁷ - total daily insulin units
⁸ - insulin units per kg of body weight

b) proportions

<table>
<thead>
<tr>
<th></th>
<th>BMI &gt;25.0 kg/m²</th>
<th>BMI &lt;18.5 kg/m²</th>
<th>SBP &gt;140 mmHg</th>
<th>DBP &gt;90 mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td>IIT¹</td>
<td>0 (%)</td>
<td>0 (0)</td>
<td>2 (10.5)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>CIT²</td>
<td>9 (27.3)</td>
<td>0 (0)</td>
<td>15 (45.4)</td>
<td>11 (33.3)</td>
</tr>
<tr>
<td>χ²-test</td>
<td>6.25</td>
<td>6.69</td>
<td>8.03</td>
<td>0.009</td>
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<tr>
<td>p value</td>
<td>0.012</td>
<td></td>
<td>0.004</td>
<td></td>
</tr>
</tbody>
</table>

¹ - intensified insulin treatment
² - conventional insulin treatment
³ - body mass index
⁴ - systolic blood pressure
⁵ - diastolic blood pressure

CONCLUSION

Intensified insulin regimen in type 1 diabetic patients is more favourable variant of treatment by which the certain level of insulin resistance, which might be present in patients treated with two higher daily dosages, is probably reduced. Therefore it improves metabolic outputs, body mass index and blood pressure values and in longer terms may have beneficial impact to development of late complications as well as to economic aspects of insulin treatment.
Table 4. Correlation coefficients (with 95% confidence limit) between the number of insulin applications (NIA) and glycaemic parameters and body mass index (BMI)

<table>
<thead>
<tr>
<th></th>
<th>FG¹</th>
<th>HbA₁c²</th>
<th>PG³</th>
<th>BMI</th>
</tr>
</thead>
<tbody>
<tr>
<td>NIA</td>
<td>r = -0.37</td>
<td>r = -0.44</td>
<td>r = -0.50</td>
<td>r = -0.49</td>
</tr>
<tr>
<td></td>
<td>(-0.37 &lt; r² &lt; 0.63)</td>
<td>(-0.29 &lt; r² &lt; 0.66)</td>
<td>(-0.14 &lt; r² &lt; 0.70)</td>
<td>(-0.19 &lt; r² &lt; 0.60)</td>
</tr>
</tbody>
</table>

¹. fasting glycaemia
². glycosylated Hb
³. postprandial glycaemia

Table 5. Correlation coefficients (with 95% confidence limit) between the number of insulin applications (NIA) and main lipid parameters

<table>
<thead>
<tr>
<th></th>
<th>LDLC¹</th>
<th>TCh²</th>
<th>TGL³</th>
<th>HDLC⁴</th>
</tr>
</thead>
<tbody>
<tr>
<td>NIA</td>
<td>r = -0.34</td>
<td>r = -0.32</td>
<td>r = -0.24</td>
<td>r = +0.20</td>
</tr>
<tr>
<td></td>
<td>(-0.37 &lt; r² &lt; 0.63)</td>
<td>(-0.42 &lt; r² &lt; 0.60)</td>
<td>(-0.47 &lt; r² &lt; 0.57)</td>
<td>(-0.24 &lt; r² &lt; 0.31)</td>
</tr>
</tbody>
</table>

¹. low density lipoprotein cholesterol
². total cholesterol
³. triglycerides
⁴. high density lipoprotein cholesterol

Table 6. Correlation coefficients (with 95% confidence limits) between the number of insulin applications (NIA) and blood pressure values and insulin requirements

<table>
<thead>
<tr>
<th></th>
<th>SBP¹</th>
<th>DBP²</th>
<th>IU³</th>
<th>IU/kg⁴</th>
</tr>
</thead>
<tbody>
<tr>
<td>NIA</td>
<td>r = -0.32</td>
<td>r = -0.42</td>
<td>r = -0.44</td>
<td>r = -0.22</td>
</tr>
<tr>
<td></td>
<td>(-0.42 &lt; r² &lt; 0.60)</td>
<td>(-0.32 &lt; r² &lt; 0.65)</td>
<td>(-0.29 &lt; r² &lt; 0.66)</td>
<td>(-0.48 &lt; r² &lt; 0.56)</td>
</tr>
</tbody>
</table>

¹. low density lipoprotein cholesterol
². total cholesterol
³. total daily insulin units
⁴. insulin units per kg of body weight
REFERENCES

(11) Dorchy H. Insulin regimen and insulin adjustments in diabetic children, adolescents and young adults: personal experience
DETRUSOR CONTRACTION DURATION
AND STRENGTH IN THE PATIENTS WITH
BENIGN PROSTATIC ENLARGEMENT

Damir Aganovic, Alden Prci
Department of Urology
Clinical Center of University of Sarajevo

ABSTRACT

Objective: examine detrusor contraction duration (DCD) in relation with obstruction grade and strength of detrusor contractility; analyze individual correlations of this parameter with urodynamic, physiological and symptoms variables in patients with benign prostatic enlargement (BPE).

Sample and methodology: 102 patients with proved BPE, underwent complete urodynamic measurements (UDM), namely uroflowmetry, cystometry and pressure/flow studies. Postvoid residual urine (PVR) was measured and the International Prostate Symptom Score (I-PSS) was fulfilled by each patient. Methodology of measurement and definitions of UDM are based on definitions and terminology defined by the International Continence Society.

Results: After grouping the patients (average age 64.7 + 8.5) related to obstruction grades according to the Schafer nomogram, ANOVA has shown a group extension of the detrusor contraction duration related to higher levels of obstruction (LinPURR O-VI; p<0.01), which is also followed by stronger detrusor contractility (Pdetmax; p<0.001). Dichotomizing of the patients with DCD cut off point 90 sec. has shown that 67% patients with underactive detrusor have DCD>90 sec, while extension of DCD and increase of the obstruction level are directly related to preserved detrusor contractility only in 20.5% cases. There is neither statistically significant difference of DCD in the patients that are not in obstruction allocated in two groups depending on detrusor contraction strength, (t=1.2, p>0.05); nor in the patients who are in obstruction range, divided on the same way (t=0.568, p>0.05). There is also no difference of the same patients groups regarding PVR (t=1.38 and t=1.17, p>0.05). Individual correlation of DCD with I-PSS has not been shown (r=0.16, p>0.05), although there is a statistically significant correlation with its obstructive subset (r=0.20, p>0.05), as well as, with LinPUR and URA nomograms (r=0.33, r=0.29; respectively, p<0.005) and with Pdetmax (r=0.26, p<0.01), PdetQmax (r=0.24, p<0.05), Qmax and Qaver (r=0.31, p<0.005). DCD does not have individual correlations with patients’ age, prostate volume and with cystometric capacity.

Conclusion: DCD is rather independent urodynamic variable, which does not correlate with I-PSS. Generally, DCD is prolonged during obstruction, while extension of DCD only partially depends on detrusor contraction strength. Practically, individual correlations of DCD with the urodynamic factors, which characterize obstructions, are modest.

Key words: BPE, UDM, detrusor contraction duration

INTRODUCTION

Detrusor contraction duration (DCD) has become a focus of interest for urodynamic investigations of bladder output obstruction after Kaplan found correlation between DCD and I-PSS. As the parameter in a function of obstruction, which can be predicted also by I-PSS Score, is based on a simple concept that a time, needed for a fluid to leave a reservoir, depends on external resistance. While this concept is correct for the reservoir with a constant volume and constant pressure, this ideal situation can not be applied to a bladder in obstruction. Resistance of a bladder outlet varies a lot during initiation and termination of micturition due to neuro-muscular mechanism, which influences the bladder output during different phases of urinating, so detrusor contraction duration can be dependent on strength, better to say on detrusor contractility, bladder capacity including additional increase of urethral resistance(2). Due to these additional factors that can influence DCD, a role of this urodynamic factor in obstruction characterization has been examined. Inter-correlations between UDM factors and the correlation of DCD with I-PSS have been determined.

SAMPLE AND METHODOLOGY

102 patients with proved benign prostate enlargement (BPE) underwent complete urodynamic measurement (UDM). Inclusive criteria were no sign of urinary infection, no bladder stones, no haemathuria and normal values of PSA, as well as preserved kidney function. Dantec 5500 apparatus was used for uroflowmetry, cystometry and pressure/flow studies. Residual urine was determined by ultrasound and International Prostatic Symptom questionnaire was fulfilled per each patient (3). Methodology and nomenclature of UDM, if not marked different, are founded on the basic definitions of the International Continence Society (4).
RESULTS

Out of 102 patients, 24 (23.5%) were not in obstruction at all, 28 (27.5%) were in mild (or equivocal) obstruction, while the rest of the patients (49%) were in a clear or advanced obstruction, based by the Schafer (linPURR) nomogram (5). Analysis of variance has determined that detrusor contraction duration is prolonged for increased levels of obstruction, what is proportionally followed by strength of detrusor contraction. (Table 1).

Distribution of patients relating to duration and strength of detrusor contraction (according to Schafer nomogram) has show that the patients with weak or very weak (GDC 0/1) and normal or strong (GDC 2/3) detrusor are rather uniformly distributed in the zones out of obstruction (linPURR 0/I) or in the zone of equivocal obstruction (linPURR II); even predomination of patients with under-active detrusor is noted. However, number of patients with GDC 0/I is decreasing in the levels of clear and advanced obstruction (linPURR >III), so there are only patients with normal and strong detrusor in the levels linLPURR V and VI (Table 2).

Related to DCD, which cut of point value is 90 seconds (6), it has been noted that detrusor contraction duration is rather uniformly distributed in the zones out of obstruction (linPURR 0-II); while only for increased levels of obstruction, extension of detrusor contraction is appearing. Analysis of 61 patients with DCD>90s, related to detrusor contraction strength, has shown that extension of detrusor contraction is appearing in 24 patients with weak detrusor (GDC 0/1), what presents 65% of the total GDC 0/1 group. 37 patients with normal or strong detrusor (GDC 2/3) have DCD>90s, what is 57% out of the total GDC 2/3 group. Patients are relatively uniformly distributed in the levels out of obstruction (LPURR 0-II) and in the level of obstruction LinPURR III (GDC 0/1 – 53.8% comparing with GDC 2/3 – 46.2%), being independent on detrusor contraction strength. Increase of obstruction level (LinPURR >IV) influences that 21 patients (95.5%) with GDC 2/3 have DCD>90s, while only one has this results in GDC 0/1 group. It means that the extension of DCD directly depends on preserved detrusor strength only for higher obstruction levels (LinPURR IV-VI); this relation has been noticed with 21 patients (20.5%) from the analyzed BPE group (102 patients).

When the patients are dichotomized in the regions in and out of obstruction by combination of Schafer and URA nomograms (7), it has shown that there is no difference in the DCD between the patients out of obstruction with weak detrusor and patients from the same group, but with normal or strong muscle (p>0.05). Also, if the patients are in obstruction, there is no significant difference of DCD relating to detrusor strength (p>0.05). There is no

Table 1: Patients allocated related to obstruction grade on Schafer nomogram

<table>
<thead>
<tr>
<th>LPURR</th>
<th>LPURR 0/1 (n=24)</th>
<th>LPURR 2 (n = 28)</th>
<th>LPURR 3-6 (n = 50)</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Arit. Sr.</td>
<td>SD</td>
<td>Arit. Sr.</td>
<td>SD</td>
</tr>
<tr>
<td>PdetQmax (cm H2O)</td>
<td>36.37</td>
<td>+9.27</td>
<td>49.65</td>
<td>+6.96</td>
</tr>
<tr>
<td>PvesQmax (cm H2O)</td>
<td>74.67</td>
<td>+20.89</td>
<td>81.72</td>
<td>+22.76</td>
</tr>
<tr>
<td>Pdetmax (cm H2O)</td>
<td>58.92</td>
<td>+19.04</td>
<td>70.27</td>
<td>+20.64</td>
</tr>
<tr>
<td>MDA (cm H2O)</td>
<td>46.42</td>
<td>+22</td>
<td>50.38</td>
<td>+25.11</td>
</tr>
<tr>
<td>DCD (Sec.)</td>
<td>87.87</td>
<td>+22.76</td>
<td>96.65</td>
<td>+24.97</td>
</tr>
<tr>
<td>GDC</td>
<td>1.62</td>
<td>+0.71</td>
<td>1.48</td>
<td>+0.63</td>
</tr>
</tbody>
</table>

Table 2: Patients distributed related to obstruction levels, depending on strength (GDC) and detrusor contraction duration (DCD)

<table>
<thead>
<tr>
<th>LPURR</th>
<th>Number of patients</th>
<th>GDC</th>
<th>DCD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-1(n=37)</td>
<td>2-3 (n=65)</td>
<td>&lt;90 (n=41)</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>17</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>20</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>IV</td>
<td>17</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>V</td>
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</tr>
<tr>
<td></td>
<td>VI</td>
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</tbody>
</table>
statistically significant difference between the patients in obstruction and out of obstruction for the group GDC 0/1 (p>0.05). The only statistically significant difference (p<0.005) is a difference of DCD for the patients out and in obstruction with normal or strong muscle (Table 3). These results have shown that DCD extends proportionally with the obstruction level, while it is less dependent on detrusor contraction strength. Now, the question is if a weak detrusor being in obstruction can effectively empty a bladder in spite of prolonged contraction duration. To examine this, a statistical analyze was performed. Student Test has shown that there is no difference in of residual urine volume between the patients with weak muscle and those with normal one (p>0.05). The difference in residual urine volume was compared with the same groups in obstruction and out of obstruction regions; no significant difference has been found (t=1.38, t=1.17, p>0.05). No significant individual correlation between DCD and postvoid residual urine was noted (r=0.009, p>0.05).

Considering aspects of lower urinary tract symptoms (I-PSS), there are few and conflicting reports about obtained correlation between IPS Score and DCD. Because of this fact, correlation of lower urinary tract symptoms (LUTS) with detrusor contraction duration was examined. A nalysis of variance has shown no correlation (p>0.05) between DCD and increase of I-PSS, so for the group with I-PS Score 0-7 (mild symptoms), the average DCD is 88.8+26.9s; for the group of patients with I-PSS 8-19 (moderate symptoms), DCD is 101.8+27.1s; while for the group with I-PSS of 20-35 scores, DCD is 106.6+32.6s. Individual correlation of DCD with I-PSS has resulted with low Pearson correlation coefficient (r=0.16, p>0.05). Also no significant correlations of DCD with prostate volume, post void residual urine, patients’ age and cystometric capacity have been reached (results not showed at all), nor with volume of voided urine (r=0.001, p>0.05).

Table 4, presents individual correlations of DCD with other urodynamic parameters. Pearson and Spearman correlation coefficient factor were used. There are statistically significant correlations with urodynamic nomograms (LinPURR, A/GNo, URA) with p<0.005 (Scattergram 1.). There are also correlations of DCD with PdetQmax and with maximal detrusor pressure (p<0.01). The best correlations of this parameter with non invasive urodynamic parameters are correlations with Qmax and Qaver (p<0.01), what theoretically responds to the fact that increase of obstruction leads to decrease of flow; this condition is followed by extension of detrusor contraction, independently on actual detrusor strength. Nevertheless, there is no correlation between DCD and I-PSS, a statistically significant correlation with its obstructive subgroup has been shown (r=0.02, p<0.05). However, practical meaning of this correlation in its absolute sense is not significant, specifically, if correlations are considered separately.

To increase the test sensibility, better to say, prediction of DCD extension, what would give better correlations with certain levels of obstruction, a multivariate regression analysis was used to define correlations between DCD and detrusor pressure at the maximal flow (PdetQmax).

### Table 3. Detrusor contraction duration related to contraction strength and obstruction

<table>
<thead>
<tr>
<th></th>
<th>Non obstructive group DCD (sec)</th>
<th>Obstructive group DCD (sec)</th>
<th>T-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>GDC (0/1)</td>
<td>95.85±26.5</td>
<td>104.52±21.14</td>
<td>t=-1.034, p&gt;0.05</td>
</tr>
<tr>
<td>GDC (2/3)</td>
<td>85.3±25.2</td>
<td>108.35±32.8</td>
<td>t=-3.1596, &lt;0.005</td>
</tr>
<tr>
<td>T-test</td>
<td>t=1.196, p&gt;0.05</td>
<td>t=0.568, p&gt;0.05</td>
<td></td>
</tr>
</tbody>
</table>

### Table 4: Correlation of DCD with urodynamic parameters

* * *<p><0.05, **p<0.01, ***p<0.005

<table>
<thead>
<tr>
<th>Correlation Coefficient</th>
<th>DCD (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I-PSS (Obst.)</td>
<td>r - 0.1993*</td>
</tr>
<tr>
<td>Tvoid.</td>
<td>r - 0.2684**</td>
</tr>
<tr>
<td>Qmax (ml/s)</td>
<td>r - 0.3092***</td>
</tr>
<tr>
<td>Qaver. (ml/s)</td>
<td>r - 0.3129***</td>
</tr>
<tr>
<td>Siroyki nomogram</td>
<td>r - 0.279**</td>
</tr>
<tr>
<td>PdetQmax (cmH2O)</td>
<td>r - 0.2362*</td>
</tr>
<tr>
<td>Pdetmax (cmH2O)</td>
<td>r - 0.2606**</td>
</tr>
<tr>
<td>LinPURR</td>
<td>r - 0.3281***</td>
</tr>
<tr>
<td>AG No</td>
<td>r - 0.3323***</td>
</tr>
<tr>
<td>URA (cmH2O)</td>
<td>r - 0.2874***</td>
</tr>
</tbody>
</table>
detrusor contraction strength and cystometric capacity. The obtained value of correlation is $R=0.341$ ($p<0.001$). However, in practical sense this is considered as a modest correlation (extension of detrusor contraction is possible to estimate in 33 % cases).

Scattergram 1: Simple linear regression for DCD and URA

Acceptable correlations between DCD and Pdetmax, as well as, with PdetQmax and with non invasive urodynamic parameters (Qmax, Qaver, Tvoid). Individual correlations with cystometric capacity and volume of the voided urine have not been shown. The presented results show that increase of urodynamic obstruction is followed by extension of detrusor contraction duration. However, detrusor contraction strength is not a factor to determine this extension, as a patient with underactive detrusor can be in obstruction with prolonged DCD. This study has shown that detrusor contraction strength influences obstruction grade and extension of DCD only for 20.5% of the examined sample. Even 67% patients of the total number patients with impaired detrusor contractility have DCD>90s. Extension of DCD depends on detrusor strength only in increased obstruction grades (linPUR IV-VI), what seems to be logical and in accordance with BOR\(^9\) diagram. Opposite to Ameda’s findings, there is no difference in DCD between non-obstructive subgroups with weak and strong detrusor; the same result has been achieved for obstructive subgroups.

