Brachial Plexus Treatment
Miguel Pirela-Cruz, Mirza Mujadžić, Enes Kanlić ............................................. 7

Updates in Flexor Tendon Repair at Zone II
Mirza Mujadžić, Miguel Pirela-Cruz, Enes Kanlić ............................................. 16

In Vitro Modeling of the Influence of FVIII Activity and
Heparin Induced Prolongation of APTT
Aida Mehmedagić, Sebnur Skrobi, Dženita Softić, Muhidin Haračić ......................... 26

Angiography Analysis of Variations of the Posterior
Segment of the Circle of Willis
Albina Voljevica, Amela Kulenović, Eldan Kapur, Ibrana Vuceković ......................... 30

The Relationship Between Suicidal Thoughts and Psychoactive Substances
Mira Spremo, Slobodan Loga .............................................................. 35

Heart Murmur and Anaemia in the Pediatric Population
Senka Mesihović-Dinarević, Jasna Ibrahimović, Edo Hasanbegović, Aida Smajić .......................... 39

Serotesting of Human Brucellosis on Wider Area of Bosnia and Herzegovina
Sadeta Hamzić, Edina Bešlagić, Šukrija Zvizdić, Mufida Aljičević, Omer Bešlagić, Sandra Puvačić .... 46

Long Term Protection After Hepatitis B Vaccination
Sandra Puvačić, Jelena Ravlija, Zlatko Puvačić .................................................. 50

Cytogenetics Findings at Turner Syndrome and
their Correlation with Clinical Findings
Amra Ćatović ................................................................. 54

Serum and Tissue Angiotensin Converting
Enzyme in Patients with Lichen Planus
Faruk Alendar, Jasminko Huskić, Nermina Babić, Nedžad Mulabegović ..................... 59

Oral Acute Toxicity of Polyenylphosphatidylcholine (PPC) in Rats
Sanja Krošnjar, Maida Todić, Sanela Bakić, Begler Begović, Irfan Zulić, Midhat Vehabović .................. 63

Branching of Main Trunk of Left Coronary Artery and Importance
of Her Diagonal Branch in Cases of Coronary Insufficiency
Almira Lujinović, Fehim Ovčina, Alma Voljevica, Aida Hasanbegović ..................... 69

Modified Pair Technique for Treatment of Hydatid Cysts in the Spleen
Erver Zeren, Amir Niahanović, Jasmin Cukić .................................................. 74

Detection of Neurovascular Structures Using Injection
Pressure in Blockade of Brachial Plexus in Rat
Ibrana Vuceković, Admir Hadžić, Faruk Dilberović, Amela Kulenović, Zakira Mornjaković, Irfan Zulić, Kučuk Alija Đosanović, Eldan Kapur, Esad Čosović, Albia Voljevica .................................................. 79

Significantly Reduced Salivary Nitric Oxide Synthesis
in Patients with Parkinson’s Disease
Jasminko Huskić, Alina Paperniku, Azra Husić, Faruk Alendar, Nedžad Mulabegović ........ 86

Instructions for preparation of manuscripts to be
published in extenso in Bosnian Journal of Basic Medical Sciences ..................... 90
EDITORIAL

Since May 2005 when the last issue of BJBMS was published, several distinguished scientists, founders of this Association that sprouted from the debris created by the tornado of human misunderstandings, made their final pass to the other side of the turbulent river of life. Over one thousand days that Sarajevo was under the siege, its proud citizens, joined more than ever through the unexpected evil, maintained all the forms of undergraduate and graduate education at the University, fully aware that any day may be their last.

Apart from participating in realization of numerous research projects, at the time supported by Open Society Fund “SOROS”, these modest great people significantly contributed to the establishment of an indexed journal. Today, it mostly publishes papers by young researchers.

In their respective environments where they acted as teachers and scientists all of them became parts of history that will be remembered by future generations. Pioneer work of these first graduate physicians, pharmacists and biologists after the World War II, set foundations for the establishment of institutions of science and education - institutes, chairs, biomedical faculties in Sarajevo and other centers.

Professor Dr. Ilduza Bubić-Huković (15 April 1927-16 May 2005) was the first graduate physician to elect anatomy as her future scientific and academic field of interest. Together with her husband, she studied morphology of isolated organs with modesty and success. With and without accompanying nerves, as politissue test models, they were generously used throughout the world in experimental research in basic medical science. Other than for her impressive scientific achievements, numerous generations remember her as an excellent educator as well as parent.

Professor Dr. Irfan Zulić (13 January 1932 - 26 May 2005), Head of the Institute for Pharmacology and Toxicology followed in the footsteps of legendary Academy members Pavao Stern and Seid Huković. He continued with modernization of scientific and academic foundations in order to provide young researchers with solid basis for competent work in the 21st century. Therefore, the title of The Humanist of the Decade, awarded to our deceased Chairman of the Steering Board and Editor in Chief by the International Ligue of Humanists, was fully warranted. In front of his office door numerous people of various academic and social status waited to see him every day, regardless of having an appointment. Most of those belonged to the group of the “humiliated and offended” and they frequented the man they trusted and envisaged as a wizard who was able to help in seemingly impossible situations. The master of human souls convincingly, gently, unobtrusively talked a person to the nirvana leaving him with a feeling that everything became less difficult, impossible made possible, irresolvable made solvable, obscure made clear, anxiety was lifted and resolution of psychological turmoil imaginable. Numerous associations, institutions, societies and citizens’ associations within our country and the world lost a distinguished member and a promoter of numerous leading scientific initiatives that served the purpose of the mankind.
Professor Dr. Branko Nikolin (20 October 1932 - 9 June 2005) began his professional career at the School of Medicine Institute of Chemistry. As one of the founders of the Faculty of Pharmacy and Head of Institute for Drugs Control he continued intensive work on education of young scientists and educators. The city of Sarajevo was fortunate to have an expert of his qualifications within its boundaries. During the Olympics he facilitated doping control of the contestants which, at the time, presented a serious issue for much wealthier countries. His internationally acknowledged name was a guaranty of quality of teaching, education and science. He is respectfully spoken of by famous scientists such as Professor Dr. J. M. van Rossum.

He was one of those who acted in the spirit of saying by Preradović "... actions - actions without words". He will be gladly remembered by all of his students and will never be forgotten by those whose academic careers he facilitated and selflessly taught the most modern methods of the complex basic science he dedicated his life to. Professionals to be proud of and solid basis for future research are his most valuable legacy.

Academician Aleksandar Nikulin (28 September 1926-18 June 2005), Head of Institute of Pathology continued modernization of the Institute, education of young professionals and establishment of solid basis for scientific work presentable at the international market. Today, the Institute is one of the most modern units of Sarajevo School of Medicine and by using telediagnostic methods of communication, it is able to provide joint consulting with the most distinguished experts in the world at any moment in time.

Professor Dr. Ljubo Radić (26 April 1937-25 June 2005) from Tuzla was one of the founders of UBMZ and the first Head of Institute for histology and embryology in Tuzla. He was engaged at the School of Medicine in Tuzla since its establishing where, acting as Vice Dean, he significantly contributed to the development of educational process.

Please be informed that at the ABMS/UBMZ Assembly Session held on 13 August 2005, after the due respects to the deceased members were paid, a new Steering Board, Editorial Board and Editorial Council were appointed and entrusted with the duty to continue publication of four issues of the Journal per year.

Sarajevo, August, 2005

EDITORIAL BOARD

This journal is indexed in: CAB Abstract / Global Health databases and Index Medicus / MEDLINE.
Dr. Irfan Zulić, retired professor of University of Sarajevo School of Medicine has taken the road of no return.

The life of professor Irfan Zulić that was lived in honor and dignity, in full recognition of his expert, scientific and academic achievements, started somewhat over 73 years ago in Čapljina and ended in Sarajevo. Within the life span of 73 years, which modern medical standards do not classify as very old age, professor Irfan Zulić exhibited organizational skills that are rarely achieved and carried out numerous duties. One of those is the position as the Head of the Institute of pharmacology and toxicology at University of Sarajevo School of Medicine where he remained for 13 years, from 1990 till his retirement in 2003.

As his first years in that position concurred with the period of war 1992-1995 it needs to be underlined that such unfortunate, unpleasant and difficult periods have only one good feature, they ensure that people, sooner or later, exhibit their true nature and show themselves in the true light. In the course of the past war, professor Irfan Zulić passed that test immaculately and with ultimate success. Thus, he proved an ancient saying that in war bad people show their worse and good people show their best. And professor Irfan Zulić was above all a good man. Therefore, when International Ligue of Humanists designated him as the Pharmacologist of the Decade in 2002 nobody was surprised. We will remember his selfless support, care and boundless compassion, regardless of the religion and nationality, throughout his mandate as Head of the Institute. During the war these qualities of his carried specific weight. Everything professor Irfan Zulić did was accompanied by unavoidable “no problem” which, uttered by him, neither did sound nor was empty or false.

Professor Irfan Zulić dedicated plenty of his time and energy to the education and development of young experts, scientists and educators. Due to his expert and academic qualities, professor Irfan Zulić was made a member to numerous boards, committees, associations and supervisory bodies within the City, Canton, Federation and the State. He was the Chairman of B&H Physicians’ Association, the last Chairman of the Union of Yugoslavian Physicians’ Associations and, until his death, he carried the duty of the Chairman of Federal Committee on Drugs.

The Ancient Romans bequeathed us with a proverb “Mors omnia aequat” - “Before death we are all equals”, that cannot be applied to professor Irfan Zulić in any way conceivable.
For, few people are able to make such a deep mark in the souls of those who had privilege, honor and pleasure to know them as professor Irfan Zulić was able to. With his personal example, high professionalism, and above all his optimism, courage and dignity during his long and difficult illness, he has left us with high moral and human standards to contemplate and admire.

It took a lot of courage and human integrity to walk that path with such a dignity, the same path that he himself selflessly assisted and supported his patients, acquaintances and friends along, knowing that at the end of that path awaits a rival named Tànatos by the Ancient Greeks. Professor Irfan Zulić fought against the same rival continuously and often successfully throughout his life and professional carrier for lives of all the people that asked for his professional advice, recommendation, support and help.

And it must have been painful and piercing, very painful and very piercing, to learn that many battles for lives of his patients, friends and acquaintances were won due to his aid and dedication while the one fought for his own life was lost. And while his life was slowly expiring and the last specks of sand were slowly running in the hourglass of his life, his lifelong rival, the unmerciful Tànatos, waited, persistent and resolute, for her ultimate victory. Unwavering, as only death can be.

Professor Dr Dubravka Potkonjak
BRACHIAL PLEXUS TREATMENT

MIGUEL PIRELA-CRUZ¹, MIRZA MUJADŽIC², ENES KANLIĆ¹

The Department of Anesthesiology, St. Luke's-Roosevelt Hospital Center, College of Physicians and Surgeons of Columbia University, New York, NY 10025, USA

1. Department of Orthopaedic Surgery at Texas Tech University Health Sciences Center in El Paso, 4800 Alberta Avenue El Paso, Texas, 79905-2700, USA
2. Christine Kleinert Institute for Hand and Microsurgery, University of Louisville School of Medicine, 225 Abraham Flexner Way Ste 650, Louisville, KY 40202-1888, USA

* Corresponding author: Miguel Pirela-Cruz, MD, FACS, Department of Orthopaedic Surgery at Texas Tech University Health Sciences Center in El Paso, 4800 Alberta Avenue El Paso, Texas, 79905-2700, USA

ABSTRACT

Brachial plexus injuries are devastating injuries that affect primarily young healthy males. For the total plexus injury, current surgical treatments have failed to achieve normal restoration of limb function but some practical goals are obtainable. This review article summarizes existing logic and approach for managing these catastrophic injuries.

KEY WORDS: Brachial plexus, treatment

HISTORY

The earliest reports of the surgical management of brachial plexus injuries appeared around the beginning of 20th centuries. Thoburn (1) in 1903 described a surgical repair of an injury to the brachial plexus. In the early 1900s Taylor (2) presented a series the surgical results of plexus surgery with significant functional improvement. Similarly, Davis, et al. (3) reviewed the surgical treatment of brachial plexus injuries and suggested that significant functional improvement could be expected after surgery. This initial enthusiasm for brachial plexus surgery in the early part of the century was followed by a pessimistic outlook as several reports documented poor results with surgical intervention (4,5). Even in 1963, Seddon (6) condemned brachial plexus surgery except for injuries to the upper trunk with complete palsies, which had a hopeless prognosis. Moreover, Yeoman (7), in 1961, proposed the amputation of the arm associated with arthrodesis of the shoulder.
Many surgeons, however, were less aggressive. Hendry (8) was definitely against amputation. He favored, when feasible, a partial reanimation of the paralyzed limb through musculotendinous transfers. Midway between these extreme attitudes of suppressing an extremity or performing limited reconstruction, at that time, there were still surgeons resorting to multiple arthrodeses such as the early fusion of the shoulder in C5,C6 lesions and, in exceptional cases, an arthrodesis of the elbow and wrist in C5, C6,C7 and C7, C8, TI lesions. Renewed enthusiasm for operative management of these injuries awaited the introduction of microneurosurgical technique and nerve grafting. Millesi pioneered the use of nerve grafts to manage these injuries (9). With the development of improved techniques, improved results followed. Kline and Nulsen (10) Narakas (11), Gilbert and Tassin (12) Brunelli (13), Merle and Deburge (14),Alnot et al (15), Allieu (16), Leffert (17) and Terzis (18) and have all made important contributions toward the operative management of brachial plexus injuries.

**DECISION MAKING**

1. **Immediate surgery** (within 24 hours) should be done in acute cases where sharp injury is present or vascular surgery is required because of major vessel injury.
   a) If the nerve is found to be sharply transected, then it should be acutely repaired. If a blunt injury has occurred, and the nerve appears injured, the stump should be tagged and a delayed repair performed at 2 to 4 weeks.
   b) Gunshot wounds should be immediately explored only if vascular surgery is required. Otherwise, observation and delayed surgery is recommended, because missile injury often leaves the nerve in continuity and many of these injuries are neuropraxias which resolve spontaneously. However, some of these lesions become neuromas in continuity and require later surgical intervention with techniques such as neurolysis, resection, or grafting.

2. **Early Period** (Less than 3 months)
   In cases where root avulsion is present, the surgical timing is important. The diagnosis and determination of root avulsion is a key step. These root avulsion injuries have a hopeless prognosis for nerve recovery; therefore, surgery should be done as soon as feasible (2 to 3 weeks after injury). Since in this situation neurotization is a reasonable option, it is of paramount importance to keep in mind that results of neurotization are dependent upon early muscle reinnervation. Delay in treatment can jeopardize the final outcome.

3. **Intermediate Period** (3 to 6 months post-injury)
   Generally speaking, surgical intervention should be performed during this period in closed traction injuries if the clinical and electrodiagnostic studies suggest no improvement in the neurological status. These injuries potentially that have the best prognosis for spontaneous recovery since there may be a spectrum of involvement (neuropraxies to neurotmetic lesions). It is felt therefore, that serial examinations is most critical during this period in determining whether surgical intervention is necessary. EMG’s and clinical examinations can provide significant information to determine if recovery is progressing or heading to a plateau. After reasonable amount of time (3 to 4 months) with no improvement in proximal muscle reinnervation, then surgical exploration should be considered.

4. **Delayed Period** (6-12 months). This is unfavorable timing for surgical intervention. Results of nerve grafting deteriorate after 6 months. There is however, some improvement that is still possible to be obtained until approximately 12 months or so.

5. **Late Period** (greater than 12 months). Majority of authors don’t advise nerve repair at this time. Treatment options in these patients to consider include:
   a) Functional free muscle transfer
      (Extraplexal neurotization)
   b) Tendon transfer(s)
   c) Joint fusion
   d) Pain management
   e) Nerve transfer(s)

**LOCALIZATION OF THE NEUROLOGICAL LESION**

Regarding the level of injury, the most important aspect (which is germane to understanding this injury) is: Is there continuity between roots and cervical spine ( CNS)? The level of lesions of the plexus can be classified as supra or infraganglionic lesions of the trunk and lesions involving the cords. Any combination of these levels may occur (19).

**Level I** Supraganglionic lesions are those proximal to the spinal ganglia. The roots are avulsed from the cervical cord and as a result, motor fibers degenerate but sensory fibers are still intact and therefore electrical conductivity is preserved for afferent impulses. As a result of this high injury level, a neuroma does not form and Tinel’s sign is absent. There are no changes in the vegetative system as the connections with the sympathetic ganglia are also intact. The deep neck muscles show evidence of denervation. This is a useful fact since electrodiagnostic testing can sample these muscles to assess their neuro-
logical status and help localize the level of the lesion(s). **Level II** Infraganglionic lesions are peripheral to the spinal ganglia. Both motor and sensory axons degenerate, electrical conductivity is lost, a neuroma forms, and Tinel sign is present. Both gray and white rami lose connection with the sympathetic ganglia. The deep neck muscles remain innervated through the intact dorsal branch. Damage may involve both the supra and the infraganglionic portion of the same root.

**Level III** In lesions of the trunks, there are signs of neuroma formation with a positive Tinel’s sign, all conductivity is lost, and vegetative functions are disturbed. Muscles, whose nerves leave the plexus more proximally, are spared i.e. levator scapalae, rhomboid, and serratus anterior.

**Level IV** Lesions of the cords are accompanied by neuromas in the supra or infraclavicular regions. The supra- and infraspinatus muscles remain intact. There may be a combination of supra- and infraclavicular lesions.

Extent of injury is also important in determining the treatment strategy. Patients may have different patterns of injuries. Most common is **upper** brachial plexus (Figure 1.). **Lower** brachial plexus injury is much less common (Figure 2). **Total** brachial plexus is present when all roots are affected, but still some function is preserved (Figure 3). **Global** brachial plexus has the worse prognosis in which all innervations have been disrupted and none of the extremity musculature demonstrates movement i.e., flail extremity (Figure 4.). In clinical practice, there can be many different variations of the injured brachial plexus. The treatment strategy therefore needs to be individualized taking into consideration the patient, time of presenta-

**Surgical Goals and Priorities**

In general, proximal muscle groups have better prognosis for recovery than distal muscle groups. There is less distance for nerve regeneration to traverse and less axons are necessary for successful reinnervation. In addition, less time to reinnervation means less muscle atrophy and less neural fibrosis. As such, attention on brachial plexus reconstruction has focused on proximal muscle groups and those groups that can provide the most useful upper extremity function. Restoration of elbow flexion is the **first** priority in the treatment of the injured plexus. Placement of the hand in a position to be useful is impossible without adequate elbow flexion. The **second** priority is shoulder stabilization and **last** is wrist and hand prehension. The order of this priority is set based on the realistic goals of success in restoration of the function rather than the actual importance in the functional need of that limb; hence, it is somewhat misleading. Probably the most important function would be the prehension, but due to the less likelihood of restoration capability many surgeons have traditionally neglected using our current repair techniques, attempt to restore this function. A combination of surgical options can be used to accomplish these goals. Surgical decision making is continued intraoperatively as the availability of donors and determination of deficits are addressed via a number of different options. The options for surgical reconstruction include microneurolysis, primary nerve repair, nerve grafts, nerve transfers, tendon transfers, free muscle transfers, and the stabiliza-
tion of joints via arthrodesis and tenodesis. The determination of which option to use is based on nerve availability, surgical goals and lastly patient’s and surgeon’s expectations. This often varies on an individual basis.

INTRAOPERATIVE EVOKED POTENTIALS

Intraoperative evoked potentials can be useful to verify a suspected nerve root avulsion or to determine whether neuroma resection and interposition nerve grafting should be performed. If direct stimulation of an exposed nerve root causes a reproducible cortical somatosensory evoked potential, then it is likely that a nerve root avulsion is not present. Another method of assessing root-to-cord continuity is via intraoperative transcranial electrical motor-evoked potentials. Intraoperative evoked potentials are also useful for addressing a neuroma in continuity. Stimulating and recording across a neuroma-in-continuity should produce a reproducible signal if axons are intact. If there is a significant demonstrable axonal continuity present then a neurolysis is all that may be required. If, on the other hand, there is no response, this usually indicates that extensive intraneural fibrosis is present, and therefore, the neuroma and adjacent nerve tissue is resected and nerve grafting is performed.

SURGICAL APPROACH

The patient needs to be placed in semi-sitting, supine position with the surgical field prepped out from the entire neck starting at the mandible on both sides and the operative shoulder, the chest wall from the midline sternum to the medial border of the scapula, and bilateral lower extremities. If you are considering the use of contralateral nerve as a donor nerve, then bilateral shoulders must be included in the sterile field. The incision for the exploration of the brachial plexus starts at the posterior border of the sternocleidomastoid muscle and then continues laterally above the clavicle (Figure 8). At the level of the coracoid process, the incision follows down the deltopectoral groove. The external jugular vein is the first important landmark. Spinal accessory nerve lies posterior to this structure. Transverse cervical artery is potential source of bleeding and good anastomosis for vascular graft. Both external jugular vein and transverse cervical artery are divided along with the omohyoid muscle in the supraclavicular fossa. The upper and middle trunks lie posterior to this muscle. Anterior and middle scalene muscles come next in the field. Between these muscles, the trunks of the plexus emerge. The phrenic nerve located anterior to the anterior scalene muscle should be identified and protected to prevent iatrogenic injury. After identification of the neural element of the C5 root, one can trace the lower nerve roots and identify the beginning of the brachial plexus (Figure 9). These neural elements can be inspected to determine whether they are avulsed, ruptured, partially injured or intact. If the lesion is extended to the infraclavicular region, the clavicle can be osteotomized and the incision extended into the deltopectoral groove. Pectoralis minor muscle detachment provides access to cords of plexus with lateral cord being most prominent. After this exploration and identification of the level, type, and extent of the lesion, an intraoperative plan is established. It is essential not to miss multilevel injuries. Sometimes, neurolysis of the nerves must be performed to truly evaluate the condition of the fascicles.
SURGICAL OPTIONS

1. Direct nerve repair is seldom possible. It is primarily indicated for acute sharp injuries.

2. Nerve repair with interpositional grafts is the most commonly used option for postganglionic injuries. The sural nerve is the most commonly used donor nerve. It can provide up to 35 cm in length. Ipsilateral cutaneous nerves of arm and forearm are the next source of neural tissue. The saphenous nerves may also be used. Vascularized grafts such as ulnar grafts have also been used for large defects and to provide larger conduit for greater quantity of axonal regeneration. Vascularized ulnar nerve can be used as a free nerve graft (epineurium is partially cut and fascicles are split into several grafts, Figure 7) (20, 21).

The vascularized ulnar nerve can also be used as a pedicled graft if the nerve is rotated on its vascular pedicle of superior ulnar collateral artery, and as a free nerve graft for bridging the long distance for performing a contralateral C7 transfer. If a ruptured nerve is found, it would be most likely accompanied by the presence of the neuroma at the distal stump. The neuroma stumps can be excised to the healthier fascicles, both distally and proximally. A difficult decision arises when there is a mixed injury within a single cord with some intact functioning nerve along with the neuroma-in-continuity. When faced with this dilemma, one must utilize the help of the microscope and attempt to separate out the intact fascicles from the
neuroma. This can be a very tedious task, but without this careful step you may downgrade the existing function and convert the partial injury to a complete one. Another technique, is to use intraoperative electrodiagnostic testing to guide the surgical decision making. Once the injured zone has been identified and excised, the interpositional nerve grafts can be used for the repairs (Figure 8). Excisions of the zone of injury and tension free repairs are critical steps regardless of repair techniques (22).

NERVE TRANSFERS

Nerve transfers are typically indicated for preganglionic lesions or when injuries are so proximal that the likelihood of recovery is extremely poor. The suitability of one nerve as a donor in nerve transfer procedures is determined by anatomic proximity, the extent of brachial plexus injury, donor nerve morbidity and the number of nerve fibers (23, 24). Numerous donors nerves that can be used for nerve transfers are available including: spinal accessory, phrenic nerve, intercostals nerves, contralateral C7 vascularized ulnar nerve, branch to the FCU (Oberlin transfer), and the 5th and 6th cervical nerve stumps. Spinal accessory nerve (SAN) use may not impact on the functional status of trapezius because of its dual innervation. Harvesting of this nerve should be performed after the take off of 1 or 2 branches to the SCM muscle to prevent paralysis of this muscle. It has approximately 2000 fibers. The SAN most commonly is used for reconstruction of the suprascapular nerve but neurotization of the musculocutaneous nerve (MCN) has also been successful (25, 26).

