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Brain mapping in hypertensive pregnant womens: ethical issues

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Abstract

Pregnant women are routinely excluded from clinical research resulting in significant knowledge gaps regarding the safety and efficacy of treatments during pregnancy. Also, this exclusion results in insufficient data to guide clinical practice for pregnant women, and hence adds risks to pregnant women and fetuses. In this article, we try to highlight ethical issues that resulted from the analysis of spatiotemporal aspects and electrical patterns of brain activity in pre-eclamptic patients and healthy pregnant control women using quantitative electroencephalogram (QEEG), also known by the acronym BEAM (Brain Electrical Activity Mapping). Ethical issues associated with our study were: the need for effective treatment and diagnostic for hypertensive women during pregnancy, foetal safety, potential harm resulting from treatment during pregnancy. On the other hand, progress in neuroscience is rapidly increasing our knowledge of brain structures and functions, and many researchers are beginning to identify brain processes that are related to experiences and concepts. Ethics of neuroscience analyze the legitimacy of technology and research projects of brain functions, and in this context quantification of brain damage in hypertension associated with pregnancy has been a challenge in terms of medical ethics.

Key words: bioethics, neuroethics, EEG brain mapping, hypertension, pregnancy.

Introduction

It is well-known that historically, women have been routinely excluded from biomedical and health research, resulting in diminished understanding of the aetiology, treatment and prevention of disease in females. This long history of excluding pregnant women from biomedical research is beginning to witness some overdue rethinking and possible reversal (1, 2). Today, the exclusion of pregnant women from research participation is not endorsed. However, a tendency still remains to exclude pregnant women from participating in research. Pregnant women are routinely excluded from clinical research resulting in significant knowledge gaps regarding the safety and efficacy of treatments during pregnancy (3, 4). Also, this exclusion results in insufficient data to guide clinical practice for pregnant women, and hence adds risks to pregnant women and fetuses (5)

On the other hand, progress in neuroscience is rapidly increasing our knowledge of brain structures and functions, and many researchers are beginning to identify brain processes that are related to experiences and concepts (6). In addition to this, Quantitative Electroencephalogram (QEEG), also known by the acronym BEAM (Brain Electrical Activity Mapping), is a non-invasive technique for topographic display and analysis of brain electrophysiological data. Once a sample of the electrical activity data of the patient's brain has been collected, the proprietary software of the acquisition device performs a computerised transformation of the raw, analog brain waves into digital form, which can be analysed by the computer. (7) From the measurement of mental processes with functional neuroimaging to their manipulation with ever more selective drugs, the new capabilities of neuroscience raise unprecedented ethical and social issues. These issues must be identified and addressed if society is to benefit from the neuroscience revolution now in progress (8, 9). Also, this development raises ethical problems whose

importance is likely to surpass even the implications of modern genetics (6, 10).

In this article, we try to highlight ethical issues that resulted from the analysis of spatiotemporal aspects and electrical patterns of brain activity in pre-eclamptic patients and healthy pregnant control women during their third trimester of pregnancy by using quantitative electroencephalogram (QEEG), also known by the acronym BEAM (Brain Electrical Activity Mapping).

EEG brain mapping in preeclampsia: a new challenge

Hypertensive disorders in pregnancy remain a leading cause of maternal, fetal, and neonatal morbidity and mortality. Pre-eclampsia is an idiopathic multisystem disorder of pregnancy, characterized by gestational hypertension and new-onset proteinuria occurring in the second half of pregnancy (11, 12) and can present as late as 4-6 weeks postpartum. Perhaps the most feared complication of preeclampsia is eclampsia itself, defined by the occurrence of one or more generalized convulsions and/or coma in the setting of preeclampsia and in the absence of other neurologic conditions (13). In this context, QEEG (Quantitative EEG) is a quantitative recording of cortical electrical activity, evidenced by different type of brain waves, based on the processing of EEG recordings. Convert characteristics of brain wave such as amplitude (shape), frequency, spatial coordinates in numbers, and their subsequent representation as statistical topographic maps of the brain (brain mapping) is achieved via a suitable software (14). These findings constitute a base of using EEG brain mapping, for the first time, in quantification of brain damage in hypertension associated with pregnancy, a noninvasive neuroimaging method with a real impact in the study of focal or diffuse brain lesions of diverse etiology.

Enrolling pregnant women in research

In our study, we recruited 40 pre-eclamptic women, in the third trimester of pregnancy, age between 18 and 40 years, from the obstetric unit. Healthy pregnant (control subjects, n=40) whose pregnancies had been uncomplicated and normotensive with normal laboratory tests were also recruited. We try to identify the patterns by which we can distinguish pathological groups from normals, the premise of implementation of this technique in study of neurological damage associated with hypertension in pregnancy.

Ethical issues associated with hypertensive pregnant women enrollment in our study were: the need for effective treatment and diagnostic for hypertensive women during pregnancy; foetal safety; harm resulting from the underuse of potentially beneficial medication and treatment during pregnancy; and the broader issues of justice and access to the benefits of research and of research participation (15, 16).

One of the problems we have encountered since the beginning of this study was the discovery that that in general researchers and institutional review boards continue to regard pregnancy as a near-automatic cause of exclusion, even in studies carrying no additional risk to the fetus (17). The 2002 International Ethical Guidelines for Biomedical Research Involving Human Subjects of the Council for International Organizations of Medical Sciences (CIOMS) contains a guideline specifi cally addressed to research involving pregnant women: "Pregnant women should be presumed to be eligible for participation in biomedical research. Investigators and ethical review committees should ensure that prospective subjects who are pregnant are adequately informed about the risks and benefi ts to themselves, their pregnancies, the foetus and their subsequent off spring, and to their fertility; research in this population should be performed only if it is relevant to the particular health needs of a pregnant woman or her foetus, or to the health needs of pregnant women in general, and, when appropriate, if it is supported by reliable evidence from animal experiments, particularly as to risks of teratogenicity and mutagenicity."

Also, one of the most important aspects of our scientific research is to prevent pregnant women and their fetuses from avoidable harms that could be caused by hypertensive disorders associated with pregnancy, an important reasons that can justify the inclusion of pregnant women in a greater number of biomedical studies than current practice allows. The most compelling reason is the need for evidence gathered under rigorous scientifi conditions, in which fewer women and their fetuses would be placed at risk than the much larger number who are exposed to medications once they come to market (16, 18).

An important consequence resulting from the exclusion of these women from clinical trials, is that pregnant women and their doctors are often forced to make difficult, anxiety filled decisions about whether to use or continue a medication in pregnancy, guessing about what medications to use, what doses to prescribe or take, or whether to use medications at all (17). There are still many unanswered questions regarding therapeutic management of hypertension associated with pregnancy, an important aspect that has motivated our research. In this context, there remain many unanswered questions regarding the pathogenesis of the cerebral manifestations of pre-eclampsia. Although numerous organs are affected by hypertension in pregnancy, cerebrovascular involvement is the direct mechanism of death in ≈40% of patients (19).

The autonomy of pregnant women is really important for many researchers. An autonomous person must have decisional capacity and his/her voluntariness must be secured. Physically or mentally disabled persons, and economically or educationally disadvantaged persons are also referred to as vulnerable populations (5). We must not forget that in some regions of the world, women are prone to be neglected or damaged due to social conditions and for many of them, participation in research projects could be the best or even the only way to have access to health assessments during pregnancy.

The CIOMS guidelines (Council for International Organizations of Medical Sciences) do not automatically consider women as vulnerable subjects, specifying separate indications for vulnerable populations and women. Specifically regarding pregnant women's decisional capacity, the Committee on the Ethical and Legal Issues Relating to the Inclusion of Women in Clinical Studies (US) has clarified that pregnant women are capable of making their own decisions (5).

Ethical Issues in Neuroimaging: Brain mapping

Ethical problems resulting from brain research have induced the emergence of a new discipline termed neuroethics (6, 20). The new methods and techniques, by laying bare neural correlates of personal identity, cause problems of individual rights on privacy, noninterference and inviolability (6). The main ethical problem that the scientific trends just reviewed pose concerns privacy: any testing method that reveals new kinds of information about an individual neurological status, it may not always be in the individual's best interest to have that information available to others (8).

Quantitative analysis of frequency EEG (QEEG) used in our study, with or without topographic mapping, is a more objective than conventional EEG interpretation, although there are a number of possible methodological pitfalls that should be avoided. Using QEEG is generally recommended only with visual EEG interpretation by an experienced observer. A report of the American Academy of Neurology and the American Society of Neuropsychology concluded role of EEG brain mappingin investigation of post-concussion syndrome , brain injury, learning disabilities, attention deficit disorder (ADHD - Attention-deficit hyperactivity disorder), schizophrenia, epilepsy, depression, alcoholism, encephalopathy diverse etiology, insomnia.

This new technologies and scientific findings create the circumstances in which ethical issues pose new challenges. Neuroethics is a relatively new discipline, on the border between neuroscience and philosophy. Researchers are not unanimous in delimiting areas belonging neuroethics. Some of them consider neuroethics as a subchapter of bioethics, with a moral evaluation of technological methods used in neuroscience. Most researchers, however, apply the term neuroethics in a much broader sense, particular focus on the relationship between new knowledge from neuroscience and moral values such as a sense of responsibility of the researcher in relation to freedom of action, rationality and human personality. Although there is a widespread notion that scientific research and especially new technologies bring new ethical problems, ethical concerns reflect the values of the society (22). Neuroethics intersects with biomedical ethics in that, broadly defined, neuroethics is concerned with ethical, legal and social implications of neuroscience research findings, and with the nature of the research itself (23).

The main issues associated with modern neuroethics were highlighted at a 2002 meeting called Neuroethics – Mapping the Field (24): 1) the implications of neuroscience for notions of the self, agency and responsibility; 2) social policy applica-

tions that make new resources such as healthcare and education available to society; 3) therapeutic intervention through advances in clinical practice; and 4) public discourse and training.

Neuroscience ethics is a philosophical discipline that aims to evaluate in terms of moral philosophy results in neuroscience research. It is very important to distinguish between general ethics and applied ethics of neuroscience. General ethics of neuroscience investigates the role that results it plays in neuroscience for understanding of the moral issues. For most authors, the freedom of decision is a prerequisite in evaluating research conducted. Ethics of neuroscience analyze the legitimacy of technology and research projects of brain functions. In this context, we try to identify the patterns by which we can distinguish pathological groups from normals, the premise of implementation of this technique – EEG brain mapping, in study of neurological damage associated with hypertension in pregnancy. Cortical and subcortical lesion complex of white matter in the form of edema, infarction and hemorrhage (petechiae and parenchymal intracerebral hemorrhage) is a common finding in patients who died due to eclampsia. However, it should be noted that although the autopsy provides information on central nervous system abnormalities in patients who die from eclampsia, this evidence is not necessarily an indicator of the type of brain injury certainly possible to have the majority of patients who survive this condition (25). Another ethical issue concerns the increasing use of neuroimaging to predict later onset brain disorders, but the complexity and plasticity of the brain, however, definitely restrict the reliability of such prognoses (26).

Informed consent

All participants of the study were informed about the non-invasive nature and purpose of the investigations, and written consent from each subject was obtained prior to the study. Type of electroencephalographic (EEG) recording used in our study , unlike other types of brain investigations is safe, painless, and does not require administration of injections or taking any substances. No known major risks associated with this type of EEG recording . This registration is non-invasive, and not affect the overall status , pregnancy outcomes, birth or lactation. This registration will not affect future childbearing ability . This procedure does not affect the fetus status in the uterine cavity, not affect the development of immediate and long-term newborn. Throughout the recording period patients were constantly monitored by medical specialists, including the obstetrician, that the occurrence of any inconvenience, discomfort, or impaired wellbeing of the pregnant woman or fetus , was able at all times to provide specialty advice and emergency aid . The physician's role is to provide adequate information and a recommended management plan, or range of possible plans, for the condition in question (27). Any new information that appeared during the study that could affect the patient's willingness to continue participation, was notified immediately.

Informed consent from pregnant womens assumes that she is properly informed of the potential benefits and / or injuries including those that directly affect the fetus. No electrical current is put into the brain. Normally, pregnancy is a physiological state in which women do not lose their ability to reason, do not become incapable of giving consent, or vulnerable to manipulation or coercion. An ethical issue is who makes decisions in such situations: the woman herself or someone else? How to prioritize a duty to protect the pregnant woman and her fetus and duty to honor a woman's right to self-determination? Fetuses require special protection as they are not autonomous agents. It may be the responsibility not only of pregnant women, but also of healthcare professionals and society to protect the fetus' well-being. Fetus should be recognized as a patient, and that maternal and fetal interests must be balanced against each other (5).

Although risk acceptance decision should be taken by the mother, in informed consent process, it is desirable that in a research directly addressing to fetus, to obtain if possible dad opinion. Especially in communities or societies in which tradition given more importance to fetus than to life of the mother, women may feel constrained to participate in research that addresses direct to benefit of the fetus. In this sense, precautions should be instituted to ensure that these women voluntarily participate in research, and investigators must create a plan for monitoring the evolution of pregnancy.

Conclusion

Quantification of brain damage in hypertension associated with pregnancy has been a challenge in terms of medical ethics. Because of the rapidly growing of neuroscience innovation and applications both within and outside of academic medicine, researchers must partner more closely than ever with physicians in many spheres of immediate importance (28). Also clinical research with pregnant women is morally challenging, but it is a challenge we must confront (17). It is in the interest of the mother and fetus to be studied pathology of pregnant women. It would be discriminatory for pregnant women to be deprived by the benefits of pathogenic mechanisms research and therapeutic effects of various drugs. Nevertheless, it might be a fundamental requirement in pregnant womens research to ensure that the informed consent process meets the highest standards regarding potential hazards to the fetus, as well as risks to their own health.

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Functional results following reconstruction with modular endoprosthesis for malignant primary and metastatic bone tumours around the hip

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Abstract

Objectives: The aim of this study was to evaluate the functional results of patients treated with endoprosthesis reconstruction following resection of primary malignant and metastatic bone tumours around the hip, and to reveal the factors which may affect these results.

Patients and Methods: A retrospective review was made of the database maintained by our university medical faculty orthopaedic oncology service and 26 consecutive patients who underwent limb salvage with proximal femoral endoprosthetic reconstruction from March 2000 to March 2012 were included in the study.

Results: The mean prosthesis life was 14.3±10.2 months (range, 4-52 months). Of the total 26 patients who were followed up, prosthesis dislocation was determined in 2 (7.6%) and prosthesis infection in 2 (7.6%). In the remaining 22 patients, no complications were determined throughout the follow-up period.

Conclusion: Due to the extent of muscle and bone resection, although it is doubtful that as much work will be seen on this topic as in total hip replacement, a proximal femur prosthetic replacement still provides better function than amputation.

Key words: Moduler endoprosthesis, malignant primary bone tumours, metastatik bone tumours, hip.

Introduction and aim

The proximal femur is a common site for primary bone tumors and the most common site for metastatic lesions.[1-2] Hip disarticulation or hindquarter amputation was the primary treatment of these lesions prior to the development of endoprostheses in the 1970s. Advances in radiation therapy and chemotherapy, enabled limb salvage to become an option in the early 1990s. One study has suggested that although there seems to be a higher incidence of local recurrence, overall patient survival rates are similar for both amputation and limb salvage.[3]

Amputation is nowadays only accepted as a relative contra-indication in cases of some recurrences, neglect, wrong treatment, mass of giant dimensions, where major neurovascular structures have been invaded and where haematoma have contaminated several compartments as a result of pathological fracture.

The development of new surgical techniques, better patient selection and improved prosthetic design have been instrumental in improving the treatment options available. However, it is still debatable as to which is the optimum method for reconstruction of the hip after resection of the proximal femur. One option, which is technically demanding, is the use of moduler prosthesis following resection of the tumor. Resection of the tumor at the level of the proximal femur results in the loss of abductors and other musculature necessary for hip stability and tissue coverage, and this then often leads to higher rates of dislocation and infection.

The aim of this study was to evaluate the functional results of patients treated with endoprosthesis reconstruction following resection of primary malignant and metastatic bone tumours around the hip, and to reveal the factors which may affect these results.

Patients and method

A retrospective review was made of the database maintained by our university medical faculty orthopaedic oncology service and 26 consecutive patients who underwent limb salvage with proximal femoral endoprosthetic reconstruction from

March 2000 to March 2012 were included in the study. The inclusion criteria for this study included all patients who underwent reconstruction of the proximal femur with a modular system using a bipolar acetabular cup for either a metastatic lesion or primary bone tumor (Figure 1).

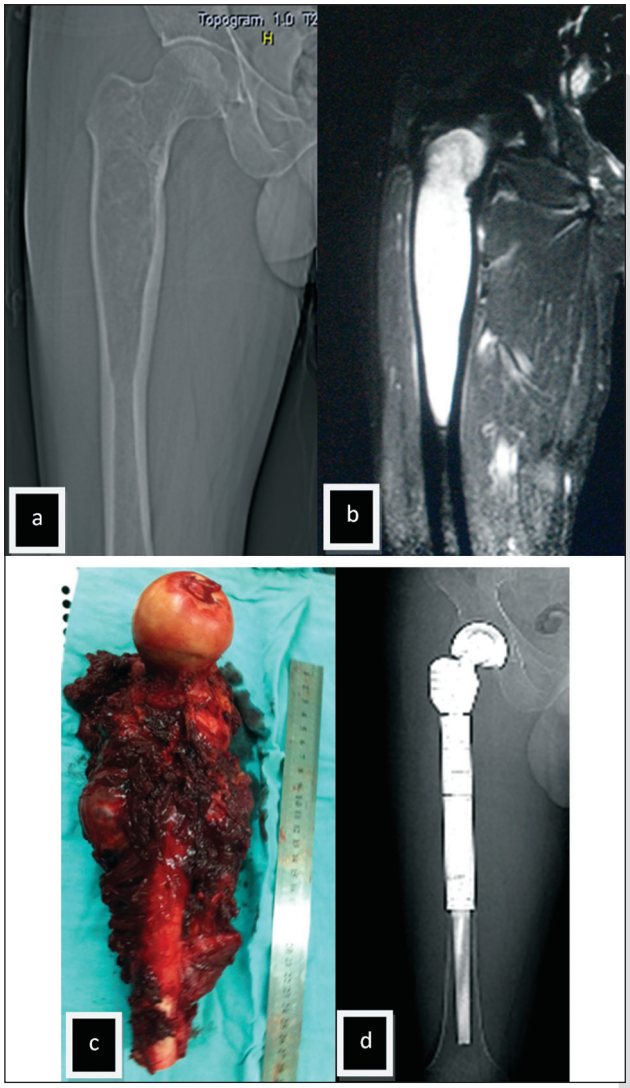

Figure 1. 57 years old, male, chondrosarcoma at proximal femur
a. Preoperative direct radiograph
b. Preoperative magnetic rezonans imaging
c. Excised tumoural tissue
d. Postoperative direct radiograph

The study comprised 9 females and 17 males with a mean age at surgery of 57.7 years (range, 33-83 years). Of the 26 patients in the study, 7 (26.9%) had primary and 19 (73.1%) had metastatic bone tumour. The diagnoses were multiple myeloma (4), lung carcinoma (8), prostate carcinoma (2), breast carcinoma (4), thyroid carcinoma (2), colon cacinma (2), renal cell cacinoma (1) and chondrosarcoma (3).

All patients had a complete tumor workup prior to surgery that included routine blood work, bone scan, CT of the chest, and MRI of the femur. All patients had an open biopsy to confirm the diagnosis. Preoperative radiotherapy and chemotherapy were administered as required.

Surgical technique

An extensile posterolateral approach was used. The sciatic nerve and femoral artery were protected and the profunda femoris artery was ligated if involved in the tumor. The gluteus medius was resected at the tendinous insertion. A T- shaped capsulotomy was performed. The femur was resected distally 4 cm below the lower margin of the tumor and a cemented or non-cemented modular megaprosthesis the exact length of the resected femur was inserted. No acetabular resurfacing was done. Capsulorrhaphy was performed and abductors were attached to the prosthesis and vastus lateralis if not resected with the tumor.

Postoperative antibiotics were administered for 72 hrs. Adequate thromboprophylaxis of low-molecular-weight heparin was administered. Postoperatively, patients were allowed full weight-bearing as tolerated immediately after surgery. No brace was applied postoperatively. Routine follow-up examinatons were performed every 3 months for the first 2 years postoperatively, then every 6 months between 2 and 5 years after treatment, and yearly thereafter.

The Musculoskeletal Tumor Society (MSTS) scoring system was used in the evaluation of the functional results.[4] The base MSTS scores were taken calculated from the final examinations of living and deceased patients. With this scoring system, a total of 6 parameters were evaluated; pain, functional capacity, emotional status, use of support, walking distance and walking manner. Each parameter was scored 0-5 according to specific scales and from the obtained result, the highest score was divided by 30 to give a percentage. The obtained MSTS scores were categorised as 100%-75% excellent, 74%-70% good, 69%-60% fair, 59%-50% inadequate, and below 50% poor.

After codification of the obtained data, computer analysis was made with SPSS 15.0 software.

The descriptive properties of the data were stated as mean±standard deviation, number and percentage. Conformity to normal distribution of measurable variables was evaluated with the Shapiro-Wilks test. In comparison between the groups of data with normal distribution, Student's t-test was used. In the comparison of counted data the Chi-square test was used. The calculation of survival rates was made using Kaplan-Meier analysis. A value of $p<0.05$ was accepted as the level of statistical significance in all the tests.

Results

The mean age of the 26 evaluated patients was 57.7±10.6 years (range, 33–83 years). The mean follow-up period was 14.1±10 months (range, 4-52 months).

The mean postoperative MSTS scores were determined as 69.1±11 (range, 46-93).

The functional results of the cases of tumours with involvement around the hip were excellent in 8 (30.8%), good in 7 (26.9%), fair in 9 (34.6%) and poor in 2 (7.7%) (Figure 2).

The patients were grouped as primary bone tumour (n:7) and metastatic bone tumour (n:19). In the metastatic bone tumour patients, the functional results were found to be excellent in 2 (10.5%), good in 6 (31.5%), fair in 9 (47.3%) and poor in 2 (10.5%) and in the primary bone tumour patients, excellent in 6 (85.7%), good in 1(14.3%) and 0 cases of fair or inadequate.

Of the total 26 patients who were followed up, prosthesis dislocation was determined in 2 (7.6%) and prosthesis infection in 2 (7.6%). In the remaining 22 patients, no complications were determined throughout the follow-up period.

The mean prosthesis life was 14.3±10.2 months (range, 4-52 months) and according to the Kaplan-Meier analysis the prosthesis survival rate was calculated as 83% at 1 year and 62% at 5 years.

Throughout the follow-up period, 13 of the 26 patients for various reasons. The histological diagnoses of the lost patients were multiple myeloma in 1, breast carcinoma metastasis in 2, thyroid medullar carcinoma metastasis in 1, lung carcinoma metastasis in 7, and prostate carcinoma metastasis in 2 cases. Mortality was due to chronic renal failure in the patient with multiple myeloma and advanced metastatic disease in the other patients. According to the Kaplan-Meier ananlysis, patient survival rates were 84% at 6 months, 61% at 12 months and 49% at 60 months.

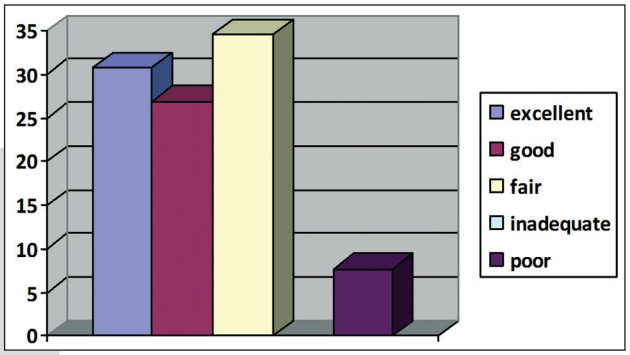

Figure 2. Functional results

Discussion

In the last 30 years, the use of modular tumour prostheses has become a method which is acceptable to many orthopaedic surgeons for reconstruction of the extensive segmentary defect, most of which include joint surfaces, formed following tumour resection for the treatment of bone tumour in the extremity. With developments in the prosthetics industry and the experience gained from the use of these implants, success rates in a 5-year follow-up of tumour endoprostheses have risen from 20% to 85%.[5]

Reconstruction with endoprosthesis has the advantages of stability, early weight-bearing, rapid restoration of function and no concerns about osteosynthesis.

Early results obtained with this method are very encouraging. However, in the long-term, the possibility of mechanical complications which may affect the prosthesis survival is the main element raising questions about the use.

When the follow-up periods are examined of patients with modular tumour resection prosthesis for a primary malignant or metastatic bone tumour with involvement around the hip, the mean follow-up periods have been reported as 20.3 months (4-51 mths) by Pannekamp et al, and 18.1 months (1-129 mths) by Menendez et al.[6-7] In the current study, the mean follow-up period for a primary malignant or metastatic bone tumour with involvement around the hip was 14.1 months (range 4-52 mths).

When the functional results of primary malignant or metastatic bone tumours with involvement

around the hip were examined, a mean MSTS score of 73% (50%-83%) was reported by Pannekamp et al and functional results of excellent in 20%, good in 26.7%, inadequate in 40% and poor in 13.3% of patients.[6] Ilbeyli et al reported a mean MSTS score of 68.6% and functional results of good in 55.5%, fair in 37.3% and poor in 7.4% of patients.[8] In the current study, the mean MSTS was found to be 69.2% (46%-93%) and functional results were obtained of excellent in 29.6%, good in 29.6%, fair in 33.3% and poor in 7.4% of patients. These results were seen to conform with the functional results in literature of tumours located around the hip.

With regard to complications in patients with modular tumour resection prosthesis applied for tumours located around the hip, Pannekamp et al reported a total complication rate of 22.7%; 13.6% prosthesis infection and 9.1% prosthesis dislocation.[6] In a series studied by Ilyas et al, complication rates were reported as 14% prosthesis infection, 7% aseptic loosening and 7% local recurrence.[9] In the current study, the complications which developed most frequently were prosthesis dislocation at rates of 7.4% and prosthesis infection at 7.4%. As these results are parallel with the figures reported in literature for prosthesis dislocation in traditional hip arthroplasty there is thought to be an association with loss of abductor muscle strength following extensive tumour resection.

When the prosthesis survival rates (Kaplan-Meier analysis) of modular tumour resection prosthesis application are considered according to tumour localisation, Zehr et al reported prosthesis survival rates of 58% at 10 years and Natarajan et al reported 66% at 5 years in patients with modular tumour resection prosthesis applied for a tumour located around the hip.[10-11] In the current study, the prosthesis survival rates were calculated as 83% at 1 year and 62% at 5 years.

Patient survival rates in cases of modular tumour resection prosthesis applied for a tumour located around the hip were reported by Hattori et al as 86% at 6 months, 54% at 1 year and 37% at 2 years, and by Chan et al as 60% at 1 year, 38% at 3 years and 30% at 5 years.[12-13] Just as with osteosynthesis in femoral neoplasms, when sufficient resection cannot be made, this constitutes an inhibition to using the extremity for a long period. When the survival of these patients is considered, the survival rates of the current study of 84% at 6 months, 61% at 1 year and 49% at 5 years favour endoprosthetic replacement treatment by reason of providing the advantages of stability, early weight-bearing and very good function.

In conclusion, due to the extent of muscle and bone resection, although it is doubtful that as much work will be seen on this topic as in total hip replacement, a proximal femur prosthetic replacement still provides better function than amputation.

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Guidelines may be the single instrument for evaluation of professional conduct?

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Abstract

The guidelines are recommendations for the health care worker behavior. However in Italy the L. 189/2012 has linked the professional responsibility to respect these without reference to the quality of the guidelines accessible.

The authors proceed to an analysis of the literature using as a measuring instrument the requirements proposed by the Institute Of Medicine finding limits of applicability of these as single instrument for evaluation of professional conduct.

Key words: guidelines, quality, professional responsibility

Introduction

The GuideLines (GL) are clinical behavior recommendations, developed through a process of systematic review of the literature and expert opinion, with the aim to help doctors and patients decide on the most appropriate care modality in specific clinical situations (1).

According to the most recently updated definition from Institute of Medicine (IOM) "Clinical Practice Guidelines are statements that include recommendations intended to optimise patient care that are informed by a systematic review of evidence and an assessment of the benefits and harms of alternative care options" (2).

In the literature, there is a strong need to measure the essential requirements of a GL, and experiences are available in many different specialist areas (3-7).

The first and most authoritative indication about the quality of the GL has been proposed by the IOM just (8).

The experiences consulted in the literature, however, ignore the use in the legal medicine that these instruments can have especially after a law (in Italy L 189/2012) that has forced the doctor compliance to the GL.

The aim of the study is to verify if and how the criteria proposed by the IOM for the quality of GL are applicable for evaluating professional conduct.

Material and Method

According to IOM the GL should be "based on a systematic review of the existing evidence; be developed by a knowledgeable, multidisciplinary panel of experts and representatives from key affected groups; consider important patient subgroups and patient preferences, as appropriate; be based on an explicit and transparent process that minimises distortions, biases, and conflicts of interest (COI); provide a clear explanation of the logical relationships between alternative care options and health outcomes, and provide ratings of both the quality of evidence and the strength of recommendations; and be reconsidered and revised as appropriate when important new evidence warrants modifications of recommendations" (8). The authors present each point by analyzing scientific literature and doctrine review.

Results and Discussion

The methodology of GL processing has affect on the applicability of the same, influencing the outcome.

In a prospective observational multicenter study conducted between November 2007 and June 2008 in Cyprus on 654 hypertensive patients, the doctor's prescription for each patient were recorded and compared with the GL 2007 of European Society of Hypertension and European Society of Cardiology. The total adherence of doctors was 70.4% (9).

All GL (sixty) developed by the Chilean program between 2005 and 2009 were evaluated independently by three experts: the '"applicability"

reported was extremely low scores (mean 23.3%, range: 0% - 72.4%) (10).

The most distinctive feature of the criteria and the requirements dictated by the IOM regards the method of preparation of the GL that should result from a systematic review of the literature and provide an assessment of the quality of evidence and power of recommendation.

In a more recent vision of the GL as a "means evidence", the need to provide a grading of recommendations and evidence in support of these well adapts to the current setting of medical professional responsibility.

The availability of a system for quantitative measurement of the recommendations (in descending order from I to VI) and testing (in descending order from A to E) overcomes the difficulties may incur in the evaluation, coming to meet, in such a way , the requirements of the law.

The cooperation of the various stakeholders in the elaboration of the GL is of fundamental importance because the management of the patient does not end in the hospital setting, where it usually proceeds to the treatment of acute, but continues and extends to involving the medicine of the territory.

For this reason it is not possible to avoid a complete and unconditional adherence by all stakeholders (11). Otherwise it would be conditioned the applicability and lose power of "usage or practice."

One of the protagonists enrolled in the drafting process of the GL should be the patient / consumer. The centrality of them, represented by caregivers, public organizations, voluntary and community or defense groups of patients (12), it is a dominant paradigm of modern health systems and a central element of evidence-based medicine (13).

It's synonymous with transparency and helps to ensure that the GL do not serve special interests different from those of the patients.

However, it is necessary to overcome this gap of knowledge that makes this hypothesis difficult and led to even groped training in skills of Evidence Based Medicine for patients and consumer representatives, such as the Cochrane Consumer Network (14). Experiences that in some cases have been shown to be feasible and much appreciated (15).

A review of the recent literature has concluded that most of the studies that have predicted the patients' participation in the development of GL have confirmed their position in favor of the patient's active involvement in the development process, despite the absence of evidence confirming the utility of this choice (16).

However, the wish of patients to want to be involved in medical decision-making (17, 18) responds to a larger pattern of "therapeutic alliance" that finds its motivation in the right to information and self-determination in itself distinct from the right to health, and as such have a guarantee regardless of lesion of the right to health.

Public access to all the test data must be ensured in order to make possible independent evaluations unrelated to any suggestion of COI.

In the literature, the responsibility of creating the GL should be borne by authors and organizations that don't have COI (19), defined in the IOM report as "Conflict of interest in medical research, education, and practice" come "a set of circumstances that creates a risk that professional judgment or actions regarding a primary interest will be unduly influenced by a secondary interest " (20).

And this is most felt where, as in relations with the industry, there are strong economic interests behind the development of GL (21) because they are intended to standardize care and therefore their freedom from bias is of utmost importance (22). So much so that some organizations to exclude authors with COI from decision-making (23).

In the study of Norris et al. (24) on a cohort of 13 GL of the National Guideline Clearinghouse (NGC) on glycemic control in diabetes mellitus type II, the percentage of authors with one or more COI varied from 0 to 94% (average 44.2%). And it was particularly high percentage in the case of two GL U.S. (94% and 91%), while in three GL more than 50% of the authors had a financial interest in patented drugs recommended in the same GL. The average 56.1% of drug producers subject to the recommendation in each GL had one or more people with an interest related to the pharmaceutical company (median 70.0%, range 0 to 100%). Three GL even had one or more authors with COI cost in all medications.

And, finally, the update. Is an essential requirement due to the legal significance of the GL. Unfortunately this does not happen very often and we are witnessing a sudden application of a therapeutic or diagnostic method immediately following the

publication of the GL (25), but in the long run population-based studies continue to show that GL produced worldwide by leading agencies for the acute and chronic diseases remain underutilized (26-30).

The World Health Organization found that for cancer, a third of cases could be prevented, another third cured, and the rest effectively managed if care consistently complied with existing GL (31).

Conclusion

Every single step that constitutes the process of decision making, whether diagnostic (32) or therapeutic (33), must be characterized by a specific and individual clinical assessment (34).

However, the GL have the ability to promote knowledge because they facilitates the process of acquisition and dissemination of the these, contributing to the process of appropriateness and, therefore, the assumption of responsibility of operators (35, 36).

Law takes as its the reasons of EBM and now the legal medicine must acquire skills and methods that enable the improvement of the assessment.

Assuming that the work of a specialist in the evaluation should not be limited to only finding of compliance with the recommendations. But it is likely that in the near future to the category is also required, a critical and scientifically objective about the methodology of processing of the instrument adopted that can and should influence the subsequent compliance by the colleagues.

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Clinical and radiographic diagnosis of proximal caries

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Abstract

Introduction: Detection of proximal caries still presents a challenge for the clinicians. Radiography, as an auxiliary diagnostic procedure is often used for early proximal caries detection.

The aim: The purpose of this study was to evaluate the reliability of the clinical diagnostic procedures for the proximal caries detection.

Material and methods: The examined group consisted of 30 patients with permanent teeth, aged 14-18. We analyzed 720 proximal surfaces in 320 lateral teeth (176 permanent molars and 184 premolars). After the radiographic examination we compared the results in regard to the presence of caries in proximal surfaces of permanent premolars and molars, gained by clinical and, subsequently, by radiographic examination. Degree of examiners' agreement and determination of the reliability of the diagnostic methods were established by the calculation of Cohen's kappa coefficient.

Results: Clinically, caries was detected in 32 teeth (8.88%), that is, in 40 proximal surfaces (5.56%). Radiographic analysis revealed the presence of caries in 44 teeth (12.22%), that is, 48 tooth surfaces (6.66%). The Cohen's kappa coefficient was higher in radiographic method of caries diagnosis.

Conclusion: The application of retrocoronary radiogram as an auxiliary diagnostic method, substantially improves the degree of reliability of caries diagnosis on these surfaces.

Key words: Proximal caries, radiography, diagnosis

Introduction

Dental caries is a disease of hard dental *tissues* that progresses centripetally and progressively, from the surface to depth and leads to the tooth destruction. Its etiology is multicausal, while the occurrence is associated with a numerous systemic and local factors, as well as internal and external circumstances. [1]. *Dental caries* is one of the *most common diseases* in the world *today*.

The surfaces of the teeth that are hardly accessible for tooth brushing and salivary flow are prone to caries. They represent a caries predilection sites. Proximal surfaces together with fissures and pits of crowns and gingival third of the vestibular and oral surfaces are the places with the highest prevalence of dental caries. The high frequency of proximal caries and his diagnosis is a common problem for the clinicians.

Due to inaccessibility of the proximal teeth surfaces for inspection and probing the clinical diagnosis is difficult, and the proximal caries is often diagnosed in an advanced stage when it destroyed most of dental hard tissue.