Figure 1. Detrusor contraction duration (DCD); DCD = 109sec, flow time=93 sec, Qura-flow, Pves – intravesical pressure, Pabd.-abdominal pressure, Pdet.- detrusor pressure, EMG – electromyography – not followed

As DCD depends on bladder strength, better to say on work necessary to prevail urethral resistance and effectively empty the bladder, very important finding is a lack of statistically significant difference of postvoid residual urine (PVR) between groups distributed relating to muscle contractility in obstructive and non obstructive groups. Generally, if the bladder outlet is obstructed, detrusor contractions can be normal at the beginning of voiding, but can also be aborted prematurely leaving larger part of total bladder capacity as residual urine. In patients with symptoms, but without obstruction, a complete emptying of bladder can be achieved, even when maximal detrusor contractility is decreased, but with appropriate contraction duration\(^10\).

DISCUSSION

Recently, Kaplan has introduced a novel urodynamic parameter, called detrusor contraction duration, what presents the time elapsed since beginning of detrusor pressure increase during voluntarily voiding, until decrease of detrusor pressure to the value of premicturition pressure, independently on duration of the observed urinary flow (Figure 1.). The same author has found statistically significant correlation with I-PS Score considering 63 patients in obstruction\(^1\). However, by now, only few reports dealing with DCD have been published. Ameda\(^2\) has not obtained a correlation between DCD and I-PS Score analyzing 58 patients. However, the same investigator has found statistically significant correlations of DCD with some urodynamic parameters (DCD with PdetQmax, $r=0.2$, $p<0.05$). A multiple regression was used to estimate extension of DCD with PdetQmax, cystometric capacity and contractility strength ($R=0.58$). The same author has found difference of DCD between patients out of obstruction with preserved contractility and patients of the same group, but with impaired detrusor contractility; as well as with patients in obstruction with normal contractility. Turner\(^8\) has published a good correlation between DCD and I-PSS analyzing patients, not medically treated yet, what shows that this parameter is important value for basic urodynamic examination of patients with BPE.

This study has not shown statistically significant correlation between DCD and I-PSS, although a correlation with its obstructive subgroup has been found. There is also a good correlation, in a practical sense, between DCD and nomograms, which determine urodynamic obstruction.
sor in patients with LUTS does not seem to be a predictive condition for urinary retention (11), what is exactly resulted by this study. These data bring us to a conclusion that patients from the selected sample extend detrusor contraction proportionally with obstruction increase and that the contraction is partially dependent on detrusor strength. The factor, which determines extension of detrusor contraction duration, is the urethral resistance. As voiding is a mechanical balance between detrusor strength and realized flow (urethral resistance is prevailed), one function determines the other (12), so decrease of flow causes extension of time needed for complete bladder voiding (Tvoid), while the time, before the stream is initiated (time for opening of urethra- Pdetop), is a part of DCD, which will be extended depending on level and type of obstruction (mechanical or dynamical). Although good correlation of DCD with I-PSS has not been shown in this paper, further investigation of doxazosin effect to decreasing urethral resistance and symptoms reduction (data not published yet), has shown a great value of post treatment success in patients with BPE who had DCD>90s before the treatment. Drastic symptoms reduction and increase of quality of life have resulted by strong decrease of DCD with a moderate reduction of obstruction levels.

CONCLUSION

This study has not shown a correlation between DCD and I-PSS, although there is a correlation of this parameter with its obstructive subgroup. DCD is increasing during obstruction, although underactive detrusor can achieve the same extension, making possible an efficient emptying of the bladder. Individual correlations of DCD with factors, which characterize obstruction, have a modest level, in an absolute sense.

REFERENCES

(7) A ganovic D: Matching degree of urodynamic nomograms in defining of the different levels of obstruction in patients with benign prostate enlargement (Part II-defining clear obstruction), Med Arch 2003; 57(2):81-86
(9) van M astriq R: Estimation of maximum contraction velocity of the urinary bladder from the pressure and flow throughout micturition, Urol Res, 1990, 18:149-154
LEFT VENTRICULAR HYPERTROPHY AND RISK FACTORS FOR ITS DEVELOPMENT IN URAEMIC PATIENTS

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4 Institute for Hygiene and Centre for Human Genetic - Medical school University of Sarajevo

ABSTRACT

Cardiovascular diseases are the major cause of mortality in uraemic patients treated by hemodialysis. Left ventricular hypertrophy (LVH) is considered to be a major cardiac risk factor.

Aim: To investigate the presence of some potential adverse risk factors in hemodialysis patients with developed LVH echocardiography verified and determine their relative contribution to the LVH in comparison with patients with normal LV.

Method: The study included 50 patients with end-stage renal disease in the first 2 years of hemodialysis treatment, who were followed up during one year. All participants have the echocardiography performed as well as serial measurements of potential modifiable cardiovascular risk factors.

Results: This investigation showed that LVH is present in high percentage (72%) in uraemic patients, even at the beginning of hemodialysis treatment. This LV morphological abnormality is statistically significantly related to anaemia (p<0.001), systolic (p<0.001) and diastolic hypertension (p<0.001), elevated mean arterial pressure (p<0.001) and hyperparathyroidism (p=0.002). A relative contribution of all of this factors in development of LVH in chronic uraemia has not yet been determined. Risk factors should be clearly identified and assessed, as their reduction might diminish overall cardiovascular morbidity and mortality.

Conclusion: Modification of existing risk factors in uraemic patients could contribute to prevention and treatment of LV hypertrophy and thus reduce cardiovascular morbidity and mortality.

Key words: left ventricular hypertrophy, uraemia, cardiovascular factors

INTRODUCTION

Left ventricular hypertrophy (LVH) is commonly present in uraemic patients and it has been recognized as adaptive mechanism of LV on volume and pressure overload (1). It is especially associated with high mortality of uraemic patients (2,3) and presents an independent risk factor of adversely impact on outcome in prae dialysis, as an in haemodialysis patients.

Left ventricular hypertrophy is resulting from hypertrophy of myocytes and hyperplasia of non-myocytes elements, especially cardiac fibroblasts (4). Myocytes are unable to replicate and consequently enlarge in hypertrophic condition, which can be a basis for distinguishing the type of LVH. In concentric hypertrophy of LV myocytes increase in thickness. The increased LV mass is associated with increased thickness of both the interventricular septum and left ventricular posterior wall, with preserved normal ventricular volume. In concentric LV hypertrophy, the relative wall thickness is higher than 45%. In eccentric LV hypertrophy myocytes grow longitudinally, and the increased LV mass is associated with increased LV volume.

End-stage renal disease includes many factors which have possible role in development of LVH. Among them, the most important are considered to be anaemia, hypertension, hypervolemia and arterio-vein fistula, hyperparathyroidism, hyperlipoproteinemia and malnutrition. A relative contribution of all of this factors in development of LVH in chronic uraemia has not yet been determined. Risk factors should be clearly identified and assessed, as their reduction might diminish overall cardiovascular morbidity and mortality.

Aim

To assess the presence of some predisponing risk factors in haemodialysis patients with echocardiographically verified LVH and in haemodialysis patients without LVH.

To compare echocardiografic features of haemodialysis patients after a 12 months period with special relations to presence of observed risk factors and their individual contribution in LVH development.

SUBJECTS AND METHODS

The study included 50 patients with end-stage renal disease, who had started haemodialysis treatment within the period of 2 years before the beginning of the investigation. All patients were dialysed under the same conditions, which consisted of three sessions of 4 hours haemodialysis treatment per week, using machines with controlled ultrafiltration, bicarbonate puffer for dialysis, as well as biocompatible polysulphone membranes, with
the same vascular access (A-V fistula) and with adequate
doses of delivered dialysis (Kt/V 1,31±0,13, urea reduc-
tion rate 63,4±2,54%). This prospective and compara-
tive study was carried out at the Institute of Nephrology
of Clinical Centre University of Sarajevo.
The study did not include patients with diabetes mellitus
and those with organic heart valves defects.
All participants had the echocardiography performed at
the beginning of the study (baseline) and after the period
of 12 months. It was done when so called «dry body
weight» of a patient was achieved, always in a period
within the 24 hours after the last haemodialysis. In that
way the interdialysis increment of internal LV diameter,
caused by increased blood volume due to fluid ingestion
and loss of excretory renal function, was avoided.
Namely, calculated LV mass index before dialysis is usu-
ally higher up to 25 g/m² as compared to postdialysis val-
ues, although the actual LV mass remains unchanged (5).
Echocardiography was performed by a cardiologist,
using ATL Ultrasound-9 echocardiograph equiped with
2.5 MHz transducer, enabled for M-mod, two-dimen-
sional and pulse Doppler measurements.
The following parameters were followed up in a month-
ly intervals: blood pressure and body weight, as well as
the laboratory tests including: haemoglobin, BUN, crea-
tinine, albumin, total cholesterol, triglycerides, calcium,
phosphorus, parathormone (PTH). For each participant
clinical and laboratory values at the beginning were con-
sidered as basal, while the average mean monthly levels
in a period of 12 months, until the second echocardiogra-
phy, were considered as comparative ones.
LV mass was calculated according the modified
Devereux’s cubic formula accepted by American
Association of Cardiologists. LV mass index was calcu-
lated as LV mass divided by body surface area:

\[ \text{LV mass index} = \frac{0.00083 \times (LVEDD+IVS+PW)^3- (LVEDD)^3 + 0.6}{\text{BSA}} (g/m^2) \]

LV volume was calculated according to Pombo’s et als.
formula:

\[ \text{LVV} = \frac{(LVEDD)^3 \times 0.001047}{\text{BSA}} \text{ (ml/m}^2) \]

The following criteria were used for distinguishing the
comparative patients groups:
LV hypertrophy: LV mass index in males >131 g/m², in
females >100 g/m² (6)
LV dilatation: LV volume >90 ml/m² (7)
concentric LVH: LV hypertrophy with normal LV vol-
ume eccentric LVH: LV hypertrophy with LV volume
>90 ml/m²

**STATISTICAL ANALYSIS**

Data were expressed as mean values with standard devi-
ation. The significance of the differences between the
mean values of the comparable groups were tested by
Student’s t test with accepted statistical significance at
the level of p<0.05. Logistic regression (Odds ratio) was
used to identify independent association of various risk
factors related to LV hypertrophy. Multilinear regression
(beta coefficient) was used to identify LV mass index
predictors.
Statistical analysis was facilitated using the statistical
programme SigmaStat, version 2.

**RESULTS**

**Echocardiography at the beginning of the study**

There were 14 (28%) patients with normal finding of the
left ventricle (LV), 19 (38%) were found to have concent-
tric LVH, while 17 (34%) showed echocardiographic
signs of eccentric LVH (Figure 1).

**Figure 1. Prevalence of the LV morphologic changes on the first echocardiography**

Regarding functional status of LV, 9 (16.7%) patients
showed impaired systolic function, 21 (38.9%) had dia-
stolic dysfunction, while the remainder of 20 (44.4%)
showed normal function of LV (Figure 2).

**Figure 2. The prevalence of the LV function status on the first echocardiography**

The age structure of participants is shown at Table 1.
They included 26 males and 24 females. It is shown that the average age was over 40 years in all groups, with lowest values in the group with normal LV function, and without statistically significant difference regarding age between the groups with concentric and eccentric LVH.

**Biochemical indicators**

**Haemoglobin**
As a main indicator of anaemia, haemoglobin (Hb) showed highly significant difference between the group with normal LV and those with concentric and eccentric LVH (Table 2). Patients with normal LV echocardiographic mass, were found to have significantly higher mean Hb values as compared to a group with systolic impairment of LV (p<0,001) and diastolic disfunction of LV (p<0,034). The average Hb values of the total sample were below the lower cut-off point of normal range for the general population (normal range Hg 120-175 g/L).

**Parathormon**
The average mean values of serum parathormone (PTH) in the group with normal LV function was significantly lower than in patients with concentric LVH (p=0,021), as well as in the group with diastolic disfunction (p=0,047), as is shown in Table 3. It is also obvious that all the groups have higher PTH values than the referent values for the general population (range 10-65 pg/ml).

In regard to other laboratory serum parameters (lipids, albumin, BUN, creatinine, calcium, phosphorus) we did not show significant differences between the groups.

**Clinical indicators**

**Systolic blood pressure**
Patients with normal LV function showed normal average systolic blood pressure (SBP) values (131 mmHg), while the patients with concentric and eccentric LVH had significantly elevated SBP (p<0,001), with the highest average values in the group with systolic impairment (170 mmHg). The results are presented in Table 4.

**Diastolic blood pressure**
Patients with normal LV function and structure, and patients with concentric LVH and diastolic disfunction had normal values of diastolic blood pressure (DBP), while the others had hypertensive diastolic values as shown in Table 5.

### Table 1. Age structure by LV functional status

<table>
<thead>
<tr>
<th>AGE (YEARS)</th>
<th>Normal LV</th>
<th>Concentric LVH</th>
<th>Eccentric LVH</th>
</tr>
</thead>
<tbody>
<tr>
<td>X ± δ</td>
<td>41,71±11,17</td>
<td>49,73 ± 11,32</td>
<td>43,45±13,72</td>
</tr>
<tr>
<td>p value</td>
<td>0,052</td>
<td>0,703</td>
<td></td>
</tr>
</tbody>
</table>

### Table 2. The mean values of haemoglobin (g/L) by groups

<table>
<thead>
<tr>
<th></th>
<th>Normal LV</th>
<th>Concentric LVH</th>
<th>Eccentric LVH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin</td>
<td>100,57±13,59</td>
<td>79,10±12,44</td>
<td>76,00 ±14,29</td>
</tr>
<tr>
<td>p-value</td>
<td>&lt; 0,001</td>
<td>&lt; 0,001</td>
<td>&lt;0,001</td>
</tr>
</tbody>
</table>

### Table 3. The mean PTH levels (pg/ml) by groups

<table>
<thead>
<tr>
<th></th>
<th>Normal LV</th>
<th>Concentric LVH</th>
<th>Eccentric LVH</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTH</td>
<td>172,53±58,19</td>
<td>293,84±179,6</td>
<td>257,58±243,6</td>
</tr>
<tr>
<td>p-value</td>
<td>0,021</td>
<td>0,212</td>
<td>0,219</td>
</tr>
</tbody>
</table>

### Table 4. The mean systolic blood pressure values (mmHg) by groups

<table>
<thead>
<tr>
<th></th>
<th>Normal LV</th>
<th>Concentric LVH</th>
<th>Eccentric LVH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sistolic BP</td>
<td>131,42±9,49</td>
<td>153,88±12,89</td>
<td>165,33±11,25</td>
</tr>
<tr>
<td>p-value</td>
<td>&lt;0,001</td>
<td>&lt;0,001</td>
<td>&lt;0,001</td>
</tr>
</tbody>
</table>
The mean arterial pressure
The lowest average values of mean arterial pressure were shown in patients with normal LV. As presented in Table 6, these are significantly different in comparison with all the other groups.

Echocardiography outcome
The changes in echocardiography findings in 12 months period by groups are presented in Table 7. We found that after 12 months period, in regard to echocardiographic morphological changes, overall 21% of patients showed normal LV, 45% had signs of concentric LVH, and 34% of eccentric LVH. Also, we noted that, after 12 months period, almost 58% of participants showed the signs of LV diastolic dysfunction, 17% of LV systolic impairment, while only 25% patients were found with normal LV function. Significant predictors of concentric LVH appearance are presented in Table 8.

### Table 5. The mean diastolic blood pressure values (mmHg) by groups

<table>
<thead>
<tr>
<th></th>
<th>Normal LV</th>
<th>Concentric HLV</th>
<th>Eccentric HLV</th>
<th>LV systolic failure</th>
<th>LV diastolic failure</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Diastolic BP</strong></td>
<td>80,00±5,54</td>
<td>83,88±7,75</td>
<td>88,75±7,18</td>
<td>91,11±7,81</td>
<td>84,73±7,72</td>
</tr>
<tr>
<td><strong>p-value</strong></td>
<td>0,229</td>
<td>0,004</td>
<td>0,005</td>
<td>0,112</td>
<td></td>
</tr>
</tbody>
</table>

### Table 6. The mean arterial pressure (MAP) values (mmHg) by groups

<table>
<thead>
<tr>
<th></th>
<th>Normal LV</th>
<th>Concentric HLV</th>
<th>Eccentric HLV</th>
<th>LV systolic failure</th>
<th>LV diastolic failure</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean AP</strong></td>
<td>95,78±6,93</td>
<td>106,90±8,61</td>
<td>112,08±10,30</td>
<td>117,32±9,11</td>
<td>108,08±10,63</td>
</tr>
<tr>
<td><strong>p-value</strong></td>
<td>&lt;0,001</td>
<td>&lt;0,001</td>
<td>&lt;0,001</td>
<td>&lt;0,001</td>
<td></td>
</tr>
</tbody>
</table>

### Table 7. Echocardiography outcome after one year follow-up by groups

<table>
<thead>
<tr>
<th>Second echography finding</th>
<th>Normal LV</th>
<th>Concentric LVH</th>
<th>Eccentric LVH</th>
<th>LV systolic failure</th>
<th>LV diastolic failure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
<td>n</td>
</tr>
<tr>
<td>First echo Finding</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal LV n=14</td>
<td>7</td>
<td>50,0</td>
<td>5</td>
<td>37,5</td>
<td>2</td>
</tr>
<tr>
<td>Concentric LVH=19</td>
<td>1</td>
<td>5,3</td>
<td>11</td>
<td>57,9</td>
<td>6</td>
</tr>
<tr>
<td>Eccentric LVH=17</td>
<td>2</td>
<td>11,8</td>
<td>5</td>
<td>29,4</td>
<td>7</td>
</tr>
<tr>
<td>LV systolic failure=8</td>
<td>2</td>
<td>22,2</td>
<td>3</td>
<td>33,3</td>
<td>3</td>
</tr>
<tr>
<td>LV diastolic failure=21</td>
<td>3</td>
<td>14,3</td>
<td>18</td>
<td>85,7</td>
<td>10</td>
</tr>
<tr>
<td>n= 50</td>
<td>11</td>
<td>20,7</td>
<td>22</td>
<td>41,5</td>
<td>17</td>
</tr>
</tbody>
</table>

**DISCUSSION**
Cardiovascular diseases are the leading cause of mortality and accounts for about 50% of overall mortality in haemodialysis patients (8,9). Pathogenesis of cardiovascular disease in these patients includes risk factors already known for general population, but as well factors related to renal failure. Relative contribution of these fac-
tors in various disorders of left cardiac ventricle is possible to determine by serial measurements of risk factors. Echocardiography presents one of the most important means among non-invasive methods for evaluation of morphological and functional heart characteristics in uraemic patients. In our study, assessment by echocardiography in haemodialysis patients showed that 72% of patients, without clinical signs of heart failure, had abnormal LV echocardiographical finding. Among them, we found concentric LVH in 38% patients, and eccentric one in 34% patients. Systolic impairment was found in 16,7%, and diastolic dysfunction in 38,9% patients. Only 28% participants did not show any echocardiographic abnormality. LV mass index was especially high in the group with diastolic LV disfunction and in patients with systolic LV impairment (higher for about 50% as compared to those with normal LV finding).