The phrenic nerve arises mainly from C4 with some contribution from C3 and C5. Pulmonary function must be carefully evaluated preoperatively. This transfer is contraindicated in age of less than 2 years. In almost 3/4 of patients, pulmonary function will be diminished postoperatively. Vital capacity usually decreases from 10 to 15%. Full recovery can be expected during first year. Coaptation with suprascapular nerve is the most effective, because interposition graft is not necessary due to proximity. The musculocutaneous (MCN) and axillary nerve (AN) can be also connected but with interpositional grafts (27). Intercostal neurotization procedures have good results if the coaption is performed directly into the recipient nerve. Coaptation with interpositional nerve graft do not have as favorable results as the direct technique. Each intercostal has 1200 fibers. Usually 3rd, 4th and 5th intercostals nerves are used (Figure 9.). Higher intercostals have more sensory fibers but problems with scapular winging make harvesting these nerves prohibitive. Higher intercostals have more sensory fibers. Most common nerve to undergo neurotization is the musculocutaneous nerve (MCN). Neurotization procedures of the median, ulnar, and radial nerve do poorly (28). Contralateral C7 transfer in recent literature has received a lot of attention. C7 root nerve allows large numbers of nerve fibers to be obtained. It is indicated in Global and Total avulsion plexus injuries. It has great capacity with 18000 to 40000 nerve fibers. The transfer is most commonly combined with a vascularized...
ulnar nerve graft (Figure 10.). It can be used partially (50%) or entire root. Before sacrificing the C7 nerve root, a diagnostic marcaine injection for assessment of the extremity functional loss should be made. With this transfer, donor deficits do not appear to be significant and the clinical results of C7 root transfer to the median nerve have shown some recovery of sensibility, but poor forearm and hand motor recovery. The increased distance and time required for axonal regeneration for distal motor recovery results in irreversible changes in the motor end plates and neuromuscular junction. Therefore, this is not a good option for distal reconstruction. On the other hand, interestingly, shortening of the arm in addition to the contralateral C7 nerve has been done and has demonstrated some distal motor recovery (29).

Ulnar nerve transfer (Oberlin transfer) is relatively simple technique used for neurotization of musculocutaneous nerve (MCN) in upper root with great return of functional elbow flexion with minimal donor morbidity. Recovery rate is excellent in 90% of patients with M4 results. Donor fascicles should be assessed intraoperatively with a nerve stimulator. The branch going to the FCU is usually selected for the transfer. The Oberlin transfer has the advantage of rapid reinnervation. The proximity of the transfer to the motor endplates of the biceps or brachialis muscles has allowed relatively early recovery of elbow flexion between two and five months. No permanent deficits are noted in the ulnar nerve distribution; however, transient paresthesia can occur (30,31). This concept has been expanded by McKinnon to include a double transfer using the Oberlin and a branch to the flexor digitorum superficialis (FDS) of the middle or ring finger and transferring it to either the brachialis or biceps and thereby innervating these two muscle groups to give very impressive clinical results with minimal clinical deficits. Hypoglossal and medial pectoral neurotization are described but rarely used in practice. Ulnar nerve and 5th and 6th cervical roots stumps nerve have shown to be effective and reliable transfers, however the best results were seen with combined donor transfers due to the increase in the quantity of the neurons, less axonal mixing, shorter distances to the motor end plates and shorter operative time.

**FREE FUNCTIONAL MUSCLE TRANSFERS**

In the past, the functional free muscle transfers have been set aside for the neglected cases of total brachial plexus palsy such as the patients who are about two or more years out from the initial injury without any treatments. However, this method of reconstruction has been more widely accepted in recent time as the primary treatment of the complete root avulsion injury, bypassing the option of nerve transfer for some surgeons. The benefit of this procedure in comparison to the other surgeries is that this is one of the only procedures that can essentially provide the patient with acceptable function of prehension after a complete brachial plexus root avulsion. This procedure is much more technically demanding and does allow a higher percentage of patients to obtain more functional prehension which is the most important function of the hand. The free gracilis innervated flap is commonly used for elbow flexion.

Restoration of elbow function is one of the first reconstructive goals. The proximal gracilis muscle is attached to the clavicle and distal portion is performed by weaving gracilis tendon into the biceps tendon (Figure 11.). The technique, popularized by Dr. Kazuteru Doi in Japan aims to restore the following four major functions (32):

a.) Independent voluntary finger flexion and extension  
b.) Independent voluntary elbow flexion and extension  
c.) Protective sensation in hand  
d.) Hand stabilization

In Doi’s reconstruction, the first free muscle transfer, the contralateral gracilis muscle, latissimus dorsi, or rectus femoris muscle, neurotized by the ipsilateral spinal accessory nerve. The free muscle transfer is spanned from the acromion, anterior to biceps, under the origin of the mobile wad as a pulley, and then tenodesed to the extensor tendons of the digits. This is to provide functions of elbow flexion and finger extension (Figure 12.). The second free-muscle transfer, neurotized by the fifth and sixth intercostal nerves is placed from the second rib, medial arm, under the flexor pronator origin and tenodesed to the flexor tendons. This transfer is to restore the flexion of the fingers once the neurotized muscle has been innervated. The third procedure is the neurotization of the denervated triceps muscle. This is performed during the second muscle transfer to restore the finger flexion. The fourth procedure, which is also performed during the second free muscle transfer, is to provide the sensibility to the hand by neurotization of the predominant sensory fibers of the median nerve with the sensory rami of the intercostal nerves or supraclavicular nerves (Figure 13.). The result of this procedure with 32 patients demonstrated 96% of satisfactory elbow function and 65% of satisfactory prehension. These
results are significantly higher than any other procedures mentioned above for regaining both the functional elbow flexion and prehension capability from the complete brachial plexus root avulsion injuries.

OTHER PROCEDURES AND APPROACHES

Reconstructive techniques such as tendon transfers, pedicle muscle transfers, joint fusions and a variety of osteotomies are indicated as secondary procedures for patients who have already had brachial plexus reconstructions to improve particular functions. These procedures are also of great use in patients who present late or those who are not good candidates for brachial plexus reconstruction. Different approaches are available in literature. Recently Millesi (33) published article summarizing five different approaches in management of brachial plexus. Besides above mentioned procedures there are several experimental works about root implantation done by Carlstedt (34) and some other authors. Although this technique seems logical and there are encouraging initial results, no clinical application has been done yet. Dorsal approach with laminectomy which is used by Kline (35) is supposed to provide better approach to the roots and more precise diagnosis about root avulsion. However there are still 15% of false negative result reported, and also there is reasonable chance for further root damage. Therefore this approach has not gained popularity. Extraplexal neurotization without exploration of plexus brachialis has been popularized from Tsuyama. Although Dr Tsuyama (36) has obtained good results using this approach there are still some opinions that this approach jeopardizes the possibility of muscle regeneration that have direct connection to the spine after the brachial plexus nerve roots are repaired. We prefer integrated concept using all options indicated above which might improve final result. Reconstructive surgeons who manage these challenging injuries should be well versed in all techniques including: neurolysis, nerve grafting, neurotization, free muscle transfer and tendon and muscle transfer and joint fusions. Moreover, a single patient may require several different options to obtain good result and a useful extremity (37,38).

POSTOPERATIVE CARE

Typical postoperative care involves strict immobilization of the neck, shoulder and elbow to protect the microsurgical repairs. This can be done with cervical collars, halos, and customized shoulder and elbow braces or slings. Immobilization is necessary for typically six to eight weeks at which point passive range of motion of the upper extremity may have begun. Once wound healing is complete, a serial neurological examination to assess recovery is performed every few months. There are multiple hand rehabilitation protocols to help maximize recovery available. The need for secondary reconstruction is determined at twelve to twenty-four months after brachial plexus reconstruction as final outcomes may take a significant time period to assess.
REFERENCES

Abstract

This article reviews the history and current management concepts of flexor tendon lacerations. Classic and contemporary repair techniques are discussed. The most popular rehabilitation protocols are also reviewed.

Key Words: Flexor tendon, injury, surgical repair

There was significant improvement in tendon repair over the last 20 years. Because of improvements in surgical technique, the material properties of suture, our understanding of the mechanisms of tendon repair and methods of aftercare, primary repair of flexor tendons has become the standard of care. Although results of primary flexor tendon repair have improved considerably there are still controversy about best methods of suture used and most effective postrepair protocol. Current series often report good to excellent outcomes in 80% of patients (1,2,3). In an effort to improve patient outcomes, researchers have recently focused on:

1. Improving the tendon strength at the repair site.
2. Decreasing tendon adhesions.
3. Increasing the motion at the repair site to improve tendon gliding and hand function.
IMPROVEMENT OF TENDON STRENGTH AT THE REPAIR SITE

According to current thinking, the aim of primary flexor tendon surgery is to use a method of repair which allows early movement without the repair rupturing, until such time as the healing tendon has sufficient strength to take over from the surgical repair. The primary problem is in bridging the enormous gap between the forces acting on flexor tendons during use (c. maximum 250N/25kg force) and the strength of the current repair techniques. At failure, older suture methods had a strength of 10 to 20 N (1-2 kg force), but newer designs achieve 50 to 80 N (5-8 kg force). In practice, lower loads are more relevant to the gap formation which restricts movement by allowing snagging of the repair on the tendon sheath edges. In the literature there is often quote the tensile force to produce a gap of 1 or 2mm at the repair site and an ultimate strength at which rupture occurs. In the clinical context, a gap is both deleterious to tendon gliding and to the final range of motion and power of the finger. How big a gap can be tolerated is not known but, probably, almost no separation is acceptable.

HISTORICAL OVERVIEW

Simpler core methods include the Kessler repair (Figure 1.) which was originally poorly described, and the Bunnell suture, which has been condemned as a cause of tendon ischaemia (Figure 2.). Kleinert and his colleagues described a variant of the Bunnell repair (Figure 3.) which was quite different (4,5). Their report also included use of a peripheral running suture, the first of its type - and also introduced a new method of rehabilitation: three new variables in one study. Since the Louisville study, it has been difficult to consider core sutures without additional epitendinous suturing, and both ideas enjoy the multiple strand principle. Tsuge and his colleagues (6), offers an easy way of locking the suture and introducing two strands in one stitch (Figure 4.).

METHODS OF TENDON REPAIR WHICH INCREASE REPAIR SITE STRENGTH

CORE SUTURE

The goal of tendon repair is to accurately coapt the tendon ends using a suture method that is strong enough to allow a functional rehabilitation program. Believing that increased repair site strength allows early active digital range of motion and improved patient outcomes, numerous investigators have devised stronger tendon repair methods.
Newer multistrand, multigrasp methods have been devised that are strong enough to allow rehabilitation programs that feature early active digital range of motion. Strickland uses combination of Tajima core suture and a matres core suture (7). This technique belongs to four strands suture (Figure 5a-d). Lim and Tsai six strands suture uses Supramed 4/0 as a suture material (Figure 6 a-h). In a study which was done by Gill et al. (8) it was shown significantly higher tensile strength than of Tsai six strand suture comparing to two strands (modified Kessler) and four strands (modified Tsuge). The 6-strand double-loop suture technique simplifies flexor tendon repair. It improves the repair’s strength and its resistance to gapping without increasing tendon handling or bulk. This increased repair strength allows using a more aggressive rehabilitation program (1,2,3).

The Teno Fix system, represents the first use of a surgical anchor system in soft tissue repair (9). Utilizing a small anchoring coil is inserted into a damaged tendon, gathering collagen fibers as it turns and harnessing the intrinsic strength of the tendon (Figure 7.). The system works by placing one anchor on each side of the repair site through a tenotomy 1.0 cm away from the cut edge. The tip of the preloaded installation instrument is placed into the tenotomy and the anchors are turned into tendon, in turn capturing collagen fibers. Although Tenofix system has been advertised as a very strong and secure, possibility of tendon rupture still exist. High costs of tendon repair using this technique, and fact that this system doesn’t appear as much simpler as conventional techniques are reasons why it doesn’t gain much popularity. This system is still in phase of initial clinical evaluation and no significant clinical experience has been cumulated yet to allow proper evaluation.

Several investigators correlated repair site strength with the gauge of suture. Taras (10) and colleagues reported that 6-0 braided polyester sutures were 8.5 stronger than a 7-0 suture. These investigators conclude that significant increase in initial strength can be achieved simply by using larger suture caliber. Using a clinically relevant canine model, basic science investigators reporting on the gliding characteristics of multiple types of tendon suture methods found a significant improvement in gliding characteristics for suture methods with internal placement of the suture knots and fewer external points of suture exposure. Chao et al. (12,13) found that resection of one slip of the FDS tendon significantly improved gliding resistance of the FDP tendon. The authors suggest that partial FDS resection may facilitate flexor tendon gliding beneath
the A2 pulley following tendon repair. Basic science investigators have also reported that increasing pulley size (venting the pulley) (14) by partial release improves gliding excursion and reduces resistance to motion. Several basic science investigators have concluded that partial A2 pulley release can be done without significant loss of finger motion. Kwai Ben and Eliott reported a clinical study which employed a distal incision in the A2 pulley at the time of zone 2 flexor tendon repair. Prospective comparative trials which have focused on the effect of sheath repair have not demonstrated improvements in outcome for patients who undergo sheath repair (15,16). Current clinical recommendations are for careful intraoperative examination of the digit to ensure satisfactory tendon excursion through the pulley tunnels. Closure of the synovial sheath is no longer considered a necessity during primary flexor tendon surgery. A survey of the world literature shows that some hand centers prefer not to repair the sheath, while others advocate repair of the sheath whenever the initial injury allows. Comparison of the clinical outcomes after sheath repair with those in which the sheath was left
open shows identical results, as was reported by Saldana et al. (17) Despite the insignificance of closure of the sheath for finger function in this report, closure of the sheath in fresh, clean-cut tendon lacerations is not wrong and actually is still popular among hand surgeons. The most important aspect, however, that we should bear in mind is not to close the sheath in patients with sheath defects, fibrosis, or obvious tendon edema.

THE CIRCUMFERENTIAL SUTURE
The epitenon is the outermost layer of the tendon within the digital sheath. To use this layer for suturing a tendon seems practically impossible - one would always include some of the fibres from the tendon itself. Therefore, the concept of an epitendinous suture is not correct, as grasping only this superficial cell layer is impossible. A better terminology is to call this suture a circumferential suture, as it runs around the circumference of the injured tendon. Alternatively, it can be seen to grasp all sides of the circumference of the tendon.

When Kleinert and co-workers presented their work they described the use of a simple continuous running circumferential suture to avoid bulging of the repair (Figure 8). In 1991, Pruitt et al. (18) showed the importance of the circumferential suture in preventing gap formation under cyclic stress testing. In 1994, Silfverskiold and Andersson (19) published their studies on a new type of circumferential suture which they named the “cross-stitch” suture (Figure 9). They described two configurations (see below) and found that the cross-stitch alone was as strong as a modified Kessler core suture with a simple circumferential suture. Since then variations of the cross-stitch has been tested in vitro and also have been compared to circumferential suturing with a Halsted type (Figure 10) (20) of suture with somewhat conflicting results with respect to the final tensile strength.

CONFIGURATION AND STRENGTH
The simple running suture is the weakest circumferential suture but the quickest to perform. The cross-stitch described by Silfverskiold and Andersson and a circumferential suture using the Halsted configuration seems to be very similar in strength (19,20). The advantage of the cross-stitch technique is that need not be very exact in the clinical setting. Silfverskiold stated that the suture bite was placed 3 to 5 mm from the cut edge. However, the addition of the cross-stitch totally eliminates the gap, making the repair smooth. While one can include as many cross-stitches across the tendon gap as one wishes, Kubota et al., (20) showed that a minimum of 4 strands increased the tensile strength.

In an ordinary tendon it is not difficult to include 4 or more strands. The disadvantage of the cross-stitch is that it may require a larger opening in the tendon sheath than is needed for a simple running circumferential suture. Another difficulty is performing the dorsal part of the circumferential suture in the region of A-4 pulley. Dorsally the surgeon has to negotiate the tails of vincula brevis, which we suggest to keep intact if possible.

DISTANCES AND DEPTH
The importance of the distance between the bites of the suture and the cut tendon surface has been studied.
very little. Tang et al. (21) recently published a study on oblique tendon lesions and the importance of the placement of the core suture. They recommended that this distance is a minimum of 7 mm. A corresponding study on circumferential sutures is lacking. Some authors do state the distance used in their studies and this varies from 3.5 to 6.8 mm. However, there is no study comparing the effect of variations of this distance on strength using the same material and configuration. Silfverskiöld used a depth of 1 mm, which is a realistic figure (19).

METHODS TO LIMIT FORMATION TENDON ADHESIONS

Prevention of adhesions after flexor tendon surgery continues to be a significant focus for basic science researchers. Adhesion formation may be related to increase in cytokine concentration which upregulates injured and uninjured synovial sheath fibroblasts. Hyaluronic acid (HA) is a glycosaminoglycan that has previously been found to be present in the normal fluid of the synovial sheath. Histologic studies of the annular pulleys have demonstrated that the cells responsible for the generation of hyaluronic acid are the cells of the inner lamina of the pulley. Previous basic science studies have suggested that hyaluronic acid may limit adhesions formation following zone 2 flexor tendon repair (22,23). Recent investigators reported in a basic science study involving chickens found that an HA membrane applied circumferentially around the tendon repair site inhibited the formation of restrictive peritendinous adhesions (24). Hyaluronic acid limits the inflammatory response associated with flexor tendon injury and limits peritendinous adhesion formation without adversely affecting tendon repair. The insertion of polyvinyl alcohol shields (PVA) have been proposed as a method for limited peritendinous adhesion formation following flexor tendon repair. The material is thought to be effective by limited cellular survival on the surface of the membrane. The material is semi-permeable allowing passage of synovial fluid nutrients to the tendon repair site. Kobayashi and colleagues (25) have reported a basic science study evaluating the effect of PVA shields on tendon repair and the formation of peritendinous adhesions. They reported that PVA shields are effective in limiting peritendinous adhesion formation but are associated with a significant rate of repair site rupture and decrease in repair site strength. Adcon T/N is an anti-adhesion barrier that is a resorbable gel, composed of gelatin and a carbohydrate polymer. The application of this biomaterial has been shown to have some value in clinical studies (26). Golash et al. (27) reported in a prospective randomized study of an anti-adhesion barrier gel (ADCON T/N). In a prospective double blind randomized study, the application of one tube to ADCON (3.5 gm) was followed by sheath closure at the time of flexor tendon repair. While the authors reported some benefit in sense of shorter period to achieve final range of motion from the application of ADCON T/N, a statistically significant improvement in function for the group treated with ADCON T/N, a significantly increased rate of peritendinous adhesion formation following tendon repair. In an animal model Augustine et al. (28) that single dose only decrease significantly synovial reaction and postoperative technique (one touch technique). In two recent studies, tendon healing was not adversely affected by the application of 5-FU. The application of 5-FU was associated with fewer peritendinous adhesions but was not associated with an increased risk for tendon rupture (29). Investigators reporting an in vitro study of tendon cell proliferation and matrix metabolism concluded that certain non-steroidal anti-inflammatory medications can limit components of matrix metabolism for tendon explants. Kulick et al. (30) found that ibuprofen selectively increase intratendinous inflammation while minimizing peritendinous scarring. It is thought that TGF-β1 contributes to the pathogenesis of excessive scar formation. In a rabbit model, Chang et al. (31) showed that intraoperative infiltration of neutralizing antibodies to the TGF-β1 diminishes scar and adhesion formation.

METHODS THAT INCREASE MOTION AT THE REPAIR SITE TO IMPROVE HAND FUNCTION (REHABILITATION)

EARLY PASSIVE MOBILIZATION

If applied with care, early passive mobilization (starting within a few days of the repair) has been shown to produce superior results, apparently because early mobilization inhibits restrictive adhesion formation, promotes intrinsic healing and synovial diffusion, and produces a stronger repair site. Moreover, early passive motion prevents the decrease in tensile strength of repairs when compared to immobi-
lized tendons as reported Duran and Houser (34). There are two basic types of early passive mobilization protocols based on the work of Kleinert (32) and on that of Duran and Houser (34). Each protocol has many variations on these two approaches described in literature. In both approaches, a forearm-based dorsal blocking splint, applied at surgery, blocks the MP joints and wrist in flexion to place the flexor tendons on slack, and the IP joints are left free or allowed to extend to neutral within the splint. Dynamic traction maintains the fingers in flexion to further relax the tendon and prevent inadvertent active flexion. It may be provided by rubber bands, elastic threads, springs, or other devices. The traction is applied to the fingernail either by placing a suture through the nail in surgery or by gluing to the fingernail a dress hook, Velcro, a piece of soft leather or moleskin.

**KLEINERT PROTOCOL**

Since the publication by Kleinert (32) of his early mobilization regimen in the 1960s, this method has been that favoured throughout USA and Europe, although its popularity in the UK has fallen. Despite some centres producing very good results using "Kleinert traction", many considered the original regimen to have significant problems both in terms of achieving poor distal interphalangeal (DIP) joint flexion and causing flexion contractures of the proximal interphalangeal Joint (PIP). Modification of the regimen by adding a palmar pulley was introduced to improve DIP flexion. Strictly speaking, the regimen should be called an active extension/assisted flexion regimen rather than an active extension/passive flexion regimen. The original protocol is no longer used as originally described. More recent adaptations are summarized as following.

**Early stage** (from 0 to 3 weeks)

In the original Kleinert protocol, the dorsal blocking splint blocked the wrist in 45 degrees of flexion and the MP joints in 10 to 20 degrees. Rubber band traction was directed to the fingernail from the wrist or just proximal to the wrist. Every hour, the patient actively extends the fingers to the limits of the splint 10 times, allowing the rubber bands to flex the fingers.

**Intermediate stage** (from 3 weeks to 5 weeks)

The rubber band from the injured digit is attached to a wrist band from 3 weeks through 5 weeks. All active movement so the wrist and hand are encourage, although the injured digit is still tethered through 5 weeks. At five weeks, gentle active flexion may begin.

**Late stage** (starting at 6 weeks)

Resisted exercise begins. Several techniques have been described to help improve the total active range of motion.

**Four-finger method.** May et al. (33) describes an early passive mobilization protocol that is a variation of the Kleinert protocol they call the "four-finger" method. The dorsal splint extends only to the PIP joints to ensure that PIP extension is not limited. With the wrist at 30 – 45 degrees of palmar flexion, and the MP at 50 – 70 degrees of flexion. All four fingers are included in traction, even if not injured. A thicker rubber band is used to ensure maximum passive flexion, and manual pressure to all four fingers unvisited to attain the final degrees of passive flexion during exercise. Patients are instructed to use the uninvolved hand to decrease resistance from the rubber bands by pulling them distally during the active extension part of the exercises. The splint is removed at 4 weeks.

**DURAN AND HOUSER PROTOCOL**

The passive extension/passive flexion regimen of Duran (34) as originally described, is little used alone. However it is often combined with other regimes to increase PIP extension. Protected passive extension is the term often used, the proximal joints being placed in maximum flexion passively and the distal joints allowed to flex by tenodesis during passive extension of the affected joint.

**Early stage** (from 0 to 4.5 weeks)

The wrist is held in 20 degrees of flexion and the MP joints in a relaxed position of flexion. Duran and Houser determined through clinical and experimental observation that 3 to 5 mm of glide was sufficient to prevent formation of firm tendon adhesions; the exercises (6 to 8 repetitions twice a day) are designed to achieve this. With MP and PIP joints flexed, the DIP joint is passively extended, thus moving the FDP repair distally, away from an FDS repair. Then with DIP and MP joints flexed, the PIP is extended: both repairs glide distally away from the site of repair and any surrounding tissues to which they might otherwise form adhesions.

**Intermediate stage** (from 4.5 weeks to 7.5 or 8 weeks) After 4.5 weeks, the splint is replaced with a wrist band to which rubber band traction is attached. Active extension exercises begin within the limitations imposed by the wrist band. Active flexion (blocking, FDS gliding, and fist) is initiated on removal of the wrist band at 5.5 weeks.

**Late stage** (starting at 7.5 to 8 weeks)

Resisted flexion starts at 8 weeks. Blocking exercises are performed 4-6 times a day with 10 repetitions.
Early Active Mobilization

Early active mobilization protocols are appropriate for alert, motivated patients who understand the exercise program and precautions. Clearly, whenever feasible, early active mobilization is preferable to early passive mobilization. The literature is growing rapidly (35,36,37,38) and contains a diversity of postoperative approaches. Based on studies indicating that early motion increases repair strength, most published protocols start motion at 24 to 48 hours after surgery. Halikis et al. (37), Gerard et al. (38) protocols use a dorsal blocking splint like those used for early passive mobilization protocols. Gerard also found (38) that early active motion doesn’t compromise concomitant digital nerve injury regeneration.