The risk of proximal caries increases in persons with previously registered lesions at age of 11-13 years. [2, 3].

The highest annual incidence of proximal caries was found in 12-year-olds, suggesting that this is a period of great risk. [4].

Radiography represents an important secondary diagnostic method, whose implementation can help in early diagnosis of proximal caries. In many cases it *provides important additional information* for final decision regarding restorative treatment [5].

Radiographic examination is based on the concept that it is very important to detect the extent of the disease, especially caries in an early stage. Because of that radiological examination should be carried out routinely, even when there are no signs of disease. [6].

Dental imaging technique is very important for successful *radiogram* and image quality. Many different types and *techniques* of recording are used in dentistry, but for an early diagnosis of proximal caries retro coronary method is most frequently used. [7]. This radiographic method is also called "Bite-wing", and it provides the visualization of the crowns of upper and lower posterior

teeth, the free edge of the alveolar bone and the bifurcations of the roots.

When used in detection of proximal caries, this method is handy for diagnosis of secondary or recurrent caries, fillings position in regard to interdental papilla and marginal parts density of the alveolar bones. The special feature of "Bite-wing" images is that both the upper and lower jaw teeth can be viewed simultaneously, from the distal surface of the canines to the end of molars, that *reduces the need* for more images and *reduces* radiation *exposure* in *x-rays* for *children*.

Aim

The aim of our study was to investigate the reliability of the clinical diagnosis of the proximal caries in tooth surfaces. In accordance with the set goal, we have determined the following tasks:

1. to evaluate the reliability of the proximal caries detection using clinical examination
2. to evaluate the reliability of the proximal caries detection with the help of radiographic examination
3. to assess the reliability and sensitivity of the overall diagnostic procedure.

Materials and methods

Clinical and radiographic examinations were performed at the Faculty of Medicine Department of Dentistry, University of Novi Sad. The examined group consisted of 30 patients with permanent teeth, aged 14-18. Before the investigation the written consent of the parents and written consent for patients participating in the survey was obtained.

The calibration of the examiners was performed before patients and radiographs examinations. The same patient was examined by a dentist with ten years of clinical experience and by two dentistry students of the fourth year. Before the clinical examination in each patient the debridment was performed using slow rotating brushes and headpiece, and fluoride containing paste (Fluorogal, ICN Galenika, Serbia). Examination was carried out with a dental probe and dental mirror, and consisted of inspection, probing, and percussion.

Inclusion criteria were defined as follows:

1. Macroscopically intact marginal ridge
2. The absence of symptoms of pulp pathology
3. The presence of proximal fillings.

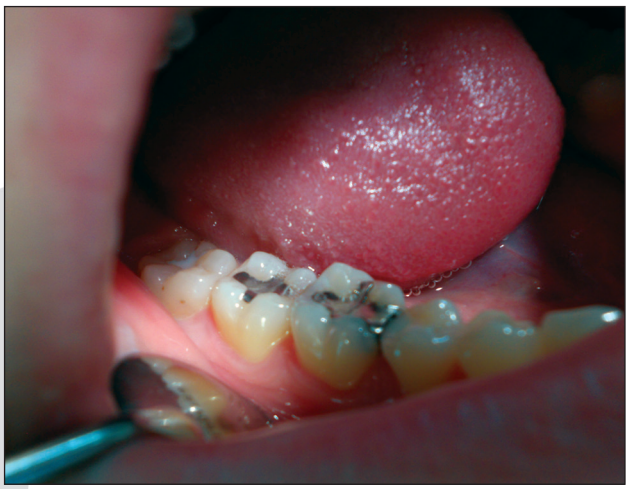


Image 1. Teeth that meet the criteria for inclusion in the study

In addition to that criteria for exclusion from the study (in regard to patient, tooth and tooth surface) were also defined and included:

1. Presence of edentulous space that allows the direct clinical examination of the proximal surfaces of
2. Extensive destruction of the tooth crown
3. Prosthetic restorations.

The patients underwent the clinical and radiographic assessment. Clinical detection of proximal caries and the overall status of the teeth were recorded in patient's charts. For the radiographic examination X-ray unit for conventional radiography (Sirona, Heliodent Vario Digital Dental Xray "2002" X-ray 1 YR WARR; 70 KV, 7MA, 120V, D3350, Germany) was used. Tube apparatus was set up so that the x-rays were directed parallel to the proximal space between the first and second permanent molars. Their direction was not allowed to form a right angle with the neck, but had to be more inclined from the dorsal direction. We used the recommended vertical angulations of +5° to +10° and distance of x-ray film tube was 100 mm, as described by Versteeg and colleagues. [8]. Retrocoronar method required film which is used together with a rubber flat mold with wings. Size of film was adapted to the age of the patient. The film included as many taped areas as possible, and at the

same time it was adapted to the size of the patient's jaw, in order to avoid excessive discomfort. The method of "tell-show-do" was used to prepare the patients for the radiographic procedure. The film was placed intraorally, in a horizontal position, and it was positioned on the oral surfaces of the upper and lower teeth, and the wings foil film was placed between the occlusal surfaces of posterior teeth. Recordings were obtained with the Ektaspeed (EP-21P) films (Eastman Kodak Company, Rochester, NY, USA) automatic processed in the device Dúrr AC245 (Dúrr Dental, Bietigheim, Germany) *according* to the *instructions* of the *manufacturer*. The exposure time was 0.5s. As a protection from the x-rays metal protective apron and thyroid collar were used. Recordings were then analyzed under a bright screen without a zoom, in a darkened room. Each of the three researchers independently analyzed the radiographs without insight into the patient's previous clinical findings. After evaluation of the "Bite-wing" images clinical and radiological results were compared.

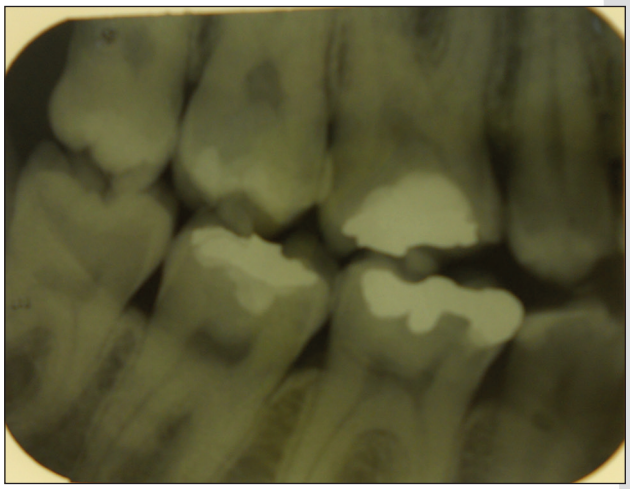


Image 2. Bite-wing image

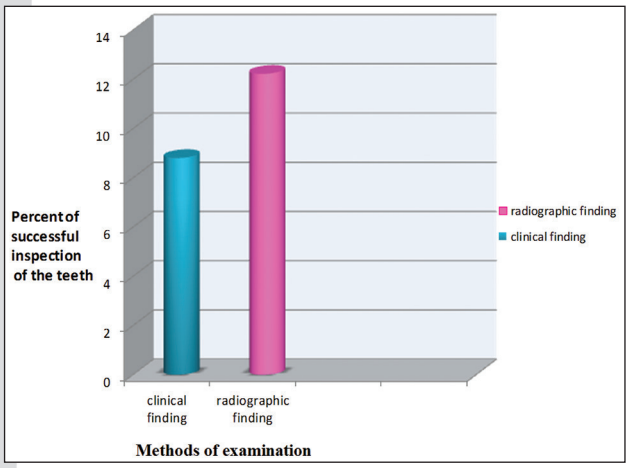
After completion a clinical and radiographic examination, a restorative treatment was carried out, where indicated.

In the data analysis standard methods of descriptive statistical analysis (rate, mean value, standard deviation) were used. Degree of examiners' agreement and determination of the reliability of the diagnostic methods were established by the calculation of Cohen's Kappa coefficient.

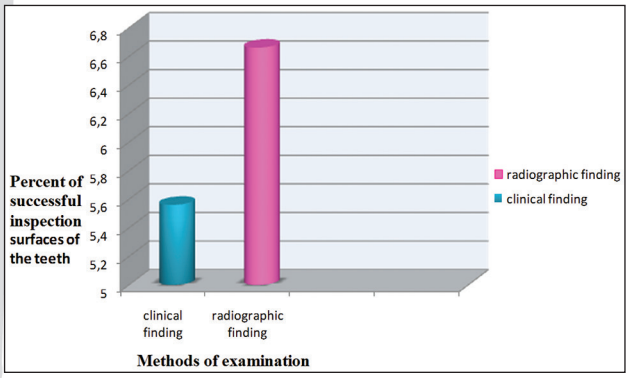
Results

The clinical and radiographic study included 30 patients aged 14-18 years (Mean 16.2, SD 2: 54, Median 16). The study included a total of 360 teeth, 184 premolars and 176 molars. Comparative clinical and radiographic analysis included 720 proximal surfaces of posterior teeth. 105 teeth were excluded from the study. Clinically, caries was detected in 32 teeth (8.88%), that is, in 40 proximal surfaces (5.56%). Radiographic analysis revealed the presence of caries in 44 teeth (12.22%), that is, 48 tooth surfaces (6.66%).

Results of clinical and radiographic findings are presented in graphs 1 and 2.



Graph 1. The rate of tooth caries detection in regard to the method of examination



Graph 2. The rate of tooth surface caries detection in regard to the method of examination

For the determination of the degree of reliability of diagnostic methods and the degree of agreement between researchers Kappa score was used and the results of the analysis are shown in Table 1.

The decision to perform the restorative treatment was based upon examiners consensus. Re-

Table 1. Reliability of clinical and radiographic diagnosis of proximal caries by researchers who participated in the study (95% percentage of confidence)

Kappa score	The clinical examination	Retrocoronar radiography
researcher 1	0.793 (0.546-0.853)	0.842 (0.548-0.912)
researcher 2	0.683 (0.448-0.726)	0.712 (0.437-0.835)
researcher 3	0.654 (0.432-0.734)	0.709 (0.452-0.798)
researcher 1 vs researcher 2	0.734 (0.465-0.769)	0.737 (0.444-0.833)
researcher 1 vs researcher 3	0.723 (0.482-0.785)	0.736 (0.428-0.842)
researcher 2 vs researcher 3	0.622 (0.421-0.678)	0.725 (0.429-0.819)

storative treatment was indicated and carried out in 32 teeth. There were no false-positive findings.

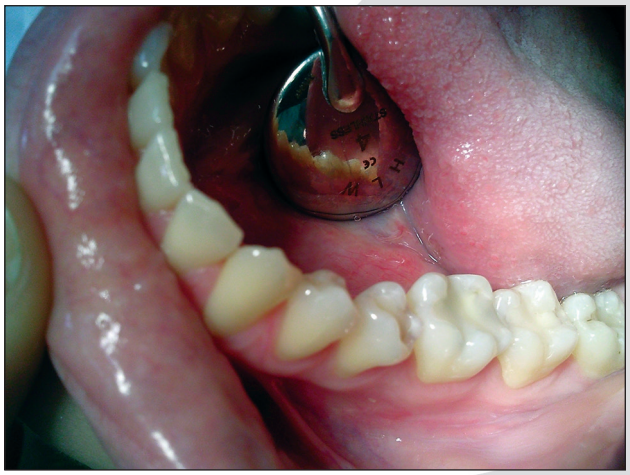


Image 3. Indication for restorative treatment in the distal surface of the second lower premolars

Discussion

Proximal caries diagnosis is rather difficult. Clinical diagnosis depends on availability of proximal surface for inspection and probing. In most cases, by regular set of teeth without orthodontic problems, caries of proximal surfaces becomes noticeable and clinically diagnosed when the most part of the hard dental tissue is already damaged. The results of this study demonstrate that clinical diagnosis of early proximal caries is not 100% reliable. It is necessary to perform some additional diagnostic methods, in order to get more reliable conclusions about the presence of caries. Radiographic methods provide more reliable and faster diagnosis and detection of caries in the early stage.

Comparison of the results obtained by clinical and radiographic diagnostic method indicates that the overall reliability of proximal caries detection is around 10%. This result is in complete agreement with many previous clinical reports and proves that it is very important to establish uniform radiographic "screening" protocol in regard to early caries detection in proximal surfaces. [9]. Of course, one should always take into account the cost, time, effort, radiation and false positive diagnoses in relation to early diagnosis of caries. [10, 11]. For these reasons, before actual radiographic screening all the factors associated with the onset of caries in proximal surfaces should be investigated in detail.

Many concerns have been raised when it comes to accuracy of interproximal radiography because of the risk of wrong diagnosis. [12, 13]. There are cases in which radiographs do not detect caries and give negative results. [14]. White and Yoon also considered the radiographic diagnosis very difficult and unreliable, and Wenzel and Hintze found that radiography is far from accurate method of diagnosing cavities, especially for small interproximal lesions. [15, 16]. Bill and Thylstrup claim that visual clinical diagnosis can be more sensitive than radiography in the detection of initial carious lesions, while the radiographic diagnosis of is more precise when the cavities are already present. [12] Espelid and Tveit found that X-ray examinations of superficial lesions did not give an accurate diagnosis due to unreliable radiographic criteria for identifying the presence of the initial damage. [17]. When evaluating certain diagnostic procedures, it is important that it exhibits a minimum diagnostic variability between measurements to ensure accuracy, consistency and repeatability. In the present investigation, the calibration of examiners was performed prior to patients' examination. Kappa scores were around 0.70, which were higher compared to the large number of published studies [18]. Kappa values

for both diagnostic methods were relatively high in all three examiners but undoubtedly demonstrated that the combination of diagnostic methods gives the best results. This finding is in full agreement with the results published by Ismail and his colleagues, who are in favor of a high degree of consistency among examiners independently from their clinical experience, provided they are given precise diagnostic criteria [19,20,21].

Histological analysis using stereomicroscope is still considered the most accurate method for determining the carious lesions, as well as the depth of the lesion. [21]. The lack of these analyzes is that they can only be performed in vitro on extracted teeth.

The presence of caries in proximal surfaces was finally confirmed after the decision to perform the restorative treatment, which was conducted in 32 teeth and there were no false positive findings. In all teeth in which restorative treatment had been carried out, the caries was actually present in the proximal surface. From the clinical standpoint the most important question to discuss is how to assess what causes more damage to the patient: to miss the chance for early diagnosis of disease due to false negative results or to treat tooth decay without lesion as a consequence of a false positive results. Definitive answer to this dilemma does not exist, but the solution may be in the holistic approach to the patient and thorough analysis of the individual risk of disease. [22]. It is therefore of utmost importance to consider the following factors: previous caries experience, caries lesion localization, diet, oral hygiene, use of fluoride, and only after all of that to make a decision about treatment. [23].

Conclusion

According to the results of the present investigation it can be concluded that neither the clinical examination nor radiographic diagnosis, are not absolutely accurate methods for detection of proximal caries.

However, the application of retrocoronary radiogram as an auxilliary diagnostic method, substantially improves the degree of reliability of caries diagnosis in these surfaces.

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Painful crises and survival of sickle cell patients

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Abstract

Background: We tried to understand whether or not there is an association between frequency of painful crises and survival of sickle cell diseases (SCDs) cases.

Methods: The study was performed in the Hematology Service of the Mustafa Kemal University on SCDs patients. Cases with a history of frequent crises, at least once a year, were put into the first, cases with rare crises, less than once a year, were put into the second, and cases with no crisis in their lives were put into the third groups.

Results: The study included 273 patients (138 males). Majority of the SCDs patients (79.1%) have frequent crises, 10.6% of them have rare crises, and 10.2% of them have no painful crisis in their lives. There was a progressive increase from the first towards the third groups according to the mean ages (28.0, 31.0, and 35.5 years), and the difference was highly significant between the first and third groups ($p<0.000$). There was a progressive increase according to female ratios in the same direction again (47.2%, 51.7%, 64.2%, $p>0.05$ between all). Parallel to the fewer crises, pulmonary hypertension ($p>0.05$), leg ulcers ($p<0.001$), smoking ($p<0.001$), chronic kidney disease ($p>0.05$), chronic obstructive pulmonary disease ($p<0.001$), cirrhosis ($p>0.05$), digital clubbing ($p>0.05$), and stroke ($p>0.05$) were all lower in females. There were 15 (5.4%) mortal patients without any gender difference ($p>0.05$), and mean ages of them were 26.1 in males and 31.0 years in females ($p>0.05$).

Conclusion: Increased frequency of painful crises may indicate shortened survival of SCDs cases.

Key words: Sickle cell diseases, painful crises, atherosclerosis, metabolic syndrome

Introduction

Atherosclerosis may be the major underlying cause of aging of human being. Probably atherosclerosis is an irreversible process that accelerated by many factors. Smoking, dyslipidemia, obesity, diabetes mellitus (DM), hypertension (HT), and various inflammatory or infectious disorders may be the accelerating causes of the systemic process. Such preventable causes of the systemic atherosclerosis are mainly collected under the heading of metabolic syndrome (1-5), which is characterized by reversible risk factors including overweight, dyslipidemia, elevated blood pressure (BP), and insulin resistance for the development of terminal diseases such as obesity, HT, DM, coronary heart disease, chronic obstructive pulmonary disease (COPD), cirrhosis, chronic kidney disease (CKD), peripheric artery disease, and stroke (6,7).

Sickle cell diseases (SCDs) are chronic hemolytic anemias. They are characterized by sickle-shaped erythrocytes which is caused by homozygous inheritance of the hemoglobin S (Hb S). Glutamic acid is replaced with valine, as a less polar amino acid in the sixth position of the beta chain of the Hb S. Presence of the less polar amino acid promotes polymerisation of the Hb S, which distorts erythrocyte into a sickle shape and decreases its elasticity. Although the polymerisation process exaggerated during various stressful conditions including operations, traumas, infections, inflammations, and emotional distress, it is probably continuously present in whole lives. The decreased elasticity of erythrocytes is the central pathology of the SCDs. Vascular occlusions induced tissue ischemia and infactions are the final consequences of the disease, so life expectancy of the SCDs cases is decreased by 25 to 30 years (8). We tried to understand whether or not there is an association between frequency of painful crises and survival of the SCDs cases in the present study.

Material and methods

The study was performed in the Hematology Service of the Mustafa Kemal University between March 2007 and October 2012. All patients with SCDs were enrolled into the study. SCDs are diagnosed by the hemoglobin electrophoresis perfor-

med via high performance liquid chromatography. Their medical histories including frequency of painful crises, regular alcohol consumption, smoking habit, leg ulcers, and stroke were learnt, and cases with a history of frequent crises, at least once a year, were put into the first, cases with rare crises, less than once a year, were put into the second, and cases with no crisis in their lives were put into the third groups. Additionally, cases with a history of one pack-year were accepted as smokers. A check up procedure including serum creatinine value on three occasions, hepatic function tests, markers of hepatitis viruses A, B, and C and human immunodeficiency virus, an abdominal ultrasonography, a Doppler ultrasonography to evaluate the portal blood flow, an endoscopy to detect esophageal varices just in suspected cases, and a computed tomography of the brain was performed. Cases with acute painful crises, infections, or any other inflammatory events were treated at first, and then spirometric pulmonary function tests to diagnose COPD, the Doppler echocardiography to measure the systolic BP of pulmonary artery, and renal and hepatic function tests were performed on the silent phase. The criterion for diagnosis of COPD is post-bronchodilator forced expiratory volume in 1 second/forced vital capacity of less than 70% (9). Systolic BP of the pulmonary artery at and above 40mmHg during the silent phase was accepted as pulmonary hypertension (10). CKD was diagnosed with a continously elevated serum creatinine level which is greater than 1.2 mg/dL on the silent phase. Cases with renal transplantation were also put into the CKD group. Cirrhosis is diagnosed with hepatic function tests, ultrasonographic findings, esophageal varices, and ascites without histologic procedure in the absence of any indication. Digital clubbing is diagnosed by determining the ratio of distal phalangeal diameter to interphalangeal diameter which is required to be higher than 1.0, and with the presence of Swamroth sign (11,12). Eventually, SCDs groups with frequent, rare, and no crises were compared in between according to the mean ages and gender distribution. Additionally, SCDs patients with regular alcohol consumption, pulmonary hypertension, leg ulcers, smoking, CKD, COPD, cirrhosis, digital clubbing, stroke, and exitus were detected and compared between the genders. Mann-Whitney U test, Independent-Samples t test, and comparison of proportions were used as the methods of statistical analyses.

Results

The study included 273 patients (138 males and 135 females). The mean age of them was 29.1 ± 9.4 (14-59) years. Majority of the SCDs patients (79.1%) have frequent crises, 10.6% of them have rare crises, and 10.2% of them have no painful crisis in their lives (Table 1). When we compared the mean ages, there was a progressive increase from the first towards the third groups (28.0, 31.0, and 35.5 years). Although the differences were nonsignificant between the first and second and between the second and third groups, probably due to the small sample sizes of the second and third groups, the difference was highly significant between the first and third groups ($p<0.000$). Similarly, although the differences were nonsignificant between the groups according to gender, probably due to the same reason above, there was a progressive increase according to the female ratio from the first towards the third groups again (47.2%, 51.7%, 64.2%, $p>0.05$ between all). Parallel to the fewer painful crises of the females, all of the terminal consequences of SCDs including pulmonary hypertension (11.1% versus 11.5%, $p>0.05$), leg ulcers (5.1% versus 16.6%, $p<0.001$), CKD (6.6% versus 10.1%, $p>0.05$), COPD (2.9% versus 10.1%, $p<0.001$), cirrhosis (5.9% versus 6.5%, $p>0.05$), digital clubbing (4.4% versus 7.2%, $p>0.05$), and stroke (3.7% versus 5.7%, $p>0.05$) were lower in females (Table 2). Although there was not any patient with regular alcohol consumption among the study cases, the smoking was significantly lower in females (4.4% versus 14.4%, $p<0.001$). On the other hand, five of the CKD cases were on hemodialysis, and one with renal transplantation. Majority of the SCDs patients (94.5%, 258 cases) have received multiple erythrocyte transfusions in their lives. The ratio of patients without any erythrocyte transfusion in their lives was higher in females (8.1% versus 2.8%, $p<0.001$). Although antiHCV was positive in two of the cirrhotic cases, HCV RNA was detected as negative by polymerase chain reaction in both. Histological diagnosis of cirrhosis was required in none of the study cases. On the other hand, there

were 15 (5.4%) mortal cases during the six-year follow-up period without any gender difference (5.0% in males and 5.9% in females, $p>0.05$), and the mean ages of them were 26.1 and 31.0 years, respectively ($p>0.05$) (Table 3).

Discussion

SCDs include a group of genetic disorders characterized by the presence of Hb S, which is known for 100 years (13). Together with the hemoglobin E, it is the most commonly seen hemoglobinopathy in the world. Hb S causes erythrocytes to change their normal elastic and biconcave disc shaped structures to a hard and sickle shaped bodies. The sickling process is probably present in whole life period of the human being, but it is exaggerated during various stressful conditions of the body. The erythrocytes can take their normal elastic shapes after normalization of the stressful conditions, but after repeated cycles of sickling and unsickling, they become a hard body, permanently, and the chronic endothelial damage and hemolysis develop. So lifespan of the erythrocytes decreases from the normal 120 days to 15-25 days. This hemolysis is responsible for the anemia that is the hallmark of the SCDs. For example, all of the SCDs cases were anemic, and majority of them (94.5%) have received multiple erythrocyte transfusions in their lives in the present study.

Painful crises are the most common and disabling symptoms of the SCDs. Although some authors reported that pain itself may not be directly life threatening (14), infections are the most common precipitating factors of the painful crises. So the risk of mortality is significantly higher during the crises. On the other hand, pain is the result of a complex and poorly understood interaction between erythrocytes, endothelial cells, leukocytes, and platelets, yet. Whether leukocytosis contributes to the pathogenesis of the painful crises by releasing cytotoxic enzymes is unknown. The adverse actions of neutrophils on endothelium are of particular interest with regard to the cerebrovascular di-

Table 1. Painful crises of the sickle cell patients

Variables	Cases with frequent crises	*p*-value	Cases with rare crises	*p*-value	Cases without crises	*p*-value*
Prevalence	79.1% (216)	<0.001	10.6% (29)	ns†	10.2% (28)	<0.001
Female ratio	47.2% (102)	ns	51.7% (15)	ns	64.2% (18)	ns
Mean age (year)	28.0 ± 8.9 (14-59)	ns	31.0 ± 9.8 (17-54)	ns	35.5 ± 10.3 (18-58)	0.000

**Difference between the first and third groups †Nonsignificant (p>0.05)*

Table 2. Characteristic features of the sickle cell cases

Variables	Prevalence	Mean age (year)	Female cases	Male cases	p-value
Pulmonary hypertension	11.3% (31)	30.4 ± 10.8 (19-56)	11.1% (15)	11.5% (16)	ns*
Leg ulcers	10.9% (30)	35.4 ± 7.4 (17-58)	5.1% (7)	16.6% (23)	<0.001
Smoking	9.5% (26)	32.9 ± 8.2 (21-54)	4.4% (6)	14.4% (20)	<0.001
CKD†	8.4% (23)	36.3 ± 9.9 (19-54)	6.6% (9)	10.1% (14)	ns
COPD‡	6.5% (18)	34.0 ± 8.4 (23-54)	2.9% (4)	10.1% (14)	<0.001
Cirrhosis	6.2% (17)	33.3 ± 11.7 (19-56)	5.9% (8)	6.5% (9)	ns
Digital clubbing	5.8% (16)	35.5 ± 11.4 (21-56)	4.4% (6)	7.2% (10)	ns
Stroke	4.7% (13)	31.6 ± 9.4 (17-47)	3.7% (5)	5.7% (8)	ns

**Nonsignificant (p>0.05) †Chronic kidney disease ‡Chronic obstructive pulmonary disease*

Table 3. Features of the mortal patients

Variables	Female cases	Male cases	p-value
Prevalence	5.9% (8)	5.0% (7)	ns*
Mean age (year)	31.0 ± 10.6 (19-45)	26.1 ± 6.7 (19-39)	ns

**Nonsignificant (p>0.05)*

seases in SCDs. For example, leukocytosis even in the absence of any infection was an independent predictor of the severity of the disease (15), and it was associated with the risk of stroke in a cohort of Jamaican patients (16). Occlusions of vasculature of the bone marrow, bone infarctions, releasing of inflammatory mediators, and activation of afferent nerves may take role in the pathophysiology of the intolerable pain. Hospital admissions for acute painful crises typically last for 4-10 days, but the time varies greatly. Because of the severity of pain, narcotic analgesics are usually required to control them (17), but according to our practice, repeated erythrocyte transfusions may be highly significant in severe painful crises, both to relieve severe pain and to prevent sudden death that may develop secondary to acute chest syndrome or sepsis induced multiorgan failures on the chronic background of the SCDs.

Because of the repeated infarctions and subsequent fibrosis, the spleen is commonly very small in adults. Eventually, a functional and anatomic asplenism develop due to the decreased antibody production, prevented opsonization, and reticuloendothelial dysfunction. The terminal consequence of the asplenism is an increased risk of infections, particularly with Streptococcus pneumoniae, Haemophilus influenzae, and Neisseria meningitidis like encapsulated bacteria. Thus, infections, especially pneumococcal infections, are common in early childhood, and are associated with a high mortality rate. The causes of death were infection in 56% of infants in a previous study (15). In another study, the peak incidence of death among children occured between 1 and 3 years of age, and the deaths among patients less than 20 years were predominantly caused by pneumococcal sepsis (18). As also observed in the present study, SCDs cases, even those who appear relatively fit, are susceptible to sepsis induced multiorgan failure and sudden death during acute crises on the ground of generalized immunosuppression.

SCDs can affect nearly all organ systems of the body (19-21). Even there was a patient with sickle cell retinopathy induced severe vision loss among our study cases. Aplastic crises, sequestration crises, hemolytic crises, acute chest syndrome, avascular necrosis of the femoral and humeral heads, priapism and infarction of the penis, osteomyelitis, acute papillary necrosis of kidneys, chronic renal failure, occlusion of retinal arteries and blindness, pulmonary hypertension, bone marrow necrosis induced dactilitis in children, chronic punched-out ulcers around ankles, hemiplegia, and cranial nerve palsies are only some of the presentation types of the disease. Eventually, the mean survival was 42 years for males and 48 years for females in the literature (8), whereas it was 26.1 and 31.0 years, respectively, in the present study. The great differences between the survival should be searched with further studies, but may be secondary to the initiation of hydroxyurea treatment in early life in developed countries. On the other hand, the lower prevalences of nearly all of the complications and the prolonged survival in females should also be searched, effectively. As a result of such a great variety of clinical presentation, it is not surprising to see that the mean body weight and body mass index (BMI) were significantly retarded in the SCDs cases (22). On the other hand, as an opposite finding to some other reports (23,24), the mean heights were nearly similar in the SCDs and control cases in the above study (22). Probably due to the significantly lower mean body weight and BMI, mean values of the low density lipoprotein cholesterol, alanine aminotransferase, and systolic and diastolic BPs were also significantly lower in the SCDs cases (22), which can be explained by definition of the metabolic syndrome (25,26).

As a conclusion, SCDs are severe inflammatory disorders with a high mortality and morbidity, and the increased frequency of painful crises may indicate the shortened survival of the cases.

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An Overview of Pharmacy Practice in the United Arab Emirates

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Abstract

Pharmacy practice has passed several rounds of advancements over the past few years. It had changed the traditional positioning criteria of pharmacists as business people into patient-centered healthcare professionals. This worldwide shift is increasingly accumulating pressure on UAE pharmacists to turn up into better level of service providing accompanied with higher demand of interpersonal skills and intellectual capabilities. This can be accomplished through stressing the significance of continuing pharmacy education in basic sciences as well as social and administrative pharmacy techniques and its collaboration in elevating the quality of pharmacy practice in the UAE.

Ethical Approval: Institutional ethics committee approvals were obtained from Universiti Sains Malaysia's Ethics Committee in Malaysia and Ajman University of Science and Technology's Ethics Committee in the United Arab Emirates.

Key words: Pharmacy practice; pharmacist; United Arab Emirates; Health Authority – Abu Dhabi; Dubai Health Authority.

Introduction

Over the last 20 years, pharmacists' role has transformed from product orientation services to patient centered services in many parts of the world [1-3]. Within the context of practice change, most of the times there is a need for the pharmacist to interact with patients and their health service provider for optimizing the delivery of pharmaceutical care services. In order to effectively perform this role, other than strong knowledge in pharmacotherapy, new generation pharmacists also need to equip themselves in fields such as sociology, management, pharmaco-economics and psychology.

Spheres of Pharmacy Practice in UAE

There are four basic spheres for any person who desires to be a part of the profession in the United Arab Emirates (UAE). The first is to master the pharmaceutical sciences by acquiring the needed knowledge and intellectual capabilities[4,5]. This can be gained through undergraduate pharmacy degree courses available at present in the UAE at 7 colleges: Ajman University of Science and Technology, Sharjah University, Gulf Medical University, Al-Ain University of Science and Technology, Dubai Pharmacy College, Ras Al-Khaimah Medical University, and Higher Colleges of Technology- Dubai Women College. Pharmacy education was first established in the UAE in the year 1992 by Dubai Pharmacy College which provided bachelor degree certificates to female students[6], and since then, many other colleges and universities started graduating a significant proportion of pharmacists per academic year needed to saturate the market domestically. However, Most of the registered pharmacists have acquired their undergraduate degrees from universities and colleges outside the country. This is due to the fact that most of the registered pharmacists in the U.A.E. are expatriates[7].

The second sphere is the presence of a national association representing all pharmacy practitioners. Emirates Medical Association (EMA) and its specialized section namely Emirates Pharmacy Society (EPS) was a trial in the direction of promoting pharmacy practice, protecting the interests of its members and end-users, and encouraging the advancement of the pharmaceutical science[8]. Nevertheless, its current role is restricted to providing certified continuous medical education (CME) credit hours required by the Ministry of Health (MOH) to renew most of the medical practitioners' licenses on yearly basis.

The third sphere relates to the professional code of conduct and ethics which guides all pharmacy practitioners. In 2001, MOH issued a guide booklet about the "Professional Code of Conduct for Primary Healthcare Staff"[9]. It contained detailed information about the definition of ethics for medical practitioners including pharmacists. A lot of the information provided was stated in articles 16 and 17 in the UAE federal law number 4 of 1983 for the pharmaceutical professions and institutions[10].

The forth sphere of a learned profession is the stipulation by its practitioners of uniform professional services and advice to the patients. This includes supplying medicines to public, in addition to providing appropriate advice to patients during the dispensing and counseling process.

Pharmacy, as a solid science profession, was almost relying on its pharmacological, chemical, and pharmaceutics scientific knowledge parts since old ages. Appearance of higher patients' expectations and development of the discipline of social and administrative pharmacy as a concept and applying it to reality brought to pharmacy practice several rounds of professional metamorphosis. As a result, pharmacy practice has been defined in a variety of ways.

A Fast Developing Country

UAE is one of the Gulf Cooperation Council states in the Middle East region. It is a constitutional federation that was established on 2nd of December 1971 [11]. The UAE constitutes 7 Emirates; Abu Dhabi (the capital), Dubai, Sharjah, Ajman, Umm Al-Quwain, Ras Al-Khaimah, and Fujairah. It lies between Oman and Saudi Arabia, and has coastal borders on the Arabian Gulf and the Gulf of Oman[12]. The UAE is a rich country and has gross domestic product (GDP), per capita, of around $52,435[13]. About one third of the GDP is gained through petroleum, oil, and gas [12]. The UAE has a diverse and a fast expanding population which was estimated to break the mark of 8 million in 2010. However, UAE nationals are considerably few compared to expatriates (non-citizens) who constitute approximately 88.5% of the total population[14]. Due to this reason, most of the work force in the UAE is made up of expatriates; who mostly come from other Arab countries, Iran, South East and South Asia (particularly from India, Pakistan, and Philippines).

The UAE has an expenditure on healthcare of about 2.8% of the GDP[15]. This relatively low percentage can be justified by the fact that the government only spends on UAE citizens in terms of delivering healthcare services in addition to the country's high income. Demands on healthcare services are continuously expanding in the country due to the dramatic influx of expatriates. This instigated the government to establish diverse, publicly-funded healthcare services by investing in private health sector and was partly a reason to form 2 semi-centralized health authorities namely Health Authority of Abu Dhabi (HAAD) and Dubai Health Authority (DHA) in Abu Dhabi and Dubai respectively. These 2 new regulatory bodies are increasingly contributing in enriching the medical practicing environment that was solely controlled by MOH in terms of licensing and controlling healthcare organizations and institutions and its professional practitioners.

The availability of 3 health regulatory authorities has provided the medical sector as a whole many advantages such as promoting and enhancing the medical professional practice plus increasing the quality of the services provided by health facilities. Despite that, some drawbacks arose like dividing delegation of authority between the MOH and the one handled by the new regulatory bodies. This particular minor conflict added an evolving need to clearly define functions among the three operating health authorities in the country in order to avoid any possible financial and effort exhaustion on some healthcare facilities caused by undefined level of command.

Pharmacy Practice Scenario

In general, a variety of views have been presented on this matter. Some consider the practice of pharmacy a profession; others look at it as a business [5,16]. There is no sharp edge description or definition on how pharmacy practice should be in the UAE. Perhaps the difficulty is because of the co-existence of both specialized and generalized professional services which the profession offers in country.

Before the existence of HAAD and DHA; pharmacy practitioners who held a bachelor of pharmacy and/or a diploma of pharmacy certificate from any accredited college or university were requested to appear in 2 scientific exams; pharmacology and pharmacy law exams by MOH. Passing these exams was a guarantee that a pharmacist or

an assistant pharmacist is allowed to work as a registered practitioner in any pharmaceutical organization across the UAE [17]. Nowadays; a pharmacist in Abu Dhabi is only requested to pass HAAD scientific exam in order to get a professional practicing license where as a pharmacist in Dubai is still required to hold both MOH as well as DHA licenses to work inside the Emirate. Every pharmaceutical organization was – and still is - required to register at least one pharmacist while licensing. In addition, few practice requirements were needed by MOH from pharmacists. Some of them were adhering to the profession code of ethics, providing proper consultation to patients when needed, renewing the professional license every year, keeping a record on semi and full-controlled medications specified by MOH and providing a monthly report on its transactions, and maintaining and monitoring inventory in the pharmacy [18].