Some other studies, using echocardiography as an evaluation method, showed prevalence of LV hypertrophy of 57% to 93% in the population of patients with end stage renal failure (10,11). Similar finding was observed in uraemic patients at the beginning of haemodialysis treatment in prospective Canadian multicentric study, which included 433 patients followed in a period of 41 months (12). Systolic dysfunction was observed in 16%, LV dilatation in 28%, LVH in 40,7% patients, while only 16% patients had normal echocardiographic finding. Parfrey et al. in 1996 confirmed that average survival of haemodialysis patients with LV abnormalities at the beginning of dialysis, is significantly poorer in comparison to those with normal echocardiographical finding (13), especially to those with systolic impairment of LV. High proportion of patients with LVH at the beginning of haemodialysis treatment indicates that predisposing factors for development of LVH are existing even in preterminal phase of chronic renal failure. After a year of follow up, we found echocardiogram abnormalities in almost 79,3% of our patients, while only 20,7% in normal echocardiogram of LV. Proportion of patients with concentric LVH increased for 6,0% in comparison to baseline findings (38% to 44%), and propor-

### Table 8. The main predictors of concentric LVH appearance

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Association</th>
<th>Odds ratio</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentric LVH on second echocardiography</td>
<td>1. Each increment of mean SBP for 1 mm Hg</td>
<td>1,127</td>
<td>0,011</td>
</tr>
<tr>
<td></td>
<td>2. Each increment of mean DBP for 1 mm Hg</td>
<td>1,342</td>
<td>0,037</td>
</tr>
<tr>
<td></td>
<td>3. Each increment of mean MAP for 1 mm Hg</td>
<td>1,229</td>
<td>0,013</td>
</tr>
<tr>
<td></td>
<td>4. Each decrement of mean Hb level for 1 g/L</td>
<td>0,695</td>
<td>0,031</td>
</tr>
</tbody>
</table>

### Table 9. The main predictors of LV mass index changes

<table>
<thead>
<tr>
<th>LV mass index (g/m²)</th>
<th>Association</th>
<th>β coefficient</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increment by 2,2 g/m²</td>
<td>1. Each increment of mean SBP for 1 mm Hg</td>
<td>0,753</td>
<td>&lt;0,001</td>
</tr>
<tr>
<td>Increment by 5,3 g/m²</td>
<td>2. Each increment of mean DBP for 1 mm Hg</td>
<td>0,675</td>
<td>&lt;0,001</td>
</tr>
<tr>
<td>Increment by 3,6 g/m²</td>
<td>3. Each increment of mean MAP for 1 mm Hg</td>
<td>0,726</td>
<td>&lt;0,001</td>
</tr>
<tr>
<td>Increment by 2,6 g/m²</td>
<td>4. Each decrement of mean Hg for 1 g/L</td>
<td>-0,614</td>
<td>&lt;0,001</td>
</tr>
<tr>
<td>Increased for 0,4 g/m²</td>
<td>5. Each increment of mean PTH for 1 pg/ml</td>
<td>0,537</td>
<td>0,002</td>
</tr>
</tbody>
</table>
tion on patients with normal finding decreased for 6% (28% to 22%). The number of patients with systolic failure is the same as at the beginning of the study, while the proportion of patients with diastolic LV disfunction increased for 47.6%. Diastolic disfunction mostly accompanies concentric LVH (in 58% of cases).

Evolution of normal to hyperthrophic LV may be under relative influence of various risk factors present at the time of echocardiography performance.

In our study we found significant differences in mean systolic, diastolic and mean arterial pressure in the group with normal LV finding as compared with the group with LV hypertrophy, both of concentric and eccentric type. We found significant independent association of systolic, diastolic and mean arterial pressure with concentric LVH. This findings clearly demonstrate the impact of hypertension on LV hypertrophy progression and the need for maintaining the target blood pressure values up to 130/80 mmHg in this population of patients. Moreover, some studies described LV hypertrophy regression by tight control of blood pressure values with combination of beta blockers, calcium antagonists and ACE inhibitors (14), with ACE inhibitor monotherapy (15) or angiotensin receptor blockers, losartan (16).

Anaemia appears to have an independent impact to echocardiographycal outcome in haemodialysis patients. We noted significant independent association between the reduction of average haemoglobin by 1 g/L with appearance of concentric LVH after a period of 1 year, as well as with the increment of LV mass index for 2.6 g/m² (p<0.001). This finding strongly indicates significance of connection between anaemia and LV hypertrophy, which was also shown in the study carried out by Foley et al. (17). Correction of anaemia by erythropoietin raises possibility for LV hypertrophy regression in this population (18, 19).

We noted significantly higher PTH levels in the group with LVH (p=0.043) as compared with the group with normal LV mass in haemodialysis patients. Pathways which can explain the role of hyperparathyreoidism in development of LV hypertrophy may include direct or indirect effects. Direct trophic effects can be mediated by increment of protein synthesis in myocardial myocytes and induction of creatin kinase BB through protein kinase C activation, via functional domain of 28-34 aminoacids (20) and by direct trophic effect to interstitial fibroblasts (21). Indirect trophic effects include increment of blood pressure values via hypercalcaemia, as well as anaemia and changes in small and intermediate blood vessels.

As LVH presents major cardiovascular risk factor in these patients, prevention of hypertrophy, its early detection and LV mass index reduction by modifying risk factors is promising process by which we may expect reduction of overall cardiovascular morbidity and mortality in patients on chronic haemodialysis treatment.

CONCLUSIONS

Echocardiographic abnormalities are commonly found in patients with end-stage renal disease at the beginning of haemodialysis treatment. Even higher proportion of the changes is found during the follow up of the treatment. Anaemia is an independent risk factor for development of concentric LV hypertrophy. Increased blood pressure values is significantly associated with LV hypertrophy. Parathormone appears to be significant LV mass index changes predictor, which gives this “uraemic toxine” the character of cardiovascular risk factor. Pharmacological impact to potentially reversible risk factors in uraemic patients, gives possibility for LV hypertrophy regression. Presence of cardiac abnormalities and its risk factors at early stage of haemodialysis treatment suggests the importance of their earlier detection and correction, even in the predialysis period of chronic renal failure.
REFERENCES

(3) Cannella G. Left ventricular hypertrophy in dialysed patients. What can be done about it? Nephrol Dial Transplant 1996; 11:418-420
(5) Harnett J.D., Murphy B., Collingwood P., Purchase L., Kent G., Parfrey P.S. The reliability and validity of echocardiographic measurement of left ventricular mass index in hemodialysis patients. Nephron 1993; 65:212
DEMONSTRATION OF DIFFERENT ENDOCERVICAL STAINING METHODS AND THEIR USEFULNESS IN THE DIAGNOSIS OF THE CHLAMYDIAL INFECTION IN EXFOLIATED CELLS

Advantages and Disadvantages

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Department of Microbiology, Faculty of Medicine, University of Sarajevo

ABSTRACT

Microscopic demonstration of chlamydial inclusions within cells offered the first laboratory procedure supporting the clinical diagnosis of chlamydial infection. Our aim is to evaluate the usefulness of different endocervical staining methods in diagnosis of Chlamydia trachomatis (CT) infection within exfoliated cells of the endocervix. The cytological test for the detection of chlamydial inclusions in genital tract infection, though not as sensitive and specific as isolation in the cell culture monolayers, is still of the diagnostic value. The present study discusses the collection of clinical smears for microscopic examination, their preparation; fixation and staining of slides by a variety of staining methods that have been used to detect Chlamydia in clinical smears and biopsies. Most of these methods such as Giemsa stain, Papanicolaou, iodine, and immunofluorescence (IF) using monoclonal antibodies, are based on the combination of dyes designed to obtain optimum differentiation of the various structures. The utilization of different endocervical smear stains together with the clinical information can be used to identify women at high risk for CT infection.

Key words: Endocervical Stain; Giemsa, Papanicolaou, Iodine, Immunofluorescence, Chlamydia Trachomatis.

INTRODUCTION

Bacterial-chlamydial genital infections are commonly included in the group of Sexually Transmitted Diseases—the second generation (STDs). Chlamydia trachomatis (CT) as bacterial agent is a cause of the huge number of genital infections spreading particularly among younger people. Almost 4 (four) million new cases of the chlamydial infections /genital tract/ are yearly registered in the USA, and 3 (three) million in the Europe [1]. Reports on the incidence of these infections demonstrate an increased number of the registered cases, which might be explain as a result of the increased number of performed tests and registrations, as well [2]. Epidemiological investigations of the large number of women in the USA and Scandinavia confirm Chlamydia to be the most prevalent STD in developed countries. In view of the prevalence of chlamydial infections, their serious consequences and huge treatment costs, screening methods that include high-risk persons have been implemented in the USA:

- Persons with anamnestic history of STDs;
- Young persons, sexually active;
- Promiscuous persons;
- Males with lymphogranuloma infection;
- Newborns;
- Reiter’s syndrome diagnosed in younger males.

Throughout years 2001 and 2002, one case of chlamydial infection was reported per a year in Bosnia and Herzegovina, while in 2003 one case was reported in July 2003 [3, 4, 5]. These data are not valid because they do not illustrate the real situation in the region, although chlamydial infections are at the list of notifiable diseases. CT is the etiological agent of cervicitis/urethritis, which are oligosymptomatic, asymptomatic, chronic and persistent infections with different complications [6]. About 70% percent of all chlamydial cervicitis are without symptoms, and 20-30% women with diagnosed chlamydial cervicitis have no clinical signs, which is important fact for the medical practitioners. Morphologically, chlamydiaceae are coccoid, small Gram-negative bacteria, non-motile, and there are obligate intracellular parasites, energy defect.

All chlamydiyas are placed into their order-chlamydiales, family-chlamydiaceae, genus: Chlamydia consisted of 4 recognized species:

C. trachomatis,
C. psittaci,
C. pneumoniae,
C. pecorum.

They possess a unique developmental cycle consisting of metabolically inactive infectious elementary bodies
(Ebs), sized about 300 nanometres, and metabolically active but non-infectious reticulate bodies (Rbs), sized about 1 micrometer.

There are different ways of chlamydial infection spreading: sexual, perinatal; although not exclusive the other ways of infection transmission include chloride water in the swimming pools, wet towels, intrahospital infections and gynaecological exams when the necessary protection measures are not applied. It is supposed according to statistics, that about 10% of women in reproductive age are infected with CT. Similarly to infection with the human papilloma virus, chlamydial infection is a very essential provoking agent of the cervical intraepithelial neoplasm with consequent bad Papanicolaou test result, salpingitis with incomplete or complete obstruction, and finally possible extrauterine pregnancy and sterility. During pregnancy, CT causes disorders and ruptures of the fertile membranes with consequent delayed spontaneous abortion or the earlier delivery.

Target population is women in reproductive age, men and adolescents. Because of that, early diagnosis and therapeutic treatment, medical education of population, especially younger, present the most important way in prevention chlamydial infection.

The aim of this study was to find a modus, one completely operative diagnostic procedure, with possibility to achieve a more applicable screening method in detection of CT in reproductively active population.

**PATIENTS AND METHODS**

There are four different laboratory procedure types for CT detection:

- **Direct microscopic slide of exfoliated cells** according to typical intracytoplasmatic inclusions;
- **Microorganism isolation in tissue culture**, method of choice, “gold standard”;
- **Detection of chlamydial antigens or nucleic acids** with immunological or hybridisation methods;
- **Detection of immune response** in sera or in secrets.

Our patients were women in reproductive age divided into two groups: moderate risk - experimental group, and low risk - control group. Moderate risk group consisted of women having some symptoms of genitourinary infections, while low risk group consisted of women without any infection symptoms. The study included 120 patients. Three endocervical swab specimens for three different screening laboratory methods (based on antigen detection using monoclonal and polyclonal antibodies) were collected per each patient. Each specimen was analysed according to following methods:

- **RIA - Rapid Immunoassay**;
- **DFA - Direct Immunofluorescence Assay**;
- **EIA - Enzyme Immunoassay**.

**RIA - use and principle**

- Rapid quantitative immunoassay based on immunocromatography;
- Intended for “in vitro” diagnosis Chlamydia antigen from endocervical swab specimen;
- If the swab specimen is positive (consist Chlamydia antigen), reaction is perceptible (a visible complex antigen - specific antibody- antibody);
- Interpretation of the results: if the test is positive, we have two same pinkie lines in the test and control regions;
- Interpretation of the results: if the test is negative, there is no visible line in the test region, but it is present in the control region;
- Interpretation of the results: if the test is invalid, there are no visible lines both in the test and control regions, the test is to be repeated;
- Do not interpret results after 15 minutes;
- The test does not make any difference among C. trachomatis, C. psittaci, and C. pneumoniae.

**DFA - use and principle**

- Intended for direct detection antigen Chlamydia cell from swab specimens in the period of acute genital chlamydial infection;
- It is based on the use fluorescent, labelled monoclonal antibodies directed to the Major Outer Membrane Protein (MOMP) that reacts to all CT serotypes;
- Chlamydial cells appear like an intensive green spots called “green apple” on dark background illustrated by fluorescence microscope (“phenomena of starry sky”);
- Test is positive if there are ten or more intensive green coloured elementary bodies on/in the red coloured epithelial cells on background;
- Every other fluorescent material or particles of irregular shapes or sizes that emitted yellow or red colour are considered as artefact and need to be thrown away.

**EIA - use and principle**

- Enzyme immunoassay is used for qualitative detection of chlamydial antigens from the endocervical swab specimens;
- It uses polyclonal antibodies directed on lipopolysaccharide antigen (LPS) that are not strictly specific for the CT species;
- A counting value of negative control, which is
divided to the whole value for three negative controls to number 3 (three);
- Presence or absences of Chlamydia antigens are established in the comparison of absorption values swab specimens with value of cut off;
- Swab specimens with absorption values same or higher than cut off is considered as positive.

STATISTICS

True positive results were those when two of three test methods were coincided.
We used descriptive statistics to determine validity of screening tests as specificity, sensitivity, and predictive values [8].

RESULTS

Table 1. Detection of Chlamydia trachomatis using Rapid Immunoassay (RIA) in experimental and control groups of the examined female population

<table>
<thead>
<tr>
<th>Examined groups</th>
<th>Total number</th>
<th>Percent (%)</th>
<th>Rapid test (+)</th>
<th>Rapid test (-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental group</td>
<td>60</td>
<td>50.00</td>
<td>4</td>
<td>56</td>
</tr>
<tr>
<td>Control group</td>
<td>60</td>
<td>50.00</td>
<td>3</td>
<td>57</td>
</tr>
<tr>
<td>TOTAL</td>
<td>120</td>
<td>100.00</td>
<td>7</td>
<td>113</td>
</tr>
</tbody>
</table>

Table 2. Detection of Chlamydia trachomatis using Direct Fluorescence Assay (DFA) in experimental and control groups of the examined female population

<table>
<thead>
<tr>
<th>Examined groups</th>
<th>Total number</th>
<th>Percent (%)</th>
<th>DFA (+)</th>
<th>DFA (-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental group</td>
<td>60</td>
<td>50.00</td>
<td>5</td>
<td>55</td>
</tr>
<tr>
<td>Control group</td>
<td>60</td>
<td>50.00</td>
<td>3</td>
<td>57</td>
</tr>
<tr>
<td>TOTAL</td>
<td>120</td>
<td>100.00</td>
<td>8</td>
<td>112</td>
</tr>
</tbody>
</table>

Table 3. Detection of Chlamydia trachomatis using Enzyme Immunoassay (EIA) in experimental and control groups of the examined female population

<table>
<thead>
<tr>
<th>Examined groups</th>
<th>Total number</th>
<th>Percent (%)</th>
<th>EIA (+)</th>
<th>EIA (-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental group</td>
<td>60</td>
<td>50.00</td>
<td>5</td>
<td>55</td>
</tr>
<tr>
<td>Control group</td>
<td>60</td>
<td>50.00</td>
<td>3</td>
<td>57</td>
</tr>
<tr>
<td>TOTAL</td>
<td>120</td>
<td>100.00</td>
<td>8</td>
<td>112</td>
</tr>
</tbody>
</table>

Table 4. Prevalence and predictive values (PPV, NPV) for Rapid Immunoassay (RIA), Direct Fluorescence Assay (DFA), and Enzyme Immunoassay (EIA) in both groups of patients

<table>
<thead>
<tr>
<th>Assay test</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
<th>Prevalence (%) (experimental and control groups)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RIA</td>
<td>100.00</td>
<td>99.10</td>
<td>5.83</td>
</tr>
<tr>
<td>DFA</td>
<td>100.00</td>
<td>100.00</td>
<td>6.66</td>
</tr>
<tr>
<td>EIA</td>
<td>100.00</td>
<td>100.00</td>
<td>6.66</td>
</tr>
</tbody>
</table>
DISCUSSION AND CONCLUSION

In our study, we tried to examine which of applicable test methods allow more rapid and supreme CT diagnosis in endocervical swab specimens collected from examined female population divided into two groups: experimental and control. The choice of the most appropriate diagnostics of chlamydial test depends on the local possibilities, proficiency, and clinical population, as well. Slides dyed by different staining method such as Gram, Giemsa, Lugol, and Papanicolaou showed a low specificity in diagnosis of chronic chlamydial infections (15-41%), so monoclonal antibodies are more frequently used.

Despite the fact that chlamydial infections are asymptomatic in 70% women (without specific symptoms of disease), gynaecological examination plus adequate laboratory treatment are the only possible ways to recognize, treat and stop chlamydial infections from spreading [6].

It is known that chlamydial infections are “enemy of the fertility” and bio-contaminant level 2 (BCL2). People infected with CT represent target population for HIV infections.

Results obtained from experimental group of women are in percentile lower than results reported by other investigators. Reasons are probably in the anamnestic data of examined patients (absence of STDs, the choice and sample size and timely period of the investigations) [9, 10]. Achieved data of the prevalence value of examined control group coincide in percentile with the results stated by other investigators. Analytical values of the applicable test methods reported by mentioned authors were between 82% (EIA) - 100% (isolation) versus 91.3% (EIA) - 95% (isolation).