Belfast and Sheffield.

The most significant change in the last 15 years has been the advent of active flexion and extension regimes, following the work by Small et al. in Belfast (39). Variants of the Belfast regimen have now become the technique of mobilization used by the most units in the UK, less commonly in the rest of the Europe, where it is only gradually becoming accepted and used. This method is the most cost effective in terms of materials and is more “user-friendly” for both patients and therapists although it appears, on first acquaintance, to have potentially more risk involved. In practice, however, this does not seem to be the case, with most units presenting rupture rates of around 5% which is the same rupture rate as those centers using Kleinert traction. Interestingly, non-compliant patients in the UK are often put back into Kleinert’s traction.

Early Stage (Up to 4 to 6 weeks)
The postoperative splint maintains the wrist at 53-degree flexion and MP joints at 83 to 93 degrees of flexion, allowing full IP extension. The dorsal splint extends 2 cm beyond the fingertips to inhibit use of the hand. A radial plaster “wing” wraps around the wrist just proximal to the thumb to prevent the cast from migrating distally. On initiation of therapy, the postoperative dressing is debulked to allow exercise. Exercises, performed every 4 hours within the splint, include all digits and consist of two repetitions each of full passive flexion, active flexion, and active extension. The first week’s goal is full passive flexion, full active extension, and active flexion to 30 degrees at the PIP joint and 5 to 10 degrees at the DIP joint. Active flexion is expected to gradually increase over the following weeks, reaching 80 to 90 degrees at the PIP joint and 50 to 60 degrees at the DIP joint by the fourth week.

Intermediate Stage (Beginning at 4 to 6 weeks)
The splint is discontinued at 4 weeks if tendon glide is poor, at 5 weeks for most patients, or at 6 weeks for patients with unusually good tendon gliding (full fist developing within the first 2 weeks). Presumably, patients continue active flexion and extension exercises, and the program progresses from this point as it would for any tendon protocol, adding light resistance first attaining tendon glide, and then stepping up resistance (late stage) for strengthening, with full function expected by 12 weeks.

Active-Hold/Place-Hold Mobilization (Strickland)
This protocol introduced by Strickland (40) is an “active-hold” or “place-hold active mobilization” protocol. The digits are passively placed in flexion, and the patient then maintains the flexion with a gentle muscle contraction.

Early Stage (Up to 4 weeks)
A dorsal blocking splint is worn most of the time, with the wrist at 20 degrees of flexion and MP joints at 50 degrees. The exercise splint has a hinged wrist, allowing full wrist flexion, but wrist extension is limited to 30 degrees. Full digit flexion and full IP extension are allowed, but MP extension is limited to 60 degrees. Every hour, patients perform the Strickland version of modified Duran exercises (15 repetitions of PROM to the PIP and DIP joints and the entire digit) in the dorsal blocking splint, followed by 25 repetitions of placehold digit flexion in the tenodesis splint. The patient extends the wrist actively with simultaneous passive digit flexion and actively maintains digit flexion for 5 seconds. The patient then relaxes and allows the wrist to flex and digits to extend within the limits of the splint.

Intermediate Stage (From 4 to 7 or 8 weeks)
Tenodesis splint is discontinued. Patient still wears dorsal blocking splint except for tenodesis exercises. The tenodesis exercises continue every 2 hours with 25 repetitions followed by 25 repetitions of active flexion and extension exercise for wrist and digits, avoiding simultaneous wrist and digit extension.

Late Stage (Starting at 7 to 8 weeks)
The splint is discontinued. Progressive resistive exercise is initiated. The patient gradually resumes activities of daily living, with no restrictions by 14 weeks.
CONCLUSION

The recent advances in zone II flexor tendon repairs a designed to increase the strength of the repair, provide safe and earlier range of motion exercises, reduce focal adhesions at the repair site and ultimately provide better function of the hand.

REFERENCES


Abstract

Anticoagulant therapy is most commonly assessed by measuring the effect of the drug on global clotting assay, such as APTT. It is known that response of the APTT to heparin may be decreased in patients with high levels of factor VIII. In this work, we have attempted to determine in vitro conditions of experiment for obtaining relationship between different concentrations of heparin and values of APTT, and to investigate influence of factor VIII on correlation between concentrations of heparin and APTT. Measurement of the effect of heparin, added in vitro in normal coagulation control plasma (NCCP) showed that heparin in concentrations from 0.1 to 1.0 IU/mL prolonged APTT from 0.735 s to 9.926 s. Linearity of the relation of natural logarithm of APTT and concentration of added heparin in plasma for concentrations from 0.5 to 1.0 IU/mL (r = 0.995), and other characteristics of the validated method (RSD = 1.17%), made possible investigation of the influence of factor VIII addition in the solution. The addition of the Factor VIII concentrate, markedly influenced these APTT results. Increased factor VIII activity shortened the APTT, having more pronounced effect in the presence of the large amounts of heparin. Increased factor VIII was associated with downward shift in the concentration - logAPTT response curve (y = 24.664 x + 30.17 vs. y = 10.864 x + 27.256). This finding suggests the possibility for modeling of ex vivo establishment of correlation between plasma activity of FVIII and needed doses of heparin for appropriate management of heparin therapy.

KEY WORDS: heparin, APTT, factor VIII
INTRODUCTION

Literature data about the relation of FVIII level in plasma with thromboembolic disease already exist (1,2). Implication of F VIII level in reoccurrence of the disease is verified and the level was set up to 234 IU/dL (3). Despite the unknown underlying mechanism which is in origin of of F VIII increased level in thromboembolic disease (2), its influence on shortening of the initial phase of the coagulation (1), and speed of thrombin generation (4), have been confirmed.

In attempt to establish mathematical correlation for modeling of relationship of increased FVIII level and its effect on heparine induced prolongation of activated partial tromboplastine time one in vitro method was validated.

MATERIAL AND METHOD

Activated Partial Tromboplastine Time (APTT) was determined by clotting method (Dialab, DiaChrom C4 combi). This investigation was performed with commercial plasma (Normal Coagulation Control Plasma – NCCP). Activity of FVIII in NCCP was determined by one-stage assay, routinely employed for this purpose in clinical practice, and measured 57,8 %, which is equivalent to 0.98 IU/mL. This plasma was after spiked with theoretical activity of 1,0; 2,0; 3,0; 4,0 and 5,0 IU/mL of commercial preparation of FVIII (Haemate®P), and in the results figures as addition of before specified activities. Basal value of NCCP – APTT was 36,8 ± 0,39 s, with 1,17% of variation of eight determinations, that was found as acceptable reproducibility located within the limits of the specification sheet (32,0-37,0 s). It was decided that all experiment should be performed within 8 hours of claimed stability of NCCP. Volumes, handling of samples and duration and temperatures of incubation, were designed in accordance with general principle of the method.

USP heparin sodium reference preparation (labeled activity of 109,5 IU) was used in the investigation. By appropriate dilution one stock solution of 13 IU/mL heparin sodium was prepared, which was utilized for further standard dilutions of 0,1 IU/mL, 0,2 IU/mL, 0,4 IU/mL, 0,5 IU/mL, 0,6 IU/mL, 0,8 IU/mL and 1,0 IU/mL. The values of APTT for each concentration level was calculated as a mean of eight determinations. On the basis of calibration of the method it was decided that the concentration of 0,5 IU/mL, located between 1,5 and 2,5 prolongation of the basal value of the APTT will be used for the investigation. APTT values for the concentrations of heparin so-

\[ y = 99,124x + 29,746 \]

The linear regression line between 0,5 and 1,0 IU/mL of heparin concentrations had coefficient of correlation 0.995, that was acceptable for comparison of influence of FVIII:C.

RESULTS AND DISCUSSION

Added amounts of FVIII:C of 1,0; 2,0; 3,0; 4,0 and 5,0 IU to NCCP (containing 0,98 IU/mL of FVIII:C) reduced APTT, as shown in Table 1. One way ANOVA-test, confirmed significant (p < 0,001) reduction in basal APTT. Post-hoc Tukey-test of multiple comparations showed that there were no significant changes in APTT between increased amount of spiked FVIII:C of 4,0 IU/mL and 5,0 IU/mL (25,27 s vs 24,54 s; p = 0,078). The influence of increasing amount of spiked FVIII:C on APTT in a presence of constant amount of heparin sodium is shown in Table 2. One way ANOVA-test showed significant (p < 0,001) difference in APTT in simulated in vitro increase of FVIII:C activity in commercial plasma from 1,0 to 5,0 IU/mL, returning to the basal value with amount of spiked FVIII:C of 4,0 and 5,0 IU/mL. Tukey’s test of multiple comparations detected insignificance difference in reduced APTT values between increased amount of spiked FVIII:C of 1,0 IU/mL and 2,0 IU/mL (43,72 s vs 42,27 s; p = 0,344), as well as between
It was decided to further investigate how the variation in heparin concentrations, at fixed VIII:C (0.98 IU/mL spiked with 5.0 IU/mL) activity, influence on APTT values. The results are presented in Table 3. One way ANOVA-test showed significant (p < 0.001) difference in APTT in simulated in vitro increase of FVIII:C activity in commercial plasma from 1.0 to 5.0 IU/mL, returning to the basal value with amount of spiked FVIII:C of 4.0 and 5.0 IU/mL. Tukey's test of multiple comparisons detected insignificance difference in reduced APTT values between increased amount of spiked FVIII:C of 1.0 IU/mL and 2.0 IU/mL (43.72 s vs 42.27 s; p = 0.344), as well as between 4.0 IU/mL and 5.0 IU/mL (34.89 s vs 33.72 s; p = 0.586). It was decided to further investigate how the variation in heparin concentrations, at fixed VIII:C (0.98 IU/mL spiked with 2.0 IU/mL) activity, influence on APTT values. The results are presented in Table 3.
CONCLUSION

As opposed to chemical spiking, biological spiking can be one very risky step, requiring appropriate piloting of the study. In this work we demonstrated the possibility for spiking of FVIII:C with plasma in one controlled way, giving possibility for further transfer of the method in clinical environment. The simple in vitro modeling of relation between FVIII:C concentration and heparin induced prolongation of APTT, primarily solved technical and mathematical aspects for the clinical ex vivo modeling.

REFERENCES

ANGIOGRAPHY
ANALYSIS OF
VARIATIONS OF THE
POSTERIOR SEGMENT OF
THE CIRCLE OF WILLIS

ALMA VOLJEVIĆ*, AMELA KULENOVIĆ,
ELDAN KAPUR, ILVANA VUČKOVIĆ

Institute of Anatomy, Faculty of Medicine, University of Sarajevo,
Čekaluša 90, 71000 Sarajevo, Bosnia and Herzegovina

* Corresponding author

ABSTRACT

Cerebral-vascular diseases present one of the leading problems of the modern mankind. They are followed by the risk of high mortality rate, and as such cause high level of disability with people who survive cerebral-vascular incident (stroke, apoplexy). Researches done so far proved that beginning, course and result of the cerebral-vascular diseases depend immensely of the possibility to establish collateral blood circulation and first of all on so called tertian level that is actually the circle of Willis. The circle of Willis, thanks to communicating segments, provide detour way to procure parts of the brain which, due to insufficiency, do not get enough quantity of blood. In this particular study by the analysis of 150 MRI patient's angiographies of the circle of Willis that had been processed at the Radiology Institute of the Clinic Center of University in Sarajevo, we tried to present the most common variations of the posterior segment of the circle of Willis with patients who did not have signs of the cerebral-vascular diseases. The analysis included two target groups (above 60 years old and younger than 34 years old) and both genders. By the analysis of the angiographies of the circle of Willis we reached following results: complete posterior configuration of the circle of Willis has been found with all patients in 54% of cases, but in some slightly higher percentage complete posterior configuration is noted with younger category of patients compared to elderly patients, and in some higher percentage with female compared to male patients. Out of variations that damage the posterior segment of the circle of Willis the first one, according to the frequency of occurring, is the variation of the type of unilateral fetal sort of posterior cerebral artery, and then the variation marked as unilateral aplasia or hypoplasia of the posterior communicating artery.

KEY WORDS: the circle of Willis, MR-angiography, variations
INTRODUCTION

The posterior segment of the circle of Willis is consisted of right and left arteria communicans posterior, final prong arteriae basilaris and initial parts of the posterior brain artery. Collateral function of the posterior segment of the circle of Willis had been described by the creator of theory, Thomas Willis in its study written in 1664 (1). The finding that was discovered that communicating segments of the circle of Willis function as valvular mechanism even during the physiological state of the organism forced many authors to research this issue. During the researches done in this field it has been found that when flexion and especially when head ex- tension, the compression of the vertebral arteries occurs and when rotation and lateroflexion, the compression of the arteriae carotis internae and arteriae carotis communis occurs. In cases of occlusion of some brain artery, the transfer of blood from one system into another might be much intensive. When the occlusion of both carotides, the blood from basilar artery transfers into front parts that were procured with carotid blood circulation, and when occlusion of the vertebral artery the blood from carotid arteries transfers into the back parts. In both cases, the most important anastomoses vessel is the arteria communicans posterior (ACoP), through which blood is transferred into one or another flow. When the occlusion of one carotid, blood comes from the same name artery of the opposite side through arteria communicans anterior and from basilar artery through arteria communicans posterior. However, this blood reshuffling can be minimal or it should not happen at all, if there are certain anatomy variations of the components of the circle of Willis. The variations of the following types like aplasia, hypoplasia and incompatibility of certain components of the circle of Willis have the highest possible importance because they cause anatomy and functional interruption of the continuity of the circle of Willis (2). Thanks to their researches, clinic staff reached common ground that patients vary in their compensatory capability, in case the change occurs in the cerebral flow. Occlusion of carotid artery with one person can be followed by temporary neurological deficit or there are no any other symptoms, while other person can get heart attack infarction of bigger part of one cerebral hemisphere. These differences are without any doubts in relation with existence or non existance of adequate cerebral blood circulation, clinic staff stated (3,4,5). Functional efficiency of the collateral blood circulation in post-occlusion states depends of the huge number of factors: the number of anastomoses and their calibre, state of complete cerebral-vascular system (existance and absence of arteriosclerosis), vascular variations and anomalies, speed of occlusion occurance (trombosis or embolia), system artery pressure, volume and viscosity of the blood (6).

STUDY’S OBJECTIVE

The objective of this study is to examine the presentation of variations of the posterior segment of the circle of Willis in percentages with the patients with regular cerebral-vascular status by application of the MRI-angiography.

MATERIAL AND METHODS

MRI – angiography analysis of the circle of Willis is performed on 150 angiographies of adults of both genders and age of 25 - 75 years old in two target groups; group of 50 patients of 25 – 34 years old, and group of 100 patients of 60 - 75 years old, who do not have any signs of the cerebral-vascular diseases, with the aim to compare the results considering the gender and age. These angiographies were done on the devices MAGNETOM IMPACT „Simens” of 1,0 Tesla technique 3D TOF- time of flight. All check-ups started with the DUAL sequence PD (TR/TE 2500-2625/16), T2 TSE (TR/TE 2500-4186/98), and T1SE sequence (TR/TE 500-693/15) for coronary angiographies in layers of 3 and 5 mm, on which we have analysed the anatomy and variations of the circle of Willis on different levels.

We analysed the types of configurations of the circle of Willis on the axial and coronary MRI scannes in T1 and T2 relaxation. Morphological analysis comprised the analysis of the anterior segment of the circle

![Scheme 1: Presentation of variation of the posterior segment of the circle of Willis, used for the development of this study.](image-url)
of Willis with the assistance of previously set forth criteria. We have used in this analysis partially modified classification that was also used by German authors Lippert H. and Pabst R. in their study published in 1988 (1), and that was result of the summarized results of the up to date researches of the circle of Willis.

RESULTS OF THE STUDY

By the analysis of variations of the posterior segment of the circle of Willis we have reached the following results presented comprehensively in Table 1. We have marked in red colour variations that means complete posterior configuration of the ring. The table itself does contain data (in percentages) on frequency of particular variations occurring, as well as representation of that variation compared to all patients. Based on the presented results, we can conclude that configuration of the posterior segment of the circle of Willis of the type a is the most common occurrence among the patients (normal configuration characterized by existence of both posterior cerebral arteries), (Figure 1.) This type of configuration is noticed in higher percentage with young patients that immensely contributed to higher representation (in percentages) of their complete back configuration compared to older patients. Regarding variations that affect the incompleteness of

<table>
<thead>
<tr>
<th>GROUP</th>
<th>a</th>
<th>b</th>
<th>c</th>
<th>d</th>
<th>e</th>
<th>f</th>
<th>g</th>
<th>h</th>
<th>i</th>
<th>j</th>
</tr>
</thead>
<tbody>
<tr>
<td>YEARS</td>
<td>25 - 34</td>
<td>19</td>
<td>13</td>
<td>0</td>
<td>12</td>
<td>3</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>n=50</td>
<td>38.0%</td>
<td>26.0%</td>
<td>0.0%</td>
<td>24.0%</td>
<td>6.0%</td>
<td>0.0%</td>
<td>6.0%</td>
<td>0.0%</td>
<td>0.0%</td>
<td>0.0%</td>
</tr>
<tr>
<td>&gt;60</td>
<td>26</td>
<td>15</td>
<td>8</td>
<td>28</td>
<td>10</td>
<td>4</td>
<td>6</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>n=100</td>
<td>26.0%</td>
<td>15.0%</td>
<td>8.0%</td>
<td>28.0%</td>
<td>10.0%</td>
<td>4.0%</td>
<td>6.0%</td>
<td>1.0%</td>
<td>1.0%</td>
<td>1.0%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>SEX</th>
<th>MALE</th>
<th>21</th>
<th>13</th>
<th>1</th>
<th>22</th>
<th>7</th>
<th>4</th>
<th>2</th>
<th>0</th>
<th>0</th>
<th>0</th>
<th>35</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=70</td>
<td>30.0%</td>
<td>18.6%</td>
<td>1.4%</td>
<td>31.4%</td>
<td>10.0%</td>
<td>5.7%</td>
<td>2.9%</td>
<td>0.0%</td>
<td>0.0%</td>
<td>0.0%</td>
<td>50.0%</td>
<td></td>
</tr>
<tr>
<td>FEMALE</td>
<td>24</td>
<td>15</td>
<td>7</td>
<td>18</td>
<td>6</td>
<td>0</td>
<td>7</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>46</td>
<td></td>
</tr>
<tr>
<td>n=80</td>
<td>30.0%</td>
<td>18.8%</td>
<td>8.8%</td>
<td>13.3%</td>
<td>7.5%</td>
<td>0.0%</td>
<td>8.8%</td>
<td>1.3%</td>
<td>1.3%</td>
<td>1.3%</td>
<td>57.6%</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>ALL</th>
<th>SUBJECTS</th>
<th>45</th>
<th>28</th>
<th>8</th>
<th>40</th>
<th>13</th>
<th>4</th>
<th>9</th>
<th>1</th>
<th>1</th>
<th>1</th>
<th>81</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=150</td>
<td>30.0%</td>
<td>18.7%</td>
<td>5.3%</td>
<td>26.7%</td>
<td>8.7%</td>
<td>2.7%</td>
<td>6.0%</td>
<td>0.7%</td>
<td>0.7%</td>
<td>0.7%</td>
<td>54.0%</td>
<td></td>
</tr>
</tbody>
</table>

TABLE 1. Presents the variations of the back segment of the circle of Willis considering the gender and age. We have marked in red colour the variations that mean complete posterior configuration of the ring. The table itself does not contain data (in percentages) on frequency of particular variations occurring, as well as representation of that variation compared to all patients.
the circle of Willis, we have marked in our study the highest percentage of variation of type d (configuration characterized by unilateral aplasia or hypoplasia of posterior communicating artery) (Figure 2). type e (hypoplasia or aplasia of both communicating arteries) and finally configuration of the type g (unilateral fetal type of posterior cerebral artery with hipoplasia or aplasia of conter-lateral posterior communicating artery) (Figure 3.).

DISCUSSION

Researches done so far showed that the most common variation that damage arteries of the circle of Willis is hypoplasia of one or both posterior communicating arteries. According to the references this variation is occurring in 22-32% of cases (8,9). Our research material show that variation marked as unilateral hypoplasia of posterior communicationg artery is on the first place according to the frequency of occurance. This variation has been noted with younger target group of patient precisely in 24% of cases and bilateral hypoplasia in 6% of cases. As the older target group of patients, unilateral hypoplasia is noted in 28% of cases and bilateral hypoplasia in 10% of cases. As male population, unilateral hypoplasia has been registered in 31.6% of cases compared to 22.5% that is registered with female target group of patients. Bilateral hypoplasia left and right of posterior communicating artery is registered in 10% of cases, males patients compared to 7.5% of cases, female patients. According to up to date researches, the variation known as fetal type of posterior cerebral artery is on the second spot according to frequency of occurance. This type of variation is characterized by carotid origine of posterior cerebral artery while posterior communicating artery has higher caliber compared to posterior cerebral artery. This variation in the studies is recorded in 15-22% of cases (10,11). Our research material show that unilateral fetal type has been registered with target group of young patients precisely in 6% of cases, and with target group of older patients in 10% of cases, where 2.9% with male patients and 10.1% registered with female patients. These results slightly deviate from the up to date results of the researches.
CONCLUSION

On the basis of the radiology-anatomy analysis of the circle of Willis we have made following conclusions:

1. Variation of posterior segment of the circle of Willis are often more presented with older patients than young patients;
2. Complete configuration of posterior segment is presented more (in percentages) with young patients than the older patients;
3. Higher percentage of the complete configuration of posterior segment has been registered with female patients than male patients;
4. According to frequency of occurrence, out of variations of the posterior segment, the variation noted as unilateral fetal type of posterior cerebral artery is registered on the first place and variation noted as unilateral aplasia or hypoplasia of the posterior cerebral artery on the second place.

REFERENCES

THE RELATIONSHIP BETWEEN SUICIDAL THOUGHTS AND PSYCHOACTIVE SUBSTANCES

MIRA SPREMO1*, SLOBODAN LOGA2

1. Department of Psychiatry Medical faculty Banjaluka, Mačvanska bb, 51.000 Banjaluka Bosnia and Herzegovina
2. Department of Medical sciences, Academy of Sciences and Arts of Bosnia and Herzegovina, Bistrik 7, 71.000 Sarajevo, Bosnia and Herzegovina

* Corresponding author

ABSTRACT

In this study is represented the relationship between suicidal thoughts and psychoactive abuse. In study were involved 202 students of Banjaluka’s secondary schools. By questioner for risky behaviour of adolescents (1) was found 28.7% of them have suicidal thoughts, and 144 haven’t suicidal thoughts. Students from both groups use alcohol but there are not statistically significant differences among groups. The representative sample was 202 students from Banjaluka’s secondary schools. The results showed that 28.7% of them have suicidal ideas. From examined students 20.2% used cannabis, and 4% other psychoactive substances, 56.9% alcohol and 35.6% smoke cigarettes. In the group with suicidal ideas, use of psychoactive substances is 8.6%, alcohol use in 63.7% cases and cannabis 36.2%, and smoking cigarettes in 48.2% cases. We may conclude that misuse of psychoactive substances is more present in group with suicidal ideas, except in case of alcohol, which was also high in group with suicidal ideas, but without statistical significances. It is also possible to conclude that there is important connection between use of psychoactive substances and presence of suicidal ideas as first step toward a suicide in adolescents.
**INTRODUCTION**

Suicide is one of the world’s main death causes in population of adolescents in age 15 – 19 years. Since 1950 the rate of suicide in population of adolescents is tripled and currently it presents third cause of death for this age group. According to the Report of WHO (2) suicide in the Europe in population of woman is fourth cause of death (9,4%), after traffic accidents, accidental injuries and tumors, and for man, third cause of death (13,6%), after traffic accidents and accidental injuries. Total percentage of committed suicides increases. Suicide, attempts of suicide and suicidal ideas, becomes broader social and medical problem. The rate of suicide vary related to age, sex, socio economical status, marital status and religion. Center for diseases control USA, in 1991, in the applied questioner for adolescents, age 14 – 17, had found that 27% of adolescents think about suicide, 16,3% had intention to commit suicide, 8,3% had attempt of suicide and 2% had attempt of suicide which requested serious medical intervention. Each year more than 250 000 adolescents attempt suicide; 8 – 10% of all children in USA try suicide in some period of childhood and 20, 5% of students had serious thoughts about suicide (3). In spite of high prevalence, for many children, risk for suicide stays discovered; suicidal ideas which may be hint verbally, involving direct statements about intention or comment in written form, or non verbally in form of art creation or behavior. Suicidal idea or thought is defined as wish or behavior, which hints ones wish to commit suicide. Suicidal thoughts are not abnormal. It is a part of normal developmental process in adolescence, when young people analyses essential life issues and try to understand life, death and sense of life. Survey of the questionnaires shows that more than half of students of higher grades of secondary schools have suicidal ideas (2). Suicidal ideas at children and adolescents become abnormal when, realization of the ideas, seems as only way out from their problems. In such situation the risk of attempt or of suicide becomes serious. Analyses show that all described factors are more often joined to attempts and commitments of suicides between children and adolescents, but they are not necessary present in each case. As, abuse of psychoactive substances is more present in youth population. In this study we paid attention to suicidal ideas in population of adolescents. Many studies show that main reason for use of psychoactive substances is to change a mood. Alcohol, marihuana, sedatives, nicotine and caffeine decrease anxiety, suffering and relax, while, stimulants and narcotics arouse positive affects. Brent et al. had found that abuse of psychoactive substances presents risky factor to commit suicide, odds ratio 8,5 (4). Kaminer reports that many studies indicate risk for suicide at adolescents with diagnosis of substances abuse. It’s known that conduct disorders and mood disturbances are joined to suicidal behaviour and substances abuse (5). Studies of the committed suicides in population of adolescents in Scandinavia, Canada, and USA show that abuse of substances is more present in population of the victims than in the general population of adolescents. Kaminer also considers that alcohol and cocaine are especially dangerous (5). Brent et al. concluded that adolescents, especially male, who abuse psychoactive substances more often use fire arms to commit suicide, than adolescents without this disorder (6). Many of those who attempt suicide abused drugs and alcohol, with reported rates 13 – 42% (7,8). Garfinkel reports that substance abuse is more often in the group of potential committer of suicide than in the control group (9). The results of this study show that five to eleven of those who attempted suicide were under influence of substances during attempt.