A typical community pharmacy in the UAE sells human medications along with other general items such as perfumes, cosmetics, baby health products, and few medical apparatus. A pharmacist is probably expected to deliver professional assistance to patients through dispensing medicines, advising patients on the proper use of it, and explaining usage frequency beside any drug-drug interaction. Additional services are consulting customers on the use of cosmetics, food supplements, and other products like diabetic care machines, weight reduction, and wound care.

Pharmacy practice in the UAE varies from one pharmacy to another. Chain-store and franchised brand pharmacies usually offer a significant proportion of non-professional services alongside the traditional professional services. Smaller independent pharmacies normally focus on professional pharmacy services. Both types are representative of private pharmacy practice in the UAE. On the other hand, pharmacy practice in the government sector is quite different. Government pharmacies are mainly available inside government hospitals. They are either in-patient or out-patient pharmacies. Pharmaceutical and medical products are available for free for UAE nationals who hold a valid UAE passport along with a serial national number. A government pharmacist mostly fills prescriptions generated by the hospital's physicians and explains the doses to be consumed by patients.

Shift in Pharmacy Practice

There are nearly 2000 private pharmaceutical organizations among the UAE which are staffed by pharmacists and assistant pharmacists from over 20 countries[18,19]. Table 1 shows the number and description of pharmaceutical organizations in the UAE till the end of April 2011[19].

Table 1. Number and description of pharmaceutical organizations in the UAE till the end of April 2011

Pharmaceutical Organization	Total Number
Private pharmacy	1481
Medical store	241
Scientific office	23
Pharmaceutical factory	13
Total	1758

Yet not enough; The number of pharmacies is rapidly growing in the country due to higher demand of healthcare services caused by fast population growth from both UAE nationals and expatriates in addition to the development of many new civilized towns and cities which required availability of advanced healthcare services. Newer statistics stated that the number of registered pharmacists was about 300 every year between the years 2005 till 2010 [19]. Table 2 shows a comparison between the number of licenses issued between 2005-2010 in Dubai and Northern Emirates [19].

In fact, the number of registered pharmacists in the UAE remains lower than the accepted worldwide number of pharmacists serving a population of 10,000 in a country. The proportion of pharmacists to 10,000 citizens in the UAE was about 4 in 2002 [20]. This proportion is about half the proportion in countries like United Kingdom and United States of America[21].

This fast growth in public demand level elevated customers' expectations about the services pharmacists must adhere to while practicing their profession. For this reason; the regulatory bodies have risen to the occasion by pushing towards enhancing quality of services provided by registered pharmacists in the country. For example, newer licensing and regulatory requirements by HAAD which currently controls Abu Dhabi, Al-Ain city, and the western region require a compulsory of 20 accredited CME credit hours per year in order to renew the professional license[22]. This requirement for practicing pharmacists is a universal trend carried out by most

Table 2. Comparison between the number of licenses issued between 2005-2010 in Dubai and Northern Emirates

License Type	2005	2006	2007	2008	2009	2010
Private pharmacy	94	80	76	65	69	82
Hospital pharmacy	1	0	5	2	3	3
Medical store	10	14	18	13	18	37
Pharmaceutical factory	0	3	2	1	0	0
Scientific office	1	2	2	1	3	3
Pharmacist	264	305	344	351	344	331
Assistant pharmacist	94	111	161	222	142	156

advanced nations and it is recently being followed out by the MOH and DHA authorities. Another new major requirement by HAAD was the introduction of "JAWDA" program which is an Arabic word that means quality [23]. This program is basically a grading system for all pharmacies within Abu Dhabi region. It consists of auditing pharmacies about 3 times per year. Each facility is ranked according to a number of checklists designed for three categories of pharmacies namely inpatient pharmacy, outpatient pharmacy, and drug store. These include employees' communication, infrastructure, dispensing and labeling systems, narcotic substances storage and records, medication reliability, and customer service and their compliance with the regulations [24]. Other new requirements by HAAD are the monthly submission of self inspection report, and availability of internet connection in each pharmacy to receive circulars and notices online and to use the e-services provided on its website like renewing the professional and practitioners licenses. The latest requirements for Abu Dhabi pharmacies are designing a patient's counseling area in each pharmacy with an accepted degree of privacy, and restricting the reach of customers to shelves containing medicines[25].

Similarly; DHA - which is controlling licensing procedures alongside the MOH in Dubai - had introduced some newer, updated requirements and operative laws and circulars which helped in enhancing pharmacy practice scenario in this busy and fast growing city. An example is mandating pharmacists to acquire malpractice insurance policies in order to backup their legal and financial rights in cases of wrong dispensing or serious complications caused by dispensing medicines with drug-drug interactions [26]. This major advancement partly eased the worries by pharmacists who sometimes felt that they did not have enough rights and protection. Health and pharmacy services in the rest five Emirates continue to be under the mandate of the MOH.

In general terms, pharmacy practice in the UAE has many strengths and prospective future. A note worthy point to mention about one of its positive features is the complete separation between physicians' and pharmacists' professions. This means that a pharmacist – in most of the cases – has a full control over the supply of medicines to patients. HAAD had moved further in this pathway; as it announced in its circular number (PHP/PHM/P0003/09) which was published in May, 2009 a new policy for prescription writing by physicians. This new regulation allowed physicians to write generic names in their prescriptions instead of writing trade names. The pharmacist carries the right to choose between trade names available in the pharmacy [27]. This new legislation provided a significant advantage towards limiting unethical marketing and selling techniques used to exist through some of the medical representatives while promoting their medicines to physicians. It also gave pharmacists more attention by principal pharmaceutical companies to their role completing the medical professional supply chain.

Pharmacy Practice in UAE: The Next Leap

At present there are only 4 documented publications about the pharmacy practice scenario in the UAE [28-31]. The study by Dameh (2009) focused on some aspects of pharmacy practice especially strengths and challenges that pharmacists face in their daily practice [28]. On the other hand, the study by Sanah et al (2011) measured some barriers to pharmacy services such as the lack of time to offer services, shortage of staff inside the pharmacy, lack of patient demand and acceptance, lack of

appropriate knowledge and skills by pharmacists, lack of financial rewards from services, underestimation to enhanced pharmacy services by physicians, and legal and regulatory constraints [29].

More studies must be pointed towards the need to standardizing the basic knowledge and skills of registered pharmacists in UAE. In fact, MOH licensing procedures which include a two years community pharmacy training requirement and examinations might not be quite enough to judge their real strengths and abilities.

Another future study area is to explore the legislations in UAE which were more comprehensive in the past in order to help in minimizing the bureaucracy in daily pharmacy transactions. An example is the regulation which restricted the sale of most of medicines without a prescription. In actuality, strict observance to the law only applies to some medicines like narcotics or any medicine that can cause dependence, and sex hormones[32].

Moreover, the relationship between pharmacists in UAE and their customers must be understood in a better way in order to measure differences among different types of customers acknowledging the fact that human being nature shows differences between people because of many factors such as culture, religion, gender, educational level, age, and income level. These factors could accumulate to form a kind of perception about health services provided by a pharmacist. By posing a spotlight on the importance of pharmacy continuing education; a pharmacist in UAE will acquire extra interpersonal and management principles required to deal with every customer as a unique case and to retain highest rate of patients' satisfaction and adherence to medications[33].

Frustration of some pharmacists across UAE might be one more point to study. According to them, reasons for such phenomena are the underestimation to their profession's importance by other medical practitioners, public, and media which sometimes frames the pharmacist as a medicine seller or a business person.

Conclusion

In conclusion, this overview work provided an insight about the pharmacy practice scenario in the UAE. It illustrated the country's demographics and pharmacists' registration requirements. In addition, this paper acknowledged the fact that there might be a number of challenges and barriers to optimized pharmacy services that can to be explored by quantitative and qualitative methods in future studies.

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Socioeconomic factors associated with overweight and obesity in young university students

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Abstract

Objective: To analyze the socioeconomic factors associated with overweight and obesity in university students.

Methods: Cross-sectional study on 638 university students from the Western Brazilian Amazon. The odds ratio of overweight and obesity by demographic and socioeconomic variables were calculated by multinomial regression.

Results: The prevalence of overweight was 16.6%, and that of obesity was 5.3%. Living with a spouse showed a 1.8 chance of overweight and a 2.2 chance of obesity as compared to students without a spouse. Women with 1 or more children showed a high magnitude of association with overweight and obesity.

Conclusion: It was shown that the main factors associated with overweight and obesity were age over 24 years, living with a spouse and having 1 or more children.

Key words: Obesity; Overweight; Epidemiologic Factors; Students; Social Class.

Introduction

The overweight and obesity condition has emerged as an important public health problem worldwide. Obesity is the sixth most important factor for the development of several diseases, and it is also associated with non-transmittable chronic diseases and mortality (1). In Brazil, the surveys performed as from the 1970s have shown increased prevalence of overweight and obesity in children and adults (2,3,4,5). In that period, different tendencies were identified in overweight and obesity increase according to gender, age, education and monthly income. The 2010 national survey showed 48.1% prevalence of overweight and obesity in the adult population, with distinction for graduate and undergraduate students, with 59.0% for males and 35.3% for females (6).

Some investigations show changes in the life habits adopted by students after entering university (7,8). In fact, the adaptation to the context and new social networks of friends can affect changes and life habits. Also, many students attend school and work, thus facing a dual daily load of activities. Hence, the long distances or short periods of time to commute between their place of work, homes and university lead students to feed on fast food and reduce their time for leisure and daily rest. Additionally, after they enter university, many students need to move to a different city from that where their parents live, which causes their financial expenses on self-care, transportation and housing to increase. Hence, this study aimed at analyzing the prevalence of socioeconomic factors associated with overweight and obesity in university students.

Methods

This is a cross-sectional study on university students in the city of Rio Branco, Western Amazon, Brazil. The investigation was conducted in the second semester of 2010. The sampling is described in detail in Ramalho et al. (9). In synthesis, the minimum sample size was considered to be the expected prevalence of 50%, with accuracy fixed for a sampling error at 0.05, a level of confidence of 95% and a design effect of 2. Two-stage cluster sampling was used to select the university students. The inclusion criterion in the study was being regularly registered and attending classes in an undergraduate program. For this study, the data collected for young individuals aged 30 years or less were analyzed. This project was approved by the Human Research Ethics Committee Acre Federal University.

A structured questionnaire containing questions related to demographic, socioeconomic and

anthropometric characteristics was used for data collection. The independent variables consisted of the demographic and socioeconomic aspects. The age ranges in years were categorized as equal to or less than 24 years and between 25 and 30 years. Social classes were established according to the criteria from the *Associação Brasileira de Empresas de Pesquisa* (Brazilian Association of Research Companies) (10) as Classes A (upper), B, C, D and E (lower). Variable marital status consisted of categories without a spouse (single, separated and widowed individuals) and with a spouse (married individuals or those with a common-law partner). The number of children was analyzed in a categorical fashion (none, 1 or more children).

Variable outcome was defined from the body mass index. Weight and height were used to estimate the body mass index (BMI: weight in kilograms divided by height in meters squared). As recommended by the World Health Organization (11), BMI values between 25 and 30 kg/m^2 were considered overweight, and the values higher than 30 kg/m^2 were classified as obesity. The reference category consisted of values less than 25 kg/m^2.

Data were entered in the *EpiData* software, and statistical analyses were performed by using the *Stata^{TM} 12* software. Prevalences and their respective confidence intervals at 95% (95%CI) were calculated for overweight and obesity.

The prevalence difference was calculated by subtracting the overweight and obesity prevalences according to categories of demographic and socioeconomic variables. The 95% confidence intervals of the prevalence differences were calculated by using the formula suggested by Newscombe and Altman (12) for comparison of independent proportions, using the *Confidence Interval Analysis* (CIA) software, version 2.2.0.

The odds ratio (OR) of overweight and obesity by demographic and socioeconomic variables were identified by multinomial regression. The associations with p values lower than 0.05 were considered to be statistically significant.

Results

Of the 638 participant university students aged 30 years or less, 63.2% were females, and 36.8% were males. Of such students, 77.0% were 24 years old or younger, and 23.0% were older than 24 years. No students belonging to social class E were identified.

Overweight prevalence was 16.6%, and that of obesity was 5.3%. The prevalence of overweight in male students were twice as high as those of females, and obesity prevalence were almost three times as high as those in the latter group. Higher prevalence of overweight and obesity were identified in the age groups above 24 years. It is noteworthy that college students who lived with a spouse and had one child or more showed high prevalence of overweight and obesity (Table 1).

The greatest differences in overweight prevalences were found between the ages equal to or below 24 years and above 24 years (difference: -18.2, 95%CI: -26.5 to -10.6) and between women with and without children (difference: -17.8, 95%CI: -28.7 to -8.5). The smallest absolute prevalence differences were observed between social classes as to overweight and obesity (Table 1).

Table 2 shows the magnitudes of associations of overweight and obesity according to demographic and socioeconomic variables. Males and those older than 24 years showed a statistically significant association ($p < 0.05$) with overweight and obesity. The situation of living with spouse showed 1.8 times greater chance of being overweight and 2.2 times greater chance of obesity in contrast to students living without spouse. It was evident that the female students who had 1 or more children showed high magnitude of association with overweight (OR: 4.5, 95%CI: 2.6 to 7.8) and obesity (OR: 4.5, 95%CI: 1.4 to 14.9). The social-class variable showed no statistically significant association ($p > 0.05$) with overweight and obesity.

*Only females. OR: Odds Ratio.

Discussion

The young university students from Rio Branco, Acre, Western Amazon, Brazil, showed worrying prevalence of overweight and obesity. The male students had higher prevalence of overweight and obesity. On international scene, the prevalence of overweight and obesity in university students from Rio Branco is lower than that identified for students in the United States of America (13) Mexico (14), Lebanon (15) and South Korea (16).

Table 1. Prevalence of overweight and obesity according to demographic and socioeconomic characteristics of young university students

		N	Overweight	Obesity
			% (95%CI)	% (95%IC)
Gender	Female (a)	403	12.9 (9.7 ; 16.8)	3.5 (2.1 ; 5.6)
	Male (b)	235	23.0 (18.0 ; 28.7)	8.5 (5.4 ; 13.0)
	Prevalence Difference (a - b)		-10.1 (-16.6 ; -4.0)	-5.0 (-9.5 ; -1.3)
Age Range	<= 24 years (a)	491	12.4 (9.8 ; 15.5)	4.2 (2.6 ; 6.8)
	>= 25 years (b)	147	30.6 (23.4 ; 38.8)	8.8 (5.1 ; 14.7)
	Prevalence Difference (a - b)		-18.2 (-26.5 ; -10.6)	-4.6 (-10.5 ; -0.4)
Social Class	Class C – D (a)	345	15.9 (13.8 ; 18.2)	4,3 (2.5 ; 7.3)
	Class A – B (b)	291	17.5 (13.8 ; 22.0)	6,1 (3.8 ; 9.8)
	Prevalence Difference (a - b)		-1.6 (-7.5 ; 4.2)	-1.8 (-5.6 ; 1.7)
Marital Status	Without a Spouse (a)	518	15.0 (12.5 ; 17.9)	4.4 (2.6 ; 7.3)
	With a Spouse (b)	118	23.7 (17.3 ; 31.6)	8.4 (5.0 ; 13.9)
	Prevalence Difference (a - b)		-8.7 (-17.6 ; -1.1)	-4.0 (-10.6 ; 0.3)
Number of Children*	None (a)	320	9.4 (6.4 ; 13.3)	2.2 (0.9 ; 5.1)
	1 or more children (b)	81	27.2 (17.6 ; 31.6)	7,4 (3.3 ; 15.6)
	Prevalence Difference (a - b)		-17.8 (-28.7 ; -8.5)	-5.2 (-13.1 ; -0.7)

**Only females.*

Table 2. Association of overweight and obesity according to demographic and socioeconomic characteristics of young university students

Variables	Overweight			Obesity		
	OR	(95%CI)	p	OR	(95%CI)	p
Gender						
Male *vs.* Female	2.1	(1.3 ; 3.4)	0.001	2.9	(1.5 ; 5.6)	0.001
Age Range						
>= 25 years *vs.* <= 24 years	3.3	(2.0 ; 5.5)	0.000	2.8	(1.3 ; 5.9)	0.005
Social Class						
Class A – B *vs.* Class C – D	1.1	(0.8 ; 1.5)	0.368	1.4	(0.8 ; 2.6)	0.167
Marital Status						
With a Spouse *vs.* Without a Spouse	1.8	(1.1 ; 3.0)	0.011	2.2	(1.0 ; 4.9)	0.042
Number of children*						
1 or more children *vs.* None	3.9	(1.9 ; 7.7)	0.000	4.5	(1.7 ; 17.8)	0.029

A population-based survey conducted in Rio Branco on adults of all age groups showed nearly twofold the prevalence of overweight and threefold the prevalence of obesity as compared to those of university students investigated in this study (17). Several studies (17,18,19,20) showed a linear increase in the prevalence of overweight and obesity in the age group of 18 to 60 years. Therefore, this divergence may have occurred due to differences in the age structures in the studies, where the university students in this study were 30 years old or younger.

The highest prevalences of overweight and obesity of male students are consistent with data for university students (13,14,15,21). It is suggested that these discrepancies result from best self-care observed for female students. In Brazil, it was shown that more than half of female students experienced periods of dieting and weight control (8,22).

The overweight and obesity condition of university students showed to be associated with the situation of living with a spouse. Similarly, the Brazilian national survey revealed a higher prevalence of excess weight and obesity in the ca-

tegory living with a spouse in contrast to that of individuals living without a partner (3). Although consistent with other studies (19,23,24,25), the mechanism of this association has not been fully elucidated.

A systematic review reported the occurrence of weight gain when couples united and of weight loss when they separated (26). Based on a longitudinal study on North-Americans, Averret et al. (27) admitted the hypothesis of social obligations of marriage, where they inferred that couples have more regular meals and participate in social events that provide foods with high energy density. Another hypothesis described by Averret et al (27) is availability for marriage. This hypothesis is coalescing with the social levy to keep up with ideal weight to attract a partner. However, after union with a spouse, BMI increase is allowed because one is in a steady relationship and because maintaining ideal BMI may be costly financially. In addition to these hypotheses, it appears that university students living with a spouse has less time for self-care related to body weight due to both the duties of marriage and the long hours dedicated to academic activities.

The situation of having one or more children associated with overweight and obesity in female university students is consistent with studies conducted in Brazil (28,29) and Turkey (30). Correia et al (29) showed that having a child increased the prevalence of overweight by 22% and the risk for obesity by 43%, while having 2 children increased the prevalence of obesity by 65%. After pregnancy, overweight and obesity may be due to the tendency to body weight retention, according to weight gain in early pregnancy, number of pregnancies and maternal age (31,32,33).

In conclusion, it was shown that male students and those older than 25 years showed the highest prevalences of overweight and obesity. Moreover, the situation of living with a spouse and women with 1 or more children were important factors associated with the overweight and obesity condition.

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Novel insights and strategies in immunosuppressive therapy in allograft transplantation

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Abstract

Long-term survival for transplant patients remains problematic despite improvement in organ preservation methods, surgical procedures, new immunosuppressive therapy regimens and intensive care administration. This has led to increased interest in alternative approaches to immunosuppression maintenance, treatment of graft rejection and monitoring of immunosuppression. The alternative approaches also aim to optimize efficacy and minimize toxicity in post-transplant cases. Improvement in long-term allograft outcomes may, thus, depend on new agents with novel mechanisms of action. Newer therapies including using Mesenchymal stem cells; phagosomes and exosomes; and apoptotic cells are increasingly being suggested as options in immunosuppreissive therapy. In addition, rapidly evolving Immunoisolation techniques, salvage therapies including photopheresis and total lymphoid irradiation have also emerged as alternate therapeutic options in transplant patients. The present review evaluates the recent clinical advances in immunosuppressive therapies.

Key words: Immunosuppression, transplantation, Immunoisolation, immune rejection, Mesenchymal stem cells, Drug Delivery

Introduction

Immunosuppression remains the backbone of therapy for successful results after transplantation. The need for ideal immunosuppression is considered essential to maintain long-term graft survival. Over the past two decades, immunosuppression for solid organ transplantation has developed to specifically target multiple immune pathways with the hope of decreasing both acute and chronic allograft rejection.

The current approach to immunosuppression is generally similar internationally although there is some variability in the medications used at different transplant centers. Maintenance regimens typically involve administration of three distinct classes of immunosuppressive agents: calcineurin inhibitors (CNIs; eg, cyclosporine, tacrolimus), antiproliferative agents (eg, azathioprine, mycophenolate mofetil [MMF], sirolimus), and corticosteroids.

Unfortunately with all the progress in medication and surgical methods, long term allograft rejection is still high and this had led to increased interest in evolving strategies to maintain immunosuppression and graft rejection treatment. Newer therapies including using Mesenchymal stemcells, apoptotic cells, phagosomes and exosomes are being considered. Furthermore, Immunoisolation techniques, salvage therapies and photopheresis including lymphoid irradiation have emerged as alternate therapeutic options. This review is aimed at evaluating recent clinical advances in immunosuppressive therapies for allograft transplantation.

Aerosolized immunosuppression, Macrolides and Statins

One of the new methods used specifically in lung transplantation is the Aerosolized immunosuppression method. In this method, immunosuppression drugs are converted to aerosols and inhaling them allows the appropriate delivery of the drug, while other parts of the body will be unaffected. In a study on 58 patients in two treatment groups with one receiving Cyclosporine aerosols and the other a placebo, the group receiving the drug indicated better graft survival[1]. The corticosteroids aerosols were also examined in a study including 30 patients with lung transplantation. The patients were divided into two groups with one group receiving Fluticasone, while the other group was administered a placebo. However, no significant differences were seen in acute rejection or survival between the two groups; although, the airway inflammatory markers decreased in the group receiving the drug[2].

Recent studies have shown that macrolides reduce the production of proinflamatory cytokines such as IL-6 and IL-8 and increase anti-inflammatory cytokines, such as IL-10[3]. They can also reduce chemotaxis and induction of apoptosis in activated neutrophils. However, their known feature is their antibacterial activity that can reduce the infection and thus lead to reduced inflammation. It was found in an associated study that receiving Azithromycin in patients with lung transplants significantly improved respiratory function and their survival[4]. Another study shows the effects of Azithromycin in reducing mortality rate in liver transplant recipients[5]. These studies led to the encouragement by some transplant centers to use this treatment method in the immediate post-transplantation period. But an important factor regarding the use of macrolides in the long-term is that they can cause antimicrobial resistance in patients[6].

Statins are known mainly with regards to their impact on cholesterol synthesis, however, recent studies have shown that these compounds are inhibitors of 3-hydroxy-3-methylglutaryl-coaenzymeA and can have anti-inflammatory action. In vitro studies have shown that Statins reduce MHCII expression in the endothelial cells and macrophages that play an important role in the onset of the inflammatory process[7]. Some studies have shown that Statins increase the proliferation of T-reg cells and inhibit the expression of pro-inflamatory cytokines[8-10]. One of the first significant research regarding Statins' positive effects on transplantation were published in relation to heart transplant and associated immunosupprisive issues in 1995.[11] In addition to lowering cholesterol levels in heart transplant patients, Statins also reduced the transplant rejection rate.

Mesenchymal stem cells

Mesenchymal stem cells (MSC) are present in most tissues such as hair follicles, teeth, bone marrow, lungs, etc[12]. These cells have been highly regarded in the past due to their potential to differentiate between endoderm and bone cells[13]. However, aside from potential differentiation feature, MSC cells also play an important role in modulating the immune system by performing this task using a variety of factors via influencing T cells and B cells. The MSC cells influence the T cell proliferation through cell-to-cell contact (PD-1 pathway) or factors such as TGF-a1, IDO, HO-1, HGF and PGE-2[14-17]. The effect of these cells on the reduction of B cell proliferation is applied through PD-1 and PD-L1 pathways. In addition, this reduction is also facilitated by partly by using soluble factors in the blood[18], reducing the expression of MHCs and related molecules such as CD86, CD83 and CD40.

Mesenchymal stem cells (MSC) inhibit the maturation of myeloid DC cells derived from monocytes and ultimately help to confront with APCs[19]. In 2002, a group of scientists showed for the first time that injecting allogeneic Mesenchymal stem cells to an animal can increase the skin graft survival[20]. Since then, several studies have been performed on immunomodulatory role of MSCs. In this context, some studies have focused on the role of MSCs in regulating tolerance against immune rejection and GVHD[21]. Some studies have pointed out to the role of MSCs in infiltration of T cells in the CNS[22]. Another related study showed that MSCs' injection can reduce peptide-specific antibodies in EAE model mice[23]. In another report, systematic injected MSCs in a rheumatoid arthritis (RA) model mouse caused the reduction of inflammatory factors such as IFNγ and activation of T-regulatory cells to confront with the inflammatory process.

Another important feature of MSCs is their engraftment characteristic that makes the migration of these cells into damaged tissues for regeneration / repair easier. This is a clinically important characteristic of these cells. Certain wound sites and pro-inflammatory environments were found capable of enhancing the engraftment of MSCs in lung fibrosis in mice induced by bleomycin in a pre-clinical model study. However, the overwhelming majority of MSCs were found in lungs after systemic administration in normal recipients, and after a while, these MSCs disappeared gradually. [24]

Recent studies point to the fact that the MSCs' allogenicity does not affect the MSCs engraftment in wound healing process. This is also an important point in conjunction with the MSCs use in therapeutic applications.

Generally, there are two ways to deliver the MSCs into the body:

1. Intravenous infusion. This method allows the use of the MSCs transference/migration features that facilitate their movement to inflammatory tissues in liver and lung transplantations[25].
2. Local injection. This method can lead to accumulation of MSCs in the damaged tissue[26].

In 2007, a phase I study showed that peripheral vein injection of MSCs can improve the survival in liver transplantation[27] cases. In 2008, United States' FDA gave green light on clinical trials for application of MSCs in MS patients and patients with cartilage defects. If successful, these trials could become potential clinical strategies for immunosuppression in the future.

Extracorporeal Photopheresis (ECP)

One of the emerging techniques to deal with GVHD and graft rejection of hematopoietic stem cells is the extracorporeal photopheresis method that is used to reduce the number of T-cells in lymphoma. This includes three steps: 1- Leukopheresis; 2-Incubation of mononuclear cells with 8MOP; and 3-Photoactivation of incubated cells with UVA radiation[28]. These cells are then returned to the body and undergo apoptosis[29]. They also increase the T-reg levels and anti-inflammatory factors[30]. In a study conducted on 80 patients divided into two groups receiving usual immunosuppressive treatments, but one group also received ECP treatment. Research results showed that the ECP group significantly showed more improvement[31].

The ECP approach seems to help in improving the graft, but it is expensive. Each 6-month treatment in the US with ECP requires 24 times of ECP therapy. Each ECP costs US $ 7000 and the insurance companies do not cover it. Also, performing every ECP procedure requires 4 hours[32].

Exosomes and Phagosomes

In the past, researchers observed that by presenting the donor MHC antigen before transplantation in mice, immune tolerance could be induced in the transplant recipient[33]. Using this feature, carriers for antigen delivery came into the focus of attention.

Exosomes are vesicles derived from the membrane with a size of 50-100 nm, which can be easily isolated from blood using ultra-centrifugation. They are formed by reverse budding of the limiting membrane of late endosomes/multivesicular bodies (MVB) fused to the plasma membrane. These exosomes are generated by a group of cells, including enterocytes, mast cells, DCs, T and B lymphocytes and tumor cells[34, 35]. Thymocyte-derived exosomes have the ability to induce Treg and immune suppression[36, 37]. In a study of heart transplantation in rats, exosomes induced a significant prolongation of allograft survival and in some recipients long-term graft survival was also seen after transplantation [33]. Other studies have shown that exosomes produced by mature DCs can activate the response of T cells and may cause skin graft rejection. (39) However, certain studies also indicate that exosomes obtained from immature DCs significantly preserved the heart transplant[38, 39]. All in all, these studies showed that the use of exosomes in antigen delivery can improve graft survival outcomes.

Recently, a protocol of alloantigen administration based on phagosomes has been developed. The phagocytosis of PLGA nanoparticles by immature DCs leads to creation of phagosomes containing PLGA. When these phagosomes are exposed to immature DCs of another strain, DCs expressed low expression levels of MHC class II and CD86 maturation markers, secreted low levels of the activating cytokines IL-2 and IL-12, and showed increased IL-10 secretion [40]. In one study, when the phagosomes were injected into mice before alloimmunization, cellular immune response and antibody levels significantly reduced[41]. The results of this study tend to suggest the use of allogeneic PLGA-phagosomes could be a suitable tool for alloantigen administration in a tolerogenic context.

Apoptotic Cells

Recently, the researchers have found that the apoptotic cells have anti-inflammatory and immunoregulatory effect on APCs[42]. A broad variety of factors are likely to determine tolerogenic or immunogenic effects of DCs after uptake of apoptotic cells. Early stage apoptotic cells are more likely to induce tolerance than late stage apoptotic cells[43, 44]. The number of apoptotic cells; molecules displayed on the surface of apoptotic cells; interactions with other cells; receptors and secreted cytokines as well as the presence or absence of

danger signals can all impact upon the determination of different types of immune responses[45]. Besides, DC maturation status can play a role in the induction of tolerogenicity or immunogenicity.

Several studies have shown that the exposure of immature DC cells (Immature) to apoptotic cells does not lead to increased DC maturation markers including MHCII, CD40, CD80, CD83, CD86, even if these cells are exposed to LPS and TNF-α[46, 47]. In addition, if DC cells are exposed to apoptotic cells in early stage, the expression and secretion of inflammatory cytokines, including IL-1α, IL-1β, IL-6 and TNF-α will reduce. In this context, the expression of anti-inflammatory factors of TGF and IL10 will increase[48, 49]. Injection of apoptotic cells having MHC molecules of the donor has been done in model animals in order to evaluate whether this prevents reaction against the transplant. These experiments have shown favorable results[50-52].

The use of the apoptotic cells includes the following advantages compared to other methods:

1. Initial apoptotic cells transfer a strong signal of immunosuppression to DC cells[53]
2. Apoptotic leucocytes are a rich source of MHC molecules[54]
3. Apoptotic cells are easy to prepare;
4. I.V. injection of apoptotic cells is relatively safe[55]
5. After intravascular injection, these cells become efficiently captured by splenic DCs[56]
6. The DCs present the apoptotic cell derived antigens to T cells[57].

In a major study, I.V. administration of the Apoptotic cells after transplantation led to reduction in B and T cell responses against the donor antigens and blocking of CD40-CD154. The result was a prolonged cardiac allograft survival in mouse models of cardiac transplantation[58].

Immunoisolation

These are quite new methods that have been developed with the aim to hide the graft cells' antigens in the body from the host. For the first time, Lind and Sun using the EPI (?) method showed that the transplantation of encapsulated islets in diabetic rats can recover the eugelycemia state[59]. In order to transplant the cells without immunosuppression there is a need for a protective cover that: firstly, does not affect the cells viability and functional properties; and secondly, keeps them out of reach of the immune system to avoid immune rejection. Biomaterials used to make these coatings should be biocompatible and allow the penetration of nutrients, hormones and oxygen into the cells. The main materials used in this context are alginate[59], chitosan[60], agarose[61] and polyethylene glycol[62]. However, these polymers allow the penetration of molecules of T cells, macrophages and cytokines such as IL-1β, TNF-α and IFN-γ into the capsules damaging and destroying cells or islets. Thus, another barrier is needed to curtail this development. In this regard, often, a poly amino acid layer is used which is placed between the cell and alginate. The positive charges of poly-amino acid and negative charges of alginate react to form a complex. The two major amino acid polymers are PLL (poly L-Lysine) and the PLO (Poly L-Ornithine). PLL is the first and most commonly used polymer. But, PLO has some additional advantages compared to PLL. These are:

1. Comparisons indicate the better performance of PLO in preventing the infiltration of immune system molecules into the capsule;
2. PLO appears to be more resistant against mechanical stresses occurring during changes in osmotic pressure.

Immunoblocking is a cutting-edge technology in which the inner surface of blood vessels is coated with a Nano-film, called NB-LVF4, to hide the endothelium antigens from the immune system. This Nanofilm is obtained from the basement membrane of cultured corneal endothelial cells and consists of corneal endothelial cell proteins including type IV collagen, vitrogen, fibronectin and laminin. The Nano-film is injected through arterial line of the organ immediately before transplantation to cover its inner surface. The Nano-film allows the passage of nutrients and oxygen[63, 64].

It was shown in a study on a renal transplant model that coating the vessels with NB-LVF4 Nano-film can reduce the stimulation index up to 99.98%[63]. In another study on an animal model, 4 renal transplants were treated with NB-LVF4 (Group I) and 4 were non- NB-LVF4 treated kidney transplants (Group II). The non-treated control group (Group II), on an average, showed re-

jection after 6 days, while the first group (Group I) showed a mean onset of rejection on day 30.

Another study to investigate the effects of Nano-film on skin grafting was performed. In this study, the skin treated by the Nano-film and the non-treated control group was evaluated. Rejection occurred after a mean time of 28 days versus 7 days in the control group (no Nano-film) [65]. However, one of the major limitations of this approach is the need to use tolerogenic regimen besides the use of Nano-films. Moreover, this method does not have an immunosuppression property and only provides a 30-day window to use the immunosuppression regimen.

Conclusion

The on-going introduction of newer methods in Immunosuppressive therapy has contributed significantly to improved survival outcomes after solid organ transplantation. Nevertheless, treatment-related adverse events and persistently high risk of chronic graft rejection remain major obstacles to long-term survival after transplantation. Enhanced understanding of the development of new agents, refinements in techniques to monitor immunosuppression as well as constantly up-dated knowledge of transplant immunobiology is essential for further improvements in post- transplant outcomes.

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Knowledge, selection and use of sunscreen products

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Abstract

Introduction: Solar ultraviolet radiation is a risk factor for various medical conditions, including skin cancer. With appropriate sun protection the risk can be limited. As the last safeguard, it is recommended to use sunscreens containing UV filters, the function of which is to absorb and/or reflect UV radiation and protect the skin. The aim of the research was to determine the level of knowledge of sunscreens and the factors that affect consumer choice and use.

Methods: A literature review was conducted to obtain basic information about UV radiation and sunscreens along with a descriptive cross-sectional study. A questionnaire composed of 20 questions was used as the sole research instrument. A total of 310 randomly selected residents of the Republic of Slovenia participated in a descriptive cross-sectional study which was being carried out from June to September 2012. The respondents were divided in two groups according to their exposure to UV radiation at the time of the survey. The response rate was 70%.

Results: 81% of the respondents use sunscreens and 55% of the respondents try not to be exposed to sunlight between 10 a.m. and 4 p.m. The majority of respondents (74%) claim that avoiding sun exposure between 10 a.m. and 4 p.m. is the first line safeguard measure and the use of sunscreens is considered to be the second most effective protection. Nearly half of the respondents (48%) use sunscreens when they are exposed to sun or while sunbathing. In 29 %, the sunscreens were applied on the skin half an hour before exposure. 95 % of sunscreen users believe that it is reasonable to reapply sunscreen.

Discussion and conclusions: Results of the study indicate that the respondents are aware of the negative effects of UV radiation and possess knowledge of safeguard measures. However, most of those surveyed do not apply them or may use them incorrectly. The choice of protection product depends on the product price, the UV filter, their friends' and acquaintances' recommendation, and product ingredients. It was also noted that the users do not clearly distinguish between sun protection factor and UV filter, and do not fully understand the composition of products.

Key words: UV radiation, sun protection, sunscreens

Introduction

Excessive exposure to solar ultraviolet radiation (UVR) has been recognized as a health risk factor causing acute or chronic health problems. Though a moderate degree of UV exposure is necessary for the production of Vitamin D essential for bone health, prolonged human exposure to solar UV radiation may result in negative health effects on the skin, eye and immune system (WHO, 2009). In the most serious cases, skin cancer and cataracts can occur. According to WHO estimates, between 2 and 3 million non-melanoma skin cancers and approximately 132.000 malignant melanomas are diagnosed worldwide each year. Some 12 to 15 million people become blind due to cataract yearly, of which 20 per cent of cataracts are caused or enhanced by overexposure to UV radiation. A total of 12.226 persons were newly diagnosed with different types of cancer in Slovenia in 2009. According to cancer incidence statistics, exposure to solar ultraviolet radiation is the major cause of non-melanoma skin cancers, which are is one of the five most common malignancies in human populations (Cancer Registry of Slovenia, 2013).