According to our results we conclude that in laboratory diagnostics of CT, in our conditions, the best choice is to use DFA, having in mind its high sensitivity and specificity [11]. It is simple in valuation of the sample quality using the specific monoclonal antibodies directed at the MOMP. This test is also a confirmed and approved by World Health Organization (WHO).

DFA is non-invasive test method in the diagnostics of chlamydial infection, which enables the unique possibility of identification both asymptomatic and symptomatic patients.

We put the accent on DFA because of its already stated advantages (use of the monoclonal antibodies, the most acceptable screening test, reference for the practical work, 100% sensitivity, 100% specificity, species specificity), having in mind the high sensitivity of EIA, and specificity of two other test methods (RIA, EIA).
REFERENCES

(2) WHO Scientific working group. Laboratory tests for the detection of reproductive Tract Infections. 1999, WHO.
(3) SFOR Communicable Diseases Annual Report for 2001
(4) SFOR Communicable Diseases Annual Report for 2002
(5) SFOR Communicable Diseases Bulletins Number 01-07/2003
Neonatal BCG vaccination reduces the risk of tuberculosis and provides protection higher than 80% against the development of meningeal and miliary tuberculosis in newborns. Tuberculosis meningitis remains a major problem and also an important cause of death in some countries. In countries with high and moderate incidence of tuberculosis, prevention from the most severe complications of tuberculosis can be achieved only with a high coverage of the universal BCG neonatal immunization, being higher than 98% in the cohort of newborns. The decrease in BCG immunization coverage within immunization program during the year 2003 in Bosnia and Herzegovina influenced the increase in tuberculous meningitis. During 2002, when coverage with BCG vaccination in cohort of newborns was 90%, the incidence rate of tuberculous meningitis was 19.04%. With the 68% decrease in BCG immunization coverage in the cohort of newborns in Bosnia and Herzegovina during the year 2003, the incidence of tuberculous meningitis raised to 33.33%. It has been proven that the 22% decrease of the neonatal BCG immunization coverage in the cohort of newborns/vaccination program of children/ caused 175 times higher number of the tuberculous meningitis cases. Newborns affected by the tuberculous meningitis were not BCG vaccinated. BCG vaccine provided effective protection against tuberculous meningitis, as well against the death of newborns caused by tuberculosis.

Key words: BCG neonatal vaccine, protective efficacy, newborns.

PROTECTIVE EFFECT OF NEONATAL BCG VACCINATION AGAINST TUBERCULOUS MENINGITIS

BCG vaccination is the only possible method of the discontinuation, or at least, transmission slowing down, since BCG is expected to prevent multiplication of Bacilli in the body and development of the new cases of tuberculosis, even though it cannot prevent primary infection. The vaccine from the Bacillus Calmette-Guerin has been in use for over 5 decades in the prevention of tuberculosis and indeed has been routinely used for the control in several countries. It is not only an extensively used, but also the most extensively studied vaccine and has been the subject of prolonged and bitter converse over its efficacy. (4)

The strategic Advisory Group of Experts strongly endorsed the continuation BCG vaccination in national immunization programs in the aim to minimize the harmful effects of tuberculosis infection during the first year of life. (5)

Vaccination of uninfected (tuberculin-negative) person

...
induces tuberculin sensitivity in > 90% of cases. Unfortunately, post-vaccination tuberculin skin test conversions or reaction sizes do not correlate with the protection.

BCG tuberculin reactivity wanes with time and it is unlikely that BCG clinical and immune effects, not necessarily Mycobacterium tuberculosis specific, are unclear. (7)

BCG vaccine is more effective when administered in childhood, compared with the vaccination of adolescents and adult populations (8, 9).

BCG neonatal vaccine is moderately effective, recent meta-analysis proved its protective efficacy as 50% against any TB disease, 64% against tuberculosis meningitis and 71%, against death from tuberculosis. (10)

Occasional cases of TB meningitis occur in children in Australia and might be prevented by BCG vaccination. (8) Japan has had a policy of universal BCG vaccination of infants against tuberculosis since 1951. (11)

Children aged from 6 to 12 years also undergo BCG revaccination, although the effectiveness of this practice is not well-established.

The average incidence of TBC has decreased from 698 per 100 000 in 1951 to 33.7 per 100 000 in 1996 (12). The paediatric incidence in Japan is much lower, at 2.1 per 100 000 compared with 3.1 per 100 000 in the USA (13).

The evaluation of BCG vaccination of Japanese infants based on an assumption of flexible vaccine efficacy /40-80%/ provided an estimation that 111-542 TB, including 10-27 of the TB meningitis, would be prevented during the 10 years of BCG vaccination among infants born in 1996.

The efficacy of BCG vaccination in newborns is well recognised and that topic has been recently reviewed by Colditz at al. (14)

It is particularly useful in the protection against the TB disseminated such as TB meningitis and miliary TB (15).

In Hong Kong, BCG vaccination was introduced in 1952 as an organized campaign by the Government (16).

The coverage for the newborn babies has been persistently over 98% since the year 1980 significantly contributing to the low rate of TB among the young people. (UNICEF/WHO)

Tuberculosis remains one of the major health problems in developing countries.

WHO estimated that in the year 1990, 7.5 millions new cases of tuberculosis were registered, 1.3 million were children under 15 years of age, of whom 450 000 died.

**MATERIAL AND METHODS**

In Bosnia and Herzegovina, as a country with the high tuberculosis incidence, BCG vaccination is being performed immediately after the birth and has the most important role in the prevention of TB meningitis in newborns.

The prospective research performed during the period from 2002 to 2003 monitoring the motions of coverage of the BCG neonatal vaccination in the Federation Bosnia and Herzegovina and tuberculous meningitis in two groups of the newborns.

The aim of research was to determine, on the basis of epidemiological and clinical methods, relation between BCG neonatal vaccination coverage and the number of tuberculous meningitis cases, in two cohort groups of newborns with the different vaccination percentage.

**RESULTS**

During the year 2002, the neonatal immunization coverage in cohort of newborns was about 90%. Tuberculous meningitis was registered in four BCG unvaccinated newborns.

The incidence rate of tuberculosis meningitis in the year of research was 19.04‰.

In the year 2003, there was a decrease in the BCG neonatal immunization coverage influencing an increase in the TB meningitis cases in newborns. (Graph 1)

During the year 2003, BCG neonatal vaccination was reported to be about 68%-decreased on the territory Bosnia and Herzegovina. The number of newborns with tuberculous meningitis increased for 7 cases in 2003.

The incidence rate of TB meningitis during the 2003 reached 33.33‰ in Bosnia and Herzegovina. This incidence rate is very high and among the highest in Europe.

The results of these researches show the importance of the high coverage with neonatal BCG vaccination in Bosnia and Herzegovina - a country with the high tuberculosis incidence, with aim to achieve the protective efficacy against tuberculous meningitis.

In the year 2003, a decrease in neonatal BCG immunization coverage in newborns /22%/ caused the increase /175 times/ in number of tuberculous meningitis cases in the cohort of newborns in Bosnia and Herzegovina.
DISCUSSION

Among transmissible diseases, tuberculosis is the second leading death cause in the world. Most cases are registered in less-developed countries, due to socioeconomic changes and decline in the healthcare system. The World Health Organization (2002) has estimated that eight million people get TB every year, of which 95% live in developing countries. Approximately two to three million people die from TB every year (17). Almost 80% of children in developing countries receive BCG vaccination by the age of two years (18).

BCG vaccination reduces the risk of tuberculosis and the effectiveness of protection last about 9 to 10 years (19). Immunization with BCG vaccine lowers the risk of serious complications of primary TB in children. The practice of BCG vaccination varies widely in different parts of the world.

The efficacy of BCG vaccination in newborns is well recognized and that topic has been reviewed frequently. It is particularly useful in the protection against disseminated TB such as meningitis and miliary tuberculosis (20, 21, 22). BCG vaccine is more effective when administered during newborn period assuring >80% protection against the development of meningeal and miliary tuberculosis (23). Protective effects against meningeal and miliary TB were higher than against pulmonary disease. Summary of the BCG protective effect against miliary or meningeal TB in randomised, controlled trials was 86%. (24)

CONCLUSION

The protective effect of BCG vaccine against meningitis and miliary tuberculosis was higher than against pulmonary disease. BCG neonatal vaccination lowers the risk of serious TB complications in newborns. BCG vaccine showed its effectiveness in reducing both mortality and long-term sequelae from disseminated meningeal and miliary tuberculosis in newborns. Decrease in the BCG neonatal immunization coverage from 90% to 68% in cohort of newborns influenced the increase in tuberculosis meningitis incidence rate from 19.04‰ to 33.33‰ in Bosnia and Herzegovina. Universal neonatal BCG vaccination with immunization coverage of more than 90% induced the protective effect against meningeal and miliary tuberculosis in cohort groups of newborns in developing countries.
REFERENCES

POSTTRAUMATIC STRESS DISORDER AMONG WOMEN AFTER THE WAR IN SARAJEVO: A RATIONALE FOR GENETIC STUDY

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ABSTRACT

An exposure to extreme trauma events leads to posttraumatic stress disorder (PTSD) in up to 14-50% of war survivors. Recent findings suggest that genetic factors could play a certain role in PTSD development. In order to illustrate this possibility, we present results of a pilot study on gender specific sample of Sarajevo civilians immediately after the war cessation. During the period 1992-1995, Sarajevo civilians experienced continuous life threatening events with a great risk of developing PTSD in such conditions.

Our study included 100 women adjusted to same socio-demographic characteristics. All women were interviewed using Harvard Trauma Questionnaire (HTQ) and divided into two groups (domestic and returnees) according to exposure length to extreme war life events of six or forty-three months. Above 50% of total analysed sample fulfilled criteria for PTSD. Regarding duration in trauma exposure no significant difference between these two groups were found. The only significant predictor found was physical abuse (p>0.01) that still cannot explain why some women develop PTSD while others not.

Several years after the war, PTSD frequencies are decreased and disorder became chronic and more severe. However, the PTSD prevalence remains high when compared to general population rates. Therefore, Sarajevo population being exposed for almost four years to extreme war life events represents unique model for comparative research on PTSD etiology within the light of latest findings in molecular genetics of PTSD.

Keywords: PTSD, women, war trauma, risk factors, trauma exposure time, physical abuse, genetics;

INTRODUCTION

Posttraumatic stress disorder (PTSD) is an anxiety disorder characterized by intrusive, avoidance and hyper-arousal symptoms. PTSD seems to be exceptional compared to other psychiatric disorders, as by definition – there is no PTSD without an extreme environmental stress. Data suggest that there is a huge discrepancy between trauma exposure rate and PTSD prevalence rate (1). The risk for PTSD is two times higher among women compared to men (2). There is evidence that severity, type and duration of trauma are significant risk factors for PTSD (3). However, described risk factors could not explain why some trauma survivors develop disorder while others do not. Recent studies suggest that certain biological mechanisms supported by specific genetic predisposition contribute to the individual variability in PTSD susceptibility (4), (5). Epidemiological and formal genetic studies point out the role of genetic factors (6) in PTSD. This could help to elucidate certain individual vulnerability to posttraumatic stress disorder.

Under almost four years siege in Sarajevo (1992-1995), whole civilian population was overwhelmingly exposed to every-day life threat (shelling, sniper-fire, massacres, eye-witnessing of injuries and deaths, loss of close family members, captivity, etc.), making this population unique in that respect. The objective of our pilot study was to demonstrate that environmental stressors, although necessary are not exclusive in etiology of this complex multi-factorial disorder. This supports the idea for research based on polymorphisms within candidate genes encoding for neurotransmission components that could be involved in biology of PTSD (5).

SUBJECTS AND METHODS

A pilot study including one hundred women (age 20-49) survived war trauma in Sarajevo area, were chosen randomly and interviewed by Harvard Trauma Questionnaire (HTQ) for PTSD, Bosnian version (7). They were chosen among women from a municipality social service in Sarajevo-Center according to time related exposure. The instrument was translated and adjusted for B & H population, but not validated at the time of sampling.

None of the interviewed had any psychiatric disorder according to Schedule for Schizophrenia and Affective Disorders - Lifetime Version (SADS-L) (8) or Anxiety disorders prior to trauma experience as confirmed by

interviewing psychiatrist. Each woman experienced some of extreme trauma events listed in the Part I of HTQ. According to the time spent under the siege, subjects were divided into two groups (A and B) as follows: Group A - women, who were exposed to extreme war life events at beginning of the war in Sarajevo (around six months), temporarily resettled to EU countries and have returned to their homes immediately after the war (returnees).

Group B - women residing in Sarajevo during and after the war (domestic) and were exposed to extreme trauma for forty-three months (1992-1996);

Interviews were made immediately after the war cessation with informed consent signed by each participant. Data presented in this paper were analyzed using standard statistical methods.

RESULTS

We found that 55 women of total sample developed PTSD that represent more than half of whole population investigated. The occurrence of PTSD was 53% and 55% in groups A and B respectively. Simple test between percentages confirms that there is not a significant difference in PTSD occurrence between these two groups (t=0.201; p>0.05). Chi squared test between frequencies of PTSD in observed groups showed no significant difference ($\chi^2=0.040$, p>0.05).

There is no significant correlation between trauma time exposure and prevalence of PTSD in the analyzed sample (correlation coefficient = -0.0201, p>0.05) (Figure 1). Out of 100 interviewed women, 11% were severely physically abused and all of them developed PTSD. Other extreme trauma events did not show to be exclusive for PTSD. A comparison of PTSD and physical abuse frequencies in total analyzed sample (N=100) shows statistically significant difference (z=6.617, p>0.05). This result indicates that although significant contributor, physical abuse is not a necessary factor for PTSD. According to a priori hypothesis that if physical abuse was an exclusive predictor, than the frequencies values for PTSD and physical abuse should be approximately the same.

DISCUSSION

PTSD prevalence range 14-50% was reported for war survivor populations across the world (9). During the period 1992-1995, Sarajevo population was under the siege and civilians were permanently exposed to war weapon fire. In our preliminary research high PTSD prevalence was found in both groups (domestic and returnees). These results could be expected since the study was performed immediately after the war cessation. Most of the interviewed woman with PTSD had good social and work function. The other reason for high figures (more than 50%) could be attributed to exclusive woman civilian sample as this gender group have two times higher risk for developing PTSD (10). Interesting results appeared when we compared PTSD frequencies between two groups; no significant difference in PTSD proportions was observed although time exposure to qualitatively same war life events was considerably different. Our results suggest that time exposure could not have crucial impact on PTSD development. However, at the moment of screening long-term exposure effects on chronicity and severity of PTSD could not be evaluated in predictive manner (11).

Two groups were also observed according to socio-demographic characteristics (age, marital status and educational level) with no difference (12). Among other specific extreme life events listed in the HTQ, physical abuse showed to be risk factor for PTSD. Although possibly important risk factor our results could not prove that

Figure 1. Correlation between PTSD and exposure time to war trauma events in two groups of Sarajevo women.
physical abuse is sufficient and necessary for PTSD development. In our study sample we tried to reduce the impact of certain range of variables caused by differences in gender, cultural background, quality of common traumatic environment, lack of pre-morbid psychiatric history which brought in front other factors involved in PTSD. Because only a proportion of war trauma survivors develop PTSD (13) as confirmed here, it raises the question of other intrinsic factors involved in protective mechanisms against PTSD. Latest findings emphasize the hypothesis on biological aspects of PTSD (14). Recently, genetic basis of neurotransmission related to PTSD susceptibility has become an interesting area of research. Association between PTSD and particular gene polymorphisms involved in dopaminergic system was found (15) although these results were not replicated in similar studies (16).

Seven years after the war in Primary Health Care setting in Bosnia and Herzegovina, the prevalence of PTSD decreased five times in comparison with result presented in this paper. Severe forms of PTSD mainly co-morbid with other anxiety disorders, depression, alcohol and substance abuse was found (Kapetanovic and Oruc 2003; unpublished data).

Having in mind the preliminary findings on PTSD in Sarajevo and current prevalence in Bosnia and Herzegovina there is a strong argument for extended research. In that sense, Sarajevo population being exposed for almost four years to extreme war life events represents unique model for comparative research on PTSD aetiology within the light of latest findings in molecular genetics of PTSD.

REFERENCES

ABSTRACT

Cervical and breast cancer are usually type of tumor that are found among women in fertile age in Bosnia and Herzegovina. Final goal was to establish frequency of risk factors that are responsible for development of those types of cancer as well as establish possibility of prevention, according to the existence of each risk factor. Research was conducted through out surveys among women which were selected by accident. The amount of questioned women is 200, and out of that number 70 (35%) were out of rural environment, 130 (65%) were from urban environment which led to statistic-processed information. Variables that were defining our interviews were: age, marital status, education level, stay during the war in B&H, number of given birth, consistency of gynecological examinations, changes that were found during the medical (gynecological) examination, number of sexual partners, usage of contraception, existence of sexual infections, usage of tobacco, existence of genetic factor.

The most important fact is that over 50% of interviewees do not visit gynecologist, and that the gynecological infections are frequent. Usage of tobacco is in high percent founded among interviewees from urban environment (85%).

Keywords: risk factors, cervical cancer, fertile age.

INTRODUCTION

With the references to the Institute for the Public health (HNK) in the county of Stolac in the year of 2002 was populated by 9861 resident. Women were presenting 48.7% of population (women in fertile age (15-49) is 33.4% - 3300 women.). Cervical and breast cancer are usually type of tumor that are found among women in fertile age in Bosnia and Herzegovina. For development of cervical cancer was discovered numerous of factors that can lead us to its founding, and the most important is neglect which is shown through out bad hygiene (1,2). Early age for the first intercourse, numerous of sexual partners, high risk of male partners (4,5) (those that are exposed to promiscuity), bad social and economical status, usage of tobacco, overconsumation of coffee, usage of contraception (6). All of these are considered factors that can lead to development of cervical cancer. Considering all of the above, we tried to conduct a research that will circulate through the risk factors in the area that is specific in B&H reality; in social, political as well as in economical sector.

Aim

To determine frequency of tumor existence among women in fertile age in County of Stolac.
To ascertained differences in development of factors in rural and urban area.
To define possibility of preventing arrangement in relation to the consistency of appearance each risk factor.

MATERIAL AND METHODS

The research was professed through out poll among women in fertile age by method of accident model. The amount of questioned women is 200, and out of that number 70 (35%) were out of rural environment, 130 (65%) were from urban environment, which led to statistic-processed information. Variables that were defining our interviewees were: age, marital status, education level, stay during the war in B&H, number of given birth, consistency of gynecological examinations, changes that were found during the medical (gynecological) examination, number of sexual partners, usage of contraception, existence of sexual infections, usage of tobacco, existence of genetic factor. The inquisition was implement during 01.11.-01.12.2002 in community, County of Stolac.