**METHODS**

This research tries to find out is there relation between suicidal ideas and use of psychoactive substances, alcohol and cigarettes, and in which proportion. The results are based on the anonym answers of 202 students of third classes of secondary schools, age 16 – 18, random sample. The research is prospective, epidemiological. In the research questioner Q |000 (1) was used, which involves and covers all aspects of adolescents’ life. The rate of abuse of psychoactive substances, alcohol and cigarettes and percentage of suicidal thoughts in this population were analysed. Microsoft Exel – Analyse it, program for data analyses was used.

**RESULTS**

**CHARACTERISTICS OF THE SAMPLE**

The examination involved 202 students, age sixteen to eighteen years, students of third class of secondary schools, by random choice. From total number of sample, males were 51 (25,2%), and females 151 (74,8%). In age of seventeen there were 18,8%, and in age of eighteen - 81,2%. It means that the sample involves mostly adolescents in age of eighteen years. Table 1 has shown percentage of positive and negative reports about exis-
The relationship between suicidal thoughts and psychoactive substances

MIRA SPREMO, SLOBODAN LOGA: THE RELATIONSHIP BETWEEN SUICIDAL THOUGHTS AND PSYCHOACTIVE SUBSTANCES

Table 1 shows percentage of cannabis abuse in population of adolescents with suicidal ideas and percentage between adolescents without suicidal ideas. There is statistically important difference between groups, $p = 0.007$.

Table 2 shows the percent of use of other psychoactive substance between adolescents with suicidal ideas and without suicidal ideas. From 202 examined total numbers of those who used psychoactive substances are seven, and from that number, five have suicidal ideas. Statically important difference between groups was found, $p = 0.0342$.

Table 3 shows the percent of use of alcohol between adolescents with suicidal ideas and without suicidal ideas. From 202 examined total numbers of those who drank alcohol are 144, and from that number, 66 have suicidal ideas. Statically important difference between groups was found, $p = 0.0026$.

Table 4 shows that bigger number of examined from both groups consume alcohol, but without statistical significance.

Table 5 represents presence of smoking of cigarettes in group with suicidal ideas and in group without suicidal ideas. In group of 202 examined, 72 reported that they smoke cigarettes, and 130 of them don’t have that habit. In group of smokers 28 have suicidal ideas and 44 are without suicidal ideas. Statistically significant difference between groups was found, $p = 0.026$.

**DISCUSSION**

The results of this study agree with conclusions of Diekstra et al. who had found that increasing in mortality and morbidity of suicide was higher in group of white, urban adolescents in North America and Europe (19). These authors connect this to the higher number of depressive disorders and percentage of abuse of psychoactive substances. In the research was shown existence of suicidal ideas in relation to use of psychoactive substances, alcohol and smoking of cigarettes. Statistical importance was found in variable of use of psychoactive substances (except cannabis), $p = 0.03$ and smoking of cigarettes ($p= 0.
since in variable use of alcohol significance wasn’t found. Use and abuse of alcohol in population of adolescents is joined to aggressive, impulsive behaviour and disphoric mood (11). Abuse or addiction about alcohol and drugs and the other psychoactive substances in group of the adolescents is very often joined with multiple psychosocial problems, psychiatric comorbidity, suicidal ideation and suicide attempts (12) and commitment of suicide (13). In longitudinal study of adolescents who had attempt of suicide, was found, that alcohol and substances abuse are of one of biggest risk for suicide. Use of substances, with other psychopathology, social demographic distress and hard experiences during childhood are related to risk of serious suicidal behaviour in population of adolescents (14). In unselected suicidal population alcohol abuse or addiction was found between at 15 – 56 % of victims same as substances abuse. Particular explanations for cigarettes use as passive way to show anger were given. Smoking of cigarettes in recent years has importantly increased. Long-term use of nicotine is related to self-medication and effort to cope with the negative emotional, neurotic and social effects of unfavourable experiences from a childhood. Problems with the depressive mood were more expressed in group of the smokers. The results of the study indicate that psychoactive substances abuse present one of risky factors for suicide in population of adolescents. Due to dates WHO (2), the abuse of psychoactive substances because changes in family relationships, school, feeling of loneliness, isolation, what generate anxiety, which may cause appearance of suicidal ideas, attempt of suicide or suicide.

**CONCLUSION**

According to the obtained data it has been concluded that one of the possible risk factors of suicidal thoughts in adolescents are the abuse of psychoactive substances. It must be taken into account in every program of prevention of suicide in adolescents.

**REFERENCES**

(3) US Public health service. The surgeon general’s call to action to prevent suicide. Washington DC, 1999
Heart Murmur and Anaemia in the Pediatric Population

Senka Mesihović-Dinarević1*, Jasna Ibrahimović1, Edo Hasanbegović1, Emina Ićindić-Nakaš2, Aida Smajić1

1. Paediatric clinic, Clinical Centre University of Sarajevo, Faculty of Medicine
   University of Sarajevo, Bolnička 25, 71 000 Sarajevo, Bosnia and Herzegovina
2. Department of Physiology, Faculty of Medicine, University of Sarajevo,
   Čekaluša 90, 71 000 Sarajevo, Bosnia and Herzegovina
* Corresponding author

Abstract

Innocent heart murmurs are hearth murmurs that occur in patients with a normal heart structure. They do not represent a disease of the heart and vascular system, and should not be treated as such. Iron-deficiency anaemia often causes, along with other symptoms, systolic heart murmurs and tachycardia. It appears in children of all ages representing a most common haematological paediatric disease. To establish the influence of iron-deficiency anaemia on genesis of innocent murmurs and to compare auscultatory and phonoelectrocardiographical findings in patients with anaemia and heart murmurs before and after iron therapy. The study includes 120 patients with innocent heart murmurs that have been auscultated at cardiorheumatic outpatient department of Pediatric Clinic of the Clinical Center of the University of Sarajevo, during the period from 01/01/2003 to 31/12/2004. Further diagnostic procedure, i.e. laboratory tests, diagnosed iron-deficiency anaemia in 30/120 patients. These patients have been followed in this study. 22/30 patients had systolic murmur I/II intensity of Levin scale; 8/30 patients had II/VI systolic murmurs of intensity by Levin. The highest number of examinees had 3–4 years of age, and in this group the number of boys was higher than the number of girls (M : F = 12:4). During the auscultatory and phono-ECG examinations of murmurs, 6 patients had haemoglobin values less than 95 g/l, which corresponds to an average and severe type of anaemia. 24 patients had haemoglobin values between 95 and 110 g/l, which corresponds to benign type of anaemia. The most numerous were patients aged between 0 – 1 year (3 patients with hemoglobin value Hb < 95 g/l, 13 patients with hemoglobin value Hb 95-110 g/l). All patients were treated with iron medicaments. After three months, clinical and laboratory re-evaluation was performed and it has demonstrated that after iron therapy 24 patients had level of a haemoglobin Hb > 110 g/l and 6 patients had haemoglobin levels between 95 and 110 g/l. By auscultatory and phono-ECG examinations, murmurs of a level of intensity I/II was registered in only one child, while in the other 29 patients there were not any registered heart murmurs. Diagnosis of anaemia in the paediatric population group delays definitive diagnosis of heart murmurs. Innocent murmurs in children with sideropenic anaemia occur as its consequence. After adequately conducted iron therapy, i.e. cured anaemia, heart murmurs were not auscultated.

Keywords: innocent murmur, anaemia
INTRODUCTION

The term "innocent heart murmur" defines heart murmur occurring in patients with a normal heart structure. This is the most appropriate term that can be understood by both child and parent. All functional murmurs appear in the conditions with high volume output, such as high temperature, anaemia, and excitement or during exercise. They are recognised by different acoustic and clinical features and can be distinguished from organic heart murmurs, which are caused by different heart and blood vessels illnesses. Patients with innocent murmurs do not have other clinical, x-ray or electrocardiographic abnormalities. They are shorter, last less than 1/2 systole, contain vibratory (musical) component and are not transmittable. Innocent heart murmurs vary in respiration, they disappear during inspiration and they change in quality depending on the body position (1,2). The clinical importance of innocent heart murmurs lies in the fact that when unrecognised and wrongly interpreted, heart murmurs can unnecessarily trigger off a complicated and long diagnostic procedure that can put both parents and children in a state of fear from heart disease (2).

Iron-deficiency anaemia often causes systolic heart murmurs and tachycardia that are consequent of accelerated circulation and/or reduced blood viscosity. Other cardiological repercussions of iron-deficiency anaemia are accentuated first heart sound on icterus, frequent and soft heart rates. Iron-deficiency anaemia appears at children of all ages and represents a most common haematological paediatric disease (3). According to the World Health Organisation (WHO), a child has anaemia if its concentration of haemoglobin (Hb) is:

- Hb<110 g/L for children in the age between 0-1, except for children between 3rd and 6th month when anaemia is diagnosed if Hb<105 g/L.
- Hb<110 g/L for children between the ages 2-5
- Hb<120 g/L for children between the ages 6-12

For children older than 12 years anaemia is usually diagnosed if the concentration of haemoglobin is 130-135 g/L depending whether it is male or a female child (3,4,5). The most frequent cause of iron deficiency in childhood is: a lack of iron depots in preterm newborns, low body mass in newborn infants, low iron supplies in food, feeding mostly with cow milk and flowery food, lack of meat in food, intensive haematoipoiesis in the period between 6th and 9th month of life, that continues on "physiological anaemia" when iron supplies in the body is reduced, difficulties in iron absorption, iron loss due to intestinal haemorrhages, frequent infections, using fresh cow milk in the first months of life may cause occult intestinal haemorrhages and iron deficiencies (3,6).

AIM

To establish the correlation between iron-deficiency anaemia and genesis of innocent murmur as well as to compare auscultation and fono electrocardiograph findings in patients with anaemia and heart murmur before and after iron therapy.

SUBJECTS AND METHODS

This study includes 120 patients with innocent heart murmurs that were auscultated at cardio-rheumatics outpatient department of the Paediatric Clinic of the Clinical Centre University of Sarajevo, during the period from 01/01/2004 to 31/12/2004. Further diagnostic procedures, i.e. laboratory tests, diagnosed iron-deficiency anaemia in 30 patients. This group of patients was followed during prospective, clinical-analytical study in period since diagnosing iron-deficiency anaemia and heart murmur, through therapeutic procedures until clinical, laboratory and phono electrocardiograph reevaluation. In this paper we used clinical examinations, laboratory tests and a questionnaire to explore risk factors for anaemia and nutrition habits.

RESULTS
Values of haemoglobin in-patients during auscultation examination of systolic murmur, prior to the start of therapy, are shown in Table 3.

<table>
<thead>
<tr>
<th>VALUE  Hb</th>
<th>Hb&lt;95 g/l</th>
<th>Hb 95-110 g/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>NUMBER OF PATIENTS</td>
<td>6</td>
<td>24</td>
</tr>
</tbody>
</table>

TABLE 3. Values of haemoglobin before iron therapy

Out of 30 patients with auscultatory findings of systolic murmur, five had haemoglobin value below 95 g/l, which corresponds to an average and severe type of anaemia. 22 patients had value of haemoglobin between 95 and 110 g/l which corresponds to a benign type of anaemia and 2 patients did not have anaemia, i.e. their haemoglobin values exceeded 110 g/l.

Age structure of patients by types of anaemia is shown on Tables 4 and 5.
Therapy:

- Medicaments of trivalent iron in daily dosage of 3 mg/kg of body weight split in three doses.
- C vitamin according to body weight of the patient.
Discussion

The most frequent murmurs in childhood are the ones, which are not caused by anatomic abnormalities of the heart. They are called: “innocent”, “benign”, “functional”, “physiological”, and “non-pathological”, “vibratory”. First description of such murmur was given by Still, around 18 years ago. (7) The Still’s murmur could be best heard alongside the left sternum edge and towards apex of the heart. Innocent murmurs vary when the position of the body is changed; its intensity decreases or disappears if patient is in standing position and reverses if the patient changes position of it’s body. This is being brought into connection with an increase of velocity of blood pumped out the left ventricle (8), or an anomalous tendons chordae of the left ventricle (9) as well as by an increase of normal vibratory activity during ventricular contraction (10). The second type of innocent ejection murmur is caused by increased blood velocity. It is audible in the second intercostal space on the left, seldom on the right side. This type of murmur is audible in children with deformed thorax – pectus excavatum (11). High heart ejection is combined with high body temperature, anaemia, thyrotoxicosis, fear and these conditions intensify these murmurs. Final evaluation of the innocent murmurs should be delayed by the time the patient is reversed to the initial hemodynamic status. Exact diagnosis of suspected innocent murmurs require evaluation of the cardio-vascular status performed by a cardiologist. Diagnosis of innocent (functional) murmurs comforts patient’s parents and the physician who sent the child to the cardiologist. Detailed history with physical examination distinct findings of innocent murmur from murmurs caused by structural abnormalities (12). It is very important that the family doctor / paediatrician, in case of suspected innocent murmurs, perform an examination of the cardio-vascular system with detailed history, by respecting all algorithms of evaluation with the aim of either confirming or excluding organic findings of the heart. Definitive diagnosis is to be given by the paediatrician – cardiologist. Innocent heart murmurs do not represent diseases of heart and vascular system, and it should not be treated as such. In childhood, over 80% of children at the age of three or four have a functional type of murmur (1). In our study 30/120 patients have been diagnosed with sideropenic anaemia. This group of patients was followed during research. The ECG’s and the X-ray’s were normal in all of the patients. We have established aus-
culty and by phono-electrocardiogram that 22 patients had systolic murmur of an intensity level I/II, and 8 patients had systolic murmur of an intensity level II (by Levin). The highest number of examinees were 0-1 years of age, and in this group the number of boys was higher than number of girls (male/female ration= 12:4).

During the auscultatory and phono-ECG findings, 6 patients had haemoglobin value less then 95 g/l that corresponds to an average and severe type of anaemia. 24 patients had haemoglobin values between 95 and 110 g/l, which corresponds to a benign type of anaemia. The majority of patients were aged between 0 – 1 year (3 patients with haemoglobin value Hb < 95 g/l, 13 patients with haemoglobin value Hb 95 -110 g/l). According to Mardešić and co-workers (13), the highest incidence of anaemia caused by iron deficiency was recorded in older infants, in the first and second year of life. Recent studies have show that 10% of healthy, term infants from different parts of Europe have levels of haemoglobin Hb<110 g/l. According to data from Scandinavian countries, 5% of Danish infants 9 months old and 10% of Norwegian children aged from 0 – 2 years have anaemia due to iron deficiency (3,7)

Data on this issue from the area of Bosnia and Herzegovina show that the percentage of anaemic children is increasing and is higher than before the aggression on Bosnia and Herzegovina, which is often being brought into connection with the poor social-economical situation. The most important factor, which affects the occurrence of anaemia due to iron deficiency, is nutrition. We have conducted research of nutrition habits in our 30 patients. The following results within the group of children aged 0 – 2 years (n=17) are:

- 2 children were breast-fed by their first year
- 1 child was breast fed till the six month of life
- 7 children were fed by cow milk since birth
- 7 children were fed by adapted milk formula since birth

Gil and co-workers (14) show incomparably better status of iron in children fed by milk formula, comparing to children fed by cow milk. Negative sides of cow milk given to infants are low iron concentration, weak absorption and micro-occult bleeding in the gastro-intestinal tract (GIT). All of the afore mentioned confirms the well-known fact that anaemia due to iron deficiency seldom occurs in children fed by natural nutrition. For children aged between 2 and 16 years, we have examined nutritional habits, i.e. ratio of iron rich foods in nutrition; the most frequent are powdery foods derived from foods of animal origin, that represents a risk for the occurrence of anaemia. However, the fact that should be taken into consideration is that the pool has been conducted within a small group. All children were treated by iron medicaments according to the following scheme:

- medicaments of trivalent iron, daily dosage of 3 mg/kg/day split into three individual doses between meals together with vitamin C,
- solutions are to be given to infants. Syrup or drops are to be given to little children,
- recommended nutrition: breast feeding in the first six months, after 6th month introduction of iron rich foods,
- different iron rich foods are recommended for children over 2 years of age.

Therapy usually lasted approximately 3 months depending on clinical status of the child and blood status that was controlled once a month. Checks on the regularity of therapy and possible negative effects of the same, have been conducted with parent / tutor of the examinees on a three-week basis. After three months, clinical and laboratory re-evaluation was performed and it has demonstrated that after iron therapy 24 patients had level of haemoglobin Hb >110 g/l and 6 patients had haemoglobin a levels between 95 and 110 g/l. By auscultatory and phono-ECG examinations, murmurs of a level of intensity I/II were registered in only one child, while in the other 29 patients there were not any registered hearth murmurs.

CONCLUSION

Heart murmur in the paediatric population require detailed evaluation of the history and physical/auscultatory findings having in mind the hemodynamic status of the patients at the moment of examination. It is necessary to obtain an expert opinion from a paediatrician – cardiologist on the cardiac status of the patient with functional (innocent) murmurs, which comforts parents, the child and the physician who sent the patient for re-evaluation as well as avoiding additional diagnostic tests. Diagnosis of anaemia in the paediatric population group delays definitive diagnosis of heart murmurs.

Innocent murmurs in children with sideropenic anaemia occur as its consequence. After adequately conducted iron therapy, i.e. cured anaemia, heart murmurs were not detected.
References

(1) Mesihović-Dinarević S. Dječija kardiologija (od fetusa do adoles-
centa), 2000.
(3) Hasanbegović E. Anemije uslijed nedostatka željeza kod dojenčadi
i malo djece, 2004.
(4) Yip R. Prevention and control of iron deficiency: policy and strat-
(5) Eden A.N. The prevention of toddler iron deficiency. Arch. Pedi-
(7) Still G.F. Common disorders and diseases of childhood, 3rd ed.
New York: Oxford University Press, 1920:495
(8) Darazs B., Hisdorffer C.S., Butterkerth A.M., Liady F. The possible
(2):341-346.
(9) Klewer S.E., Donnerstein R.L., Goldberg S.J. Still s-like innocent
murmur can be produced by increasing aortic velocity to a thresh-
(10) Park M.K. Pediatric cardiology for practitioners: history taking.
(11) Rosenthal A. How to distinguish between innocent and patho-
31:1229-1240.
(12) Lehrer S. Understanding paediatric heart sounds. Philadelphia;
(13) Boranić M. Bolesti krvi i krvotvornih organa i solidni tumori
2000: 645-647.
(14) Gil D.G., Vincent S, Segal D.S. Follow-on formula in prevention of
689.
The study involved 286 individuals from different regions of Bosnia and Herzegovina, whose sera were tested in the Laboratory for specific diagnosis of human brucellosis in Microbiology Department of Medical Faculty of University in Sarajevo, during the period from 2000 to 2003. Sera were tested using Brucelloslide Test, qualitative agglutination test Rose Bengal. Using the agglutination test, we serologically confirmed a diagnosis of human brucellosis in 89 (53.05%) seropositive individuals, whereof 53 (61.60%) men and 36 (40.66%) women. Individuals with human brucellosis were the most present in the age group of 31-40 (22.03%) and 41-50 (22.03%). One serologically confirmed death case was registered. The most seropositive individuals were from Zenica-Doboj Canton (32.20%), Sarajevo Canton (28.82%), Herzegovina-Neretva Canton (23.73%), Central Bosnia Canton (13.55%) and Una-Sana Canton (1.70%). During our four-year study, it was serologically confirmed that human brucellosis is present in Bosnia and Herzegovina and, through seropositive testing, we revealed the level of general exposition to Brucella spp. on wider area of Bosnia and Herzegovina.

**KEY WORDS:** Brucella, human brucellosis, serodiagnosis, Bosnia and Herzegovina.
INTRODUCTION

Genus Brucella is consisted of small, immobile, Gram-negative coccobacilli which are pathogenic for humans and animals. The first human cases of brucellosis were described in 1861, by J.A. Marston, physician with the British army settled in Malta (1). Brucellae are intracellular parasites that can be transmitted to a range of animal species, including humans (2). Four Brucella species, B. abortus, B. melitensis, B. suis and B. canis, can cause brucellosis when transmitted to humans. General infection routes in humans are digestive tract (consuming contaminated milk), mucosa (splashing route), skin (contact with infected animal tissues) or inhalation. Brucellosis is a system-affecting disease able to involve any of the major organ systems. Clinical disease manifestations are therefore very different. The most frequent described symptoms include periodic fever, cold, night sweating, headache, physical pains, anorexia and weakness. Additionally, infection focuses can occur in liver, reticuloendothelial system, bones and joints, urogenital tract, central nervous system, eyes, skin, lungs and heart (endocarditis) (3). The treatment of brucellosis includes a long-term antibiotic therapy, with recurrences if the therapy is discontinued too early or if microorganisms are in particularly deep focus of infection. These recurrences usually occur in 3 or 6 months after the therapy discontinuation. Controlling and supervising brucellosis is based on limited disease spreading and possible eradication of the infection in animals, pasteurizing the milk and milk products, and reducing professional risk, wherever it is possible.

PATIENTS AND METHODS

During the period from 2000. to 2003., sera from 286 individuals from different regions of Bosnia and Herzegovina, clinically suspected to have brucellosis, were tested in the Laboratory for specific diagnosis of human brucellosis in Microbiology Department of Medical Faculty of University in Sarajevo. Blood samples were collected by venipuncture, and isolated serum samples were preserved on –20°C until testing. Sera were tested using Brucelloslide Test, qualitative agglutination test Rose Bengal.