UV-C is ultraviolet radiation with wavelengths up to 290 nm, all of which is filtered out by the ozone layer and does not reach the surface of the earth. Due to high energy of rays, UV-C is the most damaging type of UV radiation with lethal effects on living organisms. UV-B is ultraviolet radiation with wavelengths between 290 and 320 nm. UV-B

penetrates into the epidermis (top layer of the skin) causing damage to the cells. UV-B radiation is responsible for sunburn, darkening and thickening of the outer layer of the skin - a significant risk factor for skin cancer, especially melanoma. UV-A is ultraviolet radiation with wavelengths between 320 and 400 nm, comprising over 95 per cent of that reaching the surface of the earth. Wavelengths in the UV-A range are by far the most abundant solar UV radiation that reaches the earth surface. UV-A rays penetrate deeply into the dermal layer of the skin. They may damage collagen and elastin fibres and consequently cause a host of irreversible skin changes resulting in premature and accelerated skin aging (wrinkling, blotchiness, etc), DNA damage and the development of skin cancer. Both UV-A and UV-B radiation can cause the formation of free radicals which can damage cell function and alter genetic material (Poljšak 2012a; Poljšak, 2012b; Kumperščck Duh, 2008; Priporočilo komisije, 2006; Lucas et al., 2006; WHO et al., 2002).

The severity of effect of solar radiation on human health depends on the amount and type of radiation impinging on the body as well as on the amount of melanin in the skin (skin phototype). Darker skin has more protective melanin pigment. Deeply pigmented skin provides important sun protection with a minimal erythemal dose (MED) 33-fold higher than fair skin. Ambient UV radiation may be weighted using an erythemal response function and expressed by the solar UV index (UVI). The minimal erythema dose has been defined as the quantity of erythema-effective energy (expressed as Joules per square meter) required to produce the first perceptible, redness reaction with clearly defined borders (Kumperščck Duh, 2008).

The UVI is an international standard measurement of the strength of the ultraviolet radiation (UV), taking into consideration the UV radiation power and the average white skin sensitivity to UV. The UVI is an important vehicle to raise public awareness of the risks of excessive exposure to UV radiation, and to alert people about the need to adopt protective measures. As UV radiation levels vary with time of day, the values of the index vary throughout the day. In reporting the UVI, most emphasis is placed on the maximum UV radiation level on a given day. This occurs during the four-hour period around solar noon. Depending on geographical location, solar noon takes place between local noon and 2 p.m. UV radiation level for Slovenia can be found on the website of the Slovenian Environment Agency (WHO et al., 2002; ARSO).

Preventive measures against solar UV radiation

Protective measures against UV radiation include avoidance of direct exposure of the skin and the eyes to sunlight between 10 a.m. to 4 p.m., the use of tightly woven, loose-fitting clothes, broad-brimmed hats, UV-blocking sunglasses and the cosmetic sunscreen products. Sun protection is best achieved by seeking shade and wearing clothes rather than applying sunscreens (Poljšak, 2012a). No sunscreen product can filter all UV radiation. Moreover, there is, to date, no conclusive scientific evidence that the use of sunscreen products prevents melanoma. Consequently, sunscreen products should not claim that they provide total protection from the risks deriving from overexposure to UV radiation. This holds particularly true for sun exposure of babies and young children. As exposure to sun during childhood is an important contributor to the development of skin cancer at a later age, sunscreen products should not give the impression that they provide sufficient protection for babies and young children. (Priporočilo komisije, 2006).

The definition of a sunscreen product according to *The Commission Recommendation of 22 September 2006 on the efficacy of sunscreen products and the claims made relating thereto (2006/647/ES)* is as follows: 'Sunscreen product means any preparation (such as creams, oils, gels, sprays) intended to be placed in contact with the human skin with a view exclusively or mainly to protecting it from UV radiation by absorbing, scattering or reflecting radiation'.

Regulation (EC) No 1223/2009 of the European parliament and of the Council of 30 November 2009 on cosmetic products give the following definition of IV filters: 'UV-filters' means substances which are exclusively or mainly intended to protect the skin against certain UV radiation by absorbing, reflecting or scattering UV radiation'. The Annex VI of this same Regulation contains a list of UV filters allowed in cosmetic products for

protection against radiation. The Regulation defines also the outer and inner packaging labelling of sunscreen cosmetic products. Labelling of sunscreen products is defined also in the *Commission Recommendation of 22 September 2006 on the efficacy of sunscreen products and the claims made relating thereto (2006/647/ES)*. In *Sections 3, 4 and 5 on the minimum efficacy of sunscreen products*, it is recommended that simple and comprehensible labelling of sunscreen products be used in order to facilitate the choice of the appropriate product for the consumer.

UV filters are substances which, contained in cosmetic sunscreen products, are specifically intended to filter certain UV rays in order to protect the skin from some harmful effects of these rays. Broadly speaking, there are two types of UV filter: organic (chemical) and inorganic (mineral) filters. The organic UV filters are aromatic, **oil soluble or water-soluble compounds** which absorb the **UV-radiation (UV-**light energy) and convert it into a small amount of heat. The inorganic filters can reflect, scatter and/or absorb the UV light depending on the size of the particles. Sunscreen products need to protect against both UV-B and UV-A radiation. Therefore, although the sun protection factor refers only to protection against the radiation which causes erythema (mainly UV-B radiation), sunscreen products should contain both UV-B and UV-A protection (Poljšak 2012b; Kumperšček Duh, 2008; Priporočilo komisije, 2006).

Sun protecting factor (SPF) of a sunscreen is a laboratory measure of the effectiveness of a sunscreen. It provides information on the efficacy of sunscreen products which should be indicated on the label by reference to categories such as 'low', 'medium', and 'high'.

Each category should be equivalent to a standardised degree of protection against UV-B and UV-A radiation. Sun protection factor means the ratio of minimum erythemal dose on skin protected by a sunscreen product to the minimum erythemal dose on the same unprotected skin (Jou et al., 2012; Kumperšček Duh, 2008; Priporočilo komisije, 2006). It should be emphasised that the increase in protection from one number to the next is negligible, particularly in the high range. Moreover, the increase in protection is only linear in the case of sunburn, that is to say, a product with sun protection factor 30 protects twice as well from sunburn as a product with a sun protection factor of 15. However, a product with sun protection factor 15 absorbs 94 % of UV-B radiation, and a product with sun protection factor 30 absorbs 97 % of UV-B radiation (Priporočilo komisije, 2006).

The importance of the link between the correct application of sunscreen products and the efficacy of the sun protection factor claimed should be accentuated. In order to reach the protection level indicated by the sun protection factor, sunscreen products have to be applied in quantities similar to the ones used for testing, i.e. 2 mg/cm2, which equals 6 teaspoons of lotion (approx. 36 grams) for the body of one average adult person (Priporočilo komisije, 2006; Poljšak, 2012a).

Wang and Dusza (2010) conducted a pilot survey with the aim to assess general sunscreen knowledge of the public and to determine factors associated with sunscreen purchasing decisions. Four hundred and twenty-three individuals completed the survey. Results of the study show that approximately 86% of the subjects knew that sunscreen use could prevent sunburn, but only 9% were aware that sunburn is caused by UV-B radiation. A total of 70% of the respondents reported that sunscreen could prevent skin cancer and 64% were familiar with the fact that it can prevent premature aging. Only 32.1% of respondents knew, however, that sunscreen should be applied 30 min before going outside, and only 30% knew the appropriate reapplication recommendation (at two hour interval). Merely 18% of respondents knew that approximately 1 ounce of sunscreen is required to cover the entire body. SPF and UV-A are the most important factors that effect purchase decision. The study results also indicate that the majority of respondents did not understand the crucial difference between a UV-A claim for a product and the SPF value listed on the product.

Gavin et al (2012) performed a study on trends in skin cancer knowledge, sun protection practices and behaviours in the Northern Ireland population. Around 3,623 randomly selected subjects participated in the study. Results of the study show that skin cancer knowledge was high (97%). The use of a SPF of 15 or over was reported by 70% of respondents who used sunscreen in 2008 (Gavin et al., 2012).

Health promotion and UV radiation protection

The National Cancer Control Programme (NCCP) of Slovenia 2010-2015 states that the activities within primary prevention in the field of UV radiation as a health risk factor should be directed towards reduction of UV radiation exposure and to early detection of all suspicious skin changes. Responsible for the implementation of tasks and activities for the period 2010-2011 was the Ministry of Health of the Republic of Slovenia, the National Institute of Public Health (NIPH), Institutes of Public Health and non-governmental organisations. The programme lists also the tasks and measures related to education of doctors, pharmacists, other healthcare providers and allied professionals in the field of oncology. The programme secures the general public the access to quality and up-to-date data on cancer, cancer prevention, early detection, treatment options and other (MZ, 2010). Defined is also the active role of non-governmental organisations in health education and cancer prevention, informing the public about cancer and enhancing participation in screening programmes. The website of the National Institute of Public Health of the Republic of Slovenia offers information about UV radiation protection along with preventive measures in case of exposure, labelling of cosmetic products, counselling on skin protection, skin types, and recommended sun protection factor (IVZ, 2010a; IVZ, 2010b). In 2009, the Institute organised education programme 'Healthy under the Sun' intended for medical school teachers and health professionals collaborating with schools. 'Healthy under the Sun' was also the motto of healthy schools in the school year 2008/2009 which referred to solar UV radiation protection as well as health lifestyle. The educational programme emphasised the need for the development of preventive programme through which healthy lifestyle would be encouraged and adopted at an early age. The positive adult role model of healthy behaviour is of paramount importance. The need for legal regulation of the use of solarium was also emphasised (IVZ, 2010c). A list of recommendations concerning avoidance of excessive exposure to UV radiation was published in the brochure 'Slovenia Goes on Holiday 2012 ' and in the article 'Slovenia Goes on Holiday 2013' (IVZ, 2012; IVZ, 2013a). In the year 2013, NIPH encouraged youth to take part in a Call for Participation within the framework of the European Week Against Cancer 2012. It was focused on sun safety, reiterating warning against excessive and potentially harmful effects of sunbathing and the use of solarium (IVZ, 2013b).

In 2007, the Institute for Public Health Celje, the Association of Slovenian Dermatovenerologists and the Cancer Society of the Celje region launched a pilot programme 'Safe with the Sun' in the kindergarten Celje. The objective of the programme was to inform the pre-school children, aged 4-6, their parents and schoolchildren on proper protection against UV radiation and the use of protective measures. Since 2008, the programme has been carried out in kindergartens all over Slovenia, and in the year 2010 it was introduced also in primary schools (ZZV Celje).

Together with Eucerin and Dialog company, the Association of Slovenian Dermatovenerologists is organising the project 'A Sun Screen Protection Day' for the third time in a row. The purpose of the project is to provide the public with information about major health problems linked to overexposure to UV radiation, sensible safety precautions and about recent significant discoveries related to sunbathing and protection against harmful effects from UV radiation exposure. Its aim is also to challenge the stereotypes, misunderstandings and *controversial opinions* on the issue. A mobile application of the 'Sun Screen Protection Day' has also been developed. It advises consumers about the necessity of sunscreen reapplication taking into consideration the skin type, environmental conditions and different values of UV index (The Association of Slovenian Dermatovenerologists).

Methods

For the purpose of the study, a systematic literature review was conducted by searching the relevant books, Medline, Cobbis.si databases, Web of Science, PubMed, WHO library database and the Official Journal of the European Union. A descriptive cross-sectional study was performed using a survey questionnaire developed for similar purposes. The questionnaire was composed of 20 que-

stions and sub-questions of open-ended, closed-ended and semi-open question type. The first three questions refer to respondents' demographic profile – gender, age, level of education. The remaining seventeen items of the questionnaire are related to the research topic. The survey was conducted from June – September 2012 in different parts of Slovenia where people are more commonly exposed to UV radiation: Ljubljana, Žusterna, Koper, Portorož, Fiesa, Strunjan, Solkan.

Eligibility criteria required that participants be the citizens of the Republic of Slovenia and aged 18 years and over. The recruited random sample of 310 individuals was divided into two groups according to whether or not they were exposed to UV radiation at the time of the study. The respondents exposed to radiation represented 59 % of the sample and those who were not represented the remaining 41%. Over half the sample (63%) was female. The data on levels of educational attainment show that 44% of respondents completed secondary school education (level V), 21% finished lower level of education (level II, III, IV) and 35% possessed higher education (level VI, VII, VIII). No answer was given on educational level of the respondents in 3%. The age of the respondents ranged from 18 – 30 (37%), from 31 – 50 (36%), and 50 years of age and over (26%). A negligible number of respondents (1%) did not report their age. As 134 addressees declined to participate, the survey response rate was only 70%. The data retrieved were analysed by Microsoft Office Excel and Word.

Results

As regards safeguard measures against solar radiation (Figure 1), 55 % of respondents avoid the direct exposure to solar radiation between 10 a.m. and 4 p.m., while 81% use cosmetic sunscreen products. A minority of all survey participants (6%) do not use any protective measure against sun whatsoever. The avoidance of UV exposure between 10a.m. and 4 p.m. increases with age while the trend in the use of sunscreen products is reverse.

On a low to high 4-point scale, the respondents (n=223) rated the avoidance of solar radiation exposure between 10 a.m. to 4 p.m. as the first line of defence against the sun's harmful ultraviolet rays (Figure 2).

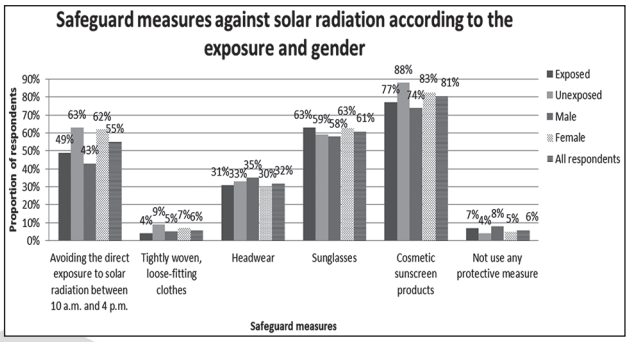

Figure 1. Safeguard measures against solar radiation according to the exposure and gender

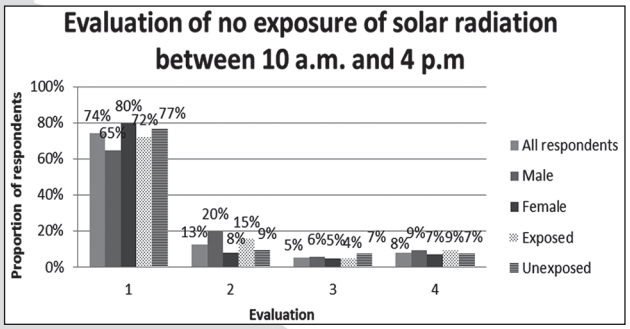

Figure 2. Evaluation of no exposure of solar radiation between 10 a.m. and 4 p.m.

39% of sunscreen users (n = 98) claim that some sunscreen ingredients should be avoided., 76 % are aware of the function of UV filters in cosmetic sunscreen products and 59% reported that they use products containing UV-A and UV-B filer (Figure 3)

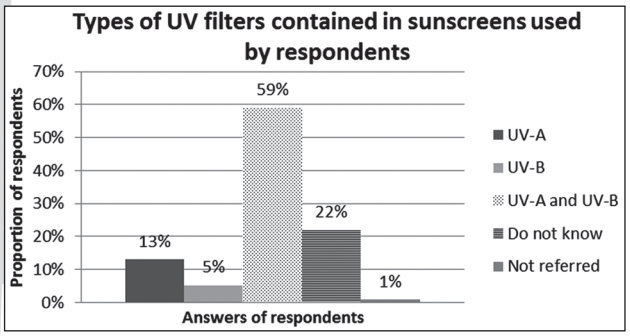

Figure 3. Types of UV filters contained in sunscreens used by respondents

Most of the sunscreen users employ the sunscreens with UV filter 15 – 29 (Figure 4).

Only 17 % of the respondents (n = 310) are aware nanoparticles in sunscreens (Figure 5).

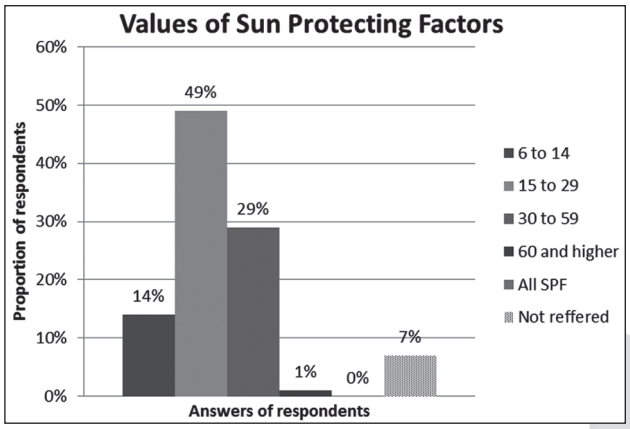

Figure 4. Values of Sun Protecting Factors

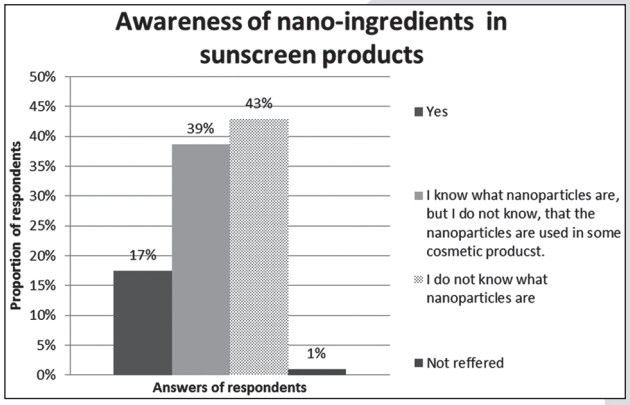

Figure 5. Awareness of nano-ingredients in sunscreen products

Discussion

The survey questionnaire assessed the respondent's familiarity and comprehension of sunscreen terminology, sunscreen application and sunscreen efficacy claims.

The current study found that over 70% of the respondents consider avoidance of solar radiation exposure between 10 a.m. to 4 p.m. as the first line of defence against the sun's harmful ultraviolet rays. It is therefore somewhat surprising that, being aware of adverse health effects, only a good half of the respondents observe this measure. The use of sunscreen was assessed as the second most important safeguard measure (40% of the respondents) which is adopted by 81% of respondents. The results of the study indicate that the use of sunscreens is the most commonly used protective measures among the respondents. Patterns of exposure and sun protection tend to vary by age and gender. Whereas 65 percent of women use sun protective cosmetic products, the corresponding proportion for men is 56 percent. The difference in the use of sunscreen was noted also between the respondents exposed to UV radiation and those who were not. In the latter group the use of sunscreens is by 10% higher than in the former. The study conducted in Ireland (Gavin et al, 2012) reports that 70% of respondents use sunscreen products.

Another important survey finding is that avoidance of UV exposure between 10 a.m. and 4 p.m. increases with age while the trend in the use of sunscreen products is reverse. A significantly low percentage of users of sunscreen products were observed in the oldest age group of respondents. The respondents who use cosmetic products (such as olive oil, Solea cream etc.) which do not absorb or reflect the sun's ultraviolet radiation) were excluded from the study.

A good half of sunscreen users usually opt for creams. It was interesting to note that some respondents who use sunscreen sprays claimed that they use creams. The study examined the behaviour patterns of the public on sunscreen use. The data gathered indicate that the general public is still not aware of the harmful effects from prolonged exposure to ultraviolet (UV) which is not limited to sunbathing by the seaside or swimming pool. Less than half of respondents use sunscreen only when sunbathing and less than 40% use these products when they are at the seaside, swimming pool or at the beach.

Only 36% of respondents follow recommendations and instructions for sunscreen use and apply the cosmetic product 20 – 30 minutes prior to exposure to radiation. In respect to the time of application, the study produced results which accord with the previous research by Wang and Dusza (2010) which found that only a small percentage of respondents (32%) apply protective cosmetic product 30 minutes before exposure.

A good half of the respondents using sunscreens reported that the 3-5 teaspoons of lotion should be applied for the body of average person (one teaspoon equals approximately 6 grams). One of the issues that emerge from these findings is that the quantity of sunscreen products applied is generally not sufficient enough to provide the necessary protection against UV radiation. The underapplication is probably the most common mistake made by sunscreen users.

The majority of sunscreen users believe that reapplication of the product is necessary and most of them would reapply the product after activities such as swimming, showering or heavy or excessive sweating. Only one-tenth of the respondents know that sunscreen must be reapplied within two hours in order to remain effective. Only a good half of them, however, comply with these recommendations.

One of the objectives of the questionnaire was also to determine the factors associated with sunscreen purchasing decisions and product selection. It was established that sun protection factor of the product, its price, ingredients and scent are the most important factors that effect purchase decision. Less important criteria are the shape of the product and the tests run by consumer organisations.

Over 70% of sunscreen users read the product labels. They pay special attention to the ingredients of the product, its sun protection factor, directions for use and UV protection factor. The fact that some respondents wrongly cited the UV factor among selection criteria shows that they are not familiar with sunscreen terminology and do not distinguish between UV filters and sun protection factors (SPF). This could also be the reason why UV filters received lower scores in the assessment of selection criteria. These results lead to a conclusion that the sunscreen ingredients are a criterion of minor importance in product selection. Some respondents are also not familiar with specific effect of certain ingredients and are not aware that some UV filters can be potentially dangerous and are therefore prohibited within the European Union (i.e. zinc oxide).

Almost two thirds of sunscreen users surveyed (76%) reported that they were familiar with the function of sunscreen UV filters. Nonetheless, only 60% of them use cosmetic products for protection against UV-A and UV-B radiation.

The respondents' familiarity and awareness of sun protecting nanoparticles in sunscreens is scarce (17%). It is expected that the users' awareness of nanoparticles included in sunscreen products will be increased by obligatory labelling of 'nano' ingredients. These expectations seem to be realistic considering the number of users who read product labels.

In addition to direct or indirect DNA damage, UVR activates cell surface receptors of keratinocytes and fibroblasts in the skin, which leads to a breakdown of collagen in the extracellular matrix and a shutdown of new collagen synthesis. It is presumed that dermal collagen breakdown is followed by imperfect repair that yields a deficit in the structural integrity of the skin, formation of a solar scar, and ultimately clinically visible skin atrophy and wrinkles. Many studies confirmed that acute exposure of human skin to UVR leads to oxidation of cellular biomolecules that could be prevented by prior antioxidant treatment and to depletion of endogenous antioxidants. The role of protective antioxidants in protecting cells against oxidative damage generated by UV radiation has not yet been elucidated. It seems that skin's antioxidative defence is also influenced by vitamins and nutritive factors (vitamin C, E, carotenoids, lycopene**,** coenzyme Q10, glutathione, zinc, resveratrol, green tea, cocoa butter and other) and that combination of different antioxidants simultaneously provides synergistic effect (Elmore, 2005; Cho et al., 2010; Pandel Mikuš et al., 2013).

Conclusion

While the knowledge and awareness of sun-protection measures is encouraging, the prevalence of their use and application remains low. The respondents are aware that the harmful effects of radiation could be prevented by reduction of skin exposure to ultraviolet radiation whether via avoidance or UV protection. Though the former is believed to be the first and most effective protection measure, only a good half of respondents avoid solar radiation and 80% use sunscreen products. Patterns of exposure and sun protection tend to vary by age and gender. The use of these products decreases with age. It should be emhpasised that products, such as olive oil, butter and similar do not provide adequate protection from harmful UV rays. Product selection and purchasing decision is most commonly based on its price, UV filter, ingredients and the advice of friends and acquaintances. Most sunscreen users read the product labels but lack familiarity and comprehension of sunscreen terminology and ingredients.

The human health effects of exposure to UV radiation can be modified by individuals' sun protection behaviour. The improvements may in part relate to changing public attitudes and behaviour to sun

exposure and sun protection where health education of general public is of paramount importance.

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Applicability of the flexible bronchoscope to the intubation of children with lung echinococcus during the period between 1995 and 2013. (Single lung ventilation)

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Abstract

Introduction: Study performed on children 1995-2013, for lung resection due to echinococcal cyst, intubated on SLV (single lung ventilation).

Methods: prospective retrospective study on children aged 4-7 who, due to the echinococcal cyst and the surgical lung resection, were intubated by the flexible bronchoscope, and by single- or double-lumen tubes. Before the echinococcal cyst resection, a detailed pre-operative preparation was conducted with a radiologically confirmed echinococcal cyst

Results: A total of 22 children with echinococcal cyst (29.333%), out of the total of 75 monitored over the period of 8 years. The children aged 4-7 were intubated via flexible bronchoscope. Thoracotomy was performed in all children patients. Echinococcal cyst was localized unilaterally, in the right-hand side in 12 children patients (54.54%), Echinococcus cysticus lobi inf.pulm.dex.compl.

Twenty patients were immediately extubated following surgery (90.90%), while two were kept in the intensive care (9.09%).

Conclusion: SLV was necessary for the lung echinococcal cyst resection. The applicability of the flexible bronchoscope to the intubation of children aged 4-7, while for the children above the age of 5 the insertion of a conventional single lumen endotracheal tube into the opposite side of the bronchus.

Key words: single lung ventilation, insertion endotracheal tube by flexible bronchoscope –intubation, lung echinococcal cyst at children**.**

Introduction

Lung echinococcus (echinococcosus pulmonis) is a common disease in Bosnia and Herzegovina. The endemic areas for our country is Herzegovina and central Bosnia, while globally it is the Middle East and South America. Echinococcal cyst studies are rare, especially in pediatrics. Our prospective retrospective study has shown the 3.5 outer diameter flexible bronchoscope application with regard to children intubation and a better insertion of the tube into the bronchus. These parasites inhabit various canine types (dog, wolf, fox). Larval development of these tapeworms takes place in secondary hosts – sheep, cattle, horses and sometimes humans as well. Echinococcosis (hydatidosis) is widely present as the most serious form of parasitic zoonosis caused by echinococcal tape worms (Echinococcus granulosus or Echinococcus multilocularis) where the human represents a transitory host where the larval form of the parasite is developed.

Every fluid-filled cyst is surrounded by a fibrous wall consisting of two walls made of parasites: the outer multi-layer chitin membrane and the internal germinal membrane. Capsules develop from the germinal membrane. Each capsule contains one or more invaginated heads (protoscolices) which may develop into adult worms if swallowed by the definitive host. The capsules and protoscolices either swim free in the hydatid fluid or attach themselves to the peduncle wall; the capsules protoscolices that swim free are known as “hydatid sand.”

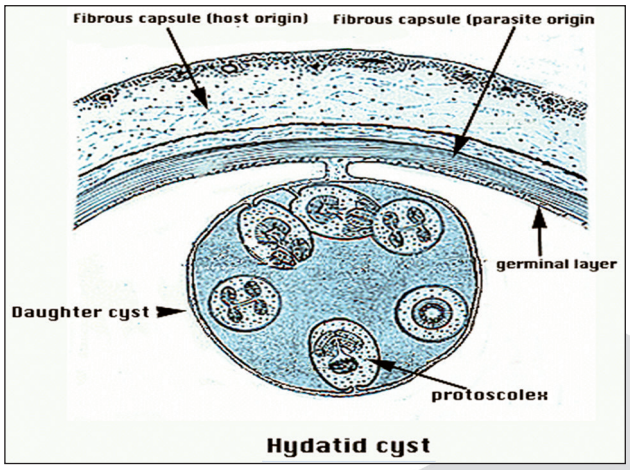
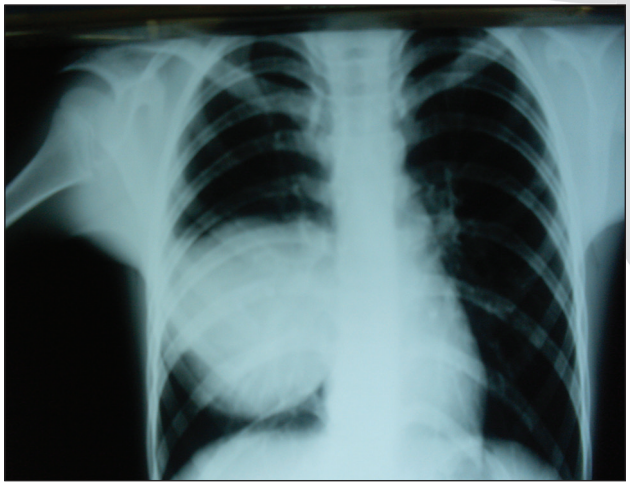


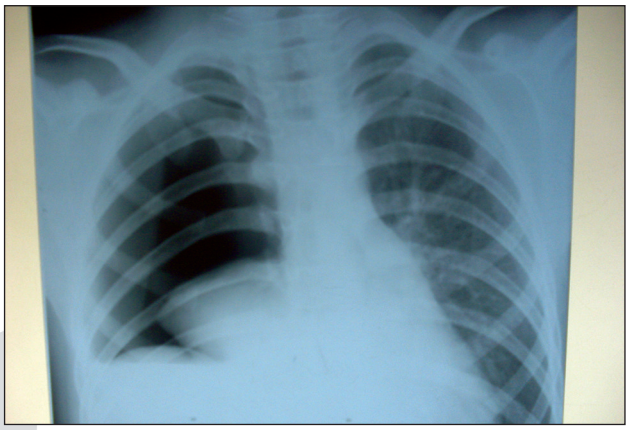
Image 1. Hydatid cyst

If the cysts rupture, new cysts may develop from the hydatid sand. Some cysts are sterile, they either never produced any capsules or become sterile after bacterial infection or calcification. Once the eggs are ingested by the hosts they are degraded by digestive enzymes, and the embryo passes along the mesenteric veins and into the portal circulation. If the embryo is not held in the liver, it passes through to the lungs, mostly localized in the right-hand lung, and also in lower lobes of both lungs. Hydatid cyst is diagnosed during a physical examination and radiology findings. Serology analyses are limited in diagnosing the hydatid lung cyst (1,2,3). If it passes through this natural filter too, it goes into the left heart and via major blood flow gets into other organs.

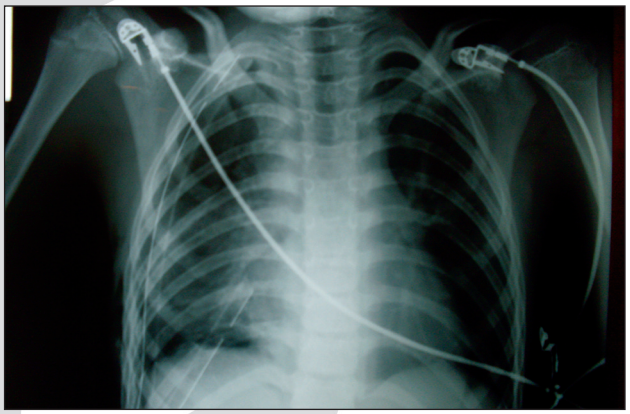
The key role in diagnosing echinococcus is played by lung radiograms, lung CTs , US and MRI.



Picture 1. Echinococcal cyst



Picture 2. Echinococcal cyst



Picture 3. Lung radiogram following surgical removal of echinococcal cyst

Method of work

Our study included 22 children who received peadiatric treatment before surgical consultations and surgical removal of echinococcal cyst from the lungs. During every visit, a detailed history of the child's disease was taken, as well as lung RTG, frontal and profile view of the infected lung, lung CT, basic laboratory diagnostics, serological analysis of antibodies for echinococcus. Upon the received findings and consultations with a thoracic surgeon and children's thoracic surgeon, the children were was prepared for surgical intervention of echinococcal cyst resection in general anesthesia. Eradication of the parasite by mechanical removal , and sterilization of the cyst cavity by injection of a scolicidal agent usually hypertonic saline.Children patients aged 4-7 were intubated via flexible bronchoscope, diameter 3.5, in the presence of an anesthesiologist in the surgery. Upon intubation and deeper insertion of the tube into the bronchus, for instance the left one, while the echinococcal

cyst was right, the left bronchus was shut off by clamping, which is to say the left lung, during the surgical resection of echinococcal cyst from the affected lung. SLV single lung ventilation. This effectively prevented the echinococcus scolex dissemination during aspiration from the echinococcal cyst. Children above the age of 5 were intubated with conventional single lung bronchial tubes placed in the good side of the bronchus (4).

The exact location of the cyst in the lungs was before the surgical intervention supposed to be in correlation with the radiology finding.

Other patients aged 8-23 were intubated with double-lumen endotracheal tube which we have clamped and which was supposed to be taken out of the ventilation function.

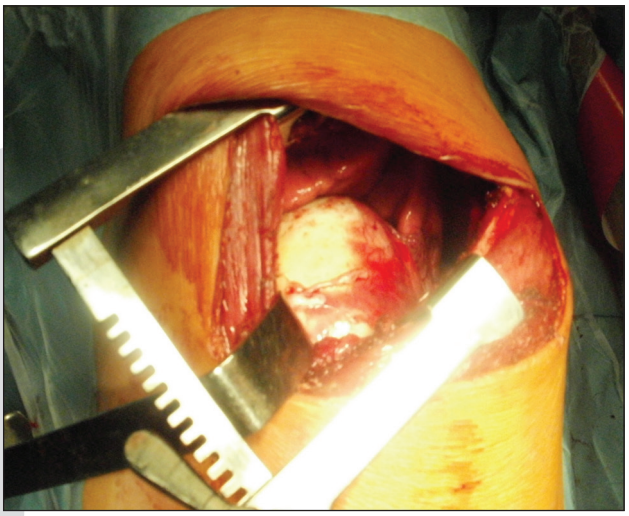
Anesthesia

The simplest technique for one-lung ventilation in infants and young children is to intubate the main stem bronchus of the non-operated lung by the conventional single-lumen tracheal tube.

The main stem right bronchus can be readily intubated by the available left beveled tracheal tube. However, it will be difficult to achieve left bronchial intubation without the help of fiberoptic bronchoscopy).

Children below 7 years of age are not intubated with double-lumen tubes for there exist no ready-made sizes less than 26 ch. Globally, the children of that age are intubated with the standard single-lumen tube with or without the fiberbronchoscope. Using the fiberoptic bronchoscope we set the tube into the adequate position. Patients above 7 years of age are intubated with double-lumen tubes (Carlins), size 26 ch. Following double-lumen catheter intubation, depending on the localization of the echinococcal cyst, the affected side is taken off ventilation by clamping. After clamping, the ventilation of one side of the lungs is determined based on the patient's age and weight, and is also based on the smaller "tidal" volume and higher frequency. During the post-operative treatment, depending on the patient's age and his pre-operative general condition, the patient may be left on mechanical ventilation over a period of 12h to 48 h, for adequate oxygenation as well as adequate post-operative pain treatment. Inadequate pain treatment disturb ventilation and tissue oxygenation, and may cause hemodynamic instability.

Analgesia includes the use of analgesics trodon or tramadol, morphine or a combination of hypnotics and opioids (dormicum) and fentanyl, to be continued with analgin and trodon.