RESULTS

Study overhaul of consistency risk factors for development tumor was operated in county of Stolac, and included 200 women in fertile age, out of those 130 (65%) from urban area, and the rest of the from rural area in the county. The research was professed through out poll among women in fertile age by method of accident model, considering non-equal (caused out many reasons) distribution of female population in this county. The result shows that the most of women from rural environment is the age group (35-44 years, 0%), and in urban environment the age group (45-49 years, 35.5%). In rural region there was 80.5% married, and in urban region was 83.9% married interviewees. Construing level of education, interviewees we pillar that major half of them from rural area has lower education, and that half of interviews from urban area has at least high school diploma.
Out of all interviewed women in rural area, 90% spent wartime as a refugee, mostly (76%) in B&H. Very similar situation is considering interviewees from urban area, because 83.8% were banished from county of Stolac. Predisposing factors we must overlook through whole spectarof possibilities physiological and potential pathological impressions and those characteristics are recorded in answers of interviewees.

Graph 1.

The biggest percentage of the inquiries from urban area (37%) has two given birth, but the most of the percentage of the inquiries from rural area (27.1%) has three and more given births. The small percentage of interviewees from rural area has one birth; while other inquirees from urban area was three given births. It is a surprising fact that occurrence of giving birth between village and town. On the other hand, the rate of non-birth is very high, especially in urban area (29.3%, graph #1).

If we observe gynecological examination, it is shown clearly that 52.8% of the interviewees from rural area don’t ever visit such kind of examination, but 37.8% did that only once in many years. In urban area situation is somewhat fortunate, even though 43.1% of the inquires never attends gynecological examinations, and 26.9% does that once a year. (graph #2)

Graph 2.

57.3% interviewees from rural area and 48.7% from urban area claim that that had one sexual partner, but the percent of those that confirmed more than five partners is very low (2.8% in rural area, and 3.6% in urban area). (Graph #3.)

Graph 3.

Usage of the contraceptive methods isn’t scientifically spread among interviewees in urban and rural area. 58.5% of surveyed women in rural area and 28.5% in urban area state that during the intercourse don’t use any kind of protection. (table #1).

Table 1.

<table>
<thead>
<tr>
<th>Usage of contraceptive methods</th>
<th>Rural Area</th>
<th>Urban Area</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number:</td>
<td>Percentage:</td>
</tr>
<tr>
<td>CONDOM</td>
<td>1</td>
<td>1.4</td>
</tr>
<tr>
<td>FOAM/JELLY</td>
<td>1</td>
<td>1.4</td>
</tr>
<tr>
<td>DIAPHRAGM</td>
<td>3</td>
<td>4.2</td>
</tr>
<tr>
<td>IUD</td>
<td>2</td>
<td>2.8</td>
</tr>
<tr>
<td>PILL</td>
<td>6</td>
<td>8.4</td>
</tr>
<tr>
<td>PERIODIC ABstinence</td>
<td>5</td>
<td>7.6</td>
</tr>
<tr>
<td>COITUS INTERRUPTUS</td>
<td>11</td>
<td>15.7</td>
</tr>
<tr>
<td>NOTHING</td>
<td>41</td>
<td>58.5</td>
</tr>
<tr>
<td>SUM</td>
<td>70</td>
<td>100</td>
</tr>
</tbody>
</table>
Among the interviewees that are using certain kind of protection, supply contraceptive pills (8.4% in village, 18.5% in town)

**Graph 4.**

![Graph showing changes found in the last gynecological examination](image)

58.6% of the interviewees from rural area are confirming pathological changes found in the last gynecological examination, while that percentage is somewhat lower in those from urban area (44.6%). Considerably greater percentage of the interviewees from rural area that are confirming existence of sexual infection that are shown through common phenomenon of colpitis (66%) related to the interviewees from urban area (33.5%) Graph # 4. & #5.

**Graph 5.**

![Graph showing existence of sexual infection](image)

Inquiring life styles within interviews, the strive was to consider consummation of tobacco and coffee as factors that are related to the appearance of the malignity, but as well as indention to the health in general. As a certificate of all the above, another fact comes into place; 53% of the interviewees are using tobacco in rural area, in the town area almost 85%, which is leading us to the conclusion that almost half of them is smoking over a pack a day. Supplemental risk factor is consuming coffee that are, by their own statement, using 2-5 times a day (50-80% out of all surveyed women). (Graph #7.)

**Graph 6.**

![Graph showing abortus](image)

Inquiring the existence of tumors in relater affinity (mother, sister, grandmother, aunts), it is representable that in 44% interviewees in rural area and 52.3% in urban area exists a “positive genetically factor” that interviewees are additionally burdened with the additional existence of another risk factors that are responsible for development of tumor for the reproductive organs.

**DISCUSSION**

According to the results, we can see that the most of the predisposing factors from cervical cancer development, exists also within women from county of Stolac, with the mildew deviations related to the village-town situation. Considering variables that were questioned, each of them was observed from the aspect of the possible connection with the development of tumor. Considering that the most number of interviewees (in town as well as in vil-
According to the so far researches, here as well as throughout the world this age group is mostly stroke by cervical cancer. When we asked a question about present during the war in Bosnia, we wanted to establish presence of stress as a risk factor for most of the tumor. Large number of interviewees (90% in rural, 83% in urban area) spent the war in refugee camps in Bosnia, so we can conclude that interviewees are from that aspect in group with high risk. Most interesting and most alarming in this research is the fact that greatest percentage of women from both areas do not visit gynecologist. It is related to the fact that women from both areas have some kind of pathological changes found within last consultation with a medical doctor. In the urban area around 44% of interviewees had more than one sexual partner while in rural area that percentage was somewhat lower (around 25%) what is applying to the possibility of HPV infection (7), which is risk factor number one for development of cervical cancer. Additional burden is a fact about existence of vaginal infections in 66% interviewees from rural area and 35% from urban area.

Considering that, each long-term chronically infection may predispose tumor. It is very interesting that in intercourse large number of interviewees does not use any kind of protection from undesired pregnancy and STD, and of those that are using, the most common "weapon" is a pill that is not providing any kind of protection. Also there are unreliable in prevention of undesired pregnancy. It is common knowledge knowledge connection between long-term usage of those methods and development of breast cancer. Researching life styles, we proved that the usage of tobacco, coffee is highly presented within interviewees from both sides. High percent of interviewees that are not using tobacco (from the village) as well as lower usage of tobacco at all, comparing to urban area. Around 50% interviewees from both sides has positive genetically factor (it is established that a tumor on reproductive organs in related affinity) whose significance is presentable.

CONCLUSION

Definitely the presence of great number of risk factors in interviewed sample of fertile population, considering side of socio-political, economical and demographical factors in county of Stolac, on the other side, are making health care as a social problem evidently. Early detection of diseases on female reproductive organs almost doesn't existed. The reasons for that are: tradition, customs, elements of health culture as well as consequences of objective reasons (dual politics and management in region functioned for almost decade), therefore there are dual health institutions.

There are no gynecological service in the area of this county, therefore, for this kind of examination, one must go Mostar or ^Apljina. We should not forget difficult existential circumstances considering for 40% of the population is a refugee that came back in last 3-4 years on the territory of this county. Considering the question of presence of each risk factor, we can see the difference between rural and urban area in: tobacco usage, consistency of gynecological examination, number of sexual partners, abort uses; however the different is minimal when it comes to genetically factor. For all that has been said, the only way out when it comes to the efficient monitoring over the health of the population would be preventing and promoting work based on organizing periodical or aimed systematical examinations which would assure overload and dearly detection all diseases as well as disease of female reproductive organ.

Something like that could provide health care teams (family doctor) considering territorial distribution (one gynecologist / 30 000 livings)

REFERENCES

6. White C.: Cancer rates in Europe are linked to overweight, experts warn, BMJ 2003; 327:700
ETIOLOGICAL FINDINGS IN ENDODONTIC-PERIODONTAL INFECTIONS

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ABSTRACT

The endodontium and periodontium are closely related and disease of one may lead to secondary disease in the other. The differential diagnosis of endodontic and periodontal disease is of vital importance, so that the appropriate treatment can be done.

Microorganisms play a primary role in endodontic and periodontal infections. The magnitude of the host response will be directly proportional to the virulence and the number of microbial cells present. Tissue damage caused by bacteria is mediated by either direct or indirect mechanisms. Direct harmful effects caused by bacteria involve their products, such as enzymes (collagenase, hyaluronidase, condroitinase, acid phosphatase), exotoxins and metabolites (bytrate, propionate, ammonium polyamines, sulphured compounds).

In addition, bacterial components such as peptidoglycan, teichoic acid, fimbriae, outer membrane proteins, capsule, and lypopolysaccharide, stimulate the development of host immune reaction capable of causing severe tissue destruction.

Key words: root canal, periodontal, microorganisms, etiological.

INTRODUCTION

The inflammatory pulpal and periapical lesions resulting from infection, trauma, inflammation and/or necrosis of the dental pulp are: reversible pulpitis, irreversible pulpitis, necrotic pulp, acute apical periodontitis, chronic apical periodontitis, suppurative apical periodontitis, apical abscess, condensing osteitis etc.

Miller (1) raised the hypothesis that bacteria are the causative factor of disease of endodontic origin. Although, he reported in 1894 the occurrence of bacteria in root canals with associated pathologic conditions, the causal relationship between microorganisms and periodontal diseases was only demonstrated in 1960’s. The search for the specific etiologic agents of periodontal diseases has been in progress since then. Regardless of the diagnostic method used, epidemiological studies have shown that more than two hundred different microbial species can be found in infected root canals, usually in combinations of four to seven species per canal (2, 3, 4). Recent epidemiological studies with molecular methods have found a relatively high prevalence of spirochetes in infected root canals, particularly Treponema denticola (Table 1), which is a putative periodontal pathogen (5).

Therefore, evidence indicates that microorganisms play a primary role in the aetiology of periodontal infections. Evidence suggests that not the particular species, but many of them possess the physiological requirements necessary to cause damage to the periodontal tissues.

The magnitude of the host response will be directly proportional to the virulence and the number of microbial cells present. Tissue damage caused by bacteria is mediated by either direct or indirect mechanisms. Direct harmful effects caused by bacteria involve their products, such as enzymes (collagenase, hyaluronidase, condroitinase, acid phosphatase), exotoxins and metabolites (bytrate, propionate, ammonium polyamines, sulphured compounds).

In addition, bacterial components such as peptidoglycan, teichoic acid, fimbriae, outer membrane proteins, capsule, and lypopolysaccharide, stimulate the development of host immune reaction capable of causing severe tissue destruction (6, 7, 8). For example, macrophages can be activated by bacterial components and can be stimulated to release chemical mediators such as cytokines (interleukin-1β, tumour necrosis factor, and interleukin-6), and prostaglandins, which are involved in the induction of bone resorption commonly observed in chronic periodontal diseases (7, 8).

The microflora found in periodontitis is complex and composed mainly of Gram-negative anaerobic bacteria (9). Moreover, studies have shown that the various clinical forms of periodontitis are associated with different microbiota (10) (Table 2, Table 3). The exact mechanisms of tissue destruction are not completely elucidated. Periodontitis is an inflammatory response to bacterial accumulation, primarily subgingival bacteria, that includes gingivitis but also spreads to deeper periodontal structure such as the gingival connective tissue periodontal ligament and supporting alveolar bone.
The endodontium and periodontium are closely related and diseases of one tissue may lead to secondary diseases in the other. The differential diagnosis of endodontic and periodontal diseases can sometimes be difficult but it is of vital importance to make a correct diagnosis so that the appropriate treatment can be provided.

**CLASSIFICATION OF ENDODONTIC-PERIODONTAL LESIONS**

Endodontic-periodontal lesions have been classified by various authors according to the primary cause of disease. A typical classification, based on the primary disease with a secondary effect, is as follows:

a) **Primary endodontic lesion with drainage through the periodontal ligament** - a deep narrow probing defect is noted on just one aspect of the tooth root. This is usually a draining sinus originating from an infected root canal system.

b) **Primary endodontic lesion with secondary periodontal involvement** - there is a more extensive periodontal pocket which has occurred as a result of the drainage from the infected canal. Long-term existence of the defect has resulted in deposits of plaque and calculus in the pocket with subsequent advancement of the periodontal disease.

c) **Primary periodontal lesion** - the periodontal disease has gradually spread along the root surface towards the apex. The pulp may remain vital but may show some degenerative changes over time.

d) **Primary periodontal lesion with secondary endodontic involvement** - progression of the periodontal disease and the pocket leads to pulpal involvement via either a lateral canal foramen or the main apical foramen. The pulp subsequently becomes necrotic and infected.

e) **Combined endodontic-periodontal lesion** - the tooth has a pulpless, infected root canal system and a co-existing periodontal defect.

A simpler classification would be to define any situation with both endodontic and periodontal diseases as being a “combined endodontic-periodontal lesion”. Periodontal diseases also include conditions such as chronic periodontitis, aggressive periodontitis, systemic disease-associated periodontitis and narcotising periodontitis (11).

---

**Table 1. Genera of putative endodontic pathogens commonly associated with different forms of periradicular diseases**

<table>
<thead>
<tr>
<th>Primary infections</th>
<th>Acute periradicular abscess (13)</th>
<th>Secondary and/or persistent infections (14)</th>
<th>Extraradicular infections (15)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chronic periradicular lesions (12)</strong></td>
<td><strong>Porphyromonas</strong></td>
<td><strong>Enterococcus</strong></td>
<td><strong>Actinomyces</strong></td>
</tr>
<tr>
<td>Bacteroides</td>
<td>Treponema</td>
<td>Actinomyces</td>
<td>Propionibacterium</td>
</tr>
<tr>
<td>Treponema</td>
<td>Fusobacterium</td>
<td>Streptococcus</td>
<td>Pseudomonas</td>
</tr>
<tr>
<td>Prevotella</td>
<td>Bacteroides</td>
<td>Candida</td>
<td></td>
</tr>
<tr>
<td>Porphyromonas</td>
<td>Prevotella</td>
<td>Propionibacterium</td>
<td></td>
</tr>
<tr>
<td>Fusobacterium</td>
<td>Streptococcus</td>
<td>Staphylococcus</td>
<td></td>
</tr>
<tr>
<td>Peptostreptococcus</td>
<td>Peptostreptococcus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Streptococcus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eubacterium</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Actinomyces</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Campylobacter</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 2. Microbial species associated with various clinical forms of periodontitis** (9)

<table>
<thead>
<tr>
<th>Species</th>
<th>Juvenile periodontitis (++)</th>
<th>Early onset periodontitis (++)</th>
<th>Adult periodontitis (++)</th>
<th>Refractory periodontitis (+ to ++)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actinobacillus actinomycetemcomitans</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>+ to ++</td>
</tr>
<tr>
<td>Porphyromonas gingivalis</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Prevotella intermedia</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Fusobacterium nucleatum</td>
<td>+</td>
<td>+</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Eikenella corrodens</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Bacteroides forsythus</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+++</td>
</tr>
</tbody>
</table>
Table 3. Bacterial species that have been associated with different forms of periodontal disease

<table>
<thead>
<tr>
<th>DISEASE</th>
<th>ASSOCIATED MICROORGANISMS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prepubertal periodontitis</td>
<td>Actinobacillus actinomycetemcomitans</td>
</tr>
<tr>
<td></td>
<td>Prevotella intermedia</td>
</tr>
<tr>
<td></td>
<td>Porphyromonas gingivalis</td>
</tr>
<tr>
<td></td>
<td>Capnocytophaga sputigena</td>
</tr>
<tr>
<td></td>
<td>Eikenella corrodens</td>
</tr>
<tr>
<td>Localised juvenile periodontitis</td>
<td>Actinobacillus actinomycetemcomitans</td>
</tr>
<tr>
<td></td>
<td>Prevotella intermedia</td>
</tr>
<tr>
<td></td>
<td>Fusobacterium spp.</td>
</tr>
<tr>
<td></td>
<td>Capnocytophaga sp.</td>
</tr>
<tr>
<td></td>
<td>Eikenella corrodens</td>
</tr>
<tr>
<td></td>
<td>Peptostreptococcus micros</td>
</tr>
<tr>
<td></td>
<td>Selenomonas spp.</td>
</tr>
<tr>
<td>Rapidly progressive periodontitis (early onset / generalised juvenile</td>
<td>Actinobacillus actinomycetemcomitans</td>
</tr>
<tr>
<td>periodontitis)</td>
<td>Prevotella intermedia</td>
</tr>
<tr>
<td></td>
<td>Bacteroides forsyts</td>
</tr>
<tr>
<td></td>
<td>Eikenella corrodens</td>
</tr>
<tr>
<td></td>
<td>Fusobacterium spp.</td>
</tr>
<tr>
<td></td>
<td>Peptostreptococcus micros</td>
</tr>
<tr>
<td></td>
<td>Treponema spp.</td>
</tr>
<tr>
<td></td>
<td>Wolinella recta</td>
</tr>
<tr>
<td>Refractory periodontitis</td>
<td>Actinobacillus actinomycetemcomitans</td>
</tr>
<tr>
<td></td>
<td>Porphyromonas gingivalis</td>
</tr>
<tr>
<td></td>
<td>Prevotella intermedia</td>
</tr>
<tr>
<td></td>
<td>Bacteroides forsyts</td>
</tr>
<tr>
<td></td>
<td>Eikenella corrodens</td>
</tr>
<tr>
<td></td>
<td>Fusobacterium spp.</td>
</tr>
<tr>
<td></td>
<td>Peptostreptococcus micros</td>
</tr>
<tr>
<td></td>
<td>Treponema spp.</td>
</tr>
<tr>
<td></td>
<td>Wolinella recta</td>
</tr>
<tr>
<td></td>
<td>Candida sp.</td>
</tr>
<tr>
<td>Adult periodontitis</td>
<td>Actinobacillus actinomycetemcomitans</td>
</tr>
<tr>
<td></td>
<td>Prevotella intermedia</td>
</tr>
<tr>
<td></td>
<td>Bacteroides forsyts</td>
</tr>
<tr>
<td></td>
<td>Porphyromonas gingivalis</td>
</tr>
<tr>
<td></td>
<td>Eikenella corrodens</td>
</tr>
<tr>
<td></td>
<td>Fusobacterium spp.</td>
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<tr>
<td></td>
<td>Peptostreptococcus micros</td>
</tr>
<tr>
<td></td>
<td>Selenomonas spp.</td>
</tr>
<tr>
<td></td>
<td>Treponema denticola</td>
</tr>
<tr>
<td></td>
<td>Pathogen-related oral spirochaete</td>
</tr>
<tr>
<td></td>
<td>Wolinella recta</td>
</tr>
<tr>
<td>Periodontal abscess</td>
<td>Porphyromonas gingivalis</td>
</tr>
<tr>
<td></td>
<td>Prevotella intermedia</td>
</tr>
<tr>
<td></td>
<td>Peptostreptococcus micros</td>
</tr>
<tr>
<td></td>
<td>Staphylococcus</td>
</tr>
<tr>
<td>Acute narcotising ulcerative gingivitis</td>
<td>Porphyromonas gingivalis</td>
</tr>
<tr>
<td></td>
<td>Prevotella intermedia</td>
</tr>
<tr>
<td></td>
<td>Treponema denticola</td>
</tr>
<tr>
<td></td>
<td>Treponema pallidum</td>
</tr>
</tbody>
</table>
TYPES OF ENDODONTIC INFECTION

There are different types of endodontic infections, which are usually associated with different clinical conditions. The root canal infection is the primary cause of acute or chronic periapical diseases. Secondary or persistent infections are the cause of secondary or chronic periapical lesions, which can result in persistent symptoms, exudation, or the failure of the endodontic treatment (Table 1).