RESULTS

Out of 286 clinically suspect serotested individuals during the four-year period mentioned above, anti-Brucella antibodies were detected in tested sera in 59 (20.62%) individuals, while the result of agglutination test Rose Bengal remained negative in 227 (79.38%) individuals (Chart 1). Serotesting results for 286 individuals clinically suspected to have human brucellosis are represented in Table 1., for the period from 2000. to 2003. During 2000., 87 individuals clinically suspected to have brucellosis, were serotested. Agglutination test was positive in 15 (17.24%) individuals, while the result remained negative in 72 (82.76%) individuals. During 2001., 27 individuals were serotested, whereof 3 (11.11%) individuals had a positive agglutination test, while the result remained negative in 24 (88.89%) individuals. Out of 61 individuals serotested in 2002., 8
(13.11%) individuals had a positive agglutination test, while the result remained negative in 53 (86.89%) individuals. During 2003, 111 individuals were serotested, whereof 33 (29.72%) individuals had a positive agglutination test, while the result remained negative in 78 (70.28%) individuals. Chart 1. represents the rate between seropositive and negative individuals in overall diagnostic specimen for the period from 2000. to 2003. Out of 59 seropositive individuals, there were 38 (64.40%) men and 21 (35.60%) infected women. The most seropositive individuals were confirmed in the age group of 31-40 (22.03%) and 41-50 (22.03%). One serologically confirmed death case was registered. Out of 15 seropositive individuals confirmed during 2000., 7 of them (46.67%) were from Mostar, 5 (33.33%) from Sarajevo, 2 (13.33%) from Gornji Vakuf, 1 (6.67%) from Blagaj. Three seropositive individuals in 2001. were from Sarajevo. During 2002., out of 8 seropositive individuals, 5 of them (62.5%) were from Zenica, while 3 (37.5%) from Sarajevo. During 2003., we had 33 seropositive individuals, whereof 8 (24.25%) from Zenica, 6 (18.18%) from Sarajevo, 6 (18.18%) from Konjic, 3 (9.09%) from Busovaca, 2 (6.06%) from Visoko, 2 (6.06%) from Travnik and 1 (3.03%) from Novi Travnik. Cazin, Breza, Zepče, Olovo and Tešanj. The results of serological diagnosis of human brucellosis for cantons, for the period from 2000. to 2003. are represented in Table 2. During the four-year period, we recorded the highest seroprevalence of human brucellosis in Zenica-Doboj Canton (32.20%), Sarajevo Canton (28.82%), Herzegovina-Neretva Canton (23.73%), Central Bosnia Canton (13.55%) and Una-Sana Canton (1.70%).

**DISCUSSION**

Brucellosis is a zoonosis of the world-wide importance, especially in developing countries. According to the World Health Organization, the annual incidence of cases reported in endemic areas varies between 1 and 78 per 100000 inhabitants, with serological diagnosis in minimum 20% of human population (4). Professions traditionally related to the higher risk include farmers, veterinarians, shamblers and butchers, as well as laboratory personnel. However, effective control steps in ranch industry, as well as more frequently consuming unpasteurized milk products, lead to the fact that brucellosis is disease most commonly caused by nutrition (5). Significant increase in the proportion of food-home transmissions is the main reason for a great need for tests that would be fast and reliable detectors of brucellae in food and environment. Through specific diagnostic of the cases suspected for human brucellosis, as well as through seroprevalent testing, we tried to reveal a level of general exposition to Brucella spp. Immediate actions should focus on public education that will help decrease the risk of brucellosis: Supervising and controlling disease outbreaks in already determined seats. Defining sources and routes of disease transmissions. Consume only boiled or pasteurized milk, or dairy products prepared from milk treated in this way. Wash hands thoroughly after handling raw meat or milk.

**CONCLUSIONS**

1. Detecting specific anti-Brucella antibodies using agglutination test Rose Bengal, we serologically diagnosed human brucellosis in 59 (20.62%) clinically suspect individuals.

2. The highest prevalence of human brucellosis was present in Zenica-Doboj Canton (32.20%).

3. Agglutination test Rose Bengal can provide a useful diagnostic information.

4. A fast diagnostic of human brucellosis has important implications of public health and laboratory security.
References


LONG TERM PROTECTION AFTER HEPATITIS B VACCINATION

SANDRA PUVAČIĆ¹, JELENA RAVLIJA², ZLATKO PUVAČIĆ¹, IVAN CURIĆ³

ABSTRACT

A survey of persistence of anti-HBs after hepatitis B vaccination has shown that five years after vaccination on a sample of 152 persons, or 82.53%, stands at >10 IU/L. Long term immunogenicity of vaccinated children remained at 88.89%, health workers 79.41% and drug addicts 64.28%. The results of these studies in Bosnia and Herzegovina show the high level of protection hepatitis B vaccine against HBV infection. Vaccination against viral hepatitis B results in immunologic memory response among the vaccinated, and even after a decrease of anti-HB level following the third vaccine dose inoculation, a booster dose is not needed. Immunity remains steady and a booster dose is not recommended.

KEY WORDS: Immune memory, hepatitis B vaccination, long term efficacy
INTRODUCTION

Hepatitis B infection is found worldwide but the prevalence varies greatly among different countries. It is estimated that a half of the world population has experienced infection and there are 350 million chronically infected individuals. Hepatitis B is responsible for 1.5 million deaths per year. Around 40% of chronically infected individuals will die as a result of their infection. Chronic HBV infection may take the form of a Chronic Active Hepatitis (CAH) or Chronic Persistent Hepatitis (CPH) or minimal hepatitis. The distinction can only be made on a histological examination of the liver. CAH is far more common in active carriers as it is indicative of active viral replication Cirrhosis and hepatocellular carcinoma (HCC) is thought to be more common in active carriers. In European countries where immunisation against viral hepatitis B is conducted, the level of incidence of viral hepatitis disease has dropped by 80% compared to the period when immunisation process was not administered (1). Data from Italy shows that in the period between 1985 and 1990 when immunisation against viral hepatitis B was introduced, the incidence of this disease was reduced by more than 80%. Research in Spain has shown that an introduction of hepatitis B immunisation reduced the hepatitis B positive markers prevalence by 46% since the rate of HBV infection reduced from 16.9% to 9.1% with the introduction of the above mentioned (2). Nine years after the inclusion of immunisation against viral hepatitis B in the regular immunisation programme for children in Saudi Arabia, the prevalence of HbsAg was significantly reduced (3,4).

SUBJECTS AND METHODS

The primary study was to compare the referring results of research on persistence of anti-HBs after hepatitis vaccination in the world, with those in Bosnia and Herzegovina. The kinetics of anti-HBs after hep-B vaccination is very similar in every vaccinated individual, irrespective of the peak antibody level after third vaccine. An anti-HBs serological test result of 10ml IU/ml indicates immunity.

RESULTS

This research encompassed 186 recipients of a vaccine against viral hepatitis B, who received three doses of the vaccine five to six years ago. Results have shown that the persistence of anti-HBs after hepatitis B vaccination >10 IU/I stands at 82,53% on average (Table 1). The largest incidence of anti-HBs >10 IU/I level was registered among the population of children vaccinated against viral hepatitis B at a rate of 88,89%, then follow health workers at a rate of 79,41%, whilst intravenous drug users show the lowest rate at 64,28%. When compared with similar findings in other countries, results in Bosnia and Herzegovina show persistence of anti-HBs after immunisation rate to reflect in similar percentages (Table 2) These results show that 5 to 11 years after immunisation, those vaccinated against viral hepatitis B display a reduction in the titer of antibodies. A present-day question arises as to whether the fall in anti-

<table>
<thead>
<tr>
<th>POPULATION</th>
<th>NUMBER</th>
<th>TIME OF VACCINATION</th>
<th>NUMBER OF ANTI-HBS &gt;10 IU/I</th>
<th>% anti-HBS &gt;10 IU/I</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHILDREN</td>
<td>90</td>
<td>5 YEARS</td>
<td>80</td>
<td>88,89</td>
</tr>
<tr>
<td>HEALTH WORKERS</td>
<td>68</td>
<td>6 YEARS</td>
<td>54</td>
<td>79,41</td>
</tr>
<tr>
<td>DRUG ADDICTS</td>
<td>28</td>
<td>5 YEARS</td>
<td>18</td>
<td>64,28</td>
</tr>
<tr>
<td>TOTAL</td>
<td>186</td>
<td>5-6 YEARS</td>
<td>152</td>
<td>82,53</td>
</tr>
</tbody>
</table>

TABLE 1. Persistence of anti-HBs after hepatitis B vaccination

<table>
<thead>
<tr>
<th>POPULATION</th>
<th>TIME AFTER VACCINATION</th>
<th>% anti-HBS &gt;10 IU/I</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAIWANESE CHILDREN</td>
<td>10 YEARS</td>
<td>85</td>
</tr>
<tr>
<td>ITALIAN CHILDREN</td>
<td>11 YEARS</td>
<td>75</td>
</tr>
<tr>
<td>ALASKAN NATIVES</td>
<td>10 YEARS</td>
<td>76</td>
</tr>
<tr>
<td>BOSNIAN-HERZEGOVINIAN CHILDREN</td>
<td>5 YEARS</td>
<td>88</td>
</tr>
</tbody>
</table>

TABLE 2. Persistence of anti-HB after hepatitis B vaccination
HB <10 IU/l among those immunised against viral hepatitis B represents a loss of immunity, and whether a hepatitis B booster vaccine is necessary to boost immunity.

Are boosters needed for lifelong hepatitis B immunity?

Recent opinions, as well as numerous epidemiologic research, show the presence of immunity among those vaccinated with three vaccines against viral hepatitis besides the reduction in the titer rate to >10 IU/l. WHO does not recommend hepatitis B vaccine booster doses based on the following:

- Many studies have shown that infants, children and adults who have responded to a complete hepatitis B immunisation. Series are protected from disease for as long as 15 years, even if they lose protective antibodies over time
- Long-term protection relies on immunologic memory, which allows a protective anamnestic antibody response after exposure to the hepatitis B virus.

Immunologic memory is made-up of a complex interplay among memory B cells, memory T helper cells, memory cytotoxic lymphocytes (CTT), and antigen/antibody complexes. In vitro studies have shown that if the hepatitis B vaccine administered in a primary series initially induces an anti-HBs titre greater than or equal to 10 IU/ml, then memory B and T-helper cells retain the capacity to generate antibodies following re-exposure to HbsAg even if the anti-HBs titre falls to less than 10 ml IU/ml later on (4,5). Some of the difficulties in determining the length of protection after hepatitis B immunisation are summarised below:

- Follow-up studies with an observation time much longer than 10 years are still rare
- The number of vaccines available for follow-up decreases with time and the data become less significant.
- In countries with low endemicity, the risk of hepatitis B infection is very low so that challenge will be rare
- Immunological memory, thus far, has been demonstrated mainly by anamnestic response to re-vaccination while reliable and sensitive cellular tests are seldom used.

DISCUSSION

Immunisation against viral hepatitis B today represents one of the most valuable preventative programmes which significantly reduces the rate if incidence of this disease. The best proof of the efficiency of this vaccine is the reduction in the incidence of the viral hepatitis B among the citizens as claimed by the USA Health Service. Taiwan was the first country in the World to introduce immunisation against viral hepatitis B in 1984. Since 1986, all children are vaccinated against it. The programme also significantly reduced the incidence of viral hepatitis B among the non-vaccinated population HbsAg positive rate, from 8.1% to 2.3% in 2000 (5). Almost all individuals adequately vaccinated against hepatitis B have shown evidence of immunity in the form of persisting anti-HBs (protective body that develops following recovery from hepatitis B virus infection or after vaccination) and/or in vitro B-cell stimulation or an anamnestic response to a vaccine challenge(6,7). Protection against HBV infection if bound to anti-HB concentration of >10 IU/l (measured 1 to 3 months after administration of the last dose of the primary schedule). Antibody persistence depends on the initial (peak) anti-HBs concentration. The kinetics of anti-HBs after hep-B vaccination is very similar in every vaccinated individual, irrespective of the peak antibody level after third vaccine. An anti-HBs serological test result of 10ml IU/ml indicates immunity. Active immunisation against HBV is indicated for groups with an increased risk of acquiring this infection. These groups include medical personnel involved in care of patients who are potential carriers of the virus, laboratory staff, people working in high risk institutions such as those for mentally handicapped, individuals requiring repeated blood transfusions and/or blood products and those who may require haemodialysis in future. The vaccine takes up to 6 months to produce adequate protection and should not be given to people who are naturally immune to HbsAg positive, or suffering from acute hepatitis B. The vaccine may be given to HIV positive individuals.
CONCLUSION

Research findings have shown that the persistence of anti-HBs after hepatitis B vaccination > 10 IU/l shows on 152 (82.52%) vaccinated persons five years after the vaccination has been administered. These research findings have also shown a high level of anti-HBs displayed among the persons vaccinated against viral hepatitis B. The lowest percentage of anti-HBs >10 IU/l was found among the group of sampled drug addicts 64.28%, with the largest among children at a rate of 88.89%. It is important to emphasise that among those immunised with three doses, an immunologic memory is manifested after the administration on the hepatitis B vaccine. This immunologic memory is long-term regardless of the level of anti-HBs persistence, therefore a booster dose vaccine is not recommended regardless of the reduction in the level of anti-HBs.

REFERENCES

Cytogenetics Findings at Turner Syndrome and their Correlation with Clinical Findings

Amra Ćatović*

Center for Human Genetics, Medical Faculty, University of Sarajevo, Čekaluša 90, 71000 Sarajevo, Bosnia and Herzegovina

* Corresponding author

Abstract

Turner Syndrome is a genetic condition in females that results from an abnormal chromosome. One of the X chromosomes is missing or misshapen in the most cells of the body. Three classics clinical symptoms of the syndrome are: incomplete sexual maturation, short stature and pterygium colli. Turner Syndrome is diagnosed by karyotyping. In the retrospective study for a twelve years period (1991-2002) correlation between clinical and cytogenetics findings was established in our Center among 47 examinees from all parts of Federation of Bosnia and Herzegovina, who had suspect clinical diagnosis of Turner Syndrome. The syndrome was demonstrated by cytogenetics examinations in 30(63.8%) examinees and excluded in 17 (36.2%) examinees. The most frequent karyotype is monosity of X chromosome (45,X) found at 63.3%, than isochromosome of Xq (46,XisoXq) found at 16.7%, mosaic form (46,XX/45,X) and deletion of Xp (46,XdelXp) both at 6.7%, than deletion of Xq (46,XdelXq) and ring of Xp (46,XX/46,XringXp) both at 3.3%. Our results suggest that promptly and exactly diagnosis of Turner syndrome is very important due to introducing growth hormone therapy and estrogen therapy at a very young age.

Key Words: Turner Syndrome; cytogenetics findings, clinical findings
INTRODUCTION

In 1938, at a medical conference, an endocrinologist named Henry Turner described cases of seven young women, aged fifteen to twenty three, with three symptoms: infantilism, congenital webbed neck and cubitus valgus (1). Polani et al. (2) and Wilkins et al. (3) demonstrated that most cases are chromatin-negative, and Ford et al. (4) first described the 45,X karyotype. Turner Syndrome is one of the most common chromosomal abnormalities. It affects approximately 1 in 2,500 live female births (5), corresponding to approximately 1.5 million women worldwide. There appears to be a high fetal wastage with only 1% of these embryos surviving to term (6). Thus, Turner Syndrome is responsible for 7–10% of all spontaneous abortions. Environmental risk factors for conceiving a child with Turner Syndrome are unknown. Turner Syndrome is not, in general, associated with advancing parental age (7). Many physical features are associated with Turner Syndrome: low birth weight and length, webbed neck, low posterior hairline, oedema of the dorsum of the hands and feet in early childhood, short stature (sexual infantilism), puberty failure, ovaries degeneration, primary or secondary amenorrhea, miscellaneous defects (heart – coarctation of the aorta, kidney – horseshoe kidney, eye), and autoimmune pathologies (such as thyroiditis, diabetes mellitus, celiac disease) are frequently observed (8).

Women with Turner Syndrome need long-term follow-up so that early medical intervention may reduce morbidity and improve life expectancy (9,10). Cytogenetics analyses explain etiopathogenesis of Turner Syndrome. Without them clinical diagnosis is uncertain. Cytogenetics findings associated with Turner Syndrome are very different. The number and severity of dysmorphic symptoms depend on these findings (11) (Figure 1).

The basic goal of this retrospective study is to evaluate cytogenetic findings in patients with suspect clinical diagnosis of Turner Syndrome and establish correlation between their clinical and cytogenetics findings.

SUBJECTS AND METHODS

The research was done as retrospective study for a twelve years period (1991-2002) and it involved 47 patients who had been send for cytogenetics analyses with diagnosis suspect Turner Syndrome in the “Center for Human Genetics” of Medical Faculty in Sarajevo from all other health institutions throughout the Federation of Bosnia and Herzegovina. Turner Syndrome is diagnosed by a blood test called karyotype. There are several cytogenetics technique used in laboratory: technique of standard, technique of metaphase and prometaphase banding. Prometaphase (high resolution) banding technique ensures better quality of cytogenetics analyses (12). The nucleus of a female cell in interphase has one dark-

![FIGURE 1. Schematic illustration of X chromosome structural abnormalities](image)

1. Normal X chromosome
2. Isohromosome Xq (long arm)
3. Short arm deletion of an X chromosome (Xp-)
4. Interstitial deletion of short arm
5. Interstitial deletion of long arm
6. Long arm deletion of an X chromosome is hard to distinguish from isochromosome Xp because bands are almost identical
7. “End to end” rearrangements Xp
staining X chromosome called Barr body (named after its discoverer, Murray Barr, Canadian researcher, who noticed these dark bodies in 1949 in nerve cell of female cats). Females with karyotype 45,X have none Barr body. Persons with abnormal numbers of X chromosomes have one fewer Barr body than they have X chromosomes per cell. Structural changes of X chromosome are manifested with misshapen Barr bodies (13).

RESULTS

The syndrome was demonstrated by cytogenetics examinations in 30 (63.8%) of 47 examinees and excluded in 17 (36.2%) examinees (Table 1). The results of research of correlation between cytogenetics findings examinees with diagnosis suspect Turner Syndrome in a twelve years period (1991-2002) were tested by χ² test. Demonstration-excluding Turner Syndrome is on the level of significance p<0.05. Cytogenetics types of Turner Syndrome are shown in Graph 1. The most frequent karyotype is monosomy of X chromosome (45,X - Figure 2) found at 63.3%, than isochromosome of Xq (46,XisoXq - Figure 3) found at 16.7%, mosaic form (46,XX/45,X) and deletion of Xp (46,XdelXp - Figure 4) both at 6.7%, than deletion of Xq (46,XdelXq) and ring of Xp (46,XX/46,XringXp) both at 3.3%. The results of research of frequency of cytogenetics types of Turner Syndrome in a twelve years period (1991-2002) were tested by χ² test and level of significance is p<0.05.

DISCUSSION

The distinction between male and female, found in most species of animals and plants, is based on the type of gametes (ova, spermatozoa) produced by individual. Gonadal development in human is determined by presence of the XX (female) or the XY (male) genotype. Combined cytogenetic, fluorescence in situ hybridization (FISH), and molecular analysis are useful in the diagnosis of sex chromosome aberrations (14). Turner Syndrome is a genetic condition in females that results from an abnormal chromosome. One of the X chromosomes is missing or misshapen in the most cells of the body. For normal development and function of ovary, two X chromosomes are necessary. Second X chromosome is necessary for ovarian maintenance, but not for its differentiation. Without that second X chromosome, ovaries, which are developed at the beginning, finally degenerate. Girls with several of the characteristic features may be suspected during infancy or early childhood. If a female adolescent does not begin puberty at a normal age she should be evaluated for Turner Syndrome. As early diagnosis has several potential advantages.


<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DEMONSTRATED TURNER SYNDROME</td>
</tr>
<tr>
<td></td>
<td>Nº</td>
</tr>
<tr>
<td>ALL EXAMINEES</td>
<td>30</td>
</tr>
</tbody>
</table>


FIGURE 2. Turner Syndrome: 45,X
a cytogenetic analysis should be considered in all girls with unexplained short stature (15). Turner Syndrome is diagnosed by karyotyping. There is a correlation between the exact cytogenetic appearance and the phenotype in Turner Syndrome. The most frequent karyotype (16), at 48% of all cases, is X monosomy (45,X). In our sample percentage is higher (63.3%), which can be in connection with the value of sample (smaller sample). They appear to be more severely affected clinically than other forms of the disorders. Structural cardiac and renal abnormalities occur more frequently in this group. Monosomy X results from nondisjunction as a result of failure of the sex chromatids to separate during meiosis in the parental gamete or in the early embryonic divisions. The latter usually results in mosaicism. According to Elsheikh et al. (16), about 11% of Turner patients are mosaic with both 45,X and 46,XX cell lines. In our sample percentage is lower. We found that 6.7% study patients were mosaic. The two populations of cells may appear in every tissue of the body or only in certain ones. Presumptive evidence for mosaics lies in a discrepancy between sex chromatin pattern and karyotype, or through observing a low percentage of chromatin-positive nuclei (5-15%) in phenotypic females. The clinical spectrum of 45,X/46,XX mosaicism is wide and may vary from cases quite typical of Turner Syndrome with many associated anomalies to cases with normal gonads and normal stature. Difference in clinical patterns may be related to the time of X chromosome loss and the particular tissues involved. Rare karyotype 46,XX is possible with one X chromosome with changed structure. Structural X chromosome abnormalities are thought to occur as a result of breakages in the X chromosome with subsequent reunion of X chromosome sequences. These changes can be more or less visible. Isochromosome Xq is the most common structural abnormality. About 18% (16) of patients having Turner Syndrome have an isochromosome Xq, i.e., replication of long arm of the late replicating X chromosome. This findings is very similar to our results (16.7%). The Barr body are larger than normal. They exhibit many of stigmata of 45,X Turner Syndrome, i.e. short stature and sexual infantilism, but are less likely to have pterygium colli and aortic coarctation. In this group risk of autoimmunity, particularly thyroiditis and deafness is increased. In study of Elsheikh et al. (16) types 46,XXp-, 46,XXq- and 46,XXr together comprise no more than 14.5% of Turner Syndrome. In our study the percent of these karyotypes is very similar (13.3%). Barr bodies in these cases are smaller than normal. These cases are with variable phenotype...
CONCLUSION

In retrospective study for a twelve years period (1991-2002) it was done the evaluations of cytogenetics analyses of patients with diagnosis suspected Turner Syndrome who were send in the “Center for Human Genetics” of Medical Faculty in Sarajevo from all other health institutions throughout the Federation of Bosnia and Herzegovina. The syndrome was demonstrated by cytogenetics examinations in 30(63.8%) examinees and excluded in 17 (36.2%) examinees. The most frequent karyotype is monosomy of X chromosome (45,X) found at 63.3%, than isochromosome of Xq (46.XisoXq) found at 16.7%, mosaic form (46.XX/45,X) and deletion of Xp (46,XdelXp) both at 6.7%, than deletion of Xq (46,XdelXq) and ring of Xp (46.XX/46.XringXp) both at 3.3%. It is very important that females with Turner Syndrome are diagnosed promptly so they may have the opportunity to receive growth hormone therapy and estrogen therapy at a very young age.

REFERENCES

SERUM AND TISSUE ANGIOTENSIN CONVERTING ENZYME IN PATIENTS WITH LICHEN PLANUS

FARUK ALENDAR\textsuperscript{1}, JASMINKO HUSKIĆ\textsuperscript{2*}, NERMINA BABIĆ\textsuperscript{2}, NEDŽAD MULABEGOVIĆ\textsuperscript{3}

1. Dermatovenerology Clinic, University of Sarajevo Clinics Center, Bolnička 25, 71000 Sarajevo, Bosnia and Herzegovina
2. Institute of Physiology and Biochemistry, School of Medicine, University of Sarajevo, Čekaluša 90, 71000 Sarajevo, Bosnia and Herzegovina
3. Institute of Pharmacology, School of Medicine, University of Sarajevo, Čekaluša 90, 71000 Sarajevo, Bosnia and Herzegovina

* Corresponding author

ABSTRACT

Serum and tissue angiotensin-converting enzyme (ACE) was measured in 20 patients with lichen planus before and after therapy, and in 20 healthy individuals. Serum and tissue ACE activity was determined by spectrophotometric method using hippuryl-l-histidyl-l-leucine as a substrate. The enzyme activity is expressed in the following units: 1 U corresponds to 1 nmol of hippuric acid released by hydrolysis of hippuryl-l-histidyl-l-leucine per minute and one liter of serum or 50 mg tissue. Before therapy, serum ACE activity was significantly increased in patients with lichen planus (35.9 ± 2.33 U/L) in comparison to healthy individuals (28.16 ± 1.7 U/L). Tissue ACE activity was increased in patients with lichen planus (2.24 ± 0.41 U/50 mg) in comparison to healthy individuals (1.86 ± 0.16 U/50 mg), but the difference was not significant. After therapy, serum and tissue ACE activity decreased and no significant difference in ACE activity was found. The determination of serum ACE activity may be a good non-specific parameter for the assessment of therapeutic effects.