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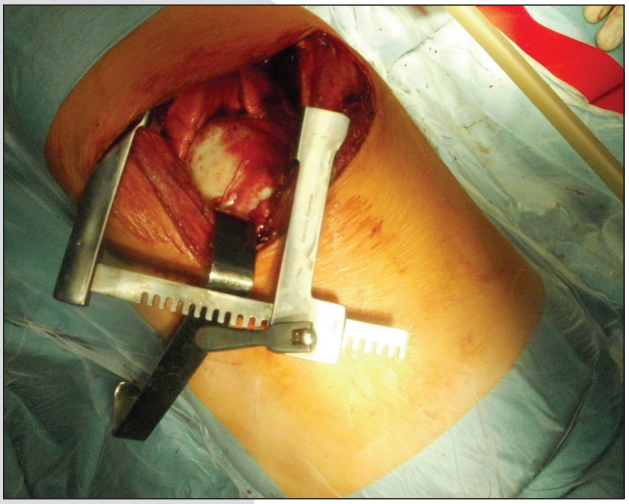

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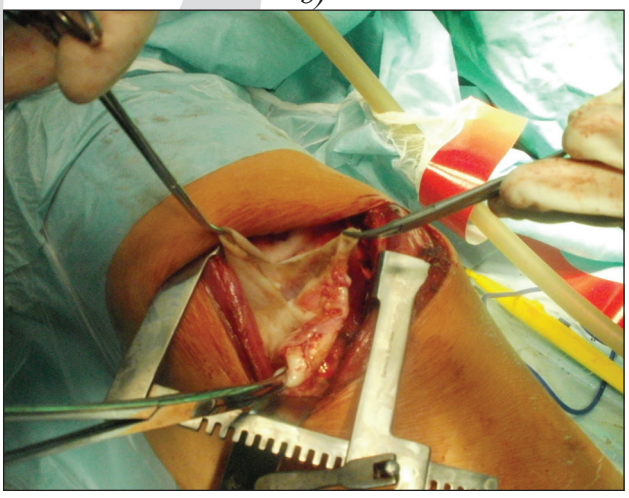

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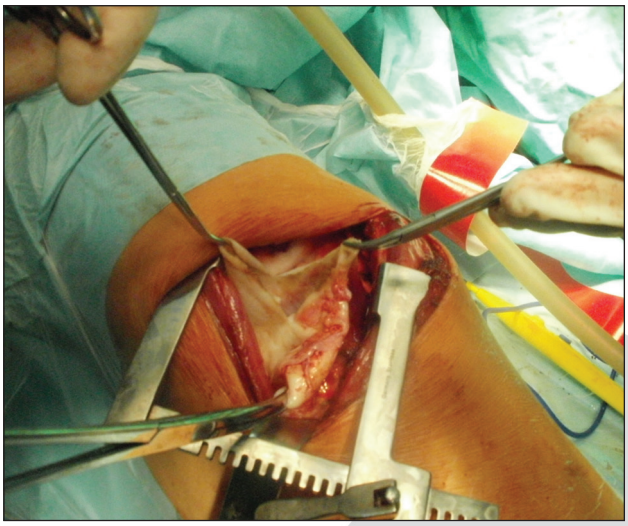

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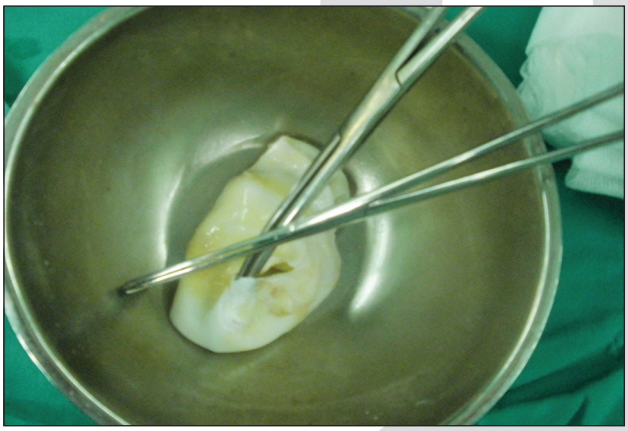

e)

Picture 4. Surgical treatment

Operative Techniques

Surgical treatment should be preferred in hydatid cyst of the lung (7).The main aim of the surgery in hydatid cyst is total excision (8,9)

A classic posterolateral thoracotomy (mostly used for muscle sparing) through the fifth or sixth intercostal space or a median sternotomy was applied after achievement of general anesthesia. A double-lumen endotracheal tube was used in older children to avoid the spillage of infected cyst material into the contralateral bronchus. After entering the hemithorax, the lung was freed from all adhesions to the chest wall. After identification of the cyst, the operative fiel and pleura were covered with wet sponges diluted with 10% povidone-iodine solution to prevent seeding of possible daughter cysts. By using needle aspiration, hydatid fluid was aspirated from the uppermost part of the cyst. Then a large suction apparatus was inserted into the cyst, and the fluid was completely aspirated. We installed a hypertonic solution. The most prominent part of the cyst was opened with scissors or electrocautery, and the cyst membrane was removed with ring forceps or another instrument. Then the residual cavity was irrigated with 10% povidone-iodine solution and was cleaned with the suction apparatus. Closure of bronchial openings was performed in all patients (10, 11, 12).

Results

Table 1. Display of good patient groups diagnosed with Echinococcus cysticus

Age	Number of patients	Percentage %
4 - 7	23	30,26316
8 - 11	17	22,36842
12 - 15	20	26,31579
16 - 19	14	18,42105
20 - 23	2	2,631579
Total	**76**	**100%**

4-7 years – Z= 1,075888 P-test= 0.2820
8-11 years Z=0,248282
12-15 Z=0,662085
16-19 Z= -0,16552
20-23 Z= - 1,82073

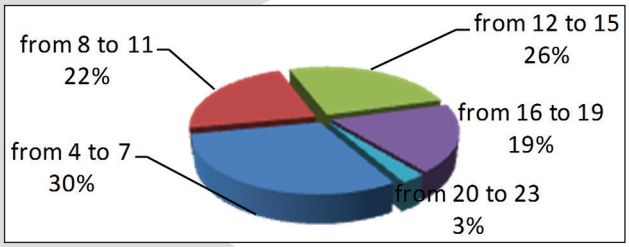

Chart 1. Patients age

Table 2. Patients gender

Gender	Number of patients	Percentage %
Male	58	76.32
Female	18	23.68
Total	76	100 %

Za MALE Z=1
Za FEMALE Z= -1

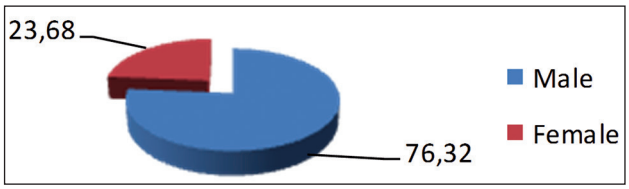

Chart 2. Patients gender

Table 3. Number of patients relative to the location of changes

Localization of changes	Number of patients	Percentage	z-score
Cystae echinococcicae pulm.bill.et hepatis.	2	2,63	-0,1118
Echinococcocosis cystica pulm.dex.	3	3,95	0,894427
Echinococcosis cystica mult. pulm.dex.compl. Fibroliquidothorax.l.dex.gravis.	1	1,32	-1,11803
Echinococcosis lobi inf.pulm.bill.compl.	2	2,63	-0,1118
Echinococcosis pulm.bill.	4	5,26	1,900658
Echinococcosis(II)lobi inf.pulm.sin.	2	2,63	-0,1118
Echinococcosis(II)pulm.dex.compl.	2	2,63	-0,1118
Echinococcosis(III) cystica lobi sup.pulm.sin.	2	2,63	-0,1118
Echinococcosis(III) cystica pulm.dex.	2	2,63	-0,1118
Echinococcus cysticus compl.lobi inf.pulm.dex.	2	2,63	-0,1118
Echinococcus cysticus complicata No II	1	1,32	-1,11803
Echinococcus cysticus complicatus lobi.sup.pulm.lat.dex.	1	1,32	-1,11803
Echinococcus cysticus lingulae permagnus.	1	1,32	-1,11803
Echinococcus cysticus lobi inf.pulm.dex.	6	7,89	3,913119
Echinococcus cysticus lobi inf.pulm.dex.compl.	2	2,63	-0,1118
Echinococcus cysticus lobi inf.pulm.dex.permagnus	4	5,26	1,900658
Echinococcus cysticus lobi inf.pulm.dex.permagnus. Echinococcosis abdominis	2	2,63	-0,1118
Echinococcus cysticus lobi inf.pulm.sin.	2	2,63	-0,1118
Echinococcus cysticus lobi inf.pulm.sin.compl.	2	2,63	-0,1118
Echinococcus cysticus lobi inf.pulm.sin.permagnus	2	2,63	-0,1118
Echinococcus cysticus lobi med.pulm.dex.et lobi dex. hepatis.	1	1,32	-1,11803
Echinococcus cysticus lobi sup.et inf.pulm.dex.et lobi dex. hepatis.	1	1,32	-1,11803
Echinococcus cysticus lobi sup.pulm.dex.	3	3,95	0,894427
Echinococcus cysticus lobi sup.pulm.dex.compl. Echinococcus lienis	2	2,63	-0,1118
Echinococcus cysticus lobi sup.pulm.dex.permagnus	2	2,63	-0,1118
Echinococcus cysticus lobi sup.pulm.sin.	2	2,63	-0,1118
Echinococcus cysticus lobi sup.pulm.sin.compl. Echinococcosis hepatis	2	2,63	-0,1118
Echinococcus cysticus lobi.inf.pulm.dex.	3	3,95	0,894427
Echinococcus cysticus lobi.inf.pulm.dex.complicata.	1	1,32	-1,11803
Echinococcus cysticus lobi.inf.pulm.sin.	2	2,63	-0,1118
Echinococcus cysticus lobi.med.pulm.dex.	3	3,95	0,894427
Echinococcus cysticus lobi.sup.pulm.dex.	1	1,32	-1,11803
Echinococcus cysticus lobin inf.pulm.sin.fertilis complicatus	2	2,63	-0,1118
Echinococcus cysticus mediastini	2	2,63	-0,1118
Echinococus cysticus complicatus lobi inf.pulm.sin.	2	2,63	-0,1118
Liquidopneumothorax l.dex.ppt rupturam cystae echinococcicae	2	2,63	-0,1118
Total number of patients	**76**	**100%**	

Table 4. Tabular display of the patients relative to the place of residence

Patients by location	Number of patients	Percentage %	z-score
Bihać	2	2,63	-0,40321
Cazin	2	2,63	-0,40321
Čapljina	2	2,63	-0,40321
Donji Vakuf	4	5,26	0,0768
Gornji Vakuf	2	2,63	-0,40321
Goražde	2	2,63	-0,40321
Fojnica	2	2,63	-0,40321
Ilijaš	1	1,32	-0,56642
Mostar	9	11,84	0,739224
Podveležˇ	1	1,32	-0,56642
Salakovac	1	1,32	-0,56642
Sarajevo	27	35,52	3,676918
Travnik	7	9,21	0,412813
Tarčin	1	1,32	-0,56642
Vareš	1	1,32	-0,56642
Visoko	7	9,21	0,412813
Zenica	5	6,58	0,086403
Total number of patients	**76**	**100%**	

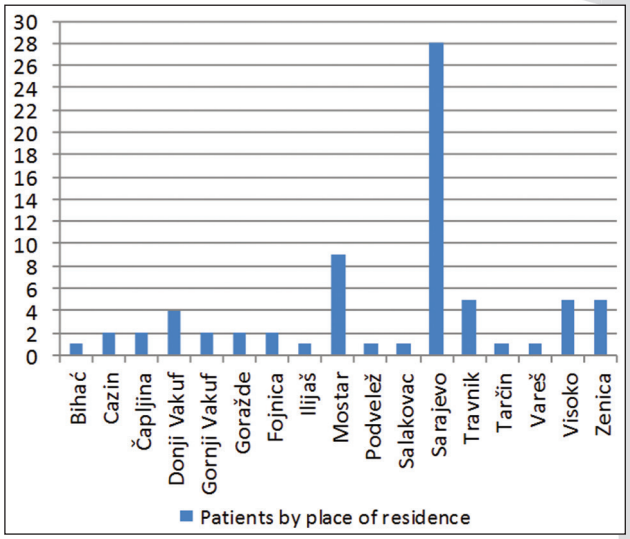

Chart 3. Patients by place of residence

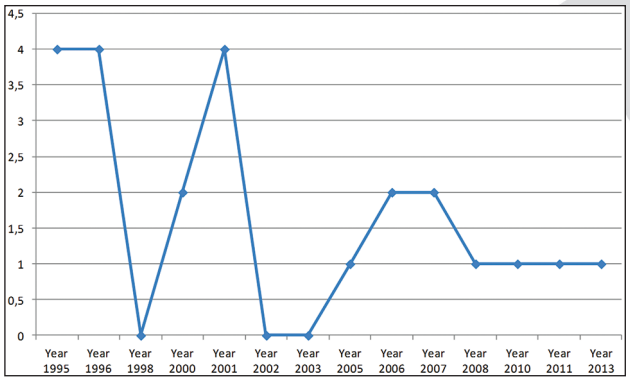

Chart 4. Number of patient aged 4-7, relative to time period of 1995-2013

Table 6. Number of patients relative to the localization of change. (Patients aged 4-7)

Localization of change	Number of patients	%	z-score
Left	11	47,83	-1
Right	12	52,17	1
Total number of patients	23	100%	

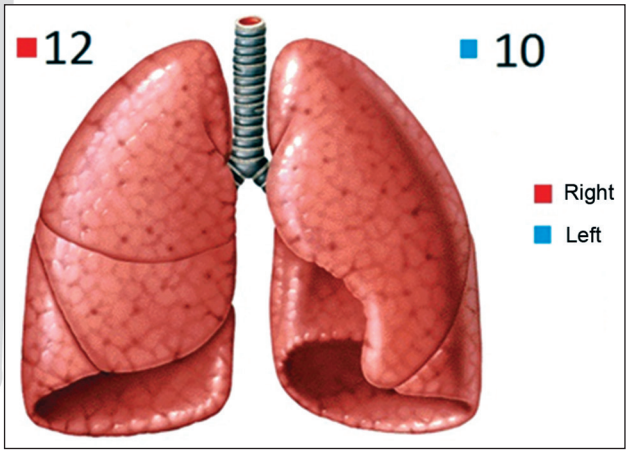

Chart 5. Number of patients relative to the localization of change

Table 7. Tabular display of localization of changes relative to the anatomic localization

Localization of change	Number of patients	Percentage	z-score
Echinococcus cysticus lobi.inf.pulm.dex.	11	47,83	1,862537
Echinococcus cysticus lobi.inf.pulm.sin.	7	30,43	0,822981
Echinococcus cysticus lobi med.pulm.dex.	1	4,35	-0,73635
Echinococcus cysticus lobi sup.pulm.sin.	2	8,70	-0,47646
Echinococcosis cystica multiplex pulm.dex.complicata.	1	4,35	-0,73635
Echinococcus cysticus lobi sup.et inf.pulm.dex.	1	4,35	-0,73635
Total number of patients	23	100%	

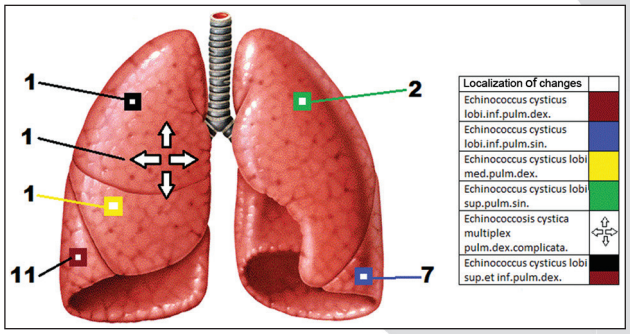

Chart 6.

Discussion

Echinococcal cyst is a public health problem in countries that are endemic echinococcus areas.

Epidemiological studies of echinococcal cyst in children are rare, which is why the objective of our study was to make an evaluation of epidemiological, clinical and age characteristics of children with echinococcal cyst in the territory of BiH during the period 2013-1995. Our intention was to demonstrate the seriousness of the echinococcal cyst in children aged 4-7 intubated via fiber bronchoscope while bringing the patient into general anesthesia in order to exclude the side not affected with the echinococcus from ventilation during its surgical removal. Other patients were intubated with a double-lumen endobronchal tube modified for the intubation of the left or right bronchus.

Conclusion

SLV (single lung ventilation) is a useful ventilation in the children with thoracal surgery procedure (echinococcal cyst resection). The SLV performed on smaller children via intubation using a flexible bronchoscope and in older pediatric population (single-lumen, double-lumen tube) showed a successful routine application, with rapid postoperative recovery without persistent atelectatic and pneumatic changes. 4-7 years – Z= 1,075888 P-test= 0.2820

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Ipratropium using an aerochamber in children with asthma attack

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Abstract

Objectives: To assess the effectiveness of ipratropium bromide (IB) administered via metered-dose inhaler (MDI) with spacer in acute asthma attack at the pediatric emergency department.

Methods: All children aged between 4 and 14 years who required treatment of acute asthma exacerbation over a two-month period (prospective, n=75) were enrolled. Nine were subsequently excluded from the study, and the remaining 66 children were assessed. Patients were divided into two groups in a random manner to receive either a routinely used acute asthma treatment protocol (n=28) or additional IB inhalation with an aerochamber (n=38). Asthma symptoms were assessed before, after and three times, every 20 minutes, during treatment.

Results: Significant post-treatment improvements ($p<0.05$) were observed in both groups. Both derived benefits from treatment, although there were no significant differences ($p>0.05$) in degree of improvement between the groups before, during or after treatment. Both therapies exhibited a similar effectiveness compared with the other.

Conclusion: The administration of IB using a MDI with spacer additionally to standard treatment of moderate asthma attack is not an effective alternative in the treatment of moderate asthma attack in children.

Key words: Ipratropium, pediatrics, metered dose inhaler, emergency, asthma.

Introduction

Until the last few years, the common treatment approach in pediatric acute asthma was the combined use of short-acting β2 mimetic and systemic steroids(1). However, many patients with acute asthma do not respond to currently accepted treatments, including oxygen, β2 agonists and corticosteroids (2, 3). The etiology of such resistance is unclear. Cholinergic hyperactivity, which causes increased bronchospasm and secretions, may be one contributory factor. The addition of ipratropium bromide (IB) to the standard acute asthma treatment has been widely studied, and the combination of a nebulized β2 agonist with an anticholinergic (IB) is known to produce produce better bronchodilation in acute asthma management than either drug alone(4). While the results of several studies carried out in pediatric populations indicate that metered dose inhalers (MDIs) with spacers are effective as nebulizers in the treatment of mild and moderate acute asthma exacerbations, bronchodilators are typically administered by means of a nebulizer (5-11).

Bronchodilators are routinely administered via inhalation when treating asthmatic crises. Nebulizers are the devices most frequently employed with children, but they are uncomfortable, expensive and potential sources of infections requiring between 15 and 20 minutes to compress the air supply in order to generate their spray. Although MDIs can be efficiently employed with patients of all ages, during normal respiration, as long as they are coupled to a spacer, permitting rapid administration, and are thus better tolerated by small children, and substances are deposited in a more even and predictable pattern than by nebulizers; there are many barriers to the successful reversal of the "nebulizer culture" in the emergency department (5-8, 12, 13).

The inhaler technique, which directly deposits an appropriate amount of drug into the correct location, is the recommended route for asthma treatment. The development of spacer devices, which are easier to use, has reduced the usage of nebulized treatments, although 3 nebulized treatment is still commonest means of administering asthmatic drugs, especially with patients suffering asthma attacks (14, 15).

The aim of this randomised this study was to examine the effectiveness of adding IB using a MDI via a mask aerochamber to moderate acute asthma treatment in children in an emergency department setting.

Subjects and Methods

The study was conducted prospectively as randomised controlled trial in a children's hospital emergency department with the permission of the hospital ethical committee. Children aged between 4 and 14 years and presenting to the emergency service over a two-month period due to moderate to severe acute asthma attack were included in the study.

Asthma diagnosis was suggested by persistency of symptoms, nocturnal attacks or symptoms produced by allergen exposure, and was supported by atopy in the patient or in the family. An acute attack was defined as an episode of increasing coughing, an inability to perform day-to-day activities, wheezing and chest recession (4, 16).

On arrival at the emergency room, a detailed history was taken, clinical examinations were performed to rule out any associated illness, and the degree of spasm was determined. The severity of the asthma episode at the beginning of and during treatment was expressed as the ''asthma score,'' and classification of asthma attack severity was made at the asthma score level. The primary outcome was noted by the improvement of asthma scores according to National Institutes of Health criteria, rating the severity of an episode as this corresponded to signs and symptoms (13, 16). The overall asthma score was the sum of such variable scores as respiratory rate, cyanosis, wheezing and accessory muscle use (Table 1). These were graded on a scale of 1 to 3 and a sum of asthma clinical severity scores assigned with a minimum score of 4 and a maximum score of 12 (Table 2)(4).Children whose symptoms were relieved after ventolin nebulizing were diagnosed as suffering mild asthma attack and were excluded from the study (n=4).In addition, children with life threatening asthma attacks characterized by one or more of the following features, cyanosis or an arterial saturation of less than 91-92, silent chest or poor air entry, marked dyspnea, the inability to speak even 3-4 words, those who had been administered a bronchodilator six hours prior to admission, and with a history of previous admission to the intensive care unit, were also excluded from the study (n=5) (16).

Nine of the 75 patients applying to the emergency department due to asthma attacks were thus excluded from the study, the remaining 66 being enrolled after meeting the inclusion criteria and then prospectively and randomly allocated into one of two groups. For this, random numbers were drawn from the random number table to decide patients group allocations well in advance.

The drugs were administered in the following dosages: Group I - Each child was nebulized with salbutamol sulfate 0.015 mg/kg/dose of 0.5% respiratory solution to a maximum of 5 mg per dose. This was diluted 1: 1 in 0.9% isotonic saline to a volume of 3 ml and nebulized via a tight-fitting face mask using a compressed air nebulizer over a period of 10 minutes. The same dose was repeated three times at 20 minute intervals on each occasion (17); Group II - Each child was treated with a combination of IB 40 μgr/dose (2 puffs from a MDI with tight-fitting face mask and aerochamber) and salbutamol sulfate (as in Group I) three times at 20-minute intervals. In both groups oxygen was administered with a nasal flow rate of 3L/minute and a single dose of 2 mg/kg (maximum 50 mg) prednisolone was administered via the oral or parenteral route at the beginning of the treatment. All the clinical parameters were recorded at minutes 0, 20, 40, 60 and 80.

Statistics: Statistical analyses were performed using SPSS-13.5

Descriptive statistics (ranged, mean, and standard deviation) were calculated for each quantitative variable.

The Mann Whitney U test was used to compare asthma scores between groups at the beginning and at the given times during treatment. The Wilcoxon paired-test was used to determine intragroup changes in asthma scores, and score parameters during treatment at all study intervals.

P values less than 0.05 were regarded as significant.

Results

Thirty-three patients were enrolled for the study and were randomized into three groups. There were 28 patients in Group 1 and 38 in Group 2. Nine pa-

tients were excluded from the study at the outset, because 5 were diagnosed as severe asthma attack (4 from Group 1 and one from Group 2) and 4 were diagnosed as mild asthma attack (all of them from Group 1). The groups did not differ significantly with regard to their present age, atopy characteristics, or asthma severity during the present episode of acute asthma. These observations are shown in Table 3. Significant ($p<0.05$) intragroup decreases in wheeze score, respiratory rate score, cyanosis score and accessory muscle score were observed in both groups at the end of treatment (Table 4). Again in each group, a significant improvement ($p<0.05$) in asthma scores was noted at the end of the study for both groups (Table 4). However, the improvement was similar in both groups and there was no significant difference ($p>0.05$) between groups in any of the study intervals (Figure 1).

Table 1. Scores of asthma parameters

Score	1	2	3
Respiratory Rate (rr/min)	<24	24-27	>27
Cyanosis	None or mild	moderate	severe
Wheezing	Terminal expiration with stethoscope	Entire expiration with stethoscope	Inspiration and expiration without stethoscope
Accessory muscle use	None or minimal intercostal	İntercostal and substernal	İntercostal, substernal and supraclavicular.

Table 2. Severity of Asthma

Severity of asthma attack	Mild	Moderate	Severe
Ranges	4-6	7-9	10-12

Table 3. Baseline characteristics of the patients in two groups

Parameter	group 1(n=14)	group 2(n=19)
Age(years)	8.68(±1.75)	9.76(±2.11)
Atopy(%)	67	63
Family history(%)	46	42
Respiratory rate(per min)	60.28(±11.82)	61.5(±19.44)
Cyanosis score	1.64(±0.95)	1.28(±0.71)
Accessory muscle score	2,3(±2.3)	2,3(±0.95)
Wheeze score	3(±0.1)	3(±0.1)
Asthma score	8,6(±0.85)	8,4(±0.83)

Table 4. Change in parameters after the treatment

	group I				group II			
	before treatment	after treatment	Z value	p value	before treatment	after treatment	Z value	p value
respiratory rate(per min)	60.28(±11.82)	42.71(±7.05)	-4,684	<0.001	61.5(±19.44)	41.8(±15.13)	-4,671	<0.001
cyanosis score	1.64(±0.95)	1(±0.1)	-3	0,003	1.28(±0.71)	1(±0.1)	-2	0,046
accessory muscle score	2,3(±2.3)	1,3(±0.49)	-3,938	<0.001	2,3(±0.95)	1,2(±0.71)	-4,564	<0.001
Wheeze score	3(±0.1)	1,7(±0.98)	-4,234	<0.001	3(±0.1)	1(±0.1)	-5,292	<0.001
Asthma score	8,6(±0.85)	7,7(±1.46)	-3,411	0.001	8,4(±0.83)	5,6(±1.34)	-4,44	<0.001

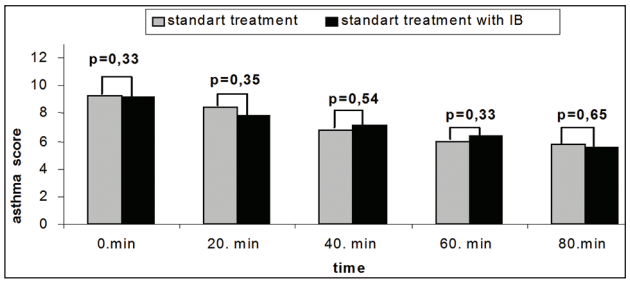

Figure 1. Asthma scores for both treatment groups at different times during treatment

No such adverse effects as tremors, vomiting or transient eye irritation were observed in either group during treatment(6).

Discussion

This study indicates that the use of MDI-spacers is not an effective alternative administration of IB in the pediatric emergency setting.

Recently, many studies have evaluated the effect of IB in the treatment of pediatric asthma attack.

Storr and Lenney reported no advantage from using the combination(18). Beck et al.(19) conducted studies in children adding a single dose of ipratropium to salbutamol and they observed a significant improvement in FEV1 at minute 60 in the ipratropium group - 20.6% vs 3.5% ($p<0.05$) – but there was no significant improvement at minute 40.

In evaluating the effect of multiple doses of ipratropium some studies (20, 21) did not report any benefit from the use of ipratropium, while others reported a better response in the combined treatment group(22, 23, 24).

Sienga Monge et al. conducted a randomized trial of 40 children with acute asthma to compare the effects of β2 agonist agents (i.e., salbutamol) that had been administered with IB (40 μg administered three times by MDI) with those of β2 agonist agents that had been administered alone. The authors concluded that the bronchodilator effects of the administration of salbutamol alone or in combination with IB were similar in intensity and in action time(25).

A meta-analysis by Rodrigo and Castro-Rodrigez included all 32 randomized controlled trials (n=3611 subjects) published before April 2005 and showed significant reductions in hospital admissions in children (RR=0.73; 95% CI 0.63 to 0.85, p=0.0001) treated with inhaled anticholinergic agents. The authors concluded that the addition of multiple doses of inhaled IB to β2-agonists is indicated as the standard treatment in children with moderate to severe exacerbations of asthma in an emergency setting (26).

Recently, the findings from different studies have clearly shown that the use of anticholinergic drugs, a combination of β2 agonist /anticholinergic therapy in acute asthma exacerbation, is associated with lower hospitalization rates and greater improvement in PEF and FEV1(4) , a conclusion also shared by us. The common feature of those suggestions and different studies is that medications were delivered using a nebulizer.

In our study, we assumed that a combination of ipratropium and β2 agonist/anticholinergic therapy is effective in acute asthma, although in contrast to other studies, we used IB via a MDI-spacer, not with a nebulizer.

The literature contains only one similar study, conducted by Chakraborti (27) et al., in which the authors concluded that the addition of IB to salbutamol administered by MDI leads to significant greater improvement in percent predicted FEF25-75 and percent predicted PEF (p=0.01 and p=0.03, respectively). However, there were some limitations to that study. No children were excluded because of poor spirometry performance. Yet the measurement of spirometric parameters is known to be not generally possible in the emergency department, and other tests such as peak expiratory flow may be difficult to perform accurately in young children, who make up a large proportion of the pediatric asthma population; in our study we used a scoring system to assess asthma attack severity. We believe that the clinical applicability of our study is thus better than that of such other studies.

Recently, different studies regarding β2 mimetic use have provided evidence of the efficacy of MDI with spacers in all age groups(5-10).

Compared with nebulizers, the use of a spacer and MDI is a more efficient means of drug delivery; a lower total dose is required for the same degree of bronchodilation, the time to delivery of a complete dose is shorter, and there are fewer side effects. Delivery by means of a spacer by improving targeting of the lung reduces the dose delivered elsewhere. In addition, it is compact, relatively

cheap, and because no external power source is required, it can be used easily in most settings(4).

In our study, we concluded that the administration of IB with a spacer and MDI in the pediatric emergency department in patients with moderate attack poses no advantages, although some points may be argued.

Inhalation therapy for asthma in children is a subject of great importance and it is essential to define the most appropriate doses and methods. However, an inappropriate dose may have been used in this study. Before reaching definitive conclusions, further studies should be carried out to determine the most efficient therapeutic doses. Using nebulizers, the oxygen flow, the distance between face and mask, tidal volume, respiratory rate and the patient's inhalation technique result in a variation in deposits in the lower airways of from 3% to 13% of the total numbers of particles available for inhalation. Using MDIs, around 20% of the particles generated reach the lower airways. Accordingly, at least 40 μr of IB administered by MDI with an aerochamber was regarded as an equally efficient dose to the nebulized form in our study, though it may not be an exactly accurate dose. The wide range reported in the literature (1: 1 to 1: 12.5) (5-10, 28)is a reflection of differing study designs and the variability in the dose delivered to the lungs.

It should also be noted that there are several limitations to our study. Since it was conducted in a single institution and with a low number of patients, the generalizability of our findings to other settings may be limited. Severe asthma attack was regarded as an exclusion criterion in our study, although studies which reported that the combination of ipratropium was safer, effective and resulted in lower hospitalization than salbutamol alone usually included children with severe asthma attack. Therefore, despite our results, it should not be concluded that combination therapy has no role to play in acute asthma. Such a study might allow subgroup analysis to define patients who would not benefit from IB via MDI-spacer.

Moreover, the bronchodilator effect of nebulized salbutamol peaks within 10 minutes, whereas that of ipratropium has been shown to take 30-120 minutes (29-31). An observation time greater than 80 minutes might have led to different results in the present study.

In conclusion, the addition of IB via MDI-spacer to the standard moderate asthma attack treatment protocol was concluded to be no more effective in terms of reducing or improving clinical scores than standard treatment alone over 80 minutes of treatment. However, we believe that such economical and practical means should be investigated in community-based studies because it is possible that the inhaler form of IB with a mask aerochamber may be effective in higher doses or over a much longer time.

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The Effect of Adding Oral Calcium Dobesilate to Laser Photocoagulation on the Macular Thickness in Patients with Diabetic Macular Edema

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Abstract

Purpose: To evaluate the effect of oral calcium dobesilate (Doxium) on macular thickness in clinically significant macular edema (CSME).

Setting: Ophthalmology Clinic, Imam Khomeini Hospital, Ahwaz, Iran.

Design: Randomized placebo-controlled single blinded trial.

Methods and Materials: Overall, 71 eyes of 40 patients with non-proliferative diabetic retinopathy and clinically significant macular edema were included. All patients were received laser treatment for macular edema. Coherence optical tomography was used to determine the retinal thickness. Patients were randomized into two groups: group A received three Doxium capsule daily and group B received three placebo capsule daily for six months.

Results: The mean macular thickness before and after treatment in the group A was 340 and 257 micrometers respectively (24.5% reduced), and in the group B was 336 micrometers and 263 micrometers respectively (21.5% reduced). Macular thickness significantly decreased after treatment in both groups and the reduction in group A is higher but the difference of reduction between the two groups was not statistically significant (P>0.05).

Conclusion: In respect to the effect of adding oral Doxium to Laser Photocoagulation on the macular thickness in patients with diabetic macular edema, this study showed no statistically significant difference between Doxium and placebo.

Key words: Calcium dobesilate (Doxium); clinically significant macular edema (CSME); macular thickness; diabetic retinopathy; macular photocoagulation (MPC)

1. Introduction

Diabetes Mellitus (DM) is a metabolic disorder of the body, in which either a sufficient amount of insulin in the body does not exist or the existing insulin may not perform his task properly [1]. Diabetic retinopathy (DR), the most important ocular complication in patients with DM is the leading cause of blindness in patients aged 20- 65 years in the United States and in individuals between the ages of 30 and 64 in the United Kingdom; in which, it accounts for nearly 12%–14% of new cases of blindness resulting from all causes [2-5]. Retinal edema threatening or involving the macula is an important visual outcome of abnormal retinal vascular permeability in DR. The diabetic macular edema (DME) is diagnosed by slit-lamp biomicroscopy of the posterior pole. DME is caused by a breakdown in the blood-retinal barrier that lead to the accumulation of fluid in the space between the layers of retina in the macular area [5]. Data from the Early Treatment Diabetic Retinopathy Study (ETDRS) showed that focal laser photocoagulation of clinically significant diabetic macular edema (CSME) substantially reduces the risk of visual loss. Focal treatment also increases the chance of visual improvement, decreases the frequency of persistent macular edema, and causes only minor visual field losses. Therefore the presence or the absence of the CSME is the most important and the only specific factor defining the need to the treatment [6]. Laser treatment prevents vision loss, but does not have a significant effect on improving vision. This problem evokes the researchers for alternative therapies. The use of intravitreal injection of the steroid compound in some studies has reported good results [7, 8]. Also intravitreal injection of the Bevacizumab (Avastin)

has showed good results [9]. Calcium dobesilate (Doxium) is an anti-oxidative and angio-protective drug that decreases edema by regulating and improvement the physiological function. Recent studies have shown that Doxium is a potent antioxidant, particularly against the highly damaging hydroxyl radical. In addition, it improves diabetic endothelial dysfunction, reduces apoptosis, and slows vascular cell proliferation [10, 11]. Doxium recently remains the object of interest in treatment of diabetic retinopathy, but various results have been reported by different studies performed on reducing the macular edema by Doxium [11-14]. Thus, this study was conducted to assess the effect of oral Doxium adding to macular laser photocoagulation on the macular thickness in patients with CSME.

2. Materials and Methods

2.1 Study design and population

A randomized, single blinded, placebo-controlled trial was conducted on patients with type II DM that had diabetic retinopathy and referred to the ophthalmology clinic, Imam Khomeini Hospital, Ahwaz, Iran from 2012-07-22 to 2013-01-19. This study was approved by Ahvaz Jundishapur University of Medical Sciences ethics committee and informed consent was obtained from all patients.

2.2 Inclusion criteria

All patients with non-proliferative diabetic retinopathy and CSME which at least have 20/200 visual acuity were included.

2.3 Exclusion criteria

Patients with the history of previous treatment for a CSME such as macular laser and intravitreal injection of Avastin, posterior segment trauma, the need for the further eye surgery such as vitreo-retinal surgery or cataract, cases of non-feasible clinical observation, optical coherence tomography (OCT) and *fluorescein angiography (*FA) such as severe opacities of the media, the existence of a previous vascular pathology of the retina or age-related macular degeneration, a history of previous intraocular surgery, visual acuity less than 20/200 and presence of macular ischemia in FA were excluded.

2.4 Intervention

The demographic profile of patients includes age, gender, occupation, history of DM and smoking, were collected. Then, visual acuity of the patients was measured using the Snellen chart. Patients were examined with a Slit-Lamp (Topcon; Tokyo Optical Co., Ltd., Tokyo, Japan) and fundoscopy was done with the Volk's 78D and 90D lenses. Intraocular pressure (IOP, mmHg) was measured using a Goldmann applanation tonometer. FA (Topcon; Tokyo Optical Co., Ltd., Tokyo, Japan) of the retina was performed for all patients to monitor the areas of leakage and the macular thickness was measured using OCT (Topcon; Tokyo Optical Co., Ltd., Tokyo, Japan). Then the patients by simple randomization were divided into two groups, group A; receiving three capsules of Doxium 500 milligram daily and group B; receiving three capsules of placebo (containing starch with same sizes and color as Doxium capsules) daily for six-month. All patients were received classic laser treatment for macular edema, and six-month later again undergone the OCT to measure the macular thickness. Macular laser therapy was performed based on the ETDRS chart and algorithm using standard protocol with green laser set up every laser point with 50 to 100 micrometers. The emission duration was 0.1 second and the power was set right with the proper laser lighting effect, and the area of the macula has exposed, so that at least 500 micrometers from the edge of the optic disk and 500 micrometers away from the center of fovea. The laser energy was started at 50 mV and gradually increased until a modest and proper white spot revealed in the macula.