Primary root canal infection

Primary root canal infection is caused by microorganisms colonizing the necrotic pulp tissue. In general, primary infections are mixed and predominated by anaerobic bacteria. Predominant species usually belong to the genera Bacteroides, Porphyromonas, Prevotella, Fusobacterium, Treponema, Peptostreptococcus, Eubacterium and Campylobacter. Facultative or microaerophilic streptococci are also commonly found in primary infections. Current evidence suggests that some Gram-negative anaerobic bacteria are closely associated with the aetiology of symptomatic periapical lesions, including cases of acute periapical abscess (3).

Secondary root canal infection

Secondary infection are caused by microorganisms that were not present in the primary infection and have penetrated the root canal system during treatment, between appointments, or after the conclusion of the endodontic treatment (4). If the penetrating microorganisms are successful in surviving and colonizing the root canal system, a secondary infection is established.

Persistent root canal infection

Microorganisms that in some way resisted the intracanal procedures of this infection cause persistent intraradicular infections. Gram-positive bacteria are the predominant in the persistent infections (11).

Extraradicular infections

The most common form is acute periapical abscess. The source of extraradicular infection is usually the intraradicular infection. Microorganisms established in the periapical tissues are inaccessible to the endodontic disinfection procedures, extraradicular infection may cause the failure of the endodontic therapy. It is recognized that oral microorganisms such as Actinomyces spp., Propionibacterium may be implicated in the extraradicular infections (12).

CONCLUSION

The inflammatory pulpal, periapical and periodontal lesions are result of infection, trauma, inflammation and/or necrosis. Regardless of the diagnostic method used, epidemiologic studies have shown that more than two hundred different microbial species can be found in infected root canals, usually in combinations of four to seven species per canal.

Recent epidemiological studies with molecular methods have found a relatively high prevalence of spirochetes in infected root canals, particularly Treponema denticola, which is a putative periodontal pathogen.

Microorganisms play a primary role in the aetiology of periapical infections. Evidence suggests that not the particular species, but many of them possesses the physiological requirements necessary to cause damage to the periapical tissues.

The microflora found in periodontitis is complex and composed mainly of Gram-negative anaerobic bacteria. Moreover, studies have shown that the various clinical forms of periodontitis are associated with different microbiota. The exact mechanisms of tissue destruction are not completely elucidated.
REFERENCES


THE EFFECTS OF MCKENZIE AND BRUNKOW EXERCISE PROGRAM ON SPINAL MOBILITY
COMPARATIVE STUDY

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3. Institute for Physical Medicine and Rehabilitation, Sarajevo, Bosnia and Herzegovina

ABSTRACT

This study encompassed 64 participants with symptoms of low back pain, 33 in McKenzie group and 31 in Brunkow group. Patients attended exercise program daily and they were asked to do the same exercise at home - five times a day in series of 5 to 10 repetition each time, depending of stage of disease and pain intensity. All patients were assessed for the spinal motion, before and after the treatment.

All parameters for spinal movements showed improvement after exercising McKenzie program for lower back pain with a significant difference of p<0.01 for all motions. Also, in Brunkow group, all of the parameters showed statistically significant improvement at the end of treatment in relation to pre-treatment values, with significant difference of p<0.01 for all motions.

Statistically comparison between McKenzie and Brunkow difference in score at the end of the treatment showed statistically significant improvement in McKenzie group, for extension, right and left side flexion, while flexion score didn't show statistically significant difference.

McKenzie exercises seemed to be more effective than Brunkow exercises for improvement in spinal motion. Both, McKenzie and Brunkow exercises can be used for spinal mobility improvement in patients with lower back pain, but is preferable to use McKenzie exercises first, to decrease the pain and increase spinal mobility, and then Brunkow exercises to strengthen the paravertebral muscles.

Key words: McKenzie, Brunkow, exercises, dynamic, isometric, spinal mobility.

INTRODUCTION

Low back pain affects 60-80 % of adults at some time of their lives, and 50% suffer from the back pain within given year. Back symptoms are among the 10 leading reasons for patient visits to emergency rooms, hospital outpatient departments and physicians’ offices. (1, 2)

Therapeutic approaches to this problem are different and very often controversial. In general, physiotherapy treatments can be sorted in “passive” treatments, such as thermo- and cryo-procedures, manipulation, massage, orthotic devices, traction and electrotherapy, and “active” treatments like kinesitherapy procedures.

There are different types of exercises for back pain, such as flexion exercises, extension exercises, or some specific exercises that are combination of these two types. Decision which type of exercises can be applied is very individual, depends of physician’s approach and there is no prescription which one is the most appropriate for each patient. (3, 4, 5, 6, 7, 8, 9, 10, 11, 12)

Exercise is typically aimed at strengthening back extensors or flexors and increasing back flexibility to reduce injury risk, improving mood and pain perception to reduce the impact of injury. (13, 14, 15, 16, 17)

One of specific exercises programs for low back pain can be McKenzie approach (18) that consists of six exercises performing in certain positions (laying in prone position, standing, laying in supine position and sitting), which gradually increase the pressure on the vertebra. These exercises can be called self-manipulation exercises and they have to be done in small sessions but frequently, during the day. During this exercise program postural correction is needed as well as observation of all changes in pain intensity and location. Number of sessions and daily frequency depended of stage of disease and pain intensity (19, 20, 21, 22).

Brunkow exercises (23) can be called “pushing exercises” and they can be done in all starting positions. Main action is isometric contraction, which started by movements of feet and/or hands, and transferring through kinetic chain to paravertebral muscles. They are starting with dynamic contraction of hands and feet with “punctum fixum” on the wrist or/and heal. Dynamic contraction from the beginning leads to isometric contraction of the group of muscles, which has to be included in the exercise. Starting positions determinates the group of muscles to be trained.

These completely different types of exercises are in daily use for patients with lower back pain in Physical Medicine and Rehabilitation Centres and the purpose of this study was to compare the effects of McKenzie and Brunkow exercises on spinal motion improvement.
METHODS

Participants
Sixty-eight patients with symptoms of low back pain were included in study, which was approved by the Ethics Committee of the Sarajevo University Faculty of Medicine. Patients were recruited from Physical Medicine and Rehabilitation outpatient Clinic in Community Based Rehabilitation Centres in Sarajevo and from University Clinical Centre Sarajevo - Institute for Physical Medicine and Rehabilitation. Subjects were referred for physiotherapy treatment from Primary Health Care physicians or from specialist Clinic (orthopaedic, traumatology, neurology etc). Patients were included in study if they had symptoms of lower back pain, without any motor or sphincter deficit. Physiatrist and physiotherapist gave instructions for exercises and provided supervision of the patients.

Of this group 34 individuals (10 men, 24 women) performed McKenzie program and 34 individuals (13 men, 21 women) Brunkow exercise program. The mean age was 50 years (SD 10.8 years) for McKenzie group and 47 years (SD 13.8 years) for Brunkow group.

Professionals who had minimal training in McKenzie and Brunkow method gave instructions for exercises and made supervision of the patients.

One patient from McKenzie group and 3 patients from Brunkow group didn't complete the treatment, thus 33 patients from McKenzie group and 31 patient from Brunkow group enrolled into the statistical analysis, total of 64 patients.

Measures
All patients were assessed before and after the treatment. Spinal range of motions were measured using centimetre and measuring a distance between top of the third hand finger and floor while patient were asked to move forward, backward and on right and left lateral side.

Exercise therapy
McKenzie and Brunkow exercises were performed individually to the need and possibility of each patient. Patients attended exercise program daily, under supervision of physiatrist and physiotherapist in the Clinic for Physiotherapy and Rehabilitation, and they were asked to do the same exercise program at home - five times a day in series of 5 to 10 repetition each time, depending of stage of disease and pain intensity.

Type of exercises and number of repetitions in each session were created individually for each patient. All exercises were followed by correction of body posture.

Starting positions for Brunkow exercise program were the same as for McKenzie program, gradually increasing pressure on vertebra (prone position, standing, supine position and sitting).

Statistics
Results were expressed as mean +/- SD. Differences in group means were examined by Student t-test.

RESULTS
A total of 64 patients participated in this study.
In McKenzie group there were 29% male and 71% female patients, and in Brunkow group exercised 38% male and 62% female patients. (Figure 1)

Figure 1. Gender structure of participants who involved in McKenzie and Brunkow program for low back pain

Mean age of patients who exercised McKenzie program was 50 years (+/- 10.8 years) and for patients in Brunkow group was 42 years (+/- 13.8 years). Brunkow group was significantly younger with t-test 2.988 and significant difference p<0.01.

In McKenzie group 23% of patients experienced first symptoms of lower back pain in the year of assessment, 27% has experienced a pain for 4 years, 23% 10 years and 27% more than 10 years.

In Brunkow group 41% of patients suffered the pain for less than one year, 15% of patients for 4 years, 20% 10 years, 24% more than 10 years. (Figure 2)

Table 1. Mean age of patients in McKenzie and Brunkow group

<table>
<thead>
<tr>
<th>Data</th>
<th>McKenzie</th>
<th>Brunkow</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>33</td>
<td>31</td>
</tr>
<tr>
<td>Mean age</td>
<td>50 years</td>
<td>42 years</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>10.8 years</td>
<td>13.8 years</td>
</tr>
<tr>
<td>Standard error</td>
<td>1.83</td>
<td>2.36</td>
</tr>
<tr>
<td>t - test</td>
<td>2.988</td>
<td>p &lt; 0.01</td>
</tr>
</tbody>
</table>

n=68
Figure 2. First symptoms of low back pain in participants in the study

According to Quebec Task Force of Spinal Disorders (24) all patients were grouped in 3 groups: acute stage of disease – pain less than 7 days, subacute stage – pain from 8 days to 7 weeks and chronic stage – pain more than 7 weeks. There were 15% of participants in acute pain, 55.5% in subacute and 29.5% in chronic pain in McKenzie group and 9% in acute stage, 50% in subacute and 41% in chronic stage in Brunkow group. (Table 2)

There was no statistically significant difference in stage of pain between McKenzie and Brunkow group before the treatment - $\chi^2 = 1.278$

In McKenzie group, patients received a mean of 15.5 days of treatments with standard deviation of 8.95.

In Brunkow group, patients received a mean of 14.9 days of treatments with standard deviation of 8.96.

One patient didn’t complete the treatment in McKenzie group and 3 patients in Brunkow group, thus 33 McKenzie patients and 31 Brunkow enrolled into the statistical analysis.

Data analysis
Table 3 shows mobility scores for McKenzie group from four movements, from the neutral position to the maximum active motion in the sagittal (flexion/extension) and coronal (left/right) phases, at 2 evaluation times.

All parameters for spinal movement showed improvement after exercising McKenzie program for lower back pain. Difference in measurements before and after McKenzie exercise program showed that flexion increased for 6.7 cm in average, extension for 4.4 cm; right side flexion for 3.5 cm and left side flexion for 3.3 cm in average. All of these parameters showed statistically significant improvement at the end of treatment in relations to pre-treatment values, with significant difference of $p<0.01$.

In second group of patients with lower back pain, who were exercising Brunkow program, all parameters for

### Table 2. Stage of pain in McKenzie and Brunkow group of patients.

<table>
<thead>
<tr>
<th>Stage</th>
<th>McKenzie</th>
<th></th>
<th>Brunkow</th>
<th></th>
<th>Total</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>%</td>
<td>No</td>
<td>%</td>
<td>No</td>
<td>%</td>
</tr>
<tr>
<td>Acute (1-7 days)</td>
<td>5</td>
<td>15</td>
<td>3</td>
<td>9</td>
<td>8</td>
<td>12</td>
</tr>
<tr>
<td>Subacute (8 days - 7 weeks)</td>
<td>19</td>
<td>55</td>
<td>17</td>
<td>50</td>
<td>36</td>
<td>53</td>
</tr>
<tr>
<td>Chronic (more than 7 weeks)</td>
<td>10</td>
<td>30</td>
<td>14</td>
<td>41</td>
<td>24</td>
<td>35</td>
</tr>
<tr>
<td>Total</td>
<td>34</td>
<td>100</td>
<td>34</td>
<td>100</td>
<td>68</td>
<td>100.0</td>
</tr>
</tbody>
</table>

### Table 3. Spinal mobility before and after McKenzie exercise program in patients with low back pain.

<table>
<thead>
<tr>
<th>Motion</th>
<th>Statistics</th>
<th>Pre treatment</th>
<th>Post treatment</th>
<th>Difference</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flexion</td>
<td>X - average</td>
<td>26.7</td>
<td>20.0</td>
<td>6.7</td>
<td>4.451</td>
<td>$p&lt;0.01$ signif.</td>
</tr>
<tr>
<td></td>
<td>SD - standard deviation</td>
<td>13.9</td>
<td>14.0</td>
<td>8.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extension</td>
<td>X</td>
<td>58.6</td>
<td>54.2</td>
<td>4.4</td>
<td>5.793</td>
<td>$p&lt;0.01$ signif.</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>12.1</td>
<td>11.1</td>
<td>4.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lateroflexion - right</td>
<td>X</td>
<td>47.6</td>
<td>44.1</td>
<td>3.5</td>
<td>4.543</td>
<td>$p&lt;0.01$ signif.</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>8.2</td>
<td>8.9</td>
<td>3.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lateroflexion - left</td>
<td>X</td>
<td>48.5</td>
<td>45.2</td>
<td>3.3</td>
<td>5.973</td>
<td>$p&lt;0.01$ signif.</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>7.8</td>
<td>8.8</td>
<td>3.1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

n=33
spinal movement also showed significant improvement at the end of the treatment (Table 4). Difference in measurements before and after treatment showed that flexion increased for 3.4 cm in average, extension for 1.7 cm; right side flexion for 2.0 cm and left side flexion for 2.2 cm in average. All of these parameters showed statistically significant improvement at the end of treatment in relations to pre-treatment values, with significant difference of p<0.01. Comparing the results in spinal movement measurements, between McKenzie and Brunkow group, it is evident that McKenzie group had better mobility score for all parameters. (Table 5)

Flexion has better score in McKenzie group for 3.3 cm, extension for 2.7 cm; right side flexion for 1.5 cm and left side flexion for 1.1 cm in average. Statistically comparison between McKenzie and Brunkow difference in score at the end of the treatment showed statistically significant improvement in McKenzie group, for extension, right and left side flexion, while flexion score didn't show statistically significant difference.

Comparing pre-treatment and post-treatment score for both group of patients, it was found that among 33 patients who did McKenzie exercise program for low back pain 6 % of patients didn’t have improvement in spinal flexion, 9 % in extension, 9 % is right side flexion and 12 % in left side flexion. In Brunkow group 13 % of patients didn’t improve in flexion score, 9 % in extension, 9 % in right side flexion and 23 % in left-side flexion. (Figure 3)

Results in this study showed that the majority of patients in McKenzie group improved from pre-treatment to post-treatment score, comparing with number of patients in Brunkow group. Statistical analysis showed that both group of patients had statistically significant improvement in spinal mobility after treatment in comparison with pre-treatment values. But, comparing inter group values, than McKenzie group had significantly better results for all spinal mobility parameters except spinal flexion. (Table 6)

**DISCUSSION**

This study investigated the use of McKenzie and Brunkow exercises for low back pain and it’s influence on spinal mobility. Kinesitherapy treatment is an “active” physiotherapy treatment that can be applied to the patients with lower back pain. Decision which type of exercises can be used is very individual, depends of physician’s approach and there is

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**Table 5. Spinal mobility in patients with low back pain after McKenzie and Brunkow exercise program.**

<table>
<thead>
<tr>
<th>Motion</th>
<th>Statistics</th>
<th>McKenzie Pre/post-treatment difference</th>
<th>Brunkow Pre/post treatment difference</th>
<th>McKenzie/Brunkow difference</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flexion</td>
<td>X mean</td>
<td>6.7</td>
<td>3.4</td>
<td>3.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>SD standard deviation</td>
<td>8.57</td>
<td>4.72</td>
<td>3.85</td>
<td>t=1.178</td>
<td>not signif.</td>
</tr>
<tr>
<td>Extension</td>
<td>X</td>
<td>4.4</td>
<td>1.7</td>
<td>2.7</td>
<td>t=3.311</td>
<td>p&lt;0.01 signif.</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>4.4</td>
<td>1.8</td>
<td>2.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lateroflexion - right</td>
<td>X</td>
<td>3.5</td>
<td>2.0</td>
<td>1.5</td>
<td>t=2.338</td>
<td>p&lt;0.05 signif.</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>3.2</td>
<td>1.6</td>
<td>1.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lateroflexion - left</td>
<td>X</td>
<td>3.3</td>
<td>2.2</td>
<td>1.1</td>
<td>t=2.595</td>
<td>p&lt;0.01 signif.</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>3.08</td>
<td>2.22</td>
<td>0.86</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

n=64
no define prescription which type of exercises is indicate for which patients and when. (3-12).

A lot of researches compared different types of exercises (12, 25, 26, 27), but there is no evidence that any of it compare effects of McKenzie and Brunkow exercises on spinal mobility. (18, 23)

These two completely different types of exercises are in daily use in Physiotherapy and Rehabilitation Centres, so our interest was to compare them and see what are their effects on improving spinal movements.

McKenzie exercises are dynamic exercises (extension – flexion type) while Brunkow exercises started as dynamic, but finishing with isometric (static) contraction of paravertebral muscles.

Patients attended exercise program daily, under supervision of physiatrist and physiotherapist in the Clinic for Physiotherapy and Rehabilitation, and they were asked to do the same exercise program at home - five times a day in series of 5 to 10 repetition each time, depending of stage of disease and pain intensity.