KEY WORDS: angiotensin converting enzyme, lichen planus, serum, therapy.
INTRODUCTION

Angiotensin converting enzyme (ACE; kininase II, EC 3.4.15.1) is widely distributed at the surface of endothelial and epithelial cells. This enzyme catalyzes the conversion of inactive decapeptide angiotensin I into active octapeptide angiotensin II, and the inactivation of the nonapeptide bradykinin (1). Subsequent investigations showing the existence of a local renin angiotensin system in the brain, kidney, adrenal gland, testis, artery walls, heart, skin and other tissues, alongside the circulatory renin angiotensin system, have pointed to a new physiological role of this very complex system (2). Investigations of ACE in dermatology are very scarce and little is known about potential role of ACE in pathogenesis of skin diseases. Recent studies have shown changes in renin angiotensin system in various skin diseases (3-5). Lichen planus is a relatively common skin disorder of unknown etiology. While most cases of lichen planus are idiopathic, some may be caused by the ingestion of certain medications (6). Recent studies have shown that angiotensin-converting enzyme inhibitors were implicated in its cause (7,8). However, the mechanism by which ACE inhibitors could flare the pre-existing disease is not clear. Raff et al. (3) reported that the serum ACE activity is increased in patients with lichen planus. However, the real value of ACE activity determination as a clinical-biochemistry test for the diagnosis of lichen planus has not been attained. Furthermore, the effect of therapy on serum ACE activity in patients with lichen planus was not studied and no data on tissue ACE activity in patients with lichen planus were published. The aim of the present study was to investigate serum and tissue ACE activity in patients with lichen planus and the possible influence of therapy on serum and tissue ACE activity.

SUBJECTS AND METHODS

1. SUBJECTS
Patients with diseases that may influence serum ACE activity (sarcoidosis, arterial hypertension, pulmonary tuberculosis, hepatic diseases, diabetes mellitus, and others) were excluded from the study. Control group consisted of 20 subjects of both sexes (10 male and 10 female) 35-45 years of age, who were healthy according to subjective and objective findings. Study group included 20 patients with lichen planus of both sexes (10 male and 10 female), 35-45 years of age, who were medically treated. The diagnosis of lichen planus was made on the basis of clinical examination and biopsy findings at the Department of Dermatology, University Hospital in Sarajevo.

2. LABORATORY AND OTHER ANALYSES
Routine laboratory analyses, including erythrocyte and leukocyte counts, erythrocyte sedimentation rate, hematocrit, hemoglobin, urea, uric acid, creatinine, triglycerides, cholesterol, and glucose levels, as well as a complete urine analysis, were performed on each patient. A biopsy of the skin was done in all patients with lichen planus for pathohystologic analysis.

3. SERUM AND TISSUE SAMPLING
Serum and tissue ACE activity was measured in patients with lichen planus before and after therapy. Blood for the determination of serum ACE activity was drawn from cubital vein. After coagulation and centrifugation at 2,000 g for 5 min, serum was frozen and stored at -20 °C until further analysis. After biopsy, all tissues were weighed and extensively washed in 0.9% NaCl solution (40°C) for blood elimination. The tissues were placed in sodium phosphate buffer (0.065 mol/L, pH 7.3, and 0.5 mol/L NaCl; 50 mg/ml) and stored at -20 °C. The tissues were homogenized in Teflon coated Potter-Elvehjem homogenizer, with one drop of a nonionic surfactant (Nonidet P 40) in each sample. After centrifugation at 4,000 g for 30 min, the supernates were frozen at -20 °C until determination of ACE activity.

4. MEASUREMENT OF ACE ACTIVITY
Serum and tissue ACE was determined by spectrophotometric method using hippuryl-l-histidyl-l-leucine (Sigma, St. Louis, Mo., USA) as a substrate (9), and Perkin Elmer 550 S spectrophotometer for optical reading. The enzyme activity is expressed in the following units: 1 U corresponds to 1 nmol of hippuric acid released by hydrolysis of hippuryl-l-histidyl-l-leucine per minute in one liter of serum or 50 mg of the tissue.

4. STATISTICS
Serum ACE activity is expressed as mean values ± SEM. Differences between the mean values were statistically compared using Student’s and paired t-tests. P-values less than 0.05 were considered significant.

RESULTS

Figure 1 illustrates that serum ACE activity was significantly increased in patients with lichen planus; the latter having a mean value by 27% higher than that found in healthy subjects (p<0.001). No signifi-
cant sex-related differences in serum ACE activity, either in the control group or in the patients with lichen planus (data not shown) were found. Following the therapy serum ACE activity decreased by 14% in patients with lichen planus when compared with the values recorded before therapy (p<0.001). Data on tissue ACE activities in the control group and in patients with lichen planus are shown in Figure 2. Tissue ACE activity was 20% higher in patients with lichen planus than in their healthy counterparts. However, there was no significant difference in the mean ACE levels. Also, no significant difference was found between the tissue ACE activity in patients with lichen planus before and after therapy.

DISCUSSION

Our study clearly showed that mean serum ACE activity is significantly increased (27% higher) in patients with lichen planus in comparison to healthy subjects. These results concur with the clinical study performed by Raff et al. (3). Accordingly, these results indicate the existence of potential role for ACE in pathogenesis of lichen planus, although we found no significant differences in tissue ACE activity in patients with lichen planus in comparison to healthy subjects. More developed and extensive lesions in patients with lichen planus may be difficult to diagnose seeing as lesions of this sort may mimic psoriasis. In this case, the determination of serum ACE activity cannot be helpful in the diagnosis of lichen planus. Accordingly, numerous studies have shown that serum ACE activity increased in patient with psoriasis in comparison to healthy subjects (3,10,11).

In the present study, we also investigated the effects of therapy on serum ACE activity in patients with lichen planus. The results showed that serum ACE activity significantly decreased in patients with lichen planus following the therapy in comparison with the values recorded before therapy (p<0.001). This suggests that the determination of serum ACE activity could be one of the discriminators used to assess the effects of therapy. Lichen planus is a skin condition of unknown origin that is frequently linked to diseases that may influence serum ACE activity [liver diseases (12,13), diabetes mellitus (14,15), sarcoidosis (16) and others]. Recent studies have demonstrated that ACE inhibitors can induce lichen planus (7,8). Unfortunately, mechanisms by which ACE inhibitors can flare a pre-existing disease are unknown. In our opinion, the investigation of this mechanism is very important because it should provide information about the pathogenesis of lichen planus. Angiotensin-II, a product of ACE activity, stimulates the release of pro-inflammatory cytokines, increases oxidant stress, and suppresses nitric oxide synthesis (17). Since ACE is present in the skin, this suggests that angiotensin-II may have a role in lichen planus, a condition in which inflammation is known to play a significant role. The ACE effectively controls bioavailability of peptide mediators released from sensory nerves and the immune and skin cells during the cutaneous response.
to endogenous or exogenous noxious stimuli (18). It is possible that the peripheral nervous system is implicated in immunopathogenesis of lichen planus and lichenoid reactions. However, this data fails to show whether an increase in serum ACE activity in patients with lichen planus is a primary process, has a pathogenic role, or is only secondary to some independent process. Obviously, this is the main question which should be answered in further investigations.

REFERENCES

Abstract

Endogen phospholipids play a major role in determining the structure and nature of cell membranes. A deficiency of phospholipids in cellular membranes makes it almost impossible for the cell membrane to perform its function as a selective barrier between what passes in and out of the cell. Polyenylphosphatidylcholine chemical structure corresponds to that of endogen phospholipids, but it possesses functional superiority because of its content of unsaturated fatty acids. Polyenylphosphatidylcholine integrates in the cell membrane and organelle systems while becoming their constitutive elements. A healthy cell membrane leads to healthy cells and then healthy tissue and then to healthy organs or body systems and finally, healthy bodies and minds. For a long time, polyenylphosphatidylcholine in combination with vitamins has been used in the treatment of numerous health problems such as liver diseases, dyslipoproteinaemias and different intoxications with consequent liver failure. The main aim of toxicology studies is evaluation of the toxic potential and risks of human exposition to the substance (1). According to the Organization for Economic Cooperation and Development (OECD) acute oral toxicity refers to those adverse effects occurring following oral administration of a single dose of a substance or multiple doses given within 24 hours. LD50 (median lethal dose), oral, is a statistically derived single dose of a substance that can be expected to cause death in 50 per cent of animals when administered by the oral route (2). Our acute toxicity study was performed on albino Wistar rats. Animals were randomised in three experimental and one control group, each of 5 males and 5 females. Study was based on the administration of a single oral dose of the test substance (polyenylphosphatidylcholine) to each experimental animal. There were three dose-levels of the test substance: 300, 500 and 1000 mg/kg. Test substance administration day was the first day of the observation period that lasted 14 days. Control animals were given milk vehicle. At the end of the study, no statistically significant differences between experimental and control animals were observed concerning the recorded parameters: body weight, respiratory rate, tremor, faeces and phonation quality, indicating the absence of the test substance acute toxicity.

Key Words: polyenylphosphatidylcholine, acute oral toxicity.
INTRODUCTION

Endogenous phospholipids are found in every cell membrane in all living matter. Endogenous phospholipids play a major role in determining the structure and nature of cell membranes. They are made up of a hydrophilic head and a hydrophobic tail. The head group has a ‘special’ region that changes between various phospholipids (Figure 1). This head group differs between cell membranes. The fatty acid tails can also differ, but there is always one saturated and one unsaturated ‘leg’ of the tail. A deficiency of phospholipids in cellular membranes makes it almost impossible for the cell membrane to perform its function as a selective barrier between what passes in and out of the cell. Endogenous phospholipids facilitate the regulation of important biochemical processes in different biological systems.

Polyenylphosphatidylyceroline (PPC), an antioxidant phosphatidylcholine mixture extracted from soybeans, consists of the highly bioavailable dilinoleoylphosphatidylcholine, restores phospholipids of the damaged membranes and reactivates their enzymes, including phosphatidylethanolamine methyltransferase, needed for phospholipid regeneration. Polyenylphosphatidylyceroline (PPC) chemical structure corresponds to that of endogenous phospholipids, but it possesses functional superiority because of its content of unsaturated fatty acids. Polyenylphosphatidylyceroline integrates in the cell membrane and organelle systems while becoming their constitutive element. A healthy cell membrane leads to healthy cells and then healthy tissue and then to healthy organs or body systems and finally, healthy bodies and minds.

Demirbilek and co-workers concluded that polyenylphosphatidylyceroline therapy might be a useful adjuvant therapy in controlling the excessive production of the inflammatory cytokines in patients with severe sepsis (3). Since polyenylphosphatidylyceroline appears to promote the breakdown of collagen, it might affect not only the progression of the liver disease, but it may also reverse pre-existing fibrosis in rats (4). Polyenylphosphatidylyceroline was found to prevent alcohol-induced steatosis and hyperlipemia in rats (5) and to exert potent antioxidant effects of possible relevance to fibrosis in baboons (6). It has been shown that polyenylphosphatidylyceroline reduced levels of transaminases in patients with hepatitis C (7).

PPC was beneficial in patients with alcoholic hepatitis, and it opposed fibrosis in heavy drinkers and decreased aminotransferases in patients with hepatitis C. Finally, replacing long-chain with medium-chain triglycerides opposed the fatty liver experimentally and clinically (8).

Authors Lee SH, et al. were performing a study in rats, found that polyenylphosphatidylyceroline expressed cytoprotective effects on pancreatic beta-cells after the diabetic induction by streptozotocin. Their results strongly suggest that polyenylphosphatidylyceroline plays important roles not only in protecting beta-cells against cytotoxicity, but also in maintaining their insulin syn-

![Figure 1: A model of phospholipid molecule](image-url)
thesis and secretion for normal glucose homeostasis (9). Aleynik SI. and Lieber CS. found that polyenylphosphatidylcholine corrected the alcohol-induced hepatic oxidative stress in rats by restoring s-adenosylmethionine and replenishing hepatic glutathione (10). Research on nerve cells has shown that polyenylphosphatidylcholine can act as a substitute for the messenger substances acetylcholine. Dietary polyenylphosphatidylcholine decreased cholesterolemia in hypercholesterolemic rabbits. Phosphatidylcholine enriched diet developed significant higher cholesterol- and triacylglycerol-lowering effects by a two-step mechanism: 1) by reducing the beta-VLDLs, 2) by enhancing the secretion of bile cholesterol indicating promising effects of soybean phosphatidylcholine at the hepatobiliary level, in the treatment or prevention of hyperlipidemia and related atherosclerosis (11). For all of these reasons, polyenylphosphatidylcholine, alone or in combination with vitamins, has been used for a long time in the treatment of numerous health problems such as liver diseases, dyslipoproteinaemias and different intoxications with consequent liver failure. The major aim of our study was to determine oral acute toxicity of polyenylphosphatidylcholine in rats.

MATERIALS AND METHODS

Our study was carried out on albino Wistar rats, during period from February 2nd until February 25th, 2003. Animals were put in 3 experimental and 1 control group, each of 5 males and 5 females (8 groups in total). Groups with male animals were signed with letter M. Groups with female animals were signed with letter F, while control groups of animals were signed with letter K. Animal groups signed with numbers 1, 2 or 3 were treated with following single doses of the test substance: 1) 300 mg/kg; 2) 500 mg/kg; 3) 1000 mg/kg. Test samples were capsules, each containing polyenylphosphatidylcholine (PPC) 300 mg, nicotinamide 30 mg, pyridoxine hydrochloride 6 mg, riboflavin 6 mg, thiamine nitrate 6 mg, tocopherol acetate 6 mg. In order to prepare test substance the content of capsules was dissolved in milk heated in the water bath up to 60 °C. Prepared test substance was administered to test animals by oesophageal intubation. Control animals were given milk vehicle. Throughout the first day following the test substance application, 4 clinical examinations were performed in 2-hour intervals. Clinical examinations and mortality check-ups were carried out daily, in constant time intervals (every morning and evening). Each animal body weight determination was performed before the test substance administration, once a week and at the end of the study.

RESULTS

The first aim of our study was to determine the effect of the test substance administration on animal body weight. Experimental animals were randomised in such manner that the mean body weight did not significantly differ between groups (p=0.5). Variations inside experimental groups of animals were within two standard deviations. Statistical analysis of the body weight variations was performed separately for males and females (Graph 1, Graph 2). T-test for independent specimen failed to show any significant difference in body weights between groups of examined animals. The second study aim was to determine the effect of the test substance administra-
SANJA KROŠNJAR ET AL.: ORAL ACUTE TOXICITY OF POLYENYLPHOSPHATIDYLCHOLINE (PPC) IN RATS

Aim of our study was to find out effect of the test substance administration on animal respiration rate. Respirator of female and male animals prior and post test substance application are presented in Graph 3 and Graph 4. Respiratory rates recorded in experimental and control groups of animals were within physiological limits for rats. Results did not significantly differ prior and post test substance administration (Table 1).

### Table 1. Descriptive statistics for respiratory rate

<table>
<thead>
<tr>
<th>GROUP</th>
<th>NUMBER OF RECORDINGS</th>
<th>MEAN</th>
<th>STD. DEV.</th>
<th>STD. ERROR</th>
<th>CI. OF MEAN</th>
</tr>
</thead>
<tbody>
<tr>
<td>3M</td>
<td>5</td>
<td>93.600</td>
<td>13.740</td>
<td>6.145</td>
<td>17.061</td>
</tr>
<tr>
<td>2M</td>
<td>5</td>
<td>74.400</td>
<td>10.431</td>
<td>4.665</td>
<td>12.951</td>
</tr>
<tr>
<td>1M</td>
<td>5</td>
<td>80.000</td>
<td>18.330</td>
<td>8.198</td>
<td>22.760</td>
</tr>
<tr>
<td>KM</td>
<td>5</td>
<td>76.800</td>
<td>12.458</td>
<td>5.571</td>
<td>15.469</td>
</tr>
<tr>
<td>3F</td>
<td>5</td>
<td>79.200</td>
<td>23.900</td>
<td>10.688</td>
<td>29.676</td>
</tr>
<tr>
<td>2F</td>
<td>5</td>
<td>74.400</td>
<td>15.126</td>
<td>6.765</td>
<td>18.782</td>
</tr>
<tr>
<td>1F</td>
<td>5</td>
<td>84.000</td>
<td>17.205</td>
<td>7.694</td>
<td>21.362</td>
</tr>
<tr>
<td>KF</td>
<td>5</td>
<td>73.600</td>
<td>26.169</td>
<td>11.703</td>
<td>32.493</td>
</tr>
</tbody>
</table>

The third aim of our study was to find out effect of the test substance administration on the appearance of clinical signs: tremor, animal phonation and faeces quality. Observed tremor was more frequent and more intensive in female animals than in male animals. Tremor was registered in 18 records from 160 records in total - 11.25%. 8 times in 3F group (in 4 animals), 6 times in 2F group (in 3 animals), and 4 times in 1F group (in 2 animals). Tremor was found to be mild in 13 cases (72.22%) or moderate in 5 cases (27.78%). In almost all cases (94.5%) tremor was registered during the first day of the test substance administration. Tremor was not observed in control group of animals. Sporadically, hoarse animal phonation was registered, predominantly in female animals. Hoarse phonation was noticed in control group of animals, as well. It was most frequently expressed during the 3rd and 4th day of the observation period. Such distribution of the changed animal phonation indicates that it was not a consequence of the test substance administration. Mildly to moderately lose faeces was observed sporadically and predominantly in male animals. Loose faeces was noticed in control animals, as well. Loose faeces were recorded 13 times in male animals (36.1%): 4 times in 3M group, 2 times in 2M group, 3 times in 1M group and 4 times in control group of animals. Loose faeces were found to be mild in 6 cases while moderate in 7 cases. Loose faeces were recorded 4 times in females (13.8%): 1 case per each group of animals. Loose faeces were found to be mild in 1 case while moderate in 3 cases (females). A statistical analysis performed on the end of our study revealed:

- **Body weight** - the test substance administration did not induce statistically significant differences in body weights between experimental and control animals.
- **Respiratory rate** - the test substance administration did not induce statistically significant differences in respiratory rates between experimental and control ani-
mals; tremor - the test substance administration did not induce statistically significant differences in observed tremor between experimental and control animals; changed phonation - the test substance administration did not induce statistically significant differences in phonation quality between experimental and control animals; loose faeces - the test substance administration did not induce statistically significant differences in faeces quality between experimental and control animals.

DISCUSSION

The main aim of toxicology studies is evaluation of the toxic potential and risks of human exposition to the substance (12). According to the Organization for Economic Cooperation and Development (OECD) acute oral toxicity refers to those adverse effects occurring following oral administration of a single dose of a substance or multiple doses given within 24 hours. LD50 (median lethal dose), oral, is a statistically derived single dose of a substance that can be expected to cause death in 50 per cent of animals when administered by the oral route. LD50 for acute oral toxicity means that dose of the material administered to both male and female young adult albino rats which causes death within 14 days in half the animals tested. The number of animals tested must be sufficient to give statistically valid results and be in conformity with good pharmacological practices. The result is expressed in mg/kg body mass. Acute oral toxicity is the adverse effects occurring within a short time of oral administration of a single dose of a substance or multiple doses given within 24 hours. LD50 (median lethal dose), oral, is a statistically derived single dose of a substance that can be expected to cause death in 50 per cent of animals when administered by the oral route. The LD50 value is expressed in terms of weight of test substance per unit weight of test animal (mg/kg) by United States Environmental Protection Agency. When the chemical substance produce toxicity only in certain circumstances, e.g. in extremely high doses, it is not necessary to evaluate the effects of irrationally high doses to demonstrate the toxicity. Information that substance in rationally high doses does not induce any lethality can be sufficient. Dose of 2 g/kg is accepted as a limit dose by the FAO/WHO Expert Committee on Food Additives, while US Environmental Protection Agency (EPA, 1994) accepts the limit of 5 g/kg. Applied doses in our study are approximations of the proposed future human single (therapeutic) doses. If one capsule contains therapeutic human dose, the applied doses in our study correspond to following: If each test sample capsule contains a single therapeutic dose of polynylphosphatidylcholine-PPC, doses administered in our study were:

- 300 mg/kg is corresponding to a 50 times higher dose than human dose, (precisely 52.6 times higher dose);
- 500 mg/kg is corresponding to a 85 times higher dose than human dose, (precisely 87.7 times higher dose);
- 1000 mg/kg is corresponding to a 175 times higher dose than human dose, (precisely 175.4 times higher dose).

Dose of 2 g/kg is accepted as a limit dose by the FAO/WHO Expert Committee on Food Additives (WHO, 1996), while US Environmental Protection Agency (EPA, 1994) accepts the limit of 5 g/kg. A substance with LD50 between 0.5 and 5 g/kg is classified as moderately toxic (11). We did not register any lethality after the 1 g/kg-dose application, so the test substance may be classified as substance with moderate toxicity, low toxicity or practically with no toxicity. The maximal tested dose in our study was 1 g/kg. Taking in consideration a fact that the overdose with a dose 200 times higher than the single therapeutic dose is hardly possible we did not find a testing of the higher test substance doses rational. Authors Gad & Changelis consider that testing of the doses 100-300 times higher than the proposed human doses is sufficient (13).

CONCLUSION

Polynylphosphatidylcholine did not express acute toxicity, when administered orally in rats in single doses of 300 mg/kg, 500 mg/kg or 1000 mg/kg. After the administration of a dose of even 1 g polynylphosphatidylcholine per kg in rats that is corresponding to 175 times higher dose than human dose, no lethality was observed. According to a definition, by which a substance whose LD50 is between 0.5 and 5 g/kg is classified as moderately toxic substance (11), our test substance, polynylphosphatidylcholine, might be classified as a substance of low toxicity or practically non-toxic substance.
REFERENCES

Abstract

Proficiency in the anatomy of coronary arteries and their variations is significant for proper interpretation of the coronary angiographies, assessment of the complexity and result of the coronary insufficiency as well as surgical myocardium revascularization.

The objective of this study is anatomy-radiology research of the methods of branching the main trunk of left coronary artery and to prove importance of the diagonal branch (ramus diagonalis) existence in the conditions of coronary insufficiency.

In this study we have analyzed 100 coronary angiographies done at the Clinic for Heart Diseases and Rheumatism of the Clinic Center of University of Sarajevo and dissected 20 human hearts from the Institute of Anatomy. In our study we have come upon two methods of branching of main trunk of left coronary artery (bifurcation and trifurcation). By the method of the angiography we have found the bifurcation in 114 of cases while 98 of cases were proved by the dissection method. Trifurcation has been discovered in 29% of cases of analyzed angiographies i.e. 35% of cases of dissected hearts. We believe that third terminal branch of the left coronary artery should be marked as ramus diagonalis. This branch, including its anastomoses, presents important pattern of the collateral blood flow, which has special meaning, under conditions of coronary insufficiency.

KEY WORDS: main trunk, left coronary artery, ramus diagonalis

Branching of Main Trunk of Left Coronary Artery and Importance of Her Diagonal Branch in Cases of Coronary Insufficiency

Almira Lujinović*, Fehim Ovčina, Alma Voljevica, Aida Hasanović

Institute of Anatomy, Faculty of Medicine, University of Sarajevo, Čekaluša 90, 71000 Sarajevo, Bosnia and Herzegovina

* Corresponding author
INTRODUCTION

Human heart in the most cases possess right and left coronary artery (a. coronaria dextra et a. coronaria sinistra), and in certain number of cases (5%) there are supernumerary heart arteries. We call them coronary because, they like a wreath encircle heart in particular groove. A.coronaria dextra originates from the right aorta sinus (sinus Valsavae dexter), headed from top toward frontal part and externally and emerges at the frontal part of heart. It passes through coronary groove and arrives at the back part of heart, where in the most cases ends at its back interventricularis groove (sulcus interventricularis posterior). A.coronaria sinistra originates from the left aorta sinus (sinus Valsavae sinister). Its main branch, after it passes by between the branches of pulmonary arteries and left aurikula, emerges at the frontal part of heart. When magisterial type of cases, here at this spot it splits on ramus interventricularis anterior and ramus circumflexus. However, there are numerous variations at the beginning, in the course and the method of branching of the heart arteries, and they appear in the most cases with left coronary artery. (2). Some hearts have the main trunk of left coronary artery containing three terminal branches. This occurrence is called trifurcation. The third branch flows on the frontal wall of left atrium and it is marked ramus diagonalis. Its vascular area is partially overlap with the area of vascularisation of ramus interventricularis anterior, and sometimes with the ramus circumflexus(3).

Diagonal branch (ramus diagonalis) is significant for collateral coronary blood flow because it Anastomoses to other branches of left heart artery with its sprigs. (4). Its importance is particularly expressed under conditions of insufficiency of ramus interventricularis anterior (5). Rarely, left coronary artery might have four or more terminal branches but then medium branches are having shorter flow than in case of trifurcation of the main trunk (6).