2.5 Outcome

Primary outcome; macular thickness

Secondary outcomes: visual acuity and intraocular pressure

2.6 Statistical analysis

After gathering information, data were analyzed by SPSS 19.0. Descriptive statistics (mean and

standard deviation) were used to describe the desired variables. Then to determine the relationship between the variable T-test and paired T-test and ANOVA were used. Significant level was considered equal or lesser than 0.05.

3. Results

Overall, 40 patients (mean age of 53.55 ± 8.8, rang of 36-72 years) were studied, which 22 patients (55%) were male and 18 patients (45%) were female. There was no significant difference between groups A and B in term of age (54 ± 9.5 vs. 53.1 ± 9.8, respectively). In this study, total of 71 eyes were studied (36 eyes in group A and 35 eyes in group B), that 33 (46.5%) and 38(53.5%) were the right and left eyes respectively. In both groups after treatment the macular thickness reduced so that in group A the reduction was 80 micrometers (24.5%) and in the group B it was 75 micrometers (21.5%). The difference of changes in the macular thickness between the two groups was not statistically significant (Table 1). Six months after treatment, nine-eye (25%) in groups A and ten-eye (28.5%) in groups B need laser retreatment. The difference between the two groups was not statistically significant. The difference of visual acuity increase and the change of the mean IOP before and after treatment had no statistically significant difference between the two groups (Table 1).

4. Discussion

Diabetic maculopathy is one of the most important causes of vision loss among people around the world today. Though laser photocoagulation remains as the gold standard therapy for diabetic macular edema, only a slight visual improvement has been reported following laser therapy. Therefore, different methods of treatment besides laser therapy are used to treat DME more effectively. *In t*his study we assessed the effect of adding oral Doxium to macular photocagulation on the macular thickness in patients with DME. In many studies the effect of Doxium on the DR has been reviewed, but the effect of this drug on the macular thickness was less evaluated. In the present study reducing macular edema evaluated with macular thickness measurement using the OCT as a more accurate method. In most studies the effect of Doxium on DR investigated and none of the studies has considered the macular thickness using OCT.

Rota et al. studied the effect of Doxium on the retinal albumin leakage in streptozotocin-diabetic rats for 10 days, and suggested that this drug stabilizes blood-retinal barrier in diabetic retinopathy [12]. Haritoglou et al. investigated the effect of Doxium in the diabetic patients with mild-to-moderate non-proliferative diabetic retinopathy, and reported that this drug did not reduce the risk of development of CSME [13]. Our study was in line with Haritoglou et al. study and revealed that Doxium didn't reduce the diabetic macular edema, but the difference was the same as our study. We measured the macular thickness using OCT before and after treatment but Haritoglou et al. just studied the effect of Doxium in terms of the risk of progression of clinical significant macular edema.

Fesharaki and Modaresi, measured the effect of Doxium on the visual acuity and surface area of retinal hemorrhage in patients with non-proliferative diabetic retinopathy, and claimed that this drug (250 mg two tablets per day for six months) may has a positive effect on visual activity [14]. In contrast with Fesharaki and Modaresi, in our study, Doxium has no effect on the visual improvement of the patients.

Table 1. Comparing the variables of interest before and after treatment with oral Doxium and macular laser

Variables	Before treatment (Mean ± SD)			After treatment (Mean ± SD)		
	Group A	Group B	P-value	Group A	Group B	P-value
Macular thickness, μm	340.8 ± 85.46	336.05 ± 81.27	0.81	257.44 ± 55.04	263.34 ± 66.62	0.68
Visual acuity, Log Mar	0.595 ± 0.192	0.567 ± 0.199	0.54	0.509 ± 0.166	0.495 ± 0.213	0.76
IOP, mmHg	13.52 ± 1.44	13.91 ± 1.59	0.28	13.00 ± 1.37	13.25 ± 1.55	0.40

Ribeiro et al. studied the effect of Doxium on the blood-retinal barrier (BRB) permeability in early diabetic retinopathy, and showed that the dosage of two grams of this drug daily for two-year has a significantly better activity on prevention of BRB disruption, with a very good tolerance [15].

Vojnikovic studied the effect of oral Doxium on the 79 non-insulin-dependent diabetic subjects with early retinopathy and open-angle glaucoma, and suggested that this drug reduces blood hyperviscosity and lowers IOP, with a beneficial effect on retinal state and visual fields [16]. Our study revealed the oral Doxium has no effect on IOP; of course we excluded the patients with a history of glaucoma and high IOP, and only the effect of this drug on the IOP of diabetic patients without a history of glaucoma were evaluated.

Stamper et al. evaluates the efficacy of Doxium for treating non-proliferative diabetic retinopathy by clinical examination, FA and fundus photography and failed to demonstrate any beneficial effect of Doxium [17].

5. Recommendations

One of the advantages of the present study was the quantitative observation of the macular thickness using OCT as a more accurate method. But the major weaknesses in our study were the short follow-up period, the high cost of supplying the drug, and no observation of the effect of the drug on the systemic blood sugar levels. Further studies in the longer follow-up period and larger sample size are recommended.

6. Conclusion

Doxium has no statistically significant effect on macular thickness, visual acuity and intraocular pressure in patients with DME treating by macular laser photocoagulation.

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Anxiety-depressive disorder´s influence on blood pressure At younger women

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Abstract

A significant increase in number of women under 40, reffered to a specialist due to high blood pressure, was noticed during the war years in Živinice. Those are mostly displaced women from eastern Bosnia and local population. All the women, were under antihypertensive therapy (were given different kinds in medication) and were reffered to a specialist due to unregulated blood pressure.

The goal of this work is to determine whether the high blood pressure at women under 40, who suffer from anxiety-depressive disorder, is an organic disease that should be treated with antihypertensive drugs or whether its a temporary increase in blood presure as a folowing state of anxiety-depressive disorder which doesn't require a specific treatment with antihypertensive drugs but it should be treated within the framewerk of the basic disease.

Trough a prospektive study, in a specific time frame, we have researched the number of appearences and gravity of the increase in blood pressure, in the targeted population.207 female subjects (from 24 to 39 years old) were divided into 2 groups: -A group constited of 105 sick women (who were diagnosed with anxiety-depressive disorder without being earlier diagnosed with hypertensive disease) and B-group constided of 102 sick women (who weren't diagnosed with an anxiety-depressive disorder or hypertensive disease).

After statistical data processing, the folowing blood pressure levels were registered: A-group (average levels of blood pressure 129/76 mmHg, SD +/- 20 mmHg) and B-group (average levels of blod pressure 131/81 mmHg, SD +/- 20 mmHg).

Altough there was, occasionally, an icnrease in levels of blood pressure in both groups and if we disregard the possibility of incorret measuring of blood pressure, the study showed that a significant statistical difference in the levels of blood pressure, in the A and B groups, doesn't exist. The study also showed that the average levels of blood pressure are within the limits of normal,and that women under 40, who suffer from anxiety-depressive disorder, do not suffer from a pemanently high blood pressure and they shouldn't be treated with antihypertensive drugs.

Key words: hypertension, anxiety-depressive disorder, women under 40.

1. Introduction

War and years after the war, loss of husbands and other family members, life in exile and uncertain future led to the permanent mental deformities of persons who are exposed to those negative impact, which is partly manifested through enhanced mental tension and consequent emergence of cardiac symptom science. Term thus, high blood pressure would probably be a consequence mentioned above and it might not result in the context of hypertensive disease in the narrow sense.

Anxiety-depressive disorder is a mental disorder that is a combination of symptoms of anxiety and depression (1), and it includes besides psychiatric symptom science (difficulty in concetration, feeling tired, irritability, worry, tearfulness, sleep disturbances, the worst expectation, pessimism, low self-esteem, feelings of worthlessness, anxiety...) and so-called SOMATIC SYNDROME (loss of appetite and weight, decreased libido, cardic symptom science: pain in the heart, choking, tightness in the chest, rapid irregular heartbeat, transient increase of blood pressure...).

2. Objective of the work

It is necessary to determine whether the blood pressure in women younger than 40 years with anxiety-depressive disorder (diagnosis verified psychiatric findings), not clinically verified as hypertensitives, organic disease should be treated

with anti hypersensitive medicines or it is a transient increase in blood pressure in the underlying disease that does not require specific treatment with anti hypersensitive medicines but are treated as a part of the underlying disease.

3. Patients and method of operation

3.1. Patients

The female subjects under the age of 40 were selected through a period of 18 months among patients referred to internal medicine specialist in Dom zdravlja (Health Centre) in Živinice. Patients who had verified anxiety-depressed disorder, were under control of neuro psychiatrist during testing and they received the appropriate individual specific therapy.

Control group was formed in the same way and consisted of the subjects who were referred to specialist examination for other reasons. All patients had their basic laboratory tests and EKG done. In both groups were not included patients who previously had clinical or outpatient verified hypertension as an organic disease.

3.2. Method of operation

In a prospective study lasting 17 months (from January 2007 to June 2008), there were 207 female subjects divided into two groups (women aged under 40): A-group (105 patients with verified anxiety-depressive disorder but without clinical verified hypertensive disorder) and B-group (102 patients without anxiety-depressive disorder and hypertensive disorder).

Testing was based on a two-month, everyday, ambulatory or home blood pressure measurement by principle: measurement of blood pressure twice a day (at 8 o'clock a.m and 8 o'clock p.m.) and recording values in the prepared form (6) with a remark that the blood pressure is always measured 3 times and to write down the last measured value. In consideration there were not included any values of blood pressure measured at any other time with the aim of eliminating other factors that may affect the blood pressure (stressful situations, annoy, painful states, increased physical exercise ...). As a criterion of increasing blood pressure it is of course taken the value of 140/90 mmHg.

Testing was conducted with the full cooperation of the patients with the assumption that the principle of measurement and recording of blood pressure mentioned above will be respected.

4. Results

After statistical analysis of the data there are registered the following values of blood pressure: A-group (average value of blood pressure 129/76 mmHg, the lowest 200/120 mmHg) and B-group (average value of blood pressure 136/82 mmHg, the lowest 90/60 mmHg, and the highest 230/120 mmHg). In the A-group there were 4 cases where the average blood pressure was above 140/90 mmHg but in the control B-group there were 7 cases like these.

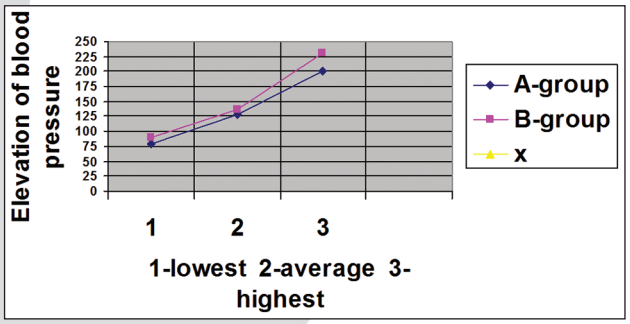

Chart 1. Comparison of the movements of blood pressure

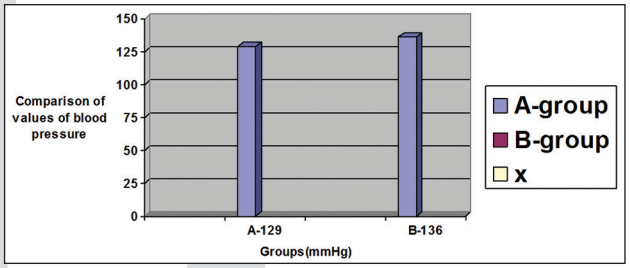

Chart 2. Comparisonofthe average blood pressure

There are no significant differences in blood pressure between the tested and control groups. Average values of blood pressure range in reference values in both groups. Occasional quite high and slightly lower values of blood pressure does not significantly interfere with the final results.

5. Discussion

A number of factors can lead to a transient increase in blood pressure. Anxious-depressive disorder is often accompanying disorder in other

chronic diseases such as resistant hypertension and diabetes which affect the reduction in quality of life (2). "White coat hypertension" leads to transient, even dramatic increases in blood pressure, and the cause is sudden the sympathetic activation of catecholamines. Occupational stress (the length of working time, job dissatisfaction, interpersonal relations, job insecurity, night shifts, exposure to physical and chemical materials ...) can lead to high blood pressure in a certain period of time. Likewise, in the sympathetic baroreceptor activation leads to transient vasoconstriction of arteries with a consequent increase in the amount of blood pressure (5,8). Headache, especially migraine in younger women, as frequent accompanying factor in anxiety-depressive disorder, can cause transient increases in blood pressure, although there is no reliable scientific evidence about a direct connection. Some drugs, such as for example - antidepressants, especially monoamine oxidase inhibitors, lead to hypertensive reactions (9). Nonsteroidal antirheumatics (especially ibuprofen and diclofenac) inhibit COX-1 and COX-2 enzymes leading to reduced synthesis of prostaglandins, which results in the retention of water and sodium, which leads to increased blood pressure. Corticosteroids, such as prednisone and hydrocortisone, also cause retention of sodium and water which results in increased blood pressure. Some other medications, such as birth control pills (9), medications for colds, decongestants, sympathomimetics and analgesics, which can consume patients of anxiety-depressive disorder, may affect high blood pressure. Stress causes an increase of interleukin-6 and fibrinogen which leads to transient hypertension (7). Excessive alcohol consumption and activation of the sympathetic can lead to high blood pressure (10). Smoking, because of elevated level of catecholamines and stimulation of vegetative ganglion, causes an increase of heart rate, stroke and cardiac output and a temporary increase in blood pressure (10). If there is a resistant hypertension in anxiety-depressive disorder it is treated by antihypertensive medications, and at the same non resistant hypertension disorders there is no need for specific treatment (4).

6. Conclusions

Occasional values of high blood pressure in women younger than 40 years old, who suffer from anxiety-depressive disorder, should observe in the context of the underlying disease but we must not exclude the possibility that such patients may suffer from hypertension (2,3). These patients were excluded in this study at the very beginning and they were not taken into consideration. Testing shows that the tested patients (except in a few cases) generally do not have hypertension in terms of organic disease and do not require specific antihypertensive treatment. It is recommended a cautious approach in the treatment of such patients to avoid the situation that the people who do not have hypertension be treated with the antihypertensive medications.

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Adherence to statin use for primary prevention in family practice

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Abstract

Objective: Large-scale, clinical end-point trials in a wide spectrum of subjects have demonstrated the universal efficacy of statins in the prevention of coronary, cerebrovascular and peripheral vascular disease in both primary and secondary prevention settings. However prescribed statins are only effective if they are taken by patient on a regular basis, known as medication adherence. Poor adherence to medical treatment severely compromises patient outcomes and increases patient mortality. The aim of the present study was to investigate the adherence and/or discontinuation rates and the reasons of non-adherence with the statin treatment in our primary care settings.

Study Design: This retrospective study was carried out at two outpatient clinics of the Department of Family Medicine of Ankara University School of Medicine and at an affiliated private family practice. Electronic medical records of the patients were filtered between January 1st, 2010 and December 31st, 2011 and patients with an ICD code of E78 were identified as potential candidates. We searched for those, first time prescribed a statin agent.

Methods: After providing informed consent, patients were questioned via face to face interviews whether they started, continued, or regularly used the statin pills and if not, the reasons for not starting and/or discontinuing. Change from one statin to another was not considered non- adherence with the therapy. The data were analyzed using the Statistical Package for the Social Sciences (SPSS), version 15.0 software. Pearson's Chi-square analyses were used to determine the association of prevalence of adherence/nonadherence to statin medication with personal characteristics.

Results: 28% (n=68) of the patients were still on treatment, 34% declared that they had never started and 38% had discontinued the therapy. Adherence to statin drugs was higher among women than men. Discontinuation of therapy was higher among males than females. News against statin drugs on Turkish media lately consisted of 34.4% of the reasons for discontinuing the medication.

Conclusion: The rate of adherence to statins is low in our primary care settings consistent with the literature. Improving adherence of the patients' to statins should be the priority of the primary care physicians. We think that it is important to involve the patients into the medical decision through patient-centered care.

Key words: primary prevention, family practice, statins.

Introduction

Hypercholesterolemia is a major risk factor for atherosclerosis and cardiovascular disease (CVD), the leading cause of death worldwide and responsible for 1/3 of all deaths in USA every year (1). CVD was found to be responsible for 4.35 million deaths across Europe in 2000, and the reason for nearly half of the deaths in both women and men (2). According to the results of TEKHARF Study of Turkey 2009, coronary artery disease (CAD) was found to be the leading cause of death in both sexes which was the highest prevalence among European countries (3). Turkey is a country with a population of 74 million and the median age is 29. CAD prevalance was unexpectedly high when compared with this young age population. Coronary mortality rate is 5 per 100 person year which is higher than many European countries (4). Amongst cardiovascular risk factors, a raised plasma concentration of apolipoprotein B-100 (Apo B), reflecting the accumulation in plasma of chiefly low-density lipoprotein cholesterol (LDL-C) particles, has the highest attributable risk for atherosclerotic vascular disease (5).

There are now several therapeutic options for regulating Apo B containing lipoproteins and lowering plasma cholesterol, including 3-hydroxy-3-methylglutaryl- CoA reductase inhibitors (statins). Large-scale, clinical end-point trials in a wide spectrum of subjects have demonstrated the universal efficacy of statins in the prevention of coronary, cerebrovascular and peripheral vascular disease in both primary and secondary prevention settings (6-14). However prescribed statins are only effective if they are taken by patient on a regular basis, known as medication adherence (15). In clinical trials, statins demonstrated benefit only after one to two years of continous treatment. Despite their well-established benefits and corresponding recommendations from expert bodies, statins are widely underused in the 'real world' of clinical practice both in patients with or without vascular disease (16,17). In primary care settings, patients were less willing to receive statins since they perceive their risks as low. Better adherence to statins is also associated with improved outcomes in primary prevention. In the West of Scotland Prevention Study (WOSCOPS) better adherence to prevastatin resulted in lower vascular morbidiy and mortality and all cause mortality (18).

The treatment of chronic illnesses commonly includes the long term use of pharmacotherapy. Although these medications are effective in combating disease, their full benefits are often not realized because approximately 50% of patients do not take their medications as prescribed. Poor adherence to medical treatment severely compromises patient outcomes and increases patient mortality (19).

During the "follow-up" of dyslipidemic patients for primary prevention at the outpatient clinics of family medicine, we realized that some of our patients were not adherent to statin medications.

AIM

The aim of the present study was to investigate the adherence and/or discontinuation rates and the reasons of non-adherence with the statin treatment in our primary care settings.

Material and method

This retrospective study was carried out at two outpatient clinics of the Department of Family Medicine of Ankara University School of Medicine and at an affiliated private family practice. Electronic medical records of the patients were filtered between January 1st, 2010 and December 31st, 2011 and patients with an ICD code of E78 were identified as potential candidates. Inclusion criteria were as follows: Age >18 yrs, at least two lipid test levels, LDL >130 mg/dL, Total cholesterol >200 mg/dL. We searched for those, first time prescribed a statin agent. Patients were excluded if they had a prior history of CVD and Diabetes Mellitus. After providing informed consent, they were questioned via face to face interviews whether they started, continued, or regularly used the statin pills and if not, the reasons for not starting and/or discontinuing. Change from one statin to another was not considered non- adherence with the therapy. The data were analyzed using the Statistical Package for the Social Sciences (SPSS), version 15.0 software. Pearson's Chi-square analyses was used to determine the association of prevalence of adherence/nonadherence to statin medication with personal characteristics.

Results

The number of the eligible patients was 240. The average age was 46.6 ± 8.2 (range 20-80). Of the patients 48,4% was female and 51,6% was male; 11% was ≤45 and 89% was over 45 years of age.

28% (n=68) of the patients were still on treatment, 34% declared that they had never started and 38% had discontinued the therapy. Adherence to statin drugs are given on Table 1.

Adherence to statin drugs was higher among women than men (39.7% vs 17.7% respectively) ($p<0,01$). 40,3% of male and 27,6 % of female had never started to take the medication. Discontinuation of therapy was higher among males than females (41,9% vs 32,8). The reasons for never starting the lipid-lowering medication according to age and sex are given in Table 2.

Reasons for never starting the therapy according to age ($p<0.001$) and sex ($p<0.015$) were statistically significant.

52.7% of the study population under 45 years of age stated that they don't want or dislike to use any medication, whereas this was 25.9% for patients ≥ 45 years (54% men, 28% women). 36.4% of patients ≥ 45 years declared that they worry

Table 1. Adherence to statin treatment

SEX	On treatment		Never started		Discontinued		Total	
	n	%	n	%	n	%	n	%
Female	46	39.7	32	27.6	38	32.8	116	100
Male	22	17.7	50	40.3	52	41.9	124	100
Total	68	28.3	82	34.2	90	37.5	240	100

Table 2. The reasons for never starting the lipid-lowering medication according to age and sex

	Not want/dislike to use		Worry about side effects		Want to lower lipids by diet and/or lifestyle modifications		Health insurance problems		TOTAL		p
AGE	n	%	n	%	n	%	n	%	n	%	**<0.001**
≤45	7	25.9	5	18.5	11	40.7	4	14.8	27	100	
>45	29	52.7	20	36.4	6	10.9	0	0	55	100	
SEX											**<0.015**
Female	9	28	10	31.2	12	37.5	1	3.0	32	100	
Male	27	54	15	30	5	10.0	3	6.0	50	100	
TOTAL	**36**	**43.9**	**25**	**30.5**	**17**	**20.7**	**4**	**4.9**	**82**	**100**	

Table 3. The reasons for discontinuing the lipid-lowering medication according to age and sex

	News on media		On-demand use		Experiencing side effects		Decrease in lipid levels		Wish to change for diet		Total		
AGE	n	%	n	%	n	%	n	%	n	%	n	%	P<0.001
≤45	5	11.6	15	34.8	7	16.2	8	18.6	8	18.6	43	100	
>45	26	55.3	5	10.6	9	19.1	4	8.5	3	6.3	47	100	
SEX													P>0.005
Female	12	31.5	8	21.0	5	13.0	8	21.0	5	13.0	38	100	
Male	19	36.5	12	23.0	11	21.0	4	7.7	6	11.5	52	100	
TOTAL	**31**	**34.4**	**20**	**22.2**	**16**	**17.7**	**12**	**13.3**	**11**	**12.2**	**90**	**100**	

about the side effects of statins and did not start the therapy, this was 18.5% under 45 years of age. Gender seemed to have no effect on this topic. A four fold increase had been detected in patients younger than 45 years of age who wished to lower lipid levels by diet and/or life style modifications and in female patients. Health insurance problems seemed an important aspect of never starting the therapy in patients <45 years. 3% of female and 6% of male patients reported the same problem.

The reasons for discontinuing the lipid-lowering medication according to age and sex are given in Table 3.

Discontinuing the lipid-lowering medication according to age groups was found to be statistically significant (p<0.001).

Recent news about statins on media had an adverse effect on adherence to the therapy. 55.3% patients ≥ 45 and 11.6% < 45 years of age discontinued their statins for this reason. % 19,1 of patients ≥ 45 discontinued their medication as they experienced side effects. On demand use, normalization of lipid levels and wish to continue by diet were seemed to be more effective for non- adherence in younger patients. There was no statistically significant difference on discontinuation of drugs according to gender (p>0.005).

Discussion

The causes of non-adherence to statins are grouped as patient, physician and health care system-related factors (15,18). Results of our study was similar to previous researches on non-adherence except we could not find any physician related factors on non- adherence. The most frequent

reason for discontinuation in our patients was negative news about lipid lowering drugs on media. This was different from other researches about non- adherence to lipid lowering drugs.

We declare that statin adherence is low (28%), consistent with the literature which reports yearly statin adherence rates from 25% to 40% (20).

The level of adherence in the West of Scotland Coronary Prevention Study (WOSCOPS) was approximately 85% at the first follow-up visit (18,21).

A Canadian study of primary prevention cases for dyslipidemia reported high discontinuation rates for statin usage of 35% and 65% at 6 months and 3 years respectively (22).

Retrospective data from a UK electronic database of 6462 diabetic patient records indicated that adherence to statin therapy was only 87% at 3 months, falling to 61% at six months and thereafter remaining stable over a follow-up period of 13 years. Only 50% of patients were fully adherent to prescribed regimen (23).

In an Israeli study of 47680 patients adherence rate was 61% at 12 months and at 6 years of follow-up this had fallen to just 10% (24).

Adherence to statin therapy was poor at 3 and 6 months in a prospective cohort study which was conducted by Mann DM and colleagues. Veterans (n=71) given their first prescription of a statin for primary prevention were interviewed at the third month, and at the sixth month regarding medication, disease, and diet beliefs along with self-reported statin adherence. It was observed that 15% discontiued statin at 3 months, 27% at 6 months. At 6-month follow-up, 55% of the cohort was non-adherent with 10% reporting never having started their statin, 50% reporting misconceptions about the duration of treatment and a median use of <2 months among those who discontinued their statin. At baseline, 79% of patients preferred to try changing their diet before starting their statin but only half reported being given such an opportunity (25). According to the results of our survey 20% of patients preferred lowering their cholesterol level by diet alone. This was the reason for never having started their statin.

Avorn J and collegues found that patients over 65 years of age failed to fill prescriptions for lipid-lowering drugs for about 40% of the study year. Patients with hypertension, diabetes, or coronary artery disease had significantly higher rates of persistence with lipid-lowering regimens. Comorbidity is a well known predictor of adherence (26). We did not enroll patients with comorbidities in our research.

In a retrospective cohort study by Chan DC et al, adherence to statin medication was measured during the year after the initial prescription. They declared that only 36.4% of patients were fully adherent. Older patient age and male gender were significant patient predictors of adherence. Having a statin prescribed by a cardiologist, or a patient's primary care physician were significant physician predictors of adherence. Lower copayments also predicted adherence (20). In contrast to this study, we found that male gender and being older than 45 years of age were predictors of non- adherence. Meanwhile patients younger than 45 years (15%) said that they did not start statin therapy because of health insurance problems.

In a study from Ontario, Cynthia et al assessed statin adherence in three seperate patient cohorts: those with recent acute coronary syndrome, those with chronic coronary artery disease and those for primary prevention. They found two-year adherence rates 40.1% for acute coronary syndrome group, 36.1% for chronic coronary artery disease group, and 25.4% for primary prevention group where all the medication costs are covered except for a small co-payment per prescription (27).

The percent of the reason of worrying about side effects for non- adherence to statins was 30.5% among our cases whereas serious side effects of statins as to discontinue the drug in chronic use is very rare (28). Although none of our cases reported serious side effects, this compliant was the reason for non- adherence in 17.7% in our cases. Desiring to lower lipid levels by diet was another attitude of our patients but it is well known that diet alone has little effect on lipid levels.

News on Turkish media about the negative aspects of statins seemed to effect the study population against these drugs. The percentage of discontinuing the medication for this reason was 36.5% among women and 31.5% among men.

The weakness of the study is that we don't know at which month the patients stopped or began to take the pills on an irregular basis.

Conclusion

The rate of adherence to statins is low in our primary care settings consistent with the literature. Causes of non- adherence are complex and can be broadly classified into three categories: patient related, physician-related and health system-related (19). Among these, patient-related factors may be the strongest (29). Also in this study patient related reasons (not want/dislike to use a pill) are mentioned as 43.9% for never starting statin medication. Improving adherence of the patients' to statins should be the priority of the primary care physicians. It is prosperous to build again the management of the patients in line with this objective. Patients should be advised of the various factors that may influence them for not taking statin medications. Not only the physicians but all healthcare staff should endeavor to improve adherence and provide the best possible patient outcomes (15). We think that it is important to involve the patients into the medical decision through patient-centered care. We must raise awareness of that "drugs don't work in patients who don't take them" (19).

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The possible relationship of Toxoplasma with risk of traffic accidents

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Abstract

Background: This study investigated the possible role of toxoplasmosis in increasing the risk of traffic accidents.

Materials and Methods: This unmatched case-control study was done on a total of 103 people (102 men and one woman) involved in a traffic accident as drivers for a time period of one year during 2011–2012.The controls were consisted of 200 people (88 men and 112 women), residents of the same region and in same age group. Blood samples were driven and all serum samples were separated and stored at -20 ℃ until the testing. All collected sera were examined using ELISA technique.

Results: For IgG seropositivity, a 2.02-fold increase (χ^2 = 7.84, P = 0.004, 95%CI: 1.23 – 3.33) in risk for traffic accident was estimated. Furthermore, IgM seropositivity could not significantly increase the risk of traffic accident, with an OR of 0.73 (χ^2 = 0.209, P = 0.461, 95%CI: 0.19 – 2.81). However, stratification analysis showed that only the individuals with age range ≤ 30-year were significantly more common in traffic accident than in controls (OR: 2.15, χ^2 = 6.91, P = 0.007, 95%CI: 1.20 – 3.85). There was no statistically significant difference between the IgM and IgG seropositivity in case and control groups (X^2 = 3.65, P = 0.09, 95%CI: 0.99 – 2.67).

Conclusion: There is an increased risk for traffic accidents for drivers owing to these high seroprevalence of latent toxoplasmosis. Latent toxoplasmosis in drivers should be taken into account while developing preventive strategies for traffic accidents in Iran.

Key words: *Toxoplasma gondii*; Latent Toxoplasmosis; Traffic accidents

1. Introduction

Accidents define as an unexpected and undesirable event causing great damage, injury or loss of life (1). Generally, accidents can be categorized into four groups including traffic accidents, home accidents, occupational accidents and sports accidents (2). Traffic accidents remain global public health problems, which were estimated that1.2 million people were killed and up to 50 million people were injured on the world's roads every year (3, 4). It was estimated in 2006 that over 27000 and 150000 people were killed and injured due to traffic accidents in Iran, respectively (5). Nearly 100 people died due of traffic accidents in Iran every day on average, but this figure is about 3000 people in the world. Although Iran has a population of nearly one-hundredth of the world, but country-specific mortality is assigned to one-fortieth of the world (3). According to the World Health Organization (WHO) estimates, in 1990 traffic accidents ranked as the 9th leading cause of death globally, and are estimated to rank sixth by the year 2020.It was reported that drivers are mostly responsible for the traffic accidents, hence they are tired, weary, sleepy and absent-minded driving, that deterioration of the drivers' mental state and the decrease of psychomotor performance are most important causes (6).

Toxoplasmosis is one of the world's most common zoonosis throughout the world, which caused by single celled parasite *Toxoplasma gondii (T. gondii)* (7). The seroprevalence of antibodies to *T. gondii* in human populations varies in different geographical regions of Iran from 19% in north to 30.2% in capital part (8, 9), while in Europe and US were reported 30%, 15.8%, consecutively (10-12). Latent toxoplasmosis can orche-

strate a significant increase in dopamine production in neural cells and could trigger changes to the chemistry of the human brain, which might affect a person's behavior (13).The parasite forms cysts within the brain cells and produces an enzyme, which is needed to make dopamine that plays a role in mood, sociability, attention, motivation and sleep patterns. Latent *Toxoplasma* infection-induced changing social behavior is not a fleeting phenomenon, and can be seen during a long time (14).

The aim of this study was to investigate the possible role of toxoplasmosis in increasing the risk of traffic accidents.

2. Materials and methods

2.1. Study design and population

This unmatched case-control study was done on a total of 103 people (102 men and one woman) involved in a traffic accident as drivers for a time period of one year during 2011–2012.The cases were chosen from people living in Ahvaz city of Iran, aged between 19 and 60 years having driving license more than a year. The controls were consisted of 200 people (88 men and 112 women), residents of the same region and in same age group, which did not show any clinical signs or symptoms of toxoplasmosis. This study was approved by local ethical committee of Ahvaz Jundishapur University of Medical Science and all parents granted informed consent.

2.2. Case and controls recruitment

People who involved in an accident and were having driving license more than a year, age range between 19 to 60 years, a driver who remained alive and was available to interview, consumption of any food or drug that will affect driving on at least 24 hours before (based on history and physical examination), and no history of neurological disorders or psychiatric medication in past 5 years, were included as the study group. People who had consumed alcohol and drug addicts were excluded. The control group was also consisted of people who were residents of the same region, with no story of a car accident, negative HIV, HBV, HCV tests or the lack of co-morbid information.

2.3. Data collection

Specific questionnaire for demographic information that includes occupation, level of education, Ethnicity, Marital status, hospitalization, initial and final diagnosis, History of psychiatric disorders, Swimming in the River or lack, predominant dietary habits, residency, were filled.

2.4. Methods

To diagnose the *Toxoplasma*-associated IgG and IgM antibodies, 5ml of each subjects' blood was used for serological assessment, after centrifugation and serum separation, samples were kept at -20° C until processed. *Toxoplasma* IgG and IgM antibody concentrations were determined by ELISA (IgG and IgM: Trinity Biotech Captia™, USA) according to the manufacturer's instructions. The samples with IgM antibody positivity index higher than one were evaluated as active toxoplasmosis and IgG antibody higher than one as latent toxoplasmosis.

2.5. Statistical analysis

Data were evaluated by SPSS 16.0 for Windows. The odds ratio (OR) used as an approximation of the relative risk in this study. The Fischer exact test between two binary variables was used to test statistical significance. $P< 0.05$ values were considered as statistically significant.

3. Results

The mean age of case and control was 29.74 ± 11.26 and 24.60 ± 4.30, respectively. Serological tests in the case group revealed that, three (2.9%) sera were IgM positive and 38 (36.9%) were IgG positive (Table 2). Hence, in control group 8 (4%) subjects were found to be IgM positive and 56 (28%) was found to be IgG positive. There was no statistically significant difference between the IgM and IgG seropositivity in case and control groups ($X^2 = 3.65$, $P = 0.09$, 95%CI: 0.99 – 2.67), which may indicate that potentially affected by toxoplasmosis has no potential risk for traffic accident. The frequency of IgM and IgG seropositivity in different genders, ages, ethnicity, education level,

job categories, history of neurological disorders and swimming frequencies in cases and controls has no significant difference, as a consequence these variables have no potential risk for traffic accident in both case and control groups (Table 1).

Table 1. Sub-groups of T. gondii total seropositivity according to demographic variables of interest

Variables	Case (n = 103) No. (%)	Control (n = 200) No. (%)
Age groups		
19 - 30	68 (66)	184 (92)
31 – 44	17 (16.5)	15 (7.5)
45 - 65	18 (17.5)	1 (0.5)
Sex		
Male	102 (99)	88 (44)
Female	1(1)	112 (56)
Education levels		
Illiterate	13 (12.6)	0 (0)
Before high school	59 (57.3)	3 (1.5)
Diploma	27 (26.2)	21 (10.5)
Post-diploma	0 (0)	34 (17)
Bachelor	2 (1.9)	125 (62.5)
Master and PhD	2 (1.9)	17 (8.5)
Job categories		
Jobless	17 (16.5)	14 (7)
Worker	29 (28.2)	17 (8.5)
Employee	10 (9.7)	36 (18)
Student	13 (12.6)	110 (55)
Other	34 (33)	23 (11.5)
History of Neurological disorders		
Yes	1 (1)	4 (2)
No	102 (99)	196 (98)
History of physical illness		
Yes	10 (9.7)	33 (16.5)
No	93 (90.3)	167 (83.5)
Contact with cat		
lives indoors	1 (1)	3 (1.5)
lives outdoors	55 (53.4)	63 (31.5)
other	47 (45.6)	134 (67)

For IgG seropositivity, a 2.02-fold increase ($\chi 2$ = 7.84, P = 0.004, 95%CI: 1.23 – 3.33) in risk for traffic accident was estimated (Table 2). Furthermore, IgM seropositivity could not significantly increase the risk of traffic accident, with an OR of 0.73 ($\chi 2$ = 0.209, P = 0.461, 95%CI: 0.19 – 2.81) (Table 2). However, stratification analysis showed that only the individuals with age range ≤ 30-year were significantly more common in traffic accident than in controls (OR: 2.15, $\chi 2$ = 6.91, P = 0.007, 95%CI: 1.20 – 3.85) (Table 2).