Type of exercises and number of repetitions were created individually for each patient.

All patients were assessed before and after the treatment and their spinal movements are measured (flexion – extension – right and left side flexion).

There were no significant differences between the groups of patients with a low back pain that participate in this study. In both groups majority of participants were females, aged between 42 and 50 years, first pain episode in last 5 years, last pain episode one month before treatment and treatment period of 15 days. There was no statistically significant difference in stage of pain between McKenzie and Brunkow group before the treatment.

All patients showed some evidence of restricted ROM before the treatment, mostly because of pain limitation. Spinal mobility measurements were used as a predictor for functional evaluation and pain reduction (more pain relief, better functional results), so this functional test functioned as a pain provocation test.

An improvement in all parameters of spinal motion was seen in both groups, either they exercised McKenzie or Brunkow program, (comparing pre treatment and post treatment measurements), although there are two completely different types of exercises. Reduction of the pain after training has also been reported in some trials involving the low back, cervical pain etc. (28) Activities related pain can be decreased by increasing endorphins that occurs after training. Strong muscle contractions activate muscles’ ergo-receptors (stretch receptors). (29) The afferents from the receptors cause endogenous opioids to be released and also cause the release of beta-endorphin from pituitary. These secretions may cause both - peripheral and central pain to be blocked. (30)

Low back pain is frequently associated with persistent joints stiffness from capsular, ligamentous, or pararticular muscle and tendon contracture, and that is another reason for limited spinal mobility in our participants. Comparison between spinal mobility measurements in both groups of patients after exercise program showed that McKenzie group showed better results in all parameters in relation with Brunkow group. Statistical analysis, also, showed better values for all parameters in McKenzie group, except for spinal flexion. This can be explained by a nature and technique of McKenzie exercises, which are at the beginning of treatment, preferable extension type and later as pain decreases, continues with flexion movements. Terminal extension and flexion in the same time would stretch some spinal structures while strengthening of the others, and on that way increasing spinal flexibility at all. Through stretching and active physical training, some pain can be relieved as stiffness improves. (31).

On the opposite side, Brunkow exercises are isometric exercises, which can slightly decrease the pain, but mostly can be used for strengthening the spinal muscles. Assisting patients to maximize mobility before they start a strength-training program is a key principle of functional restoration (31, 32, 33).

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### Table 6. Significant difference in spinal mobility after exercise program for lower back pain.

<table>
<thead>
<tr>
<th>Motion</th>
<th>McKenzie group</th>
<th>Brunkow group</th>
<th>McKenzie/Brunkow difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>t-test</td>
<td>signif (p)</td>
<td>t-test</td>
</tr>
<tr>
<td>Flexion</td>
<td>t=4.451</td>
<td>p&lt;0.01</td>
<td>t=4.593</td>
</tr>
<tr>
<td>Extension</td>
<td>t=5.793</td>
<td>p&lt;0.01</td>
<td>t=5.118</td>
</tr>
<tr>
<td>Right lateroflexion</td>
<td>t=4.543</td>
<td>p&lt;0.01</td>
<td>t=6.909</td>
</tr>
<tr>
<td>Left lateroflexion</td>
<td>t=5.973</td>
<td>p&lt;0.01</td>
<td>t=5.519</td>
</tr>
</tbody>
</table>

n=64
CONCLUSION

McKenzie exercises seemed to be better than Brunkow for improvement of the spinal motion. Spinal mobility in patients with lower back pain, can improve by performing exercises for lower back pain either McKenzie or Brunkow program. For better functional restoration, patients with lower back pain first have to increase spinal mobility and then to start with strength training program. Both, McKenzie and Brunkow exercises can be used for spinal mobility improvement in patients with lower back pain, but is preferable to use first McKenzie exercises to decrease the pain and increase spinal mobility, and then Brunkow exercises to strengthen the paravertebral muscles.

REFERENCES

(3) Faas A. Exercises: which ones are worth trying, for which patients, and when? Spine 1996; 21(24): 2874-8.
(17) Jette D., Jette A. Physical therapy and health outcomes in patient with spinal impairments. Phys ther 1996; 76(9): 930-945.
(18) McKenzie. Treat your own back. Spinal publications LTD., New Zealand


MORPHOGENESIS OF THE RAT FOREBRAIN
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ABSTRACT
Background and Purpose: Developmental process that leads to final forebrain shaping is a result of complex histogenetic and morphogenetic events. Comprehensions about brain development are based on observations carried out on ontogenetic successive stages. Microscopic analysis of brain together with analysis of serial sections gives information about shape the of some forebrain parts and basic relations between them. The aim of this study was to analyse morphogenesis in the earliest stages of rat's forebrain development.

Material and Methods: Rat brains used in this study were obtained from Fisher inbred rats with accurately timed pregnancies. The investigation was carried out on serial frontal sections of rat embryonic heads from the 12th (E12) to the 16th (E16) day of gestation. Gestation was considered to have begun early in the morning when sperm was found in the vaginal smear. Histological paraffin and plastic sections were systematically inspected with regard to morphogenetic changes of the forebrain parts telencephalon and diencephalon.

Results: E12: neural tube is completely closed in its cranial part. Rostral part of forebrain shows telencephalon vesicles origins as slightly paired enlargements of neuroepithelial wall. Between telencephalic vesicles origin and in direction to caudal there is an origin of diencephalon. E13: rostral part of forebrain shows well expressed and divided areas of telencephalons vesicles as basal, basolateral, dorsal and medial telencephalon. Central area between paired vesicles is a telencephalon impar. In diencephalon optic vesicles appeared. Epithalamus, thalamus and hypothalamus origins are slight enlargements of its neuroepithelial wall. E14: telencephalic vesicles spread above telencephalon impar into rostral direction and above diencephalon in rostrodorsal direction. Their basolateral parts of are very thickened and become folded. Sulcus telodiencephalicus appears. E15: the main event is the appearance of the origins of plexus chooroideus in the area of telencephalon impar as fingerlike processes. E16: all forebrain parts, especially telencephalic vesicles-origin of brain hemispheres and processes of plexus chooroideus, are progressively growing and shaping.

Conclusions: Our morphologic analysis describes significant morphogenetic changes in the forebrain shape. The forebrain changes from a relatively simple tubular structure with thin walls surrounding a large ventricular system to a thick-walled brain with a highly convoluted but reduced ventricular system.

INTRODUCTION
Neural tube formation occurs in three stages: a) formation and thickening of neural plate by elongation of neuroepithelial cells, b) bending of neural plate along the embryo midline, resulting in the elevation of neural folds and appearance of the neural groove, and c) curling over and fusion of neural folds to generate a closed neural tube. (1, 2, 3, 4)
The spectrum of shapes displayed by the developing of the neural tube of mammalian embryos is complex. In the mouse and rat, for example, the neuroepithelium exhibits rather abrupt and dramatic regional variations in size and shape. (2, 3, 5, 6, 7)
Morphogenesis and histogenesis of rat brain are much more faster than in human brain taking in consideration 21 days of rat development. Rat embryo's neural tube is already completely closed at the cranial end at 12th day of pregnancy. (8) From this time, the forebrain changes from a relatively simple tubular structure with thin walls surrounding a large ventricular system to a thick-walled brain with a highly convoluted but reduced ventricular system. (9) Comprehensions about brain development are based on observations carried out on one after another ontogenetic successive stages. Microscopic analysis of the brain together with analysis of serial sections and model reconstruction of them gives the information about shape of some telencephalon parts and basic relations between them. (10, 11) Three-dimensional reconstructions of the normal rat embryonic (E) neocortex on days E15, E17, E19 and E21 show that the neocortical ventricular zone shrinks rapidly in the medial direction during cortical morphogenesis. (12)
The aim of this study was to analyse morphogenesis in the earliest stages of rat's forebrain development.

MATERIAL AND METHODS
Foetal brains used in this study were obtained from "inbred" Fisher rats with accurately termed pregnancies. The day on which early in the morning sperm was found in the vaginal smear was considered as the first day of embryonic development. We have used 12 (E12) to 16 (E16) days' embryos. Each embryo was removed under anaesthesia through a separate incision in the uterus of a pregnant female. Fixation was performed by immersion of embryos in 1% glutaraldehyde and 1% paraformaldehyde in 0.15M sodium phosphate buffer. Heads of each investigated embryonic day E12-E16 (n=20) were embedded in paraffin and cut into 6µm frontal serial sec-
tions through whole developing rat heads and brains. Some of E12 embryos were embedded in Epon-Araldite and cut on serial frontal 1µm plastic sections. All histological sections were stained by toluidine blue and systematically inspected with regard to morphogenetic changes of the forebrain parts telencephalon and diencephalon. The representative sections of each investigated embryonic day were projected onto a screen of microscope «Visopan» (Reichert) and brain contours were crossed out.

RESULTS

At 12th day of gestation (E12) neural tube is completely closed in its anterior cranial part. That cephalic end of the neural tube shows slight enlargements, dilatations that are primary brain vesicles origins: the forebrain (prosencephalon), the midbrain (mesencephalon) and the hindbrain (rhombencephalon). Rostral part of forebrain shows telencephalic vesicles origin as paired slightly enlargements of neuroepithelial wall (Fig. 1a). Between telencephalic vesicles origin and in direction to caudal there is origin of diencephalon. Two lateral prominences on each side of the diencephalons neuroepithelial wall are optic vesicles (Fig 1b). Cavities inside brain vesicles are origin of the primitive ventricles.

Figure 1. Schematic drawings of rostral (a) and caudal (b) frontal sections of the rat brain at E12

Lateral ventricles are inside telencephalic vesicles. The cavities of the telencephalon and diencephalon contribute to the formation of the third ventricle, although the diencephalon cavity contributes more.

At 13th day of gestation (E13) rostral part of prosencephalon shows already well-expressed and divided areas of telencephalon. Paired lateral well-expressed prominences are telencephalic vesicles. They are primordia of the future cerebral hemispheres (Fig. 2a). Central area between paired telencephalic vesicles is telencephalon impar. Neuroepithelial wall impar telencephalon is very thin, particularly in lamina epithelialis area. Some parts of neuroepithelial telencephalon wall are more thickened in comparison with the others, so they can be divided on basal, basolateral, dorsal and medial telencephalon. Particularly, basal area of telencephalon named ganglionic hill is thicken. It is corpus striatum origin. Origins of hypothalamus, thalamus and epithalamus appear as slight enlargements of diencephalon neuroepithelial wall. Neuroepithelial wall of diencephalon’s area of optic vesicle differentiates in optic cup and recessus opticus (Fig. 2b).

Figure 2. Schematic drawings of frontal sections in area of telencephalon (a) and diencephalon (b) of the rat forebrain at E13.

As basolateral parts of telencephalic vesicles are very thickened, neuroepithelial wall is protruded to ventricular cavity, and lumen of ventricular becomes folded. That part of telencephalon is ganglionic hill. The neuroepithelial wall has uniform wideness in lateral and dorsal parts of telencephalon. Medial part of telencephalon, telencephalon impar, is elongated structure between telencephalic vesicles. Telencephalon impar is together with surrounding mesenchymal positioned in the front of ganglionic hill. Telencephalic vesicles spread above telencephalon impar in rostral direction. The neuroepithelial wall in medial telencephalon is considerably thinner than in the other parts of telencephalon. The thinnest dorso-medial part of telencephalons neuroepithelium is lamina epithelialis. Immediately above the lamina epithelialis the wall of the hemisphere is thickened, thus forming limbus, the origin of hypopocampus. From that side in caudal direction towards diencephalon thin wall of cerebral hemispheres protrudes to lateral ventricle. It is origin of the future plexus choroides that is a abundantly vascularised epithelo-mesenchymal structure. It is also called area epithelialis. Ventricle cavity near telencephalon impar is still very wide. The line of demarcation between telencephalon and diencephalon becomes

Figure 3. Schematic drawing of frontal section of the rat telencephalon at E14.
complex. A groove, the telodiencephalic sulcus, divides forebrain into a ventral and dorsal region and telencephalon and diencephalon, respectively. This sulcus telodiencephalicus appears from ventricular side. For the first time cerebral hemispheres partially cover diencephalon in rostrodorsal direction. Neuroepithelial wall of diencephalon shows thickenings in the zone of hypothalamus origin and that a downward extension, the infundibulum, and the mammillary body which forms a distinct protuberance on the ventral surface of the hypothalamus on each side of midline. In the infundibular part of diencephalon origin of neurohypophysis appears for the first time.

At 15th day of gestation (E15) telencephalic vesicles are growing in their basal, lateral and dorsal parts. Interhemispheric groove becomes deeper and origins of brain hemispheres are better expressed because of that. Neuroepithelial wall, on the bottom of interhemispheric sulcus, is extremely thin. It is a very thin lamina tectoria s. area epithelialis in area of telencephalon impar, on the tectorial lamina and limbus of hemisphere connecting site, mesenchyme together with thin neuroepithelial wall spread in direction to telencephalic ventricular cavity. Mesenchymal blood vessels form a rich capillary plexus that lies close against the thin neuroepithelium and push it into the ventricular lumen as a fingerlike processes. It is origin of plexus choroideus (Fig. 4). Basolateral part of telencephalic vesicles (ganglionic hill) is thicker than in E14. It is origin of basal ganglia. Telencephalon partially covers basomedial and posterior part of diencephalon. Sulcus telodiencephalicus is situated between telencephalon and diencephalon. Origins of thalamus and epithalamus are well expressed in diencephalon. At 16th day of gestation (E16) all forebrain parts, especially telencephalic vesicles (origin of the brain hemispheres) and processes of plexus choroides, are progressively growing and shaping. Fingerlike shaped processes of plexus choroides partially fill ventricle cavities (Fig. 5). Mesenchymal stroma with numerous blood vessels follows epithelial folds. In the area of diencephalon, thalamus, epithalamus and hypothalamus are well expressed. Thalamus is still morphologically undifferentiated.

**DISCUSSION**

The formation of the vertebrate nervous system during embryogenesis is a contingent in a close relation between the invaginating chorda-mesoderm and the overlying ectoderm. As a result, the ectoderm is determined to become the neuroectoderm-precursor of the nervous system. This sort of interaction, in which one tissue directs another to differentiate in a way it otherwise would not, is called induction. The induction of the neuroectoderm by chorda-mesoderm is only one link in a cascade of inductive interactions involved in determining neural structures and associated tissues. (13) In rats embryos neural tube is already completely enclosed on its cranial part on 12th day of the development. Once the neural tube is closed, its walls are subject of the pressure of contained fluid providing the formation of fluid to be greater than absorption. The fluid may result in rostrocaudal enlargement and widening of the brain. Such a mechanism would be expected to contribute to the shaping of the neural tube and to preserving it from collapsing, although the main contribution to growth is from mitotic activity. From three prominent vesicles on its cranial part brain develops. Rostral vesicle, prosencephalon, divide onto telencephalon and diencephalon. Primarily, telencephalon is a vesicle on the rostral end of the neural tube, but very soon, lateral prominences begin to develop on its both sides as future cerebral hemispheres. (14) During development, the structure of the brain, especially telencephalon, is dramatically transformed by region-specific proliferation and differentiation of the neuroepithelial cells. (15) Changes of the external and internal forebrain shape are results of the complex histogenetic events and internal forming of the brain vesicles’ wall. (16) Our morphologic analysis describes significant morphogenetic changes in the forebrain shape. The forebrain changes from a relatively simple tubular structure with thin walls surrounding a large ventricular system to a thick-walled brain with a highly convoluted, but reduced ventricular system.
REFERENCES

(16) Kostović I. Razvitak i građa mo`dane kore. JUMENA, 1979; Zagreb.
ABSTRACT

Endodontic pathology is a bacterial disease. It is well established that periapical disease is the result of bacteria, their product, and the host response to them. Periradicular disease will occur after microorganisms and their metabolic products affect the periradicular tissue. Aim of using antibiotics as part of a treatment regimen is to achieve, within the periodontal environment, a concentration of the drug that is sufficient either to kill (bactericidal) or arrest the growth (bacteriostatic) of pathogenic microorganisms. There are two possible approaches to improve the drug action: sustained and controlled drug release to reduce or eliminate side effects by improving the therapeutic index and site-specific drug delivery to minimize systemic effects. These two strategies have been explored by the association of drugs with different vehicles, either naturals or synthetics.

A wide variety of specialized local delivery systems (i.e. intrapocket devices) have been designed to maintain the antibiotic in the GCF (gingival crevicular fluid) at a concentration higher than the MIC (minimum inhibitory concentration). Fibres, films, strips and microparticles made of biodegradable or non-biodegradable polymers have been reported as effective methods to administer antibacterial agents for periodontal therapy. Together with these solid devices, semisolid adhesive or non-adhesive formulations have also been proposed.

Key words: local delivery, antibiotics, delivery devices, periodontal, root canal.

INTRODUCTION

Endodontic pathology is a bacterial disease. It is well established that periapical disease is the result of bacteria, their product, and the host response to them. Histological studies in general, have not been able to demonstrate viable bacteria in periapical lesions. These findings persist to the present time. Nowadays, evidence indicates that many of these lesions are indeed infected before, and after endodontic treatment. Iwu showed that 88% or 14 of 16 periapical granulomas were positive for bacteria when they were homogenized and cultured (1).

In 1992 Wayman (2) studied 58 cases of periapical lesions. He cut these lesions in half and examined one half histologically and cultured the other half. In only 8 of 58 cases could he demonstrated bacteria histologically. However, when the other half lesion was cultured 51 of 58 cases were positive. He found 133 isolates, of which 87 were strict anaerobes, 37 were facultative anaerobes and only 9 were aerobes. The bacteria (3) were found not only in periapical abscess but also in granulomas and cysts.

Microorganisms vary in their pH tolerance ranges, and most human pathogens grow well within a range of 5 to 9 pH (4). Some strains of Escherichia coli, Proteus vulgaris, Enterobacter aerogenes and Pseudomonas aeruginosa can survive in pH 8 or 9 (5). These bacterial species have occasionally been isolated from infected root canals, usually causing secondary infections (6). Certain bacteria, such as some enterococci, tolerate very high pH values, varying from 9 to 11. Fungi generally also exhibit a wide pH range, growing within a range of 5 to 9 pH (5). It has been demonstrated that enterococci and fungi are highly resistant to calcium hydroxide (7, 8). Since these microorganisms are commonly found in cases of endodontic failure, the routine use of calcium hydroxide should be questioned.