Proficiency in anatomy of coronary arteries and their variations is significant for proper interpretation of the coronary angiographies, assessment of the complexity and result of the coronary insufficiency as well as surgical myocardium revascularization (2).

OBJECTIVE

The objective of this study is anatomy-radiology research of the methods of branching of the main trunk of left coronary artery and to prove importance of the diagonal branch (ramus diagonalis) existence under conditions of coronary insufficiency.

MATERIAL AND METHODS

As supporting material, we have used in this study 100 coronary angiographies taken at the Clinic for Heart Diseases and Rheumatism of the Clinic Center of University of Sarajevo and 20 human hearts taken from the Institute of Anatomy. In our work, we used the method of coronary angiography and dissection method. All research described in submitted publication involving human subjects and material derived from human subjects complied with principles outlined in Helsinki Declaration. The method of coronary angiography, as well as use of appropriate contrast substance enabled that we were capable to visualize the heart blood vessels. Judkins left catheter was introduced through a.femoralis for left coronary artery and Judkins right catheter for right coronary artery. These catheters are thin, elastic and it is possible to introduce them through huge blood vessels to the heart. Blood pressures, content of gasses in the blood had been measured and iodine non-ion contrast substance was introduced that provides the image of blood vessels and heart cavities. Recording was done in LAO projection (left frontal projection) and in RAO projection (right frontal inclined projection). During the same act, the ventriculography of the left heart ventricle has been done allowing us to monitor its function, ejection capability and eventual disorder of its kinetics. The method itself lasts about half an hour; it is painless for patient who cooperates during the act with the team that perform the catheter process.

During the process of the analysis of coronary angiographies, special attention was drawn to the method of branching of the main trunk of left coronary artery. In the analysis of statistic data, the percentage of representation of different methods of division of main trunk of left coronary artery has been determined and results had been compared depending to the gender of patients. In cases of simultaneous presence of ramus diagonalis and insufficiency ramus interventricularis anterior, the presence of homocollateral as well as the function of left ventricle was monitored. Functional state of left ventricle was assessed based on the value of ejected fraction (EF). The dissection method enabled the image of heart blood vessels as well as monitoring of their flow and branching. The heart samples were as fresh as possible; they were conserved from 3 to 5 days in 10% solution of formaldehyde. Upon this, fat tissue was removed and by careful dissection arterial blood vessels were prepared. The method of branching of the main trunk of left coronary artery was monitored as well as the course of terminal branches.
RESULTS

We have analyzed 100 coronary angiographies in our work out of which 60 angiographies belonged to male persons while 40 angiographies belonged to female persons. On the angiographies taken in LAO and RAO projections, we monitored carefully the spots where coronary arteries were split as well as their course and division. There has been a presence of main trunk of the left coronary artery on all analyzed angiographies and we did not have any occurrence of independent emerging of ramus interventricularis anterior and ramus circumflexus from left sinus aorta. Also, we did not notice any other anomalies of emerging of these arteries’ branches. We have noticed on the analyzed angiographies two methods of branching of the main trunk of left coronary artery: on two branches (bifurcation) and on three branches (trifurcation). Bifurcation, i.e. branching of the main trunk of left coronary artery on two terminal branches (ramus interventricularis anterior and ramus circumflexus) has been found in 71% of tested cases (Figure 1). Out of 60 analyzed angiographies of male persons, bifurcation has been found in 73.33% of cases while remaining 40 analyzed angiographies of female persons bifurcation was present in 67.5% of cases (Table 1). Branching of the main trunk of left coronary artery on three terminal branches has been stated in 29% of cases.

<table>
<thead>
<tr>
<th>GENDER</th>
<th>BIFURCATION</th>
<th>TRIFURCATION</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEMALE</td>
<td>67.5%</td>
<td>32.5%</td>
<td>100%</td>
</tr>
<tr>
<td>MALE</td>
<td>73.33%</td>
<td>26.67%</td>
<td>100%</td>
</tr>
</tbody>
</table>

TABLE 1. Presentation of appropriate ramification methods of left coronary artery depending on gender (in percentages)
out of total number (100) of analyzed angiographies (Figure 2a and 2b). Trifurcation is common with female persons (32.5%), while 20.75% with male persons. Not single analyzed coronary angiographies presented branching of the main trunk of left coronary artery on four or more terminal branches. Regarding changes on the ramus interventricularis anterior that cause its insufficiency, we have found strong collateral connections (Figure 3) with sprigs of ramus diagonalis, that resulted with good function of left ventricle (ejected fraction >50%). By the dissection method we were able to determine the presence of the main trunk of left coronary artery in all examined cases. Its average length is 16 mm (range from 5 to 22 mm).

**DISCUSSION**

Numerous authors studied the method of branching of the coronary arteries and their variations: Angelini (1), Hadžiselimović (4), James (6), Džavahisvili, Komahidze (7) and other. As the left coronary artery, quite often we find variations in the source, in the course and method of branching than it is the case of right coronary artery (2). James (6) emphasis that left coronary artery in most of cases is divided on two branches, and rarely there is bigger number of terminal branches. If left coronary artery is divided on two branches than we deal with magisterial type of artery, but if it ends with bigger number of terminal branches then we deal with diffusive type of artery (4). Reig (8) used dissection of 100 samples of hearts; he discovered that bifurcation of main trunk of left coronary artery is presented in 65% of cases, trifurcation was presented in 31% of cases and squarefurcation in 4% of cases. Cavalcanti (2), Džavahisvili (7), Šečerov (9), Surucu (10), Wieloposh (11) and others reached approximate results in their researches. The frequency of the trifurcation of main trunk of left coronary artery determined during our researches confirmed results achieved by the above mentioned authors. We could not find division of left coronary artery into 4 or more branches with the dissection method or by analyzes of the coronary angiographies.
In the studies done by Garg (12) and Yamanaka (13), it is pointed out that some persons (1%) do not have main trunk of left coronary artery and ramus interventricularis anterior and ramus circumflexus have independent beginning originating from left aortic sinus (sinus Valsavae sinister). We have not found any of such cases in our studies as well as some other anomalies originated in left coronary artery and its branches. We faced with different names for third terminal branch of the main trunk of left coronary artery in the literature. Some authors mark it as ramus diagonalis (4,3), other call it medium left artery (10) or ramus intermedius (14). We think that the most appropriate name for this branch is ramus diagonalis. Ramus diagonalis flows over frontal wall of left ventricle and its vascular area is partially covered with the vascular area of ramus interventricularis anterior. Due to this reason, in cases of occlusion of the anterior interventricular branch or its lateral sprigs, diagonal branch softens ischemia of anterior wall of left ventricle (3,10).

This is realized by collateral blood flow i.e. anastomoses of sprigs of anterior interventricular branch (ramus interventricularis anterior), with sprigs of diagonal branch, and sometimes with sprigs of arch branch (4,10,15). The importance of this inter artery connections under conditions of the coronary insufficiency are stressed by other researchers (5,16). This has been confirmed in our researches because in cases of the simultaneous presence of ramus diagonalis and insufficiency ramus interventricularis anterior, we noticed developed inter artery connections and good function of left ventricle.

CONCLUSIONS

- We found two methods of branching of main trunk of the left coronary artery in the researches we have conducted (bifurcation and trifurcation).
- The bifurcation has been found by method of angiography in 71% of cases while it has been proved by dissection method in 65% of cases.
- Trifurcation has been declared on 29% of analyzed angiographies i.e. 35% of dissected samples of heart.
- We found higher percentage of trifurcation of the left coronary artery with females (35,8%), while 26,67% with males.
- Considering the branching from the main trunk of the left coronary artery, we find that the most appropriate name for its third branch would be ramus diagonalis.
- Diagonal branch with its anastomoses present an important way of coronary collateral blood flow under conditions of coronary insufficiency.

REFERENCES

(9) Šećerov D., Dilberovic F., Ovčina F. Individualne karakteristike krvnih sudova srca i koronarnoj bolesti, Sarajevo 1983.
ABSTRACT

The aim of this study was to evaluate the results of single-session sclerotherapy with mixture of alcohol and polidocanol and a subsequent injection of albendazole for devisceration of hydatid cysts in the spleen. Eight patients (four women and four men, average age 22.9±11.4 with hydatid cyst in the spleen were treated with 10 minutes of exposure to mixture of ethanol 95% and polidocanol 1%. After that, 2 to 5 ml of albendazole was injected into the cyst cavity. Two patients had 2 cysts. At follow-up the patients were examined with clinical and biochemical examinations, ultrasonography, and serologic test for echinococcal antibody titres. The mean hospital stay was 2.5±0.93 days. During the follow-up period, mean cyst diameter decreased from 16.4±13.66 mm to 13.6±16.26 mm. In all ten cysts, a reduction of post procedural recolection of fluid over 40% was observed. Five cysts (50%) disappeared during the follow-up period. All cysts (5) smaller then 50 mm in diameter disappeared during follow-up period. After an initial rise, the echinococcal-antibody titres fell progressively and at the last follow-up were negative (<1:160) in 7 (88%) patients. No complications were observed, except for pain, fever and urticaria during the first 24-hours after the procedure. Sclerotherapy using only one session and 10 min time of exposure to the mixture of ethanol and polidocanol, and a subsequent injection of albendazole solution represents an effective treatment of hydatid cysts in the spleen. This procedure is even more efficacious for hydatid cyst with diameter smaller then 50mm.

KEY WORDS: Spleen hydatid cyst, PAIR, Interventional ultrasound
INTRODUCTION

Hydatid disease is a worldwide zoonosis produced by the larval stage of the Echinococcus tapeworm. Echinococcosis is a zoonosis transmitted by dogs in livestock-raising areas and accidentally affects humans. Human infection is acquired from ingestion of the parasite eggs from infected animals. Echinococcus granulosus causes cystic echinococcosis in humans, a condition that is found throughout the world, particularly in the great grazing regions of the world as Africa, South America, the Mediterranean region, the Middle East, Australia and New Zealand (1, 2). The most frequent site of hydatid cysts is in the liver (50% to 70% of cases), followed by the lungs (20% to 30%), and less frequently, the spleen, kidneys, heart, bones, central nervous system, and elsewhere. The ultimate goal of treatment is elimination of the germinal layer of the hydatid cyst. Currently, three treatment options are available: surgery, medical treatment and percutaneous drainage. Surgery was the only treatment option available until the mid 1980’s (2, 3). However, drug therapy with mebendazole and albendazole (4, 5, 6, 7) and percutaneous drainage have been introduced as alternative treatments. Percutaneous treatment was called PAIR, Puncture-Aspiration-Injection-Reaspiration. Percutaneous drainage is minimally invasive and very effective in the treatment of hydatidosis (8, 9, 10, 11). Later, PAIR-derived technique was introduced for treatment of complicated hydatid cysts, cysts containing non-drainable material and cysts with difficult approach for intervention (12, 13, 14). We now report on a prospective study of modified PAIR technique for percutaneous treatment of hydatid cysts in the spleen.

PATIENTS AND METHODS

Patients that were admitted to our hospital with hydatid cyst in the spleen between May 2000 and February 2003, were included. Patients that were enrolled had symptoms, such as pain in left hypochondrium, or were asymptomatic at the time the cyst in the spleen was diagnosed. Spleen infestation with Echinococcus granulosus was confirmed serologically (IgG >1:160) in all cases. Hydatid cyst was diagnosed in four female and four male patients, with a mean age of 22.9±11.4. Patients signed written informed consent. On ultrasonographic examination, six patients had univesicular cysts, which were rounded with well-defined borders and contained pure fluid, and two had multivesicular cysts, with pure fluid in each vesicle. All patients undergoing procedure were treated with albendazole, administrated orally in a dose of 10 mg per kilogram of body weight per day. Albendazole prophylaxis was started one week before the procedure and continued for three weeks thereafter. The procedure was performed in three steps. In step one, the cyst was punctured under ultrasound guidance, using 18-gauge needle, and the fluid content of the cyst was subtotally aspirated. In step two, the cyst cavity was nearly filled with a mixture of 95% alcohol and 1% polidocanol (ethoxysclerol), which was left in the cavity for 10 minutes. In step three, the cyst was aspirated completely, and 2 to 5 milliliter of albendazole was injected into the cavity depending on the cyst size. The cyst fluid was subjected to cytologic and microbiologic examination. After the procedure, the patient was observed for 24 hours and then discharged from the hospital. Examinations were performed at the time of enrollment, at one and four months, and subsequently every three months during the follow-up period. The ultimate goal of treatment was the disappearance of the cyst (Figure 1 and 2). Other important parameters of efficacy were the size of the cyst over time, the length of the hospital stay, and any complications related to the procedure. A secondary parameter of efficacy was the serum echinococcal-antibody titer over time. Differences in cystic diameter before and after therapy were examined using the Wilcoxon signed-rank test. All p values were two-tailed.

RESULTS

Ten spleen hydatid cysts in eight patients were treated by single-session sclerotherapy performed with a 10 minute time of exposure to alcohol 95% and polidocanol 1% mixture. Consequently, 2 to 5 ml (depending on cyst volume) of albendazole solution was injected into the cyst cavity. All the patients received the assigned treatment. The mean hospital stay was 2.5±0.93 days, and it was significantly shorter in relation to other procedures. The procedure was successful in all eight patients in relation to deiscervation of the cysts. The mean diameter of the cyst was decreased from 46±16.4 mm before treatment to 13.6±16.26 mm at the last examination. This represents a reduction of diameter of 50%-100% (median 92%, p < 0.001). The time of observation was 7-24 months (Figure 3). After the procedure, the maximal diameter was reduced in all cysts. In the two, the maximal diameter was reduced over 40%, and in all others over 50% compared with the one before the procedure. Five of ten cysts disappeared between 4 and 24 months after the treatment (Table.
All of them had initial diameter smaller than 50 mm. Two patients (25%) had a fourfold rise in antibody titres in the first four months after the procedure. At the last follow-up examination, all patients had smaller levels of antibody titres than before treatment, and seven of them (88%) had negative antibody titres (< 1:160). There were no complications during procedures, except for the pain. Two patients (25%) had fever within 24 hours after the procedure, one patient had transient hypotension, and one had urticaria. Cultures of cyst fluid obtained from the cysts during puncture failed to document a microbial cause of fever. All complications disappeared within 48 hours after the procedure.

DISCUSSION

Percutaneous therapeutic treatment of hydatid cysts has long been discouraged because of the risk of anaphylaxis and intraperitoneal seeding. Nevertheless, accidental

<table>
<thead>
<tr>
<th>No</th>
<th>Age (Years)</th>
<th>Gender</th>
<th>Initial Diameter</th>
<th>Final Diameter</th>
<th>Follow Up (Month)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6</td>
<td>M</td>
<td>41</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>M</td>
<td>28</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>17</td>
<td>M</td>
<td>58</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>4</td>
<td>17</td>
<td>M</td>
<td>19</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>27</td>
<td>F</td>
<td>34</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>6</td>
<td>32</td>
<td>F</td>
<td>56</td>
<td>9</td>
<td>24</td>
</tr>
<tr>
<td>7</td>
<td>23</td>
<td>M</td>
<td>57</td>
<td>25</td>
<td>24</td>
</tr>
<tr>
<td>8</td>
<td>43</td>
<td>M</td>
<td>68</td>
<td>40</td>
<td>24</td>
</tr>
<tr>
<td>9</td>
<td>21</td>
<td>F</td>
<td>54</td>
<td>32</td>
<td>24</td>
</tr>
<tr>
<td>10</td>
<td>14</td>
<td>F</td>
<td>65</td>
<td>30</td>
<td>24</td>
</tr>
<tr>
<td>MEDIAN VALUES</td>
<td>23</td>
<td>46</td>
<td>14</td>
<td>24</td>
<td></td>
</tr>
</tbody>
</table>

TABLE 1: Ten cysts in eight patients treated with single-session sclerotherapy with mixture of alcohol and polidocanol and injection of albendazole solution thereafter.
and intended diagnostic puncture of what appeared to be hydatid cysts happened to occur uneventful (15). In early 1990’s, a systematic percutaneous technique was introduced, at the first as treatment for liver hydatid cysts (8). To prevent intraabdominal seeding, the hydatid cyst is punctured through normal tissue. The tissue collapses when needle or catheter is removed and thus serves as protection against leakage of the cyst fluid (12, 16). This technique was based on the one introduced for percutaneous treatment of non-parasitic cysts with 95% alcohol (17) and called PAIR. Usually albendazole prophylaxis is started one week before PAIR and continued for three to eight weeks thereafter (10, 11, 18, 19). Many studies demonstrated that PAIR was safe, successful in 90%-100% of cases, and had few relapses (0-4%) (9, 10, 11, 16, 19, 20, 21, 22). The WHO Informal Working Group on Echinococcosis reported the results of 765 hydatid cysts treated in multiple centres. Success rate was 99.7%, failure rate 0.26%, relaps rate 1.57%, and complication rate 14.8%. Anaphylactic shock (one patient died) and spillage occurred in 0.52% each. Minor complications were observed in 13.7% (10, 16, 23). Hydatid cysts in the spleen have a specificity to be found in smaller organ than the liver and lung cysts, hidden by the rib arch and therefore difficult to approach for introducing of catheter. Hydatid cysts of the spleen are usually smaller then 50 mm in diameter. Some authors suggest that small (< 50-60 mm) and large (> 50-60 mm) cysts should be treated differently (24, 25, 26). It has been suggested that cysts smaller then 50-60 mm in diameter should not undergo catheterization after the initial drainage. For these, after the initial PAIR procedure, only single-session sclerotherapy with a 10 minute time of exposure to the mixture of alcohol and polidocanol, and injection of albendazole solution thereafter is enough for devisceration. As a result of our study, hydatid cysts smaller then 50 mm had regression faster and completely in relation to the ones greater then 50mm in diameter. Some patients in our study showed an increase of antibody titres soon after the intervention. After follow-up period, all patients had antibody titres less than before the procedure, and most of them had negative antibody titres. Our results of level of antibody titres are similar to the results of other studies (19, 27). Routine culture of the aspirated fluid to detect possible bacterial infection or contamination is also done in more investigations (19, 24, 25, 26, 27). In our study, microbiologic examinations were negative. There were no serious complications from the interventions. The reason is probably that modified PAIR is less agressive in relation to classic PAIR procedure and surgery treatment.

CONCLUSION

Modified PAIR (with injection polidocanol and albendazole) can be performed safely and results in the disappearance of the hydatid cysts in the spleen. The efficacy of this procedure is similar to that of standard PAIR procedure (especially in cysts smaller then 50 mm), and treatment with cystectomy, in terms of reducing the size of the cyst and causing its disappearance over follow-up period of two years. The advantages of modified PAIR include a shorter hospital stay and a lower complication rate.
ENVER ZEREM ET AL.: MODIFIED PAIR TECHNIQUE FOR TREATMENT OF HYDATID CYSTS IN THE SPLEEN

References

Detection of Neurovascular Structures Using Injection Pressure in Blockade of Brachial Plexus in Rat

Ilvana Vučković1*, Admir Hadžić2, Faruk Dilberović1, Amela Kulenović1, Zakira Mornjaković3, Irfan Zulić4, Kučuk-Alija Divanović3, Eldan Kapur1, Esad Čosović3, Alma Voljevica1

1. Institute of Anatomy, Faculty of Medicine, University of Sarajevo, Čekaluša 90, 71000 Sarajevo, Bosnia and Herzegovina
2. St. Luke’s-Roosevelt Hospital, 1111 Amsterdam Avenue, New York, USA
3. Institute of Histology and Embryology, Faculty of Medicine, University of Sarajevo, Čekaluša 90, 71000 Sarajevo, Bosnia and Herzegovina
4. Institute of Pharmacology, Clinical Pharmacology and Toxicology, Faculty of Medicine, University of Sarajevo, Čekaluša 90, 71000 Sarajevo, Bosnia and Herzegovina
5. Clinic of Surgery with Onychology and Ophthalmology, Faculty of Veterinary Medicine, University of Sarajevo, Zmaja od Bosne 90, 71000 Sarajevo, Bosnia and Herzegovina

* Corresponding author

ABSTRACT

In the last few decades there has been a great development of regional anesthesia; all the postulates are defined and all the techniques of usage are perfected. However, like any other medical procedure, the block of brachial plexus carries a risk of certain unwanted complications, like possible intraneural and intravascular injections. The reason for great discrepancy between the injury of brachial plexus and other periphery nerves while performing the nerve blockade is the frequent usage of this block, but also the specific proximity of neurovascular structures in axilla. The purpose of this work is to determine the values of pressures which appear in paraneural, intraneural and intravascular injection applications of local anesthetic, and to compare those values in order to avoid cases of intraneural and intravascular injections in clinical practice with consequential complications. In experimental study there have been used 12 Wistar rats of both genders. After anesthesia with ether and midhumeral access to the neurovascular structures in axilla, the injection of 2% lidocain with epinephrine was performed with the help of automatic syringe charger. The needle was at first placed paraneural, and then also intraneural and intravascular. During every ap-
INTRODUCTION

In the last few decades there has been a great development of regional anesthesia: all the postulates are defined and all the techniques of usage are perfected. The world trend of favoring various techniques of regional anesthesia is the result of advantages that the regional anesthesia comes with, especially in comparison with the general anesthesia, like avoiding chemical and instability and lung complications and enabling faster mobilization and earlier release of the patients to their homes (1). One of the most frequently used techniques of regional anesthesia is the block of brachial plexus, which can be performed in all cases when the surgery is necessary on upper extremities or in therapy of postoperative pain (2). However, like any other medical procedure, the block of brachial plexus carries a risk of certain unwanted complications. To this also contributes the fact of specific anatomic proximity of neurovascular structures in axilla, which makes the branches of brachial plexus even more vulnerable (3). It is known, from the researches so far, that the unwanted complications during the nerve blockade can happen after unintentional intraneural or intravascular injection of local anesthetic in the surrounding neurovascular structures. Intraneural injections can be followed by the consequential paresis or the paralysis of certain nerve, while the intravascular injections of the local anesthetic can result in the appearance of the symptoms of toxicity of cardiovascular system or of central nervous system, with possible cardiac arrest, and even death. As prevention from intraneural injections and consequental complications today are in use two methods: the method of causing paresthesia or the method of using the stimulator of periphery nerve; however the injury can still occur, independent from the used techniques, even in the hands of the most experienced executer. In prevention from intravascular injection exists the method of aspiration before and during the application; but even the negative aspiration test does not always signify that the injection is placed out of artery or vein. The other possibility is the usage of epinephrine (addition to the local anesthetic) as a marker of intravascular injection. The reasoning behind this is that in 20 seconds after the injection the pulse is increasing up to 30% and this appearance can be a verification of intravascular injection. However, the epinephrine test also does not give enough security to exclude the intravascular injection (4,5). How to prevent mentioned complications are the themes that are most frequently discussed on the congresses of anesthesiologists.

OBJECTIVE

The purpose of this work is to determine the values of pressures which appear in paraneural, intraneural and intravascular injection application of local anesthetic, and to compare those values in order to avoid cases of intraneural and intravascular injections in clinical practice with consequental complications. Purpose is also the standardization of the mentioned method.

MATERIALS AND METHODS

In experimental study there have been used 12 Wistar rats of either sex. After anesthesia with ether and midhumeral access to the neurovascular structures in axilla (Figure 1.), with microscopic guidance, the injection of 2% lidocain with epinephrine was performed with the help of automatic syringe charger. The automatic syringe charger was set so that the speed of application is 3ml/min, and the volume of the injected solution is 4ml (Figure 2.). The needle, with the diameter of 26 G (Microlens) and under the angle of 45 degrees, was at first placed in close proximity to neurovascular structures (paraneural), and then into a nerve (n.medianus-intraneural) (Figure 3.) and into
blood vessels of axilla (a. and v. axillaris- intravascular). During every application the pressure values were monitored (expressed in PSI; 1 PSI=6.98 kPa) using the manometer (PG 5000; PSI-Technologies Inc, Tulare, CA) (Figure 4.), that is connected to a computer with analogous digital converter (DAQ card 6023 National Instruments), and then they were analyzed by special software program - BioBench 1.2. National Instruments. BioBench program is designed for registration and analysis of data obtained in medical researches and is used for educational requirements as well. Using National Instruments for getting data, we can get data form any physiological type of linear voltage source. The program itself has the ability to use the obtained data and analyze it integrating the personal computer with physiological monitoring systems, and thus saves a great amount of time and money. Using the Bio Bench program we facilitated the registration, analysis and adequate evaluation of an enormous number of data which we got during our research.

RESULTS

Generally speaking, all the injections were characterized by initial pressure, which was followed by a quite similar, but lower pressure in the remaining part of injecting. All paraneural injections resulted with the pressure between 13.96-27.92 kPa. The majority of intraneural injections were combined with the injection pressure greater than 69.8 kPa, while the intravascular injections were combined with injection pressure less than 6.98 kPa (Graphic 1, 2, 3). The average value of intraneural pressures was 86.55±13.262 kPa, in comparison to 25.128±3.49 kPa for paraneural injection and 7.0498±2.094 kPa for intravascular injection. Statistic analysis was executed using SPSS program - version 11.5. The test was used for determining significances during paraneural, intraneural and intravascular injections. The obtained results showed that p-value < 0.05, which is considered significant (intraneural-paraneural ap-
application \( p < 0.001 \); intravascular-intraneural application \( p < 0.001 \); intravascular-paraneural application \( p < 0.001 \).

**DISCUSSION**

The incidence of permanent nerve damages during periphery nerve blocks varies between 0.02% and 0.04%, depending on the type of damage and the amount of time spent observing (6,7). The incidence of persistent neurological damage decreases with time. The proofs of neurological abnormality can be found in \( 19\% \) of patients in first 24 hours, then they are decreasing to 3-8% through 4-6 weeks, and in 1 year they are reduced to 1% (8).

Cheney and his associates were investigating American Society of Anesthesiologist Closed Claims data base in order to determine the frequency of nerve damages based on filed claims. Out of 4,183 reviewed claims, 670 (16%) were because of the nerve damages that had some connection to anesthesia. The most frequent areas of damage were n. ulnaris (28%), brachial plexus (20%), lumbosacral nerve roots (16%) and spinal cord (13%) (9).

Auroy and his associates made a prospective study grading the incidence and the characteristics of serious complications connected to regional anesthesia. Total of 103,730 techniques of regional anesthesia, including 71,053 of neuroaxial anesthesia, 21,278 periphery nerve blocks and 11,229 intravenous regional anesthesia, were carried out through a 5 month period. Neurological complications connected to techniques of regional anesthesia were present in 34 patients. Out of 34 neurological complications, 24 (70%) happened during spinal anesthesia, 6 (18%) during epidural anesthesia, and 4 (12%) during the blockade of periphery nerves. The additional complications that happened during the blockade of periphery nerves include the cardiac arrest (0.01%), death (0.005%), brain stroke (0.08%) and radiculopathy (0.02%) (10).

Based on the available data it can be noticed that so far none of the methods of prevention of unwanted complications of regional anesthesia can insure the avoidance of intraneural and intravascular injection of local anesthetic. There are many discussions about how to prevent intraneural injection and nerve damage coupled with periphery nerve block, and all debates are focused on two methods of nerve localization (paresthesia versus nerve stimulator). Many anesthesiologists intentionally cause paresthesia during the execution of periphery nerve blockade in order to reliably localize nerve structures. This partly emerged from an ancient saying «no paresthesia, no anesthesia» (11). Even though causing paresthesia can represent a direct trauma with needle and theoretically increased risk of neurological injury, there are no prospective clinical randomized studies that are able to definitely support or negate this (6, 7).

Selander and associates reported a high incidence of nerve damages in patients that had parasthesia that was intentionally caused during the axillary block, in comparison to those patients on whom the perivascular technique was applied (2.8% versus 0.8%). Auroy
The concern comes out of the fact that paresthesia can be lessened for parasthesia during the placement of the needle without real contact with the nerve (21,22,23). However, recent studies of Choyce and associates showed that this maybe isn’t the case. In their study they explored the relation between subjective parasthesia and objective motor response, caused with periphery nerve stimulator, in patients that had interscalene and axillary blockade (15,24). During the procedure the not insulin needle was entering all until the parasthesia was caused. In that moment the current of the nerve stimulator started to gradually increase until reaching the adequate motor response. It is interesting that after achieving parasthesia, for 25% of the patients the current greater than 0.5 mA was needed for achieving manifesting motor response. The place of initial parasthesia matches the place of consequential motor response in 81% of the cases, indicating the inconsistency in provoking the motor response, in spite of the fact that the needle was probably close to the nerve. This concern is confirmed further by the reports of nerve damage after using low current (less than 0.5 mA) of periphery nerve stimulator and intramedullary injection during interscalene block of patients under general anesthesia (25). Stimulator with such low current is usually joined with parasthesia on injection, which can indicate possible intraneural placement of the needle. Therefore, the claim that the periphery nerve stimulator allows clinical staff to get close to nerve structures without a risk of mechanical trauma does not seem valid. It should be pointed out that the nerve stimulators used in blockade of periphery nerves quite vary in their characteristics, like stimulating frequencies, maximal production of voltage, duration of stimulus and their precision (26). Because of this the nerve stimulators undergo the tests of preciseness. Unfortunately, the majority of manufacturers make the tests using the current of 1.0 mA. It would be much more efficient if they would do these tests with clinically relevant current range from 0.1 to 0.5 mA. In the contemporary clinical practice there is no consensus about a technique or a method that reduces a risk from intraneural injection. The proximity of the brachial plexus to the vascular structures can contribute to intravascular injection of local anesthetic. Stan and others reported that the frequency of unintentional intravascular injection is 0.2% in 996 patients that had to undergo the axillary blockade, in spite of the negative test for aspiration (27). Intravascular complications were also noted during the interscalene block, where a deep insertion of a needle into interscalene groove can result with puncture or unwanted injection of local anesthetic into a.vertebralis, when local anesthetic gets directly to the brain (28). Unfortunately,
the minimal quantities of local anesthetic (0.5 cm³) can cause extremely high concentrations of anesthetic in the central nervous system, which brings to a strong toxic response. Other, just as risky, vascular structures are a. carotis communis and v. jugularis externa (interscalene block), a. and v. subclavia (supra or infra clavicle block) and a. and v. axillaris (axillary block). Similar complications due to intravascular injections were noted after giving the penicillin G to gluteal region, with consequential cardio respiratory arrest and death because of lung embolism caused by insoluble substances (29,30). One needs to be especially mindful of this because of frequent administration of penicillin into gluteal region, mainly to children, during the therapy of pharyngitis, impetigo or the infection of the middle ear. Clinical watchfulness, periodical aspiration, the usage of epinephrine as vascular marker, and continuous observation in search for symptoms and signals of toxicity of local anesthetic are presently the key variables for prevention from these complications, even though we should be mindful that even this technique is not absolutely dependable. One study on small animals showed that the intraneural injection can be joined with high injection pressure. The earlier studies carried out on rabbits showed that generally higher pressure (higher than 11 psi) is needed in order to inject local anesthetic into intraneural space, in comparison to paraneural application (31). Anesthesiologists often rely on subjective estimate of abnormal resistance to injection during the performance of periphery nerve block, knowing that intraneural injection results with bigger resistance to injection. Hadžić and associates showed that the perception of the resistance can rather vary among the anesthesiologists and that this method is inconsistent and can be affected by different designs of needles (32). In the study that used dogs, Hadžić and associates showed that the intraneural injection into n.ischiadicus is joined with high injection pressure with consecutive persistent neurological deficit (33).

**CONCLUSION**

Based on our research it is obvious that the measuring of pressure during the nerve blockade is very important in order to decrease the risk of neurological and possible systematic complications. It is also clear that a small, mobile, and financially quite available apparatus for pressure measurement can help in differentiation between paraneural, intraneural and intravascular injection. Avoiding high injection pressure prevents from lodging the needle into intraneural space, while avoiding a very low injection pressure prevents from lodging the needle into intravascular space followed by consequential complications. The usage of this apparatus can find its application in other blockades of periphery nerves, and in other branches of medicine as well, for example in everyday practice of giving intramuscular injections of different medicines (antibiotics-penicillin, corticosteroids and similar) into gluteal or deltoid region, because the application into different tissues results with different values of injection pressures, which greatly depends on structure, compactness and extensibility of the tissue. The method of monitoring application pressure in detection of neurovascular structures is still in its developmental stage, and the clinical experience of its usage is limited. However this study shows that there exists a great potential in improvement of block performance resulting in better successfulness and lesser risk of lesions of nerves and blood vessels. In the near future the monitoring of injection pressure might exist in order to avoid intraneural injection and to more objectively document the procedure of periphery nerve block, and to analogously document the blood pressure. Applying these results to clinical practice, during periphery nerve blocks, the risk of unwanted complications can be reduced. It should be pointed out that none of the techniques can be a substitute to a good knowledge of anatomic relations.

Supported by:
Ministry of Education and Science, Bosnia and Herzegovina
Bosnalijek Pharmaceuticals, d.d. Sarajevo, Bosnia and Herzegovina
Department of Anesthesiology at St. Luke’s Roosevelt Hospital, New York, USA
REFERENCES


BOSNIAN JOURNAL OF BASIC MEDICAL SCIENCES 2005; 5 (3): 79-85

85
Significantly Reduced Salivary Nitric Oxide Synthesis in Patients with Parkinson’s Disease

Jasminko Huskić*, Alma Paperniku2, Azra Husić3, Faruk Alendar4, Nedžad Mulabegović3

1. Institute of Physiology and Biochemistry, School of Medicine, University of Sarajevo, Čekaluša 90, 71000 Sarajevo, Bosnia and Herzegovina
2. Institute of Pharmacology, School of Medicine, University of Sarajevo, Čekaluša 90, 71000 Sarajevo, Bosnia and Herzegovina
3. Clinic for Gastroenterology, University of Sarajevo Clinics Center, Bolnička 25, 71000 Sarajevo, Bosnia and Herzegovina
4. Dermatovenerology Clinic, University of Sarajevo Clinics Center, Bolnička 25, 71000 Sarajevo, Bosnia and Herzegovina

* Corresponding author

ABSTRACT

In order to study concentration of nitric oxide (NO) in the saliva of patients with Parkinson’s disease (PD), we measured the concentration of its stable metabolite nitrite (NO2-) in the saliva of these patients and healthy subjects. We analyzed saliva flow rate and salivary NO concentrations in 16 subjects with Parkinson’s disease and in 16 healthy subjects. Concentration of nitrite was determined by colorimetric method using Griess reaction. Saliva flow rate was significantly lower in patients with Parkinson’s disease (0.23±0.03 mL/min; X±SEM) than in healthy subjects. Salivary NO2- concentration was significantly lower (5.02±0.64) than in healthy individuals (22.39±1.24; p<0.0001).

KEY WORDS: nitric oxide; Parkinson’s disease; saliva
INTRODUCTION

Nitric oxide (NO) is a prod-uct of nitrogen metabolism with short life. NO is synthesized from L-arginine by NO synthase (NOS) which is made up of at least three isoforms (1). Although initially investigated and characterized as endogenous vasodilator, NO is now known to perform a wide range of physiological and pathophysio-logic functions. (2). Previous studies have shown that NO is present in human saliva. However, the physiological role of NO in saliva is not clear. Clinical investigations of salivary NO concentration have shown that the production of this free radical changes in various diseases. It was reported that salivary NO production is significantly lower in smokers than in non smokers (3). Furthermore, some authors found that salivary NO levels were significantly decreased in healthy subjects after a 2 day course of the broad spectrum antibiotics then prior to using medication (4). On the other hand, oral NO increases during de novo deposition of dental plaque (5). Also, it has been proven that salivary NO levels change in patients with periodontitis (6,7) and gingivitis (8). Further investigations showed that salivary concentration of NO in patients with Sjogên’s syndrome significantly increased in comparison to healthy subjects (9). Recently, we have also found a decrease in salivary NO concentration in patients with Sjogren’s syndrome significantly in creased in comparison to healthy subjects (3). On the other hand, oral NO increases during de novo deposition of dental plaque (5). Also, it has been proven that salivary NO levels change in patients with periodontitis (6,7) and gingivitis (8). Further investigations showed that salivary concentration of NO in patients with Sjogên’s syndrome significantly increased in comparison to healthy subjects (9). Recently, we have also found a decrease in salivary NO concentration in patients with Sjogren’s syndrome significantly increased in comparison to healthy subjects (9). One of the leading symptoms of Parkinson’s disease, as well as Sjogren’s syndrome and diabetes mellitus, is the reduced secretion of saliva. However, there is no data on salivary NO concentration in patients with Parkinson’s disease. Therefore, the aim of the present study was to investigate saliva flow rate and salivary concentrations of NO in patients with Parkinson’s disease as well as to establish correlation between salivary flow rate and salivary concentration of NO in those patients.

SUBJECTS AND METHODS

1. SUBJECTS

Study group included 16 patients with Parkinson’s disease of both sexes (8 male and 8 female), 60-70 years of age, who were medically treated. The diagnosis of Parkinson’s disease was made on the basis of clinical examination at the Neurology Clinic, University of Sarajevo Clinics Center. Control group consisted of 16 subjects of both sexes (8 male and 8 female), 60-70 years of age, who were healthy according to their subjective and objective findings. A medical history was obtained for all subjects and they received a complete dental check-up.

2. MEASUREMENTS OF NON-STIMULATED SALIVA FLOW RATE

Whole non-stimulated saliva samples were collected into tubes during a 15 minute period. Saliva flow rate was determined by dividing the volume of saliva by the collection time.

3. SALIVA SAMPLING

In all subjects, NO concentrations were determined after 2 days of low NO2- diet for. Subjects were told not to eat or smoke for 1 hour before the examination. Non-stimulated saliva was collected after the subjects washed their mouths with tap water for one minute in order to reduce bacterial contamination. Freshly secreted saliva was collected within the first 30-60 seconds after mouth washing. The samples were then refrigerated until essay.

4. NITRITE MEASUREMENT

NO concentration in the saliva was determined by measuring nitrite concentration, a stable metabolic product of NO with oxygen, as determined by Griess reaction (11). Briefly, equal volumes of saliva and Griess reagent were mixed at room temperature. After 5 min, the absorbance was measured at 570 nm using Perkin Elmer 550 S spectrophotometer. Nitrite concentration was determined by a standard curve prepared with sodium nitrite (1-200 UM).

5. STATISTICAL ANALYSES

The measurement results were expressed as mean ± SEM. Differences between means were statistically compared by Student’s t-test, and differences at P<0.05 were considered significant. Correlation coefficients were determined by employing Spearman’s test.

RESULTS

Non-stimulated saliva flow rates in patients with Parkinson’s disease and healthy subjects are presented in Figure 1. The saliva flow rate was significantly lower in patients with Parkinson’s disease (0.2±0.03 mL/min; X±SEM) than in healthy subjects (0.55±0.02; p<0.0001). Results of salivary NO2- concen-trations are presented in Figure 2.

Samples obtained from patients with Parkinson’s disease had significantly lower salivary NO2- concen-trations (5.02±0.64) than the healthy persons’ samples (22.39±1.24; p<0.0001). There were no significant sex-related differences in salivary NO2- concentrations, either in the control group or in the group of patients with Parkinson’s
disease (data not shown). No correlation was found between salivary NO\textsubscript{2} concentrations and saliva flow rate in patients with Parkinson’s disease (data not shown).

**DISCUSSION**

Our study clearly shows that the mean saliva flow rate in patients with Parkinson’s disease is significantly higher than the one found in healthy subjects. Various studies conducted earlier, presented incongruency of data on the excretion of saliva in Parkinson’s disease. Certain studies reported hypersalivation while others reported a decrease in saliva secretion (12). According to the most authors on the subject matter, a very common symptom of Parkinson’s disease (almost 75% of the recorded cases), is the condition of hypersalivation. This has instigated numerous research projects aimed at discovering an efficient means of dealing with this symptom. Moreover, further research confirmed that the symptom of hypersalivation in Parkinson’s disease is not the result of overproduction in the salivary glands, but cause is merely in the difficulty of swallowing (13). Beside that, further studies have shown that the quantity of non-stimulated saliva excretion is significantly decreased in patients with Parkinson’s disease who did not use any medication as well as in patients who were on medications for longer periods of time (14). Moreover, the studies have confirmed statistically non-significant differences in the quantity of secreted saliva between medicated and non medicated patients. The results of our quantitative study on the secretion of non-stimulated saliva in patients with Parkinson’s disease are in accordance with the results of the above study. In our study, all patients whose saliva secretion was examined were on antiparkinson’s drugs for longer periods of time. The results indicate that medication is not the only agent responsible for decreased saliva secretion. Taking into account that when Parkinson’s disease is concerned there is a deficiency of dopamine, we conjecture that this deficiency is precisely the reason for the increased quantity of saliva secretion. It was proven that reduced saliva secretion is a symptom of Sjogern’s syndrome. The reduction, in this disease, is a consequence of the structural destruction of the secretory, acinar parenchyma caused by lymphocytic infiltration of the salivary gland. On the other hand, numerous studies have shown that in the case of the latter disease, an increase of NO concentration in the saliva exists. It is believed that this increase is the result of an increased activity of iNOS which is induced by cytokines from the lymphocytes. The production of NO induced by iNOS is permanent and brings about the production of large concentrations of NO, which may cause tissue destruction. It is precisely this that leads scientists to believe that the reduced saliva secretion in Sjogern’s syndrome results from the destruction of acinar parenchyma. Our results in investigating NO concentrations in patients with Parkinson’s disease have shown that NO concentrations are significantly reduced. We were unable to compare our results with other studies for in the literature at hand, we were unable to find any indication of previous works on this issue. In contrast to Sjogern’s syndrome, the reduced saliva secretion in Parkinson’s disease is not the result of a disrupted salivatory gland, and in all probability does not lead to the induction of iNOS. The concentration of NO in Parkinson’s disease is partially instigated by ingested nitrates, and partially from acinar cells. NO from acinar cells is brought about partially by the activity of the constitutive isoforms of NOS, and partially from endogenous production. Endogenous production of NO in acinar cells is stimulated by beta-adrenal stimulation. Taking into consideration that the concentration of dopamine is reduced in Parkinson’s disease, it is thought that
this reduction may be responsible for the decreased NO production in acinar cells. Moreover, our results show no significant statistical correlation between the NO concentrations in the saliva of patients with Parkinson's disease and that of the measured unstimulated saliva rate flow. Due to the unavailability of literary data, we were unable to compare our findings. The oral cavity is inundated with pathogenic bacteria. Saliva plays an active role in defending the organism from these pathogens, destroying them and reducing their number, due to its unique chemical composition. Accordingly, reduced quantities of saliva may bring about worrisome changes in the oral mucous membrane and instigate the development of caries. Since bacterial activity in the oral cavity, in reacting with nitrates, forms NO, the reduced saliva secretion may be the result of an increased concentration of NO in the saliva. However, the results of research are inconsistent so far. Decreased concentrations of NO in the saliva are found in the use of wide spectra antibiotics, in smokers, and in patients exhibiting good oral hygiene. All of this may be related to the reduced amounts of bacteria in the oral cavity. The results of several studies on the oral health and dental status in patients with Parkinson's disease presented better dental statuses compared to the control group (15). On the other hand, in the cases of inflammatory processes in the oral cavity, both increased (6) and reduced (7) concentrations of NO, were confirmed, despite the presence of large amounts of bacteria. It is thought that certain factors from the saliva itself either stop the synthesis or cause decomposition of the formed NO. This is supported by studies which have shown that saliva in patients with parodontitis or gingivitis stops or even blocks NO synthesis in polymorph nuclear leukocytes, which is not the case with healthy patients (16). Currently, it is not clear whether the reduced concentration of NO in inflammatory diseases simply reflects increased inflammatory activity and tissue destruction, or results from pathogenesis. Which implores the question: are NO concentrations in the saliva, except in the case of parodontal diseases, of diagnostic concern?

REFERENCES

SUBMISSION: Only papers written in correct English are considered. Manuscripts must be typewritten in triplicate (with three sets of illustrations of which ones 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. Full-length articles should not exceed 8 printed pages (4400 words including tables and references, or about 20 typewritten double-spaced 8½” x 11” sheets, with wide left-hand margins). Please include the word count on cover letter and title page of manuscript. Subject matter should be organized under suitable headings and subheadings such as Abstract, Introduction, Materials and Methods, Results, Discussion and References.

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 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. View-points 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.

COMMENTARIES AND LETTERS to the Editor concerning work published in the journal may also be submitted. They should not exceed 2 manuscript pages including one table or figures.

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.

One of the criteria considered in reviewing manuscripts is the proper treatment of animals. In particular, the use of painful or otherwise noxious stimuli must be carefully and thoroughly justified. Papers that do not meet these criteria will not be accepted for publication.
GENERAL REQUIREMENTS FOR PREPARATION OF MANUSCRIPTS

FIRST PAGE - A concise but informative full title of the article. Avoid abbreviations and colloquialisms.

SECOND PAGE - Name(s) of author(s). Write first names in full. Complete address of the laboratory (institution) for each author (i.e., department, section or unit of an institution, hospital or organization, city, state and/or country where it is located). Corresponding author should be designated with an asterisk.

THIRD PAGE - footnotes to the title, if any. List of any non-standard abbreviations.

FOURTH PAGE - An Abstract (no more than 250 words) 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.

Key words: A maximum of 6 key words or short phrases suitable for indexing should be supplied.

CONTENT OF MANUSCRIPT

Number the remaining pages consecutively and type the author’s(s) last name(s) at the top of each page.

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 Materials and Methods section to enable other investigators to repeat the experiments. Also, note that all research described in submitted publication involving human subjects and material derived from human subjects complied with ethical principles outlined in World Medical Association Declaration of Helsinki - Ethical Principles for Medical Research Involving Human Subjects (initiated in June 1964, amended in October 1975; October 1983; September 1989; October 1996; October 2000).

Report Results clearly and concisely. Do not present the same results in tables and illustrations. Exceptionally, results and discussion can be combined in a single section. Data should be expressed in SI units.

In the Discussion interpret the results, state their meaning and draw conclusions. Do not simply repeat the results.

The Conclusion(s) state(s) the significance of the results. Start each section on a separate sheet.

EDITORIAL STYLE

Arabic numerals should be used for weights, measures, percentages and degrees of temperature. Weights and measures should be abbreviated according to the International System of Units: kg, g, mg, mmol, μmol; m, cm, mm, μm, nm, A, cm², ml, μl, M, mM, μM, nM; N; h, min, s, ms, μs. Use % after numerals throughout. Give generic names of all pharmaceutical preparations. Give the manufacturer’s name and address (in parentheses) following names of any instruments or equipment cited by brand name. Please use third person tense. Manuscripts not adhering to Instructions may be returned to author.
SUPPLEMENTS TO MANUSCRIPT

FOOTNOTES: Avoid footnotes

TABLES AND ILLUSTRATION(S): 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”.

Each table should be titled, numbered (with Arabic numerals), and typed on a separate page. Only standard, universally understood abbreviations should be used. Authors should prepare tabular material in an easily readable form, eliminating tables presenting information that can easily be incorporated into the text. *Tables should be referred to in the text as “Table 1.” etc. and approximate position indicated.*

Colour illustrations 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. *Figures should be referred to in the text as “Figure 1.” etc. and approximate position should be indicated.*

Please, note that art material (tables, drawings, graphs, photographs) has to be submitted separately from the text, numbered and with the text appearing on it. The preferably resolution of the illustration (for the electronic version) should be 250 lines/inch and the size should be adjusted to the size of the Journal, otherwise the illustrations might be illegible; JPG format of illustrations is recommended. Figures must be stored on the disk in a separate file.

ABBREVIATIONS: Introduce an abbreviation only when the same term occurs three or more times.

REFERENCES: References are to be cited on a separate page at the end of the article, numbered *consecutively in the order in which they are mentioned in the text*, and marked by Arabic numerals in round brackets. A citation in the text is marked by the reference related number. All authors are mentioned, if six or less, while if more than six, only three first ones are cited adding et al. Type references on a separate sheet. Cite in sequence authors names and initials, title of article, name of journal, year of publication, volume number of journal, and first and last pages (*Vancouver style*).

The surnames of the authors followed by initials should be given. There should be no punctuation other than commas to separate the authors.

*Abbreviate journal titles* according to the International List of Periodical Title Word Abbreviations, Current Contents or according to Index Medicus. Material submitted for publication but not yet accepted should be noted as unpublished data and not included in the reference list.

Text references should be indicated by Arabic numerals in round brackets: “the incidence is similar to that in other reports (1,2,3). Davies et al. (4) have reported...” To avoid any delays in the editing process, authors must make every effort to see that each reference is correct and complete. Incomplete references will be returned to the principal author for completion before the manuscript is edited.
Upon appraisal by the B&H Federal Ministry of Education, Science, Culture and Sports logged as 04-15-1589/03 of 29 May 2003 Bosnian Journal of Basic Medical Sciences is classified as product described in article 18, provision 10 of the Law on products and services exchange taxation and consequently exempt from products exchange tax.