4. Discussion

Following the presence of HIV, increase in organ transplantation and complications caused by immunosuppressive therapies of malignancies during the past decades, there is an obvious increased in the studying the influence of latent toxoplasmosis. Traffic accidents may happen due to many reasons such as drivers' mental state (16-18) and decrease of psychomotor performance (16). Latent toxoplasmosis can be located in neural or muscular tissues and cause prolonged reaction times of the muscles, in which lead to deceleration of the reflexes that itself could be a major cause of traffic accidents (19). First traffic accident case caused by toxoplasmosis, an AIDS case with acute toxoplasmosis, was reported from, USA in1998 (20).

Significant changes in personality and psychomotor performance with the neurotropic cysts or secretion of certain metabolites of *Toxoplasma* in infected rodents have been observed (21). The changes in cerebral neurotransmitter due to latent toxoplasmosis could be a component of the alteration in personality attributes of individuals, which can be caused by either the process of pathogenicity or the host and parasite interaction. Many studies were reported high seroprevalence of active or latent toxoplasmosis among populations. This seroprevalence among pregnant women in Finland (22), Denmark (23), Italy (24), France (25), India (26) and Turkey (27) was reported as 20%, 27%, 40%, 84%, 17.2%, and 29.4%, respectively. Flegr et al. observed changes in personality profiles in acute *Toxoplasma*-infected women and found that *T. gondii* induced changes in their personality profiles (28).

It was also reported that latent toxoplasmosis could cause prolonged reaction times and could increase the risk of accidents, especially the traffic accidents. According to this statement, Flegr et al. compared the seroprevalence of latent toxoplasmosis in subjects involved in traffic accidents with general population living in the same area and showed that the subjects with latent toxopla-

Table 2. Distribution of T. gondii seroprevalence according to sex and age group

Variables	Case (n= 103)	Control (n = 200)	OR	95%CI	χ^2	P-value
IgM						
Total	3	8	0.73	0.19 – 2.81	0.209	0.461
Age groups						
19 - 30	2	7	0.55	0.11 – 2.70	0.554	0.364
31 – 44	1	1	1.91	0.11 -30.87	0.215	0.571
45 - 65	0	0	----	----------	------	------
Sex						
Male	3	5	1.2	0.28 – 5.14	0.640	0.533
Female	1	3	0.64	0.66 – 6.26	0.146	0.581
IgG						
Total	38	56	2.02	1.23 – 3.33	7.84	0.004 *
Age groups						
19 - 30	30	49	2.15	1.2 – 3.85	6.91	0.007*
31 – 44	5	5	0.83	0.18 – 3.72	0.057	0.555
45 - 65	3	1	0.12	0.009 -1.74	2.94	0.155
Sex						
Male	38	29	1.20	0.66 – 2.19	0.383	0.321
Female	0	27	1.51	0.13 -17.38	0.114	0.582
Comparing the Igs seropositivity						
IgM	3	8	1.62	0.99 – 2.67	3.65	0.09*
IgG	38	56				

** Significant difference; OR, Odds ratio; Igs, Immunoglobulins*

smosis have significantly increased risk (OR:2.65, 95%CI: 1.76 - 4.01) of traffic accidents than the toxoplasmosis-negative subjects (19). Yereli et al. investigated the incidence of *T.gondii* among the population who were involved in a traffic accident while driving and reported significantly higher rates of seroprevalence in the subjects with latent toxoplasmosis than the toxoplasmosis-negative subjects (29). They also reported higher risk in male than female and different age groups for IgG seropositivity. In our study, significantly higher risk of IgG seropositivity was found in ≤30 age group compared to control. In agreement with the present study Flegret al. also showed significant antibody seropositivity in 15-29-year age group (19). Hence, Yereli et al. reported significant increased risk in more than 30-years age also. One of main reason of our finding returns to cultural difference between Iran and western countries. Increasing number of stray cats and environmental infection, Nutritional behavior and life style may increase chance of toxoplasmosis therefore personality changes due to increased accidents happen.

5. Conclusion

There is an increased risk for traffic accidents for drivers owing to these high seroprevalence of latent toxoplasmosis. Latent toxoplasmosis in drivers should be taken into account while developing preventive strategies for traffic accidents in Iran.

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Identification and determination of the study of *Pneumocystis* in lungs of immune competent laboratory rats using Nested PCR

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Abstract

Pneumonia caused by *pneumocystis* species is a serious disease with high mortality in most immunodeficiency mammals. The use of immunosuppressed rat as an animal model is of particular importance in experimental studies. Immunosuppression of laboratory rates predisposes them to PCP (*Penumocystis carinii* pneumonia). The purpose of this study was to detect and determine the study of *pneumocystis* in the natural cycle of immune competent laboratory rat in experimental studies. 35 rats of Sprague Dowley race were providing by Razi Institute for Serum and Vaccine. The rats were autopsied according animal ethic committe, and their lungs were isolated in sterile conditions. PCR was conducted after purification of DNA by specific primers for Pneumocystis species. *Pneumocystis* DNA was positive in primary amplification with a 346bp product using PAZ-102-E and PAZ102-H primers in 10 rats (28.57%). Nested PCR was performed on negative PCR products using PAZ-102-X and pAZ102-W primers, and the *pneumocystis* genome was present in 8 (24.24%) rats. Genome amplification of Pneumocystis wakefieldiae was positive with a 251bp product in 10 rats (28.57%) by RR1 and RR2 primers. The results also showed that 18 rats (51.42%) were infected with *Pneumocystis wakefieldiae* and *Pneumocystis carinii.* Statistical analyses showed that there wasn't a significant correlation between the existence of *Pneumocystis carinii* and *Pneumocystis wakefieldiae genomes* with sex and weight ($p < 0.05$).

Laboratory rats are naturally infected with *Pneumocystis carinii* and *Pneumocystis wakefieldiae*. Compromised cellular immunity in these rats can cause PCP infection. There is a high incidence of *pneumocystis* infestation in laboratory rats. In order to eliminate the interfering factor of lethal pneumonia due to this organism it is recommended to use anti-*pneumocystis* prophylaxis in studies in which immunosuppressed rats are used.

Key words: *Pneumocystis carinii*, *Pneumocystis wakefieldiae*, Laboratory Rats, Nested PCR

Introduction

To date, rodents such as mice and rats comprise more than 90% of animals used in laboratory research. Rats and humans are both mammals, and there are many similarities in characteristics and function of their genetic structure. Small size, easy transport, rapid reproduction, low price and easy handling of these animals has caused rats to be the most frequently used as experimental animals (1). *Pneumocystis* was described for the first time in 1909 in Brazil by Carlos Chagas during his study of *T.cruzi* infection in human and animal models (2). The five species of *Pneumocystis* identified in mammals can cause life-threatening opportunistic pneumonia in animals and immunocompromised humans (3). Animal models of *Pneumocystis* are important in research on this organism. As there is no stable in vitro reproduction system for this organism, the researchers have relied on animal models in order to have a source of organism to evaluate pharmacological responses. Similarity in pathogenesis and biology of this organism with humans is a reason for using of rat animal model in basic research (4). Studies are showed that im-

paired cellular immune system is a risk factor for P C P (5). Impaired cellular immune response by administration of immunosuppressive agents such as corticosteroids, and malnutrition or wounds from fights with rats or other animals in natural ecosystem can predispose these rodents to PCP(6). In our country, like other countries of the world, the use of laboratory rats in experimental studies is universally accepted. The aim of this effort was to diagnose and determine the study of *Pneumocystis* molecularly in natural cycle of laboratory rats used in a variety of experimental studies in one of the reference laboratories of Iran.

Materials and Methods

Seventeen female and eighteen male Wistar rats of Sprague Dowley race weighting 150-200 grams were provide by Animal Production Center of Razi Institute of Vaccine and Serum. Under sterile conditions according animal ethic committe, the rats were autopsied, followed with lung biopsy. Twenty five grams of left lung of each rat was isolated, and was kept at -20°C.

Homogenizing lung tissue and DNA extraction

The samples were homogenized using Heidolph DIAX 600 Homogenizer by 5 ml sterile PBS, and the resulting homogenate was passed through a three-layer sterile gauze to clear the homogenate. The collected liquid was poured into a test tube. 2.5ml dithiotritol was added to it, and then incubated for 15 min at 37 °C. For lysis of host cells and RBC, 2.5 ml of 0.85% ammonium chloride (PH = 6) was added and incubated in laboratory temperature for 3 min. Then, the liquid was centrifuged for 15 min in 3000 rpm. 1 ml of the sediment in the bottom of tube was decanted to extract DNA. Pneumocystis genome was extracted using Bionner Company kit.

PCR

MtLSUrRNA Gene amplification was done in primary PCR using PAZ-102-E and PAZ102-H primers, and PAZ-102-X and pAZ102-W primers in secondary PCR of both *Pneumocystis carinii* and *Pneumocystis wakefieldiae* (Table 1).

Nested PCR was performed using RR2 and RR1 primers of primary PCR product. The final reaction mixture and thermal protocol of the reaction were according Palmer and et al in 2000(7).

Positive and negative controls: The isolated and sequenced positive control of Pneumocystis in rat animal model with suppressed immune system with gene bank number GenBank: EF646865.1 was obtained from Razi Vaccine and Serum Institute. Deionized distilled water was used instead of template as negative control.

Statistical test

X^2 and T- test was used as significance tests to assess primary amplification and nested PCR with weight and sex of laboratory rats of the study.

Results

In this study, there were 18 male (51.4%) and 17 female (48.6%) rats in a total of 35 rats. 9 rats weighted 210-233 kg, 11 rats 224-237 kg and 15 rats 238-251 kg.

In primary amplification of *Pneumocystis* DNA by PAZ-102-E and PAZ102-H primers for both *Pneumocystis carinii* and *Pneumocystis wakefildiae*. Among 35 rats, 25 (71.4%) were negative and 10 (28.67%) were positive for *Pneumocystis* in respect to 346bp product.

Nested PCR was conducted using PAZ-102- X and pAZ102-W primers from primary PCR product to detect both *Pneumocystis carinii* and *Pneumocystis wakefildiae*. The results showed that in Nested PCR amplification by a 261bp product

Table 1. Characteristics of the primers used in this study

Primer	Sequence	TM	Primer type	Amplified band
pAZ102-H	GTGTAC GTT GCA AAG TAC TC	55.3	Forward	347bp
pAZ102-E	GAT GGCTGT TTC CAA GCC CA	59.4	Reverse	
pAZ102-X	GTG AAA TAC AAA TCGGAC TAGG	59	Forward	261bp
pAZ102-W	TCAATAACAATTTTGGAACTTTATA	57.4	Reverse	
RR1	GTAGATAGCTTAATAAGGATG	49.3	Forward	251bp
RR2	TTCTTGACTGTCTATGAAGT	51	Reverse	

18 rats (51.4%) were positive for *Pneumocystis carinii* and *Pneumocystis wakefildiae* but 17 rats (48.6%) were negative.

Nested PCR using species-specific RR2 and RR1 primers was done to detect *Pneumocystis wakefildiae*. Of the 35 laboratory rats, 10 (28.6%) were positive for *Pneumocystis wakefildiae* by a 251bp product but 25 rats (71.4%) were negative for this product (Figure 1).

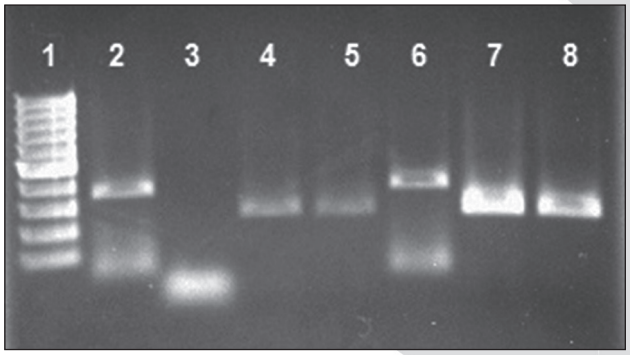

Figure 1. Lane 1: 100 b DNA ladder; Lane 2: positive control; Lane 3: negative control; Lane 5 and 6: positive Pneumocystis sample in secondary amplification, Lane 7&8: positive samples of Pneumocystis wakefildiae

Statistical analysis showed no significant difference between primary and secondary amplification of *Pneumocystis* species with weight and sex of laboratory rats.

Discussion

Pneumocystis carinii and *Pneumocystis wakefieldiae* are two distinct species found individually or jointly in the lungs of rats, and have the ability to cause fatal pneumonia in immunosuppressed rats (8). Detection of *pneumocystis* species is often done by routine staining techniques such as gomori methenamine silver staining as the gold standard to detect PCP (9). Molecular methods have higher sensitivity than microscopic methods to detect *Pneumocystis* (10). According to previous studies, the sensitivity of lung homogenates samples for molecular diagnosis of *Pneumocystis* has been higher than oral swab and BAL lavage samples. (11). we used lung homogenate samples from immune competent laboratory rats in our study. In a study of molecular diagnosis, Cushion et al showed that 80%, 98% and 100% of newborn rats acquired Pneumocystis from environmental sources within 2, 24 and 48 hours after birth, respectively. The main source of Pneumocystis to infect newborn rats is outdoor air (12). *Pneumocystis* DNA amplification in aerosols where laboratory rats are kept promotes air transfer of *Pneumocystis carinii* (13). Increased animal age and development of the immune system seems to restrict the function of Pneumocystis in animal body.

Obtained Lung samples of wild rats in natural ecosystems are negative using staining technique for *Pneumocystis carinii* but these rats are susceptible to PCP in case of immunosuppression (14). Molecular methods can identify Pneumocystis wakefildiae and *Pneumocystis carinii* in the natural cycle of wild rats. Infection by *Pneumocystis wakefildiae* and *Pneumocystis carinii* can be found alone or together in the lungs of wild rats (15). In the study of Wakefield et al, *Pneumocystis carinii* was observed in lung homogenate of 8.33% of immune competent laboratory rats, and *Pneumocystis wakefieldiae* was confirmed in 83.33% of them by Nested PCR (7). In view of infection by a single strain of *Pneumocystis*, *Pneumocystis wakefieldiae* is more prevalent, which is consistent with our results. In immunosuppressed rats afflicted with PCP, the disease control and treatment is done in case of appropriate prophylaxis(16). Our study shows that *Pneumocystis carinii* and *Pneumocystis wakefieldiae* are naturally present in lung alveoles of immune competent laboratory rats. Both strains can opportunistically cause Pneumocystis carinii PCP in these rodents. Therefore, proper prophylaxis to control *Pneumocystis* is recommended in studied in which requiring suppression of the immune system to prevent PCP and to eliminate the interference factor of this disease.

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Clinical outcomes following posterior cruciate ligament reconstruction in multi-ligament knee injuries; A midterm follow up study

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Abstract

Background: Posterior cruciate ligament injury is one of the most severe injuries to lower extremities and is associated with high morbidity. The purpose of this study was to evaluate midterm results of patients with multi-ligament knee injuries that were undergone posterior cruciate ligament reconstruction with or without repair or reconstruction of other ligaments.

Methods: Twenty nine patients with isolated PCL or multi-ligament knee injuries were enrolled in this retrospective study. Demographic data, type of trauma, trauma to surgical interval, type of ligamentous injury (anatomical classification) and surgical techniques were collected. Activity level in compare to pre-injury level, patient return to work and sport activities , performance of reconstructed knee in comparison with to healthy opposite knee, degree of knee pain, amount of remaining posterior laxity and clinical score (based on HSS) were assessed at the last follow-up.

Results: At latest follow-up evaluation, HSS knee ligaments rating scale mean value was 74.2 in all patients. There was no pain in 13 patients (44.8%), but remaining patients experienced various degrees of pain. Except for one patient (3.4%), all others had no restrictions regarding activities of daily living and work (96.6%) while only 3 patients (10.3%) regained their previous competitive level in sports. Despite reconstruction, mean activity level (based on IKDC scoring system) had significant difference compared to pre-injury activity level. Regarding knee performance, no patient achieved to 100% of *opposite normal knee level*, but 68% of patients reported over 70 %. Ten patients (34.5%) were rated as grade 0 on posterior drawer testing, 12 patients (41.4%) in grade 1+, 5 patients (17.2%) in grade 2+ and 2 patients (6.9%) in grade 3+. With regard to post-operative laxity, at least one degree of posterior laxity was left in 66% of patients.

Conclusions: Although in multi-ligament knee injuries, more than half of the patients have satisfactory results; many patients remain with some degree of ligamentous laxity, postoperative pain and reduced ability in sports and activities of daily living.

Key words: Knee dislocation, multi-ligament knee injuries, posterior cruciate ligament.

Introduction

Posterior cruciate ligament (PCL) injuries account for 3% to 23% of all knee *ligament* lesions [1-4]. *Isolated PCL injury* occurs less common and more often presents in combination with other ligament injuries [2-4]. *Multiple ligament knee injuries* (MLKI) are very serious injuries of lower extremity that *usually occur following* knee dislocation and lead to major morbidities before and after surgical intervention. These dislocations are typically caused by high energy motor vehicle accidents or low energy sport traumas. Determining the natural history of injury is intended to assist in *making* the best *decision.* Recent recommendations for a diagnostic and treatment *algorithm have been published* in previous literature. But much *controversy exists regarding* the optimal treatment [2-6]. Conservative treatment is very effective in treating patients with partial *PCL injuries*, while more *severe* injuries; especially those combined with other ligament involvement are the indications for operative treatment *[7].*

Finding the role of PCL in *knee biomechanics and stability,* most *studies* reported the *results* of *PCL reconstruction techniques.* However despite advances in surgical methods, complications such as residual posterior knee laxity after PCL reconstruction are not unusual[8]. The optimal PCL reconstruction is challenging. To restore the best

function of a normal knee and functional outcome is of high importance; therefore, we attempt to review our experience of PCL reconstruction in patients with multi-ligament knee injuries regarding functional outcome and complication rate.

Materials and methods

We retrospectively reviewed the records of all 29 patients who had isolated PCL or combined PCL and other knee ligaments reconstructions between 2003 and 2010. Data were collected from the department of knee surgery in the Shafa Yahyaeean hospital over a period of 7 years. Inclusion criteria were PCL tear and minimum 1 year of follow up. The exclusion criteria were fractures around the knee joint and neurovascular injuries. All patients were recalled specifically for this study. This study was approved by our Institutional Review Board and all subjects signed informed consent. A single orthopaedic surgeon who was not involved in the treatment process, examined all patients in their last follow-up visit. Demographic data, type of trauma, trauma to surgical interval, type of ligamentous injury (based on anatomical classification) and surgical techniques were collected. Degree of knee dislocations was classified base on anatomic classification of knee dislocations proposed by Wascher[9]. Activity level in comparison with pre-injury level (based on IKDC scoring system), patient return to work and sport activities, performance of reconstructed knee compared to healthy opposite knee, degree of knee pain, amount of remaining posterior laxity on posterior drawer test and clinical score based on hospital for special surgery knee ligament score (HSS Score) were assessed at the last follow-up visit.

Descriptive statistics consisted of means, ranges, and standard deviations were reported. Indipendnt ant paired *t* tests, Mann Whitney U test and chi-square tests were used for data analysis.

Results

Demographic data

Twenty nine patients aged between 18–51 (mean, 27.86) years at the time of surgery included in this study. There were 6 females and 23males. Twenty three patients (79.3%) sustained high-energy trauma and 6 (20.7%) had low-energy trauma during sports activities. Of all, 5 had isolated PCL injury, and 24 had combined PCL and other ligaments injuries as follows: with ACL (n=2); ACL and PLC (n=12); ACL and MCL (n=4); PLC (n=3); MCL (n=2); and ACL, PLC and MCL (n=1). Based on anatomical classification, 11 (37%) of patients were KD I, 1 (3.4%) KD II, 13 (55%) KD III and 1 (3.4%) KD IV. Transtibial or Inlay methods were used for ligament reconstruction. Surgical techniques were open inlay in 16 of patients (55.2%), and arthroscopic transtibial in the 13 others (44.8%). Of 29 patients, 13 patients underwent isolated PCL reconstruction and the 16 underwent concomitant ACL (n=3), MCL (n=1), and PLC (n=12) reconstruction. Eight patients 27.5% underwent surgery within three months from injury and 21 (72.5 %) after 3 months. The average ±SD of follow-up duration was 32.9±21.47 months (range, 12–88 months, median=27.00). PCLs were reconstructed with Achilles tendon allograft in 22 cases (75.9%), Hamstring autograft in 3 cases (10.3%), Tibialis anterior allograft in 3 cases (10.3%) and Tibialis posterior allograft in 1 case (3.4%). All patients in Inlay group received Achilles tendon allograft. Demographic and follow up characteristic of patients have shown in table 1.

Follow-up evaluation

At latest follow-up evaluation, HSS knee ligament rating scale mean value was 74.2 ±١٥,٢ in all patients. The mean value of HSS was 72.6±8 in transtibial group and 75.5±16.7 in Inlay patients (P value=0.62). High energy group had mean value of 72.78+/-16.2 HSS and low energy group had 79.6+/-10.4 (P value=0.33). The mean scores for patients operated within and after 3 month were 67.7 and 76.6 respectively (P value=0.16). HSS scores according to KD class were as following: 81, 60, 69, and 73 in KD I to KD IV respectively. Mean HSS score in patients with isolated PCL injury was 75.20±8.40 and in MLKI group was 74.00±16.47 (P value=0.88).

With regard to symptoms, there was no pain in 13 patients (44.8%), 8 patients had suffered pain after stressful sports, 2 (6.9 %) patients complained of pain with daily activities and 6 (20.7%) had occasional pain. None of patients experienced

Table 1. Demographic and follow up characteristic of patients

Patient nomber	Age	SSex	TType of trauma	Trauma to surgery interval	Surgical technique	Follow up duration	Anatomic classification	Injured ligament	Type of graft	Reconstructed ligament	Pain	Comparing opposite Knee	Return to work	Return to sport	PDT	HSS score	Pre- injury activity level	Post reconstruc-tion avtivity level
1	29	M	H.E	9	Transtibial	36	KD I	PCL	Tibialis anterior allog.	PCL	Stress activity	80%	Full	Same, decrease	2 +	88	2	1
2	22	F	H.E	1	Transtibial	23	KD III L	PCL,ACL,PLC	Achilles allograft	PCL,PLC	Stress activity	60%	Full	No return	1 +	60	1	1
3	30	M	H.E	0.5	Inlay	87	KD IV	PCL,ACL, PLC,MCL	Achilles allograft	PCL,PLC	Stress activity	90%	Full	Same, decrease	0	73	3	3
4	51	M	H.E	2	Inlay	14	KD III L	PCL,ACL,PLC	Achilles allograft	PCL,PLC	occasional	50%	Full	Different	2 +	48	3	2
5	30	M	S	8	Inlay	48	KD I	PCL,PLC	Achilles allograft	PCL,PLC	Stress activity	80%	Full	Same, decrease	1 +	86	3	2
6	22	M	H.E	25	Inlay	60	KD III L	PCL,ACL,PLC	Achilles allograft	PCL,PLC	No pain	75%	Full	Same, decrease	2 +	87	3	2
7	19	F	H.E	7	Inlay	34	KD III L	PCL,ACL,PLC	Achilles allograft	PCL,PLC	No pain	70%	Full	Different	1 +	58	3	2
8	24	M	S	1.5	Inlay	46	KD III L	PCL,ACL,PLC	Achilles allograft	PCL	No pain	90%	Full	Different	1 +	73	2	2
9	32	M	H.E	11	Inlay	33	KD I	ACL,PLC	Achilles allograft	PCL,PLC	occasional	95%	Full	Full	0	94	3	2
10	36	M	H.E	10	Transtibial	39	KD III L	PCL,ACL,PLC	Tibialis anterior allog.	PCL	occasional	40%	Unable	No return	0	68	3	1
11	21	M	H.E	8	Transtibial	13	KD III L	PCL,ACL,PLC	Hamstring autograft	PCL	No pain	80%	Full	Different	3 +	73	3	1
12	30	F	H.E	4	Inlay	12	KD I	PCL,MCL	Achilles allograft	PCL	occasional	95%	Full	Full	0	89	2	2
13	46	F	H.E	13	Transtibial	36	KD III M	PCL,ACL,MCL	Achilles allograft	PCL,MCL	Daily activity	60%	Full	Different	1 +	41	2	1
14	19	M	H.E	7	Transtibial	13	KD III M	PCL,ACL,MCL	Achilles allograft	PCL,MCL	No pain	50%	Full	Different	1 +	79	2	1
15	22	M	S	36	Inlay	57	KD III L	PCL, ACL,PLC	Achilles allograft	PCL,ACL	No pain	60%	Full	Different	0	75	3	1
16	35	M	H.E	12	Transtibial	27	KD III M	PCL,ACL,MCL	Achilles allograft	PCL,ACL	Stress activity	90%	Full	Same, decreased	2 +	83	2	1
17	22	M	S	12	Inlay	18	KD I	PCL,MCL	Achilles allograft	PCL	No pain	80%	Full	Same, decrease	2 +	93	3	2
18	41	F	H.E	24	Transtibial	13	KD I	PCL	Tibialis anterior allog.	PCL	No pain	80%	Full	Same, decrease	0	74	3	2
19	23	M	H.E	9	Inlay	12	KD I	PCL	Achilles allograft	PCL	occasional	85%	Full	Full	1 +	72	2	2
20	28	M	H.E	55	Inlay	63	KD I	PCL,PLC	Achilles allograft	PCL,PLC	Stress activity	70%	Full	No return	1 +	75	3	2
21	23	M	S	9	Transtibial	26	KD III L	PCL,ACL,PLC	Hamstring autograft	PCL	No pain	80%	Full	Different	1 +	86	3	2
22	30	M	H.E	2	Inlay	12	LD III L	PCL,ACL,PLC	Achilles allograft	PCL,PLC	Stress activity	50%	Full	No return	1 +	38	2	1
23	22	M	S	9	Inlay	13	KD I	PCL	Achilles allograft	PCL	Stress activity	40%	Full	Same, decrease	3 +	65	3	1
24	21	M	H.E	36	Inlay	27	KD I	PCL,PLC	Achilles allograft	PCL,PLC	No pain	70%	Full	Different	0	87	3	2
25	25	M	H.E	5	Transtibial	24	KD II	PCL,ACL	Hamstring autograft	PCL	Daily activity	50%	Full	No return	0	60	3	1
26	18	F	H.E	3	Transtibial	29	KD III M	PCL,ACL,MCL	Tibialis posterior allo.	PCL	occasional	70%	Full	Different	1 +	65	3	2
27	20	M	H.E	1	Transtibial	40	KD III L	PCL,ACL,PLC	Achilles allograft	PCL,PLC	No pain	80%	Full	Different	0	90	3	1
28	45	M	H.E	5	Transtibial	12	KD I	PCL	Achilles allograft	PCL	No pain	80%	Full	Different	0	77	2	2
29	22	M	H.E	O.5	Inlay	88	KD III L	PCL,ACL,PLC	Achilles allograft	PCL,ACL,PLC	No pain	80%	Full	Different	1 +	95	3	2

continuous pain. Pain in the transtibial group was not significantly different from inlay group patients (P value=0.27).

Subjectively, except for one patient (3.4%), all others had no restrictions regarding activities of daily living and work (96.6%), while only 3 patients (10.3%) regained their previous competitive level in sports. Thirteen patients (44.8%) changed their sports, 8 patients (27.5%) got back to the same but decreased level of sports and 5 others (17.2%) ceased sport activities.

Subjectively, the mean rank activity levels based on IKDC score were 2.62 and 1.62 pre-injury and after reconstruction respectively (P<0.00). Regarding knee performance, no patient reached to 100% of *opposite normal knee level*, however 68% of patients reported over 70% of performance.

At 90° of knee flexion and the tibia in a neutral position 10 patients (34.5%) were rated as grade 0 on posterior drawer testing, 12 patients (41.4%) in grade 1+, 5 patients (17.2%) in grade 2+ and 2 patients (6.9%) in grade 3+. With regard to postoperative laxity, at least one degree of posterior laxity was left in 66% of patients. Inseventeen patients with combined PCL/PLC injury, 12 of the were reconstructed their PCL and PLC and 5 only for their PCL. Of 12 combined reconstructions, 4 achieved to normal PDT and 5 rated in grade 1+ and in only PCL reconstructed group 2 got normal PDT, 2 rated in 1+ and 1 in 3+.

Discussion

In the current study PCL reconstruction improved subjective knee scores. By HSS scoring, 68% of our patients achieved to excellent or good results after 12-88 months follow up. Also based on patients self-reported scoring, except for one patient (3.4%), all others related no restrictions regarding activities of daily living and work (96.6%), however we didn't achieved to satisfactory return to athletic activities. Despite reconstruction, activity level had significant difference with before injury. Regarding knee performance, no patient achieved to 100% of *opposite normal knee level*, however 68% of patients reported over 70%. With regard to objective outcome, at least 1 degree of posterior laxity was present in 19 patients (66.5%). Although , severe swelling in acute PCL injuries disturb the reliability of PDT [10] It is a sensitive test to assess chronic PCL lesions [11].

There was no significant difference between transtibial and Inlay groups and low and high energy groups in terms of the HSS score, the PDT and the functional outcome. Also HSS score did not differ significantly between patients reconstructed within 3months versus those operated after 3 months.

Mean value of HSS knee ligament rating scale was 74.2 in our patients post operatively. We did not study preoperative score of HSS. We had no significant difference between isolated and combined group's results based on HSS scoring. In a study by Garofalo et al [12] grade III isolated chronic PCL tears in 15 patients reconstructed by double-bundle trans-tibial technique. They reported improved HSS scoring scales from 23.06 to 80.7 after 2-year follow-up. In another study by Fanelli and Edson [13] 35 patients with MLIK treated by arthroscopically assisted single-bundle PCL reconstruction. They reported postoperative mean HSS knee score as 86.8 after 2 to 10- year follow-up. Other studies also reported PCL reconstruction result to improvement by Lysholm scores and International Knee Documentation Committee scoring [12, 14-15]. However, the optimal treatment of PCL injuries remains still controversial and it seems in depends on surgeon decision considerably.[16]

Traditionally, arthroscopically assisted single-bundle transtibial technique was used to reconstruction of PCL tears. Although posterior knee laxity can improve by 1 grade in this procedure for isolated PCL lesions, but residual posterior knee laxity and degenerative osteoarthritis were not unusual by this method.[2] Therefore double-bundle PCL reconstruction developed. This technique has been shown to more closely restore normal knee laxity and biomechanics in vitro.[17] But, there is no definite evidence to show superiority of the double bundle PCL reconstruction to the single bundle technique.[18] The both single-bundle and double-bundle transtibial technique result a sharp turn of graft, the "killer turn". Because of this occurrence, the tibial Inlay technique was developed to decrease weakening of the graft.[3, 19-21] Less anterior-posterior laxity in the tibial Inlay group after cyclic loading of the knee were observed by Bergfeld et al[21] and Markolf et al[19] . McAl-

lister et al[22] did not observed any significant differences in knee laxity fixation between tibial tunnel and inlay reconstructions at the time of initial graft fixation, but there was more posterior laxity after cyclic loading in first group.

In this study we didn't found any significant difference between transtibial and Inlay group regarding PDT after 12-88 months follow up. In a systemic review Papalia et al [23] encountered the lack of published randomized clinical trials and reported findings to determine whether the tibial inlay for PCL reconstruction is reliable alternative to transtibial techniques.

The debate also exists in the superior graft source for PCL reconstruction. Allogeneic and autogeneic tissue have their own advantages and disadvantages. A small number of data exists comparing allogeneic and autogeneic PCL reconstruction.[24] The most commonly used graft in the North America for PCL reconstruction is the Achilles tendon allograft.[18] In Japan, autografts of the Bone-Tendon-Bone and hamstring are generally used.[25] From our patients 22 (75.9%) were received Achilles tendon allograft. Although all combined cases were received Achilles tendon allograft, there were no significant difference in results. It may be indicate the superiority of this graft. Also one patient who experienced three ligament (PCL/ACL/PLC) reconstruction using an Achilles tendon allograft had 95 scale based on HSS. Fanelli et al [18] reported good long term results with the use of this graft for combined PCL and PLC reconstruction . 70% of patients present normal posterior drawer test in their study and 1 grade of laxity was reported in 27% at latest follow up. While we had only 32% of grade 0 PDT in as Achilles group and 45.5% of 1 grade laxity. Shi et al used this graft in the patient with combined ACL/PCL injuries and followed them up for 38 months. Despite they showed good results, any patients achieved to a normal PDT at least follow up. 86.7% had a grade 1 and 13.3% grade 2 posterior drawer tests in their study.

In the setting of a combined PCL/PLC injury, untreated PLC injury has reported to increase load in the PCL graft and result in PCL graft failure. By the same, reconstruction of both PCL/PLC indicated better restore normal knee kinematics [26-27]. Of our 17 patients with combined PCL/PLC injury, in 12 their PCL were and PLC reconstructed and 5 only for PCL. Because of limited number of subjects, we didn't assess this outcome between two groups.

Limitations

Because having the small number of patients and heterogeneity of injury, it was difficult to compare the subgroups. Furthermore, various surgical reconstruction techniques and different type of grafts made comparisons difficult.

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Study the status of peripheral eosinophilia in patients infected with intestinal parasites in the city of Shushtar, Southwest Iran

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Abstract

Aim: Intestinal parasitic infections are still the major health problems in many parts of the world. Most of the infection agents, almost exclusively helminth parasites elicit eosinophilia. Although peripheral eosinophil is common in some of the intestinal helminthic infections, but some report showed association of intestinal and tissue protozoa and eosinophilia. The purpose of this study was evaluation of peripheral eosinophil in patients infected with intestinal parasites in the city of Shushtar southwest Iran.

Methods: Stool samples of 300 individuals referred to the central laboratory of Shushtar Khatamolanbia hospital were examined by the method of wet mount smears using normal saline and working Iodine. Peripheral blood samples of patients infected with intestinal parasites were collected and prepared smears stained with Wright- Eosin- methylene blue and examined for eosinophil count.

Results: Fifty (16.6%) out of 300 cases were infected with intestinal parasite (26 male and 24 female). 38 (12.6%) cases were infected with *Giardia lamblia*, 5 (1.6%) with *Entamoeba histolytica/ dispar*, 4 (1.3%) with *Entamoeba coli*, 1 (0.3%) with *Cryptosporidiun Sp*, 1 (0.3%) with *Trichomonas hominis* and 1 (0.3%) with *Hymenolepis nana*. Blood eosinophil was observed in 38% of infected patients. The range of observed eosinophilia was from 2- 7% and the average of 7.7%, interestingly that eosinophil in patient infected with *Cryptosporidium* was17%, in *Hymenolepis nana* 5%, and the average of eosinophil in *Entamoeba hitolytica/ dspar* 10%, in *Entamoeba coli*, 9% and in *Giardia lamblia* was 4%.

Conclusion: The results of this study indicated that the rate of intestinal parasitic infection decline significantly in southwest of Iran, but Giardia lambli is still prevalent and proved that intestinal protozoa can induce local and peripheral eosinophilia and in individuals with hypereosinophilia, protozoa infection should be considered as well.

Key words: Eosinophilia, parasite, infection, Iran

Introduction

Intestinal parasitic infections are still the major health problems in many parts of the world and about 3.5 billion people are infected by intestinal parasites [1, 2]. Although most of the intestinal parasites, especially the helminthes are reduced significantly, but those who are transmitted directly from man to man are still prevalent [3]. Eosinophils are leukocytes derived from bone marrow and their development and terminal differentiation are under the control of several cytokines that are responsible for eosinophilopoesis, Eosinophilia, defined as 450-500 eosinophils / ul is normally measured by sampling peripheral blood, (4). The degree of eosinophilia in parasitic infections is depend on migration, development and distribution in the host as well as by the host' s immune response and their products come into contact with the tissue cells. Most of the infection agents, almost exclusively helminth parasites elicit eosinophilia [5-7]. Although peripheral eosinophil is common in some of the intestinal helminthic infec-

tions, but some report showed association of intestinal and tissue protozoa and eosinophilia very rare in infection with protozoa [8]. In this study the rate of peripheral eosinophil in patients with intestinal parasitic infection in Shushtar city is evaluated.

Methods

Stool samples of 300 individuals referred to the central laboratory of Shushtar Khatamolanbia hospital were examined by the method of wet mount smears using normal saline and working Iodine. Peripheral blood samples of patients infected with intestinal parasites were collected and prepared smears stained with Wright- Eosin- methylene blue and examined for eosinophil count.

Results

Fifty (16.66%) out of 300 cases were infected with intestinal parasite (26 male and 24 female). Thirty-eight (12.6%) cases were infected with *Giardia lamblia*, 5 (1.6%) with *Entamoeba histolytica/ dispar*, 4 (1.3%) with *Entamoeba coli*, 1 (0.3%) with *Cryptosporidiun Sp*, 1 (0.3%) with *Trichomonas hominis* and 1 (0.3%) with *Hymenolepis nana*. Blood eosinophil was observed in 38% of infected patients. The range of observed eosinophilia was from 2 to 17% and the average of 7.7%, interestingly that eosinophil in patient infected with *Cryptosporidium* was17%, in *Hymenolepis nana* 5%, and the average of eosinophil in *Entamoeba hitolytica/ dspar* 10%, in *Entamoeba coli*, 9% and in *Giardia lamblia* was 4% [Table 1].

Discussion

Intestinal parasitic infections cause clinical problems in all around the worlds and are responsible for morbidity and mortality in adults and children and many epidemiological data are available for these areas [9]. The prevalence in the communities may be altered because of changes in social behavior and life styles during years. Different epidemiological studies of such infections will provide better understanding of the health status of these countries. In recent years, several researches have been conducted in different parts of Iran to reveal the status of prevalence of intestinal parasitic infections. All these studies indicated that there is a sharp decline in the prevalence of intestinal parasites compared to those studies of previous 3 decades or before that [10- 15]. Non- pathologic functions of eosinophils and the cationic enzymes of their granules include mediating parasite defense reactions, allergic response, tissue inflammation, and immune modulation [16, 17]. Although, eosinophilia is associated with many disorders, limiting its usefulness as a diagnostic tool in screening expatriates for parasite infections. In addition, only tissue-invasive helminthic parasites cause eosinophilia [18] Protozoa infection such as giardiasis associated with eosinophilia has been reported (19- 21]. Akhlaghi et al in 2006 indicated that 50% of allergic children infected with *Giardia lamblia* were hypereosinophilic [22]. In this study, the majority of cases were infected with *Giardia lamblia,* while, infection with other parasites was lower. The rate of eosinophilia in patients infected with helminthes was lower than those infected with protozoa. In one patient who was infected with *Cryptosporidium* the rate of eosinophil was17%, while in the case infected with *Hymenolepis nana* was 5%.

The results of this study indicated that the rate of intestinal parasitic infection decline significantly in southwest of Iran, but Giardia lambli is still prevalent and proved that intestinal protozoa can induce local and peripheral eosinophilia and in individuals with hypereosinophilia, protozoa infection should be considered as well.

Table 1. Frequency of intestinal parasites in infected individuals and the average of eosinophil

Parasite	No of infected (%)	Eosinophil average (%)
Giardia lamblia	38 (12.67)	4
Entamoeba histolytica/dispar	5 (1.67)	10
Entamoeba coli	4 (1.33)	9
Trichomonas hominis	1 (0.33)	2
Cryptosporidium Sp	1 (0.33)	15
Hymenolepis nana	1 (0.33)	5

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Evaluation of skeletal myotoxicity in statin treated rats receiving local anesthesia with bupivacaine

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Abstract

Objective: To investigate the effect of bupivacaine use in anesthesia on myotoxicity in rats receiving statin therapy.

Design: Experimental animal study in rats.

Setting: Experimental Medical Research Institute, Istanbul University Istanbul Faculty of Medicine, Turkey.

Animals: Thirty adult male Sprague-Dawley rats were divided into 5 groups including control (C), bupivacaine (B), atorvastatin (A), atorvastatin-bupivacaine (AB) and vehicle (M) groups.

Interventions: Blood samples were taken for determination of serum levels for creatine kinase (CK), CK-MB and myoglobin, following 12-day treatment with atorvastatin (A, AB), vehicle (M) or no treatment (C). Bupivacaine hydrochloride (B, AB) or dry (A, C, M) injections were made on day 12, while gluteus maximus muscles were harvested for histopathological examination on day 13. On day 14, rats were sacrificed through cervical dislocation and blood samples were collected by cardiac puncture for the measurement of serum levels for CK, CK-MB and myoglobin in each rat for the second time. **Measurements and Main Results:** Experimental groups were compared in terms of serum levels for CK, CK-MB and myoglobin and histopathological scores for myotoxicity. No significant difference was observed in serum levels for CK, CK-MB and myoglobin in atorvastatin (group A), bupivacaine (group B) and atorvastatin plus bupivacaine (group AB) groups compared to controls.

Conclusions: Our findings revealed no significant effect of atorvastatin, bupivacaine or atorvastatin plus bupivacaine on biomarkers of muscle toxicity (CK, CK-MB and myoglobin) compared to controls and no difference between atorvastatin and bupivacaine treatment groups (A, AB, B) in terms of histopathological markers of myotoxicity.

Key words: Atorvastatin, bupivacaine, muscle, rat, myopathy, myotoxicity

Introduction

Since the publication of "The 4S Study" in 1994, statins have been widely used as cholesterol reducing drugs in the prevention of cardiovascular diseases[1] as well as in primary and secondary prevention of coronary events and stroke.[2] Within the last two decades, use of the statins has increased to more than 100 million prescriptions in a single year.

Statins competitively inhibit 3-hydroxy-3-methlglutaryl-coenzyme-A (HMG-CoA) reductase, the rate-limiting step of cholesterol formation in the liver and decrease the concentrations of low-density lipoprotein cholesterol in blood by blocking the rate-limiting step of the mevalonate pathway.[3]

While the long term statin therapy has been considered to be generally well-tolerated, statin-induced myotoxicity (SIM) has been reported amongst the most important clinical adverse events associated with statin therapy with severity ranging from mild myopathy to serious rhabdomyolysis[3] and for this reason; cerivastatin was withdrawn from markets in 2001.[4]

Increase in serum levels for mevalonic acid derivatives, breakdown of the T-tubular system and subsarcolemmal rupture have been indicated as the possible cellular mechanism underlying the development of SIM.[3,5,6]

Being a frequently used statin derivative worldwide, atorvastatin therapy was also reported to be associated with development of SIM including 0.1% of patients under therapy.[7]

Bupivacaine is a local anesthetic (LA) drug having an amide structure, a short latent time and long effect with higher efficacy in creating sensory than motor block. Due to this feature, it has become a popularly used, safe and effective agent in interventions such as peripheral nerve block, birth analgesia and postoperative analgesia along with chronic pain treatment.[8-12] Moreover, peripheral or/and santral nerve blocks with bupivacaine are used commonly in peripheral arter diseases' pain treatment.[13-15] However, development of myotoxicity under bupivacaine was also demonstrated in rare instances.[10-12]

Based on the statement that myopathic effects of statins can clearly increase depending on the combined drug use,[16] the present experimental study was designed to evaluate the biochemical and histopathological determinants of myotoxicity in skeletal muscles of statin treated rats to receive local anesthesia with bupivacaine. In this way, we searched for the answers of such questions: "Is the use of long-term statin required to be questioned in preoperative period?", "Does the combination of bupivacaine and statin has a negative effect on the skeletal muscle?" and "Should use of statin be paused in the preoperative period when anesthesia with bupivacaine is considered?"

Methods

Animals

Male Sprague-Dawley rats (262-322 g) were housed in 5 separate cages with 6 rats in each cage in an air-conditioned room at a constant temperature of 22±2 ˚C with 12:12 h light/dark cycle and fed a standard diet and water ad libitum. All experiments were conducted at Istanbul University Istanbul Faculty of Medicine Experimental Medical Research Institute. Experimental protocol was approved by the Istanbul University Faculty of Medicine Animal Care and Use Committee (Project no: 28.02.2013/ 25).

Experimental groups

Rats (n=30) were divided into 5 groups including control (group C; n=6, no atorvastatin or bupivacaine treatment), bupivacaine (group B; n=6, no atorvastatin but bupivacaine treatment), atorvastatin (group A; n=6; atorvastatin but not bupivacaine treatment), atorvastatin-bupivacaine (group AB; n=6, atorvastatin plus bupivacaine treatment) and vehicle (group M; n=6, methylcellulose without atorvastatin or bupivacaine treatment) groups. Experimental groups were compared in terms of serum levels for CK, CK-MB and myoglobin as well as histopathological scores for myotoxicity and sensory and motor block rates.

Study procedures

For the first 12 days of the experiment, rats in group C and B received no treatment. A and AB group rats received atorvastatin (Ator® 10 mg film tablet, solved at a dose of 1 mg/kg in 0.5% methylcellulose, Sanovel®, Turkey) at a dose of 10 mg/kg (5 ml/kg) through oral access, while rats in M group received 5 ml/kg 0.5% methyl cellulose (atorvastatin solvent) through oral access for 12 days. A 12-day of drug application period was selected based on the fact that a 12-day span in rat life corresponds to a year in human life.[17]

On the thirteenth day, blood samples were taken from each rat following administration of intraperitoneal sodium pentobarbital (40 mg/kg), for determination of serum levels for creatine kinase (CK), CK-MB and myoglobin (first measurement). After taking blood samples, 1mg/kg 0.25% (0.2 ml) plain bupivacaine hydrochloride (Marcaine® 0.5% injectable solution, AstraZeneca®, Turkey) was injected through the left sciatic nerve in Group B and AB rats, while a dry injection was applied for rats in Group C, Group A and Group M.

On the fourteenth day, left gluteal areas of all rats were shaved under general anesthesia provided with 50 mg/kg intraperitoneal ketamine hydrochloride. Gluteus maximus muscles (approximately 1x1x1 cm) were harvested in each rat for histopathologic examination and kept in 10% neutral formalin solution until the day of analysis. In C group rats, right gluteus maximus muscles were also sampled for histopathological examination.

Thereafter rats were sacrificed through cervical dislocation and blood samples were collected by cardiac puncture for the measurement of serum levels for CK, CK-MB and myoglobin in each rat for the second time.

Bupivacaine hydrochloride injection

For perineural/intramuscular injection; the sciatic nerve of both hind extremities was exposed with

a lateral incision over the thigh and division of the superficial fascia as described before. [18,19] Following the dissection, the sciatic nerve was clearly determined at a point proximal its bifurcation. Under direct vision, all rats received injection of bupivacaine (0.2 ml) into the perineural space under the clear fascia covering the nerve and proximal to the bifurcation of the sciatic nerve. A non-absorbable muscle fascia suture was placed at the midpoint of the injection site as a marker for subsequent nerve removal. The suture was placed in the muscle fascia of the gluteus maximus below the subcutaneous tissue and was neither directly touching nor surrounding the nerve. The incisions were closed.

Sensory and motor block

Sensory processing was evaluated in paw withdrawal response to forceps pinch of the lateral foot/toe. The pinch was limited to a maximum of one second to avoid direct paw tissue trauma. The sciatic nerve block used did not compromise the motor nerves to the hip muscles, and the rats were, thus, able to withdraw the tested paw in return for the pain.[20,21] Sensory responses were evaluated by the withdrawal reflex or vocalization to pinch and quantified as 0= vigorous paw withdrawal to pinch (normal sensory function), 1= moderate withdrawal, 2= minimal withdrawal, 3= full sensory block/no response to pinch.[16,22] Motor function was also determined using the 0-3 scale.

Motor function was quantified as 0= normal motor function, 1= normal dorsiflexion ability and the rat walking with curled toes, 2= moderate dorsiflexion ability and the rat walking with curled toes, 3= no dorsiflexion ability and the rat walking with curled toes.[20,21] Sensory and motor function were evaluated every 30 min until the complete resolution of blockade. All injections were made using an insulin syringe and a 23-gauge needle.

Biochemical analysis for CK, CK-MB and myoglobin values

CK, CK-MB and myoglobin values were biochemically measured again in blood samples. CK (normal range: 33-211 U/L) and CK-MB (normal range: 0-25 U/L) were studied with a Siemens® Advia 1800 biochemistry device through the International Federation of Clinical Chemistry (IFCC) method. Myoglobin (normal range: 0.01-110 ng/mL) was studies with a Siemens® Advic Contour XP device through chemiluminescence method.

Histopathological evaluation of myotoxicity

The histopathological analyses were carried out in the Pathology Department of Istanbul Bagcilar Training and Research Hospital. Histopathological examination was performed by using light microscopic analyses (Olympus®; BX-51). The samples obtained from the muscle tissue were fixed in 10% neutral buffered formalin solution for 2 days. Tissues were washed in running water, and were dehydrated with increasing concentrations of ethanol (50%, 75%, 96% and 100%). After dehydration, specimens were placed into xylene to obtain transparency and embedded in paraffin. Embedded tissues were cut into 5 μm-thick sections and were stained with hematoxylin and eosin (HE). Histopathologic examinations were performed by a pathologist blinded to the study groups. The samples stained with HE were examined for necrotic fibrillar focus, vacuolization, and the presence of hypercellularity (peripheral nuclei) was evaluated in the HE stained samples using a semi-quantitative scoring system. Scoring was as follows: 0_none, 1_mild, 2_moderete, 3_severe of degerations.[23]

Statistical analysis

Statistical analysis was made using computer software (SPSS version 21.0, SPSS Inc. Chicago, IL, USA). Chi-square (χ^2) and Fischer's exact tests were used for the comparison of categorical data, Kruskal-Wallis and Mann-Whitney U tests in the analysis of quantitative data and Wilcoxon test in the analysis of repeated measurements. Data were expressed as "mean± standard deviation (SD)", minimum-maximum and percent (%) where appropriate. $p<0.05$ was considered statistically significant.

Results

First and second measurements for CK

No significant difference was determined between experimental groups in terms of CK levels in the first as well as second measurements. There was a significant decrease from first to second measurement in mean±SD levels for CK in group C (from 1050±495 to 891±498, with -16.6

Table 1. Comparison of first and second measurements for creatine kinase (CK) in the experimental groups

	CK (first)			CK (second)			Variation (%)	p
	Mean ± SD	Median	Min. - Max.	Mean ± SD	Median	Min. - Max.	Mean ± SD	
Group C	1050 ± 495	934	412 - 1666	891 ± 498	680	386 - 1527	-16.6 ± 10.8	0.028
Group A	1182 ± 363	1025	883 - 1751	1121 ± 422	1123	657 - 1715	-6.6 ± 13.5	0.225
Group AB	881 ± 484	901	182 - 1644	891 ± 148	858	760 - 1158	49.6 ± 134.8	0.917
Group B	831 ± 302	871	444 - 1273	778 ± 301	840	307 - 1209	-7.3 ± 13.9	0.116
Group M	819 ± 278	763	519 - 1330	680 ± 196	713	416 - 956	-16.0 ± 9.8	0.028
p	0.362			0.343			0.356	

C: control group (no atorvastatin or bupivacaine), A: atorvastatin group (atorvastatin but not bupivacaine), B: bupivacaine group (no atorvastatin but bupivacaine), AB: atorvastatin-bupivacaine group (atorvastatin plus bupivacaine), M: vehicle group (methylcellulose without atorvastatin or bupivacaine)
Kruskal-Wallis (Mann-Whitney U test) / Wilcoxon test

±10.8% change, p=0.028) and M (from 819±278 to 680±196, with -16.0 ±9.8% change, p=0.028), while the first and second measurements revealed similar findings in other experimental groups. Experimental groups were similar in terms of percent variation in CK levels from first to second measurement (Table 1) (Figure 1).

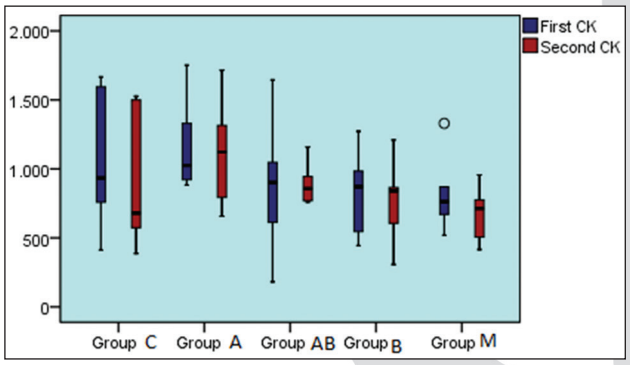

Figure 1. First and second measurements for creatine kinase (CK) in the experimental groups

First and second measurements for CK-MB

No significant difference was determined between experimental groups in terms of CK-MB levels in the first measurement, while the second measurement revealed significantly higher mean ±SD values for CK-MB in the A (2140±827) and AB (1558±284) group rats when compared to M (1003±248) group (p<0.005 for each). There was a significant decrease from first to second measurement in mean±SD levels for CK-MB in group C (from 1999±968 to 1599±869, with -20.6 ±9.2% change, p=0.028) and M (from 1583±546 to 1003±248, with -34.9 ±8.1% change, p=0.028), while first and second measurements revealed similar findings in other experimental groups. Percent variation in CK-MB levels from first to second measurement was significantly lower in A (-7.7±12.1%) and B (14.3±14.7%) groups when compared to M (34.9±8.1%) group rats (p<0.05 for each) (Table 2) (Figure 2).

Table 2. Comparison of first and second measurements for creatine kinase (CK)-MB in the experimental groups

	CK-MB (first)			CK-MB (second)			Variation (%)	p
	Mean ± SD	Median	Min. - Max.	Mean ± SD	Median	Min. - Max.	Mean ± SD	
Group C	1999 ± 968	1809	805 - 3168	1599 ± 869	1229	689 - 2776	-20.6 ± 9.2	0.028
Group A	2277 ± 693	1994	1682 - 3334	2140 ± 827*	2090	1254 - 3339	-7.7 ± 12.1*	0.225
Group AB	1928 ± 502	1892	1176 - 2643	1558 ± 284*	1464	1374 - 2121	-15.8 ± 20.5	0.075
Group B	1494 ± 503	1558	838 - 2266	1299 ± 547	1240	573 - 2265	-14.3 ± 14.7*	0.075
Group M	1583 ± 546	1475	996 - 2589	1003 ± 248	961	691 - 1443	-34.9 ± 8.1	0.028
p	0.177			0.030			0.037	

C: control group (no atorvastatin or bupivacaine), A: atorvastatin group (atorvastatin but not bupivacaine), B: bupivacaine group (no atorvastatin but bupivacaine), AB: atorvastatin-bupivacaine group (atorvastatin plus bupivacaine), M: vehicle group (methylcellulose without atorvastatin or bupivacaine)
Kruskal-Wallis (Mann-Whitney U test) / Wilcoxon test
** p<0.05 compared to rats in group M*

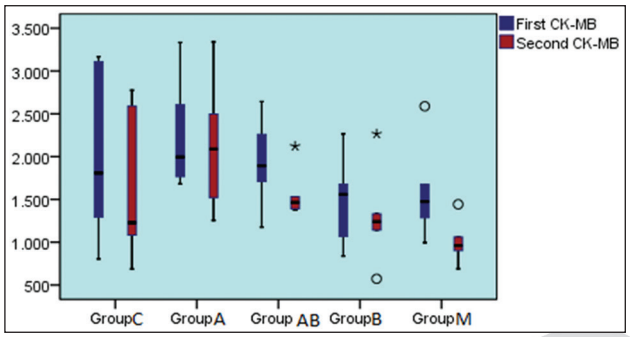

Figure 2. First and second measurements for creatine kinase CK-MB in the experimental groups

First and second measurements for myoglobin

No significant difference was determined between experimental groups in terms of myoglobin levels in the first measurement, while the second measurement revealed significantly higher mean ±SD values for myoglobin in the AB (6.71±0.07), B (6.83±0.14) and M (6.69±0.06) group rats when compared to A (6.59±005) group ($p<0.005$ for each). In each group, no significant difference was noted between first and second measurements for myoglobin.

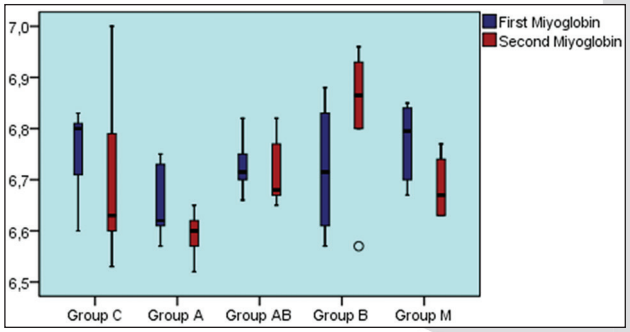

Figure 3. First and second measurements for myoglobin in the experimental groups

Percent variation in myoglobin levels from first to second measurement was also similar between groups (Table 3) (Figure 3).

Necrotic fibrillar focus and vacuolization scores

Based on pathologic scores, percentage of rats without necrotic fibrillar focus was significantly higher in control rats (83.3%) compared with rats in groups A (0.0%), AB (0.0%) and B (16.7%) ($p<0.005$, for each). Mild-moderate necrosis was less common in Group C compared to Group A, Group AB and Group M ($p<0.05$, for each) (Table 4). Similarly, percentage of rats without findings related to vacuolization was ere identified in significantly significantly higher in control rats (83.3%) compared with rats in groups A (0.0%), AB (0.0%) and B (16.7%) ($p<0.005$, for each). Mild-moderate vacuolization was less common in Group C compared to Group A, Group AB and Group M ($p<0.05$, for each) (Table 4). There was no significant difference regarding histopathological myotoxicity findings between the right and left specimens of muscle tissue in Group C (Table 4).

Sensory block rate

Sensory block rate in Group AB and B in the 30^{th}- 60^{th}- 90^{th} – 120^{th} – 150^{th} – 180^{th} – 210^{th} and 240^{th} minutes was significantly higher than the rate in groups C, A and M ($p<0.05$ for each), while no difference was observed between groups in the 270^{th} minute. No significant difference was noted in sensory block rate between Group AB and B in the 30^{th}- 60^{th}- 90^{th} – 120^{th} – 150^{th} – 180^{th} – 210^{th} and 240^{th} minutes (Table 5)

Table 3. Comparison of first and second measurements for myoglobin in the experimental groups

	Myoglobin (first)			Myoglobin (second)			Variation (%)	p
	Mean ± SD	Median	Min. - Max.	Mean ± SD	Median	Min. - Max.	Mean ± SD	
Group C	6.76 ± 0.09	6.80	6.60 - 6.83	6.70 ± 0.17	6.63	6.53 - 7.00	-0.9 ± 2.2	0.248
Group A	6.66 ± 0.08	6.62	6.57 - 6.75	6.59 ± 0.05	6.60	6.52 - 6.65	-0.9 ± 1.8	0.345
Group AB	6.73 ± 0.06	6.72	6.66 - 6.82	6.71 ± 0.07*	6.68	6.65 - 6.82	-0.2 ± 1.4	0.6
Group B	6.72 ± 0.12	6.72	6.57 - 6.88	6.83 ± 0.14*	6.87	6.57 - 6.96	1.7 ± 2.7	0.173
Group M	6.78 ± 0.07	6.80	6.67 - 6.85	6.69 ± 0.06*	6.67	6.63 - 6.77	-1.3 ± 1.6	0.115
p	0.325			***0.032***			0.183	

C: control group (no atorvastatin or bupivacaine), A: atorvastatin group (atorvastatin but not bupivacaine), B: bupivacaine group (no atorvastatin but bupivacaine), AB: atorvastatin-bupivacaine group (atorvastatin plus bupivacaine), M: vehicle group (methylcellulose without atorvastatin or bupivacaine)

*Kruskal-Wallis (Mann-Whitney U test) / Wilcoxon test , *p<0.05 compared to rats in group A*

Table 4. Pathological scores related to necrotic fibrillar focus and vacuolization in the experimental groups

	0		Mild		Moderate		p
	n	%	n	%	n	%	
Pathologic scores for necrotic fibrillar focus							
Group C	5	83.3	1	16.7	0	0.0	p<0.05
Group A	0	0.0*	3	60.0	3	50.0	
Group AB	0	0.0*	6	100.0	0	0.0	
Group B	1	16.7*	5	83.3	0	0.0	
Group M	6	100.0	0	0.0	0	0.0	
Group C / right	5	100	0	0.0	0	0.0	
Pathologic scores for vacuolization							
Group C	5	83.3	1	16.7	0	0.0	p<0.05
Group A	0	0.0*	3	50.0	3	50.0	
Group AB	0	0.0*	5	83.3	1	16.7	
Group B	1	16.7*	4	66.7	1	16.7	
Group M	5	83.3	1	16.7	0	0.0	
Group C / right	5	83.3	1	16.7	0	0.0	

C: control group (no atorvastatin or bupivacaine), A: atorvastatin group (atorvastatin but not bupivacaine), B: bupivacaine group (no atorvastatin but bupivacaine), AB: atorvastatin-bupivacaine group (atorvastatin plus bupivacaine), M: vehicle group (methylcellulose without atorvastatin or bupivacaine)
*Chi-square (Fischer Exact) / *p<0.05 compared to rats in group C*

Motor block rate

Motor block rate in groups AB and B in the 30^{th} – 60^{th} – 90^{th} – 120^{th} – 150^{th} minutes and only in group AB in the 180^{th} minute was significantly higher as compared to groups C, A and M ($p<0.05$ for each), while no significant difference was observed between groups in the 210^{th}, 240^{th} and 270^{th} minutes. Motor block rate in groups AB and B in the 30^{th} – 60^{th} – 90^{th} – 120^{th} – 150^{th} minutes did not demonstrate any significant difference (Table 6).

Discussion

Our findings related to evaluation of skeletal muscle myotoxicity in rats under statin and/or bupivacaine treatment revealed no effects of atorvastatin, bupivacaine or atorvastatin plus bupivacaine on the biomarkers of muscle toxicity including serum levels for CK, CK-MB and myoglobin. While significantly higher scores for histopathological markers of myotoxicity (necrotic fibrillar focus and vacuolization) were noted in rats received atorvastatin and/or bupivacaine than rats in the control group, no difference was noted between atorvastatin and bupivacaine treatment groups (A, AB, B) in terms of histopathological myotoxicity.

Albeit not significant compared to control rats, significantly higher values for CK-MB were noted with atorvastatin therapy than vehicle administration regardless of the concomitant bupivacaine treatment, whereas significantly higher values for myoglobin were noted in rats with bupivacaine as well as atorvastatin plus bupivacaine therapies compared to rats with atorvastatin therapy per se. Being similar in B and AB groups, bupivacaine was associated with a longer term effectiveness in sensory (up to 270 minutes) than motor (up to 150 minutes) block.

Although multiple pathophysiological mechanisms including membrane excitability, mitochondrial function, ubiquinone depletion, impairment of calcium homeostasis, induction of apoptosis and genetic determinants have been suggested to play a role in the development of SIM in patients under statin therapy,[24] the exact mechanism is not yet fully understood. Past studies on the subject revealed inconsistent results. Several studies reported beneficial effects of statins in improvement of endothelial function and functional muscle re-innervation after complete nerve section, stabilization of plaques, stimulation of neovascularization and postoperative cognitive function and reduction in morbidity

Table 5. Sensory block rates in the experimental groups

Sensory block score		Group C	Group A	Group AB	Group M	Group B	p value
		n(%)					
Minute 30	0	6(100.0)*#	6(100.0)*#	0(0.0)	6(100.0)*#	0(0.0)	p<0.05
	1	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	
	2	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	
	3	0(0.0)	0(0.0)	6(100.0)	0(0.0)	6(100.0)	
Minute 60	0	6(100.0)*#	6(100.0)*#	0(0.0)	6(100.0)*#	0(0.0)	p<0.05
	1	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	
	2	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(17.0)	
	3	0(0.0)	0(0.0)	6(100.0)	0(0.0)	5(83.0)	
Minute 90	0	6(100.0)*#	6(100.0)*#	0(0.0)	6(100.0)*#	0(0.0)	p<0.05
	1	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	
	2	0(0.0)	0(0.0)	0(0.0)	0(0.0)	6(100.0)	
	3	0(0.0)	0(0.0)	6(100.0)	0(0.0)	0(0.0)	
Minute 120	0	6(100.0)*#	6(100.0)*#	0(0.0)	6(100.0)*#	0(0.0)	p<0.05
	1	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	
	2	0(0.0)	0(0.0)	3(50.0)	0(0.0)	6(100.0)	
	3	0(0.0)	0(0.0)	3(50.0)	0(0.0)	0(0.0)	
Minute 150	0	6(100.0)*#	6(100.0)*#	0(0.0)	6(100.0)*#	0(0.0)	p<0.05
	1	0(0.0)	0(0.0)	0(0.0)	0(0.0)	3(50.0)	
	2	0(0.0)	0(0.0)	6(100.0)	0(0.0)	3(50.0)	
	3	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	
Minute 180	0	6(100.0)*#	6(100.0)*#	0(0.0)	6(100.0)*#	0(0.0)	p<0.05
	1	0(0.0)	0(0.0)	0(0.0)	0(0.0)	6(100.0)	
	2	0(0.0)	0(0.0)	6(100.0)	0(0.0)	0(0.0)	
	3	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	
Minute 210	0	6(100.0)*#	6(100.0)*#	0(0.0)	6(100.0)*#	0(0.0)	p<0.05
	1	0(0.0)	0(0.0)	6(100.0)	0(0.0)	6(100.0)	
	2	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	
	3	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	
Minute 240	0	6(100.0)*#	6(100.0)*#	1(17.0)	6(100.0)*#	1(17.0)	p<0.05
	1	0(0.0)	0(0.0)	5(83.0)	0(0.0)	5(83.0)	
	2	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	
	3	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	
Minute 270	0	6(100.0)	6(100.0)	6(100.0)	6(100.0)	6(100.0)	p<0.05
	1	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	
	2	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	
	3	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	

C: control group (no atorvastatin or bupivacaine), A: atorvastatin group (atorvastatin but not bupivacaine), B: bupivacaine group (no atorvastatin but bupivacaine), AB: atorvastatin-bupivacaine group (atorvastatin plus bupivacaine), M: vehicle group (methylcellulose without atorvastatin or bupivacaine)
Chi-square test (Fischer test)
*Compared to rats in * group AB, # group B*

and mortality after cardiac and major non-cardiac surgery along with evidence for neuroprotective, antithrombotic, anti-inflammatory and immunomodulatory effects of the drug.[25-27]

On the contrary, given the demonstration of a significant increase in plasma myoglobin concentrations in patients receiving statin treatment, at 5th and 20th min after succinylcholine application that

Table 6. Motor block rates in the experimental groups

Motor block score		Group C	Group A	Group AB	Group M	Group B	p value
		n(%)					
Minute 30	0	6(100.0)*#	6(100.0)*#	0(0.0)	6(100.0)*#	0(0.0)	p<0.05
	1	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	
	2	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	
	3	0(0.0)	0(0.0)	6(100.0)	0(0.0)	6(100.0)	
Minute 60	0	6(100.0)*#	6(100.0)*#	0(0.0)	6(100.0)*#	0(0.0)	p<0.05
	1	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	
	2	0(0.0)	0(0.0)	2(33.3)	0(0.0)	5(83.0)	
	3	0(0.0)	0(0.0)	4(67.0)	0(0.0)	1(17.0)	
Minute 90	0	6(100.0)*#	6(100.0)*#	0(0.0)	6(100.0)*#	0(0.0)	p<0.05
	1	0(0.0)	0(0.0)	0(0.0)	0(0.0)	3(50.0)	
	2	0(0.0)	0(0.0)	4(67.0)	0(0.0)	3(50.0)	
	3	0(0.0)	0(0.0)	2(33.3)	0(0.0)	0(0.0)	
Minute 120	0	6(100.0)*#	6(100.0)*#	0(0.0)	6(100.0)*#	0(0.0)	p<0.05
	1	0(0.0)	0(0.0)	3(50.0)	0(0.0)	6(100.0)	
	2	0(0.0)	0(0.0)	3(50.0)	0(0.0)	0(0.0)	
	3	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	
Minute 150	0	6(100.0)*#	6(100.0)*#	0(0.0)	6(100.0)*#	1(17.0)	p<0.05
	1	0(0.0)	0(0.0)	4(67.0)	0(0.0)	5(83.0)	
	2	0(0.0)	0(0.0)	2(33.3)	0(0.0)	0(0.0)	
	3	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	
Minute 180	0	6(100.0)*	6(100.0)*	1(17.0)	6(100.0)*	1(17.0)	p<0.05
	1	0(0.0)	0(0.0)	5(83.0)	0(0.0)	5(83.0)	
	2	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	
	3	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	
Minute 210	0	6(100.0)	6(100.0)	6(100.0)	6(100.0)	6(100.0)	p<0.05
	1	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	
	2	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	
	3	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	
Minute 240	0	6(100.0)	6(100.0)	6(100.0)	6(100.0)	6(100.0)	p<0.05
	1	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	
	2	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	
	3	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	
Minute 270	0	6(100.0)	6(100.0)	6(100.0)	6(100.0)	6(100.0)	p<0.05
	1	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	
	2	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	
	3	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	

C: control group (no atorvastatin or bupivacaine), A: atorvastatin group (atorvastatin but not bupivacaine), B: bupivacaine group (no atorvastatin but bupivacaine), AB: atorvastatin-bupivacaine group (atorvastatin plus bupivacaine), M: vehicle group (methylcellulose without atorvastatin or bupivacaine)

Chi-square test (Fischer test)

*Compared to rats in * group AB, # group B*

succinylcholine-induced muscle injury has been suggested to be slightly worse in patients receiving statins as compared to those not taking such drugs, while the difference seems unlikely to be clinically significant.[28]

It has been suggested that diseases causing mutations and certain variants in the RYR1 gene may contribute to underlying genetic risk for non-anesthesia-induced myopathies and should be included in genetic susceptibility screening in patients

with severe statin myopathy and in patients with non-SIM of unknown etiology.[29]

Drug interactions, especially use of drugs metabolized via cytochrome P-450 system,[30] were reported to increase the risk for SIM[2] with the incidence ranging from 1.5 to 5.0% in patients receiving statin therapy combined with multiple medications.[31] In most cases, SIM was reported to occur without CK elevation along with no abnormal electromyography findings and normal histopathological evaluation,[32] whereas myalgia, muscle tenderness, weakness, cramps and muscle aches have been suggested as unnoticed SIM related symptoms.[2,32]

SIM related interactions of statin with various medications such as gemfibrozil, sildefanil, colchicine, fusidic acid, omeprazole, macrolide antibiotics, azole antifungals, and cyclosporine were previously reported.[2,32] Using statin therapy as a part of polypharmacy, older age, renal or hepatic failure, personal or family history of muscular symptoms, hypothyroidism, corticosteroid usage, type-1 diabetes, female gender, low body mass index, elevated CK levels and being of Asian population are amongst the risk factors reported for the development of SIM.[32,33]

Owing to rapid increase in use of statins worldwide with 1 out of almost 100 people prescribed with this therapy,[34] an increasing proportion of surgical patients have become receiving simultaneous statin treatment during anesthesia practice with higher likelihood of anesthesiologists to face with a patient under statin treatment.[28] In fact, in most of these patients, statins will be only one class of concomitantly used drugs.

LAs themselves are also known to be associated with myotoxicity and myonecrosis.[35] However, data available on the clinical significance of LA-induced myotoxicity (LAIM) is limited and still unclear with consideration of the likelihood of associated damage not to be clinically significant since the muscle itself regenerates normally.[36,37]After LA administration, the affected muscle fibers were reported to undergo an intrinsic degenerative phase with an increase in pycnotic myonuclei and pathologically condensed chromatin. Nonetheless, the cellular mechanisms, particularly the apoptotic pathway(s) involved in LAIM, are still to be characterized.

Emphasizing the need for a better understanding of the mechanisms of LAIM in order to develop efficient clinical strategies to protect against LA related adverse outcomes, many anesthesiologists do not consider LAIM as a genuine clinical problem given the skeletal muscle injuries after the application of these drugs remain clinically in-apparent in most cases and are expected to be reversible within several weeks.[38] In the present study, we chose the adaptation of accustomed conventional bupivacaine dose in the regional anesthesia in rats along with use of atorvastatin at treatment doses. Atorvastatin was selected in the present study based on the fact that it is the most commonly used statin derivative in Turkey besides its long half-life.

In our study, atorvastatin or bupivacaine treatment was not associated with an increase in biomarkers of muscle toxicity including CK, CK-MB and myoglobin. While significantly higher scores for histopathological markers of myotoxicity were noted in rats received atorvastatin and/or bupivacaine when compared to rats in the control group, no difference was noted between atorvastatin and bupivacaine treatment groups (A, AB, B) in terms of histopathological myotoxicity.

Albeit not clinically