Endodontic infections are polymicrobial, and no known medicament is effective against all the bacteria found in infected root canals. In addition, the medicament should ideally reach microorganisms located in distant areas of the root canal system in lethal concentrations. Antibiotic therapy has been used for years as an adjunct to periodontal treatment. One of the most promising recent advances in periodontal therapy has been the development of sustained-release delivery systems to administer antibiotics directly to the periodontal pocket. Locally delivered antibiotics overcome many of the disadvantages that we see with systemic drugs. There are specific guidelines and indications for the use of locally delivered antibiotics as adjuncts to periodontal therapy in dental practice.

Periradicular disease will occur after microorganisms and their metabolic products affect the periradicular tissue. The magnitude of the host response will be directly proportional to the virulence and the number of microbial cells present. Tissue damage caused by bacteria is mediated by either direct or indirect mechanisms. Direct

ENDODONTIC-PERIODONTAL LOCALLY DELIVERED ANTIBIOTICS

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harmful effects caused by bacteria involve their products, such as enzymes (collagenase, hyaluronidase, condroitinase, acid phosphatase), exotoxins and metabolites (bytrate, propionate, ammonium polyamines, sulphured compounds). In addition, bacterial components such as peptidoglycan, teichoic acid, fimbriae, outer membrane proteins, capsule, and lypopolysaccharide, stimulate the development of host immune reaction capable of causing severe tissue destruction (9, 10, 11). For example, macrophages can be activated by bacterial components and can be stimulated to release chemical mediators such as cytokines (interleukin-1b, tumours necrosis factor, and interleukin-6), and prostaglandins, which are involved in the induction of bone resorption commonly observed in chronic periradicular diseases (10,11). Recently, it has been demonstrated that bacterial DNA may activate macrophages and dendritic cells triggering release of pro-inflammatory cytokines (12). A nother example refers to the tissue damage associated with the acute periradicular abscess. Host defense mechanisms against bacteria aggressing from the root canal appear to be the most important factor involved in the pus formation associated with acute periradicular abscess. Formation of oxygen derived free radicals, such as superoxide and hydrogen peroxide, together with the release of lysosomal enzymes by polymorphonuclear neutrophils, such as elastase, collagenase, and gelatinase, induce the destruction of the extracellular matrix, leading to the pus formation (13). Therefore, bacteria can exert indirect destructive effects, which seems to be more significant in the tissue damage associated with acute and chronic periradicular lesions.

**DRUG DELIVERY DEVICES**

There are two possible approaches to improve the drug action:

- sustained and controlled drug release to reduce or eliminate side effects by improving the therapeutic index;
- site-specific drug delivery to minimize systemic effects.

These two strategies have been explored by the association of drugs with different vehicles, either naturals or synthetics.

Drug delivery systems can be classified according to the mechanism controlling drug release. We distinguish three categories:

- “solvent controlled” matrix systems based on macromolecular matrix permeability to small molecules after matrix swelling into hydrated medium;
- “reservoir systems” controlled by drug diffusion across a polymeric membrane;
- “chemically controlled systems” where the rate of drug release is controlled by the rate and extent of degradation of chemical bonds and the erosion of the polymeric matrix.

For all these systems, the basic polymer can be of natural origin such as proteins (14) or collagen (15), semi-synthetic such as cellulose derivatives (16,17) or synthetic, all of which must preferably degrade during use. Natural polymers have been considered as biodegradable carriers (18). However, most of them have disadvantages inherent to their structure, including limited half-life, complexity of composition and immunogenicity due to the polymer itself or to its degradation by-products. Many polymer-based systems for antibiotic delivery in the treatment of periodontal diseases have been studied and evaluated in vitro and/or in vivo.

**PERIODONTAL LOCAL DELIVERY DEVICES**

Local delivery devices were widely studied for various applications. Table 1 includes a list of advantages and potential disadvantages of controlled release devices. Regardless of the carrier system used, a candidate polymer for the design of a controlled delivery system must comply with a range of characteristics valid for most biomaterials:

- it must be free of elutable impurities, additives, stabilizers, catalyst residues, and emulsifiers;
- with the exception of bioerodable systems, the physical, chemical, and mechanical properties of the polymer should not be altered by the biological environment;
- it must have sufficient mechanical and thermal stability;
- it must be able to be readily processed, cast, or moulded in films, rods, tubing systems, and so forth;
- the material should not be carcinogenic, toxic, or inflammatory;
- the system must be able to be sterilized or prepared under aseptic conditions.

A wide variety of specialized local delivery systems (i.e. intrapocket devices) have been designed to maintain the antibiotic in the GCF (gingival crevicular fluid) at a concentration higher than the MIC (minimum inhibitory concentration). Fibres, films, strips and micro particles made of biodegradable or non-biodegradable polymers have been reported as effective methods to administer antibacterial agents for periodontal therapy. Together with these solid devices, semisolid adhesive or non-adhesive formulations have also been proposed.
Table 1. Main advantages and potential disadvantages of controlled delivery systems (CDS) for the treatment of periodontitis

<table>
<thead>
<tr>
<th>Advantages of CDS</th>
<th>Disadvantages of CDS</th>
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<tbody>
<tr>
<td>Maintenance of drug levels in a therapeutically desirable range</td>
<td>Toxicity or lack of biocompatibility of the polymer material</td>
</tr>
<tr>
<td>Reduction or elimination of harmful side effects of drugs</td>
<td>Pain caused by the presence of the implant</td>
</tr>
<tr>
<td>Protection from degradation of drugs with short in vivo half-lives</td>
<td>Production of harmful by-products from a polymer if it is biodegradable</td>
</tr>
<tr>
<td>Improved patient compliance</td>
<td>Need of surgical procedure to implant the device in the appropriate location</td>
</tr>
<tr>
<td>Elimination of patient discomfort compared to parenteral administration</td>
<td>Expense of a particular polymer-drug formulation</td>
</tr>
<tr>
<td>Improved drug administration in geographic areas with low medical supervision</td>
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Root canal therapy
The most important elements of root canal preparation are effective access and aseptic biomechanical preparation. Medicaments such as Ledermix paste and calcium hydroxide pastes have been recommended as routine intracanal medicaments.

Early investigations evaluated two antibiotic-containing preparations: Grossman’s polyantibiotic paste, which contains penicillin, bacitracin or chloramphenicol and streptomycin (19) and the other a mixture of neomycin, polymixin and nystatin (20). Both of these had some efficacy as intracanal medicaments. A more recent study has shown that clindamycin gave no advantage as a root canal dressing when compared with conventional root canal dressings (21). Further in vitro investigations have produced more favorable results with antibiotic mixtures such as ciprofloxacin, metronidazole and minocycline that were used as topical root canal agents (22, 23).

However, the consensus of clinical opinion is that calcium hydroxide is the most appropriate agent for the purpose of controlling bacterial activity (24). Even though, it has been demonstrated that enterococci and fungi are highly resistant to calcium hydroxide (7, 8). Since these microorganisms are commonly found in cases of endodontic failure, the routine use of calcium hydroxide should be questioned.

Medication used inside root canals:
Kenacomb: corticosteroid, antibiotic cream (Bristol-Myers Squibb Company, Cairo, Egypt) purchased on the open market. Each gram of the cream contained the following:
- Nystatin (mycostatin), 100 000 units
- Neomycin (as neomycin sulphate), 2.5 mg
- Gramicidin, 0.25 mg
- Triamcinolone acetonide 1.0 mg
These ingredients were combined in an aqueous cream base.

ADVANTAGES AND DISADVANTAGES OF LOCAL DELIVERY

<table>
<thead>
<tr>
<th>Advantages of local delivery when compared to systemic delivery of antimicrobial agents:</th>
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<tbody>
<tr>
<td>higher concentration</td>
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<tr>
<td>fewer side effects</td>
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<tr>
<td>sustained / controlled delivery</td>
</tr>
<tr>
<td>patient compliance</td>
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<table>
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<tr>
<th>Disadvantages of local delivery when compared to systemic delivery of antimicrobial agents:</th>
</tr>
</thead>
<tbody>
<tr>
<td>more expensive</td>
</tr>
<tr>
<td>more time consuming</td>
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<tr>
<td>no effect on bacterial reservoirs</td>
</tr>
</tbody>
</table>

TYPES OF LOCAL ANTIBIOTIC THERAPY WITH SLOW RELEASE DEVICES

A. Tetracycline impregnated fibres
- FDA approved as Actisite (Alza Corp., USA) in the United States (25)
- controlled release-10 days
- demonstrated to decrease pocket depth, increase attachment gain and reduce periodontal disease recurrence
- non-resorbable
- comparison-tetracycline
Local delivery
12.2 mg / fibre
Ten days
Total: 12.5 mg
Concentration > 1,300 µg / ml

B. Doxycycline gel for subgingival delivery.
- FDA approved as Atridox (Atrix, The Block Drug Co.) for use in the United States
- Sustained release: 27 days resorbable
- 8.5% doxycycline; 420 µg/ml in GCF

C. Metronidazole gel for subgingival delivery
- Sustained release: one day resorbable
- Elyzol (Durnex, Denmark)
- 25% metronidazole; 24 hrs > 1 µg/ml in GCF

D. Minocycline gel or powder (2%) for subgingival delivery
- Dentomycin- Great Britain, Periocline - Japan (Cyanamid International)
- Sustained Release: resorbable
- 2% Minocycline

E. Indications for Local Delivery of Antibiotic Agents
- Adult periodontitis - localized pockets 5 mm with bleeding
- Pocket in anterior area of mouth, where if periodontal surgery done, it may pose all aesthetic problem
- Recurrent/Refractory periodontitis
- Medically compromised patients where periodontal surgery is not indicated

**Tetracyclines**
The tetracyclines are a group of broad-spectrum antibiotic agents that were introduced into clinical practice in the late 1940s. There are now numerous compounds on the market, all based on the congeneric derivatives of the polycyclic naphthacene carboxamide (26). Tetracycline, doxycycline and minocycline are used extensively in the management of periodontal diseases. They are bacteriostatic antibiotics, which interfere with bacterial protein synthesis and also inhibit tissue collagenase activity (27). They have a broad spectrum of activity inhibiting both Gram-negative and Gram-positive organisms, including the beta-lactamase producing strains which occur in approximately 50% of 6-7 mm deep periodontal pockets and against which penicillins are ineffective. Tetracycline analogues such as doxycycline and minocycline, although more expensive, have a number of theoretical advantages over tetracycline. They exhibit greater oral absorption, they have more prolonged half-lives, and they show enhanced lipid solubility, which is important for their antibacterial action (26). The inhibitory effect of tetracycline on oxygen radicals may also prevent a wider spectrum of tissue destruction. Thus, tetracyclines may have general antiproteolytic properties.

**Anticollagenase inhibition**
In addition to the antibiotic effects of tetracyclines, a further mechanism has been proposed to explain their efficacy in the treatment of periodontal disease, notably their anticollagenase action (28). This action appears to be related to the source of the enzyme and the tetracycline used. Doxycycline is the most potent tetracycline for collagenase inhibition. Collagenases derived from neutrophils (mature metalloproteinases-8) are more susceptible to a tetracycline-induced inhibition while collagenases derived from human fibroblasts or gingival cervical fluid collagenase harvested from deep periodontal pockets appear to be more resistant to the drug.

Tetracycline inhibition of collagenase may relate to the drug’s ability to bind with calcium and zinc ions (28). Zn$^{2+}$ are located at the active site of the enzyme, whilst Ca$^{2+}$ are on an exogenous co-factor. A further mechanism may be associated with the ability of the tetracyclines to scavenge reactive oxygen radicals (e.g. hydroxyl groups or hypochlorous acid) produced by PMNs. These oxygen radicals activate latent collagenases. Inhibition of collagenase may result in further antiproteolytic effects such as inactivation of a-1 proteinase inhibitor and neutrophil elastase.

**Metronidazole**
Among the antibiotics that have been considered for periodontal treatment, metronidazole has often been chosen because of its selective efficacy against obligate anaerobes (29). Metronidazole acts by inhibiting DNA synthesis. It is known to convert into a reactive reduced form and affects specifically anaerobic rods and spirochetes in the subgingival microflora. Metronidazole has also been successful in refractory and advanced cases when used for a 1-week period (16). Other studies reported that adjunctive metronidazole therapy was more effective in adults with deep pockets than with less advanced periodontitis (30).

**Clindamycin**
Clindamycin has been investigated for treatment of periodontal disease in a limited number of studies (31, 32). Systemic clindamycin therapy, as an adjunct to scaling, decreased the incidence of active disease from an annual rate of 8.0 to 0.5% of sites per patient (33, 34).
Following gel insertion of clindamycin in conjunction with subgingival scaling, motile rods and spirochetes were not detected after 1 month. Prevotella intermedia and Porphyromonas gingivalis were eliminated or below detectable levels after 1 week.

CONCLUSION

Antibiotic therapy has been used for years as an adjunct to periodontal treatment. One of the most promising recent advances in periodontal therapy has been the development of sustained-release delivery systems to administer antibiotics directly to the periodontal pocket. Locally delivered antibiotics overcome many of the disadvantages which we see with systemic drugs. There are specific guidelines and indications for the use of locally delivered antibiotics as adjuncts to periodontal therapy in dental practice.

Endodontic infections are polymicrobial, and no known medicament is effective against all the bacteria found in infected root canals. In addition, the medicament should ideally reach microorganisms located in distant areas of the root canal system in lethal concentrations.

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REFERENCES

(10) Henderson, B., Poole, S., Wilson, M. Bacterial modules: A novel class of virulence factors which cause host tissue pathology by inducing cytokine synthesis. Microbiol. Rev. 1996; 60: 316-341
A plication of a local drug delivery system to periodontal therapy. I. Development of collagen preparations 

81: 533-543.

(17) Paquette D.W., Waters G.S., Stefanidou V.L., Lawrence H.P., Friden P.M., O’Connor S.M., Sperati J.D., 
Oppenheim, F.G., Hutchens L.H., Williams R.C. Inhibition of experimental gingivitis in beagle dogs with 

(18) M.Cole A.D., Tolentino L., Tozer T.N. 
Glucocorticoid-dextran conjugates as potential prodrugs for colon-

(19) Curson I. 
120: 381-383.


(21) Molander A., Reit C., Dahlen G. 

(22) Hoshino E., Kurihara A ndo N., Sato I., Uematsu H., Sato M., Kota K., Iwaku M. 
In vitro antibacterial susceptibility of bacteria taken from infected root dentine to a mixture of ciprofloxacin, metronidazole and 

(23) Sato T, Hoshino E, Uematsu H, Noda T. 

(24) Fava L.R.G., Saunders W.P. 

(25) Niderman R., Abdelshehid G., Goodson J.M. Periodontal therapy using local delivery of antimicrobial 

(26) Seymour R.A., Heasman P.A. 

(27) Yu Z., Ramamurthy N.S., Leung M., Chang K.M., M Namara T.F., Golub L.M. 

(28) Seymour R.A., Heasman P.A. 

(29) Noyan U., Yilmaz S., Kuru B., Kadir T., A car O., Buget E. 

(30) Fiorellini J.P., Paquette D.W. 

(31) Higashi K., Matsushita M., M orisaki K., Hayashi S.I., M ayumi T. 

(32) Sauvetre E., Glupczynsky Y., Yourassowsky E., Pourtois M. 
The effect of clindamycin gel insert in periodontal pockets, as observed on smears and cultures, Infection 1993; 21: 245-247.

(33) Gordon J., Walker C., Hovilas C., Socransky S. 

J ung J., Clark W.B. 
21: 628-637.
Instructions for preparation of manuscripts to be published in extenso in Bosnian Journal of Basic Medical Sciences

Submission: Only papers written in correct English are considered. Manuscripts must be typewritten in triplicate (with three sets of illustrations of which one is an original), double spaced on one side of the paper with a 2.5 cm wide margin on top, bottom and both sides, accompanied by the identical file on a diskette.

Original research papers: Submitted manuscripts should be fully documented reports of original research. They must contain significant and original observations to be critically evaluated.

Short communications: These manuscripts should not exceed 2 printed pages (i.e. 5 manuscript pages), including an abstract essential references and not more than 3 tables or figures. Short communications should represent complete, original studies and should be arranged in the same way as full length manuscripts.

Leading articles and editorials are solicited by the Editorial Board with the aim to bring to the general readership pressing topics in life sciences and related environmental and bioethical dilemmas.

Review articles and viewpoints: Authors who wish to contribute a manuscript to this category are encouraged to contact the Editor-in-Chief. Reviews should be focused on topics of current interest. Viewpoints should offer a more personalized perspective on a topic that will be of interest to the general readership. All contributions to those categories will be subject to editorial review.

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CONDITIONS: All manuscripts will be reviewed by the editor and two referees in order to expedite review, authors may suggest three to five potential reviewers with their addresses. Manuscripts are received with the explicit understanding that they are not under simultaneous consideration by any other publication. A cover letter with the name, postal address, postal code, telephone, fax and E-mail numbers of the corresponding author must accompany each manuscript. This letter must include a statement confirming that all authors concur with the submission. The contents of BOSNIAN JOURNAL OF MEDICAL SCIENCES may be reproduced without permission provided that credit is given to the journal. It is the author’s responsibility to obtain permission to reproduce illustrations, tables, etc. from other publications.

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First page - A concise but informative full title of the article. Avoid abbreviations and colloquialisms.

Second page - A condensed title (running title) of no more than 70 letters and spaces. Name(s) of author(s). Write first names in full. Complete address of the laboratory (institution) for each author.

Third page - footnotes to the title, if any. List of any non-standard abbreviations.

Fourth page - An Abstract (no more than 250 words) must be divided into four separate sections: background and purpose, materials and methods, results and conclusions, and should be a factual condensation of the entire work including a statement of its purpose, a clear description of the findings and finally a concise presentation of conclusions.

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Number the remaining pages consecutively and type the author’s(s) last name(s) at the top of each page. Write in the first person (except summary) and the active voice whenever possible.

Keep the INTRODUCTION brief, stating clearly the purpose of the article and its relation to other papers on the same subject. Do not give an extensive review of literature.

Provide enough information in the MATERIAL AND METHODS section to enable other investigators to repeat the experiments.

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Start each section on a separate sheet.
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Tables and illustration: Tables and illustrations (both numbered in Arabic numerals) should be prepared on separate sheets. Tables require a heading, and figures a legend. Only good drawings and original photographs can be accepted: negatives or photocopies cannot be used. On the back of each illustration, indicate its number, the author’s name, and “top”. Colour illustrations can now be integrated within the text and are reproduced at the author’s expense. Each table and illustration must have all the necessary information to be understood independently of the text.

Submit a drawing twice the final size. Lettering and identifying marks should be clear and eligible after reduction. We prefer either an original drawing in India ink on white drawing or tracing paper or an electronic printout. Submit glossy prints of good quality. Write lightly in pencil the author’s name and figure number (indicate top) on the back of each illustration.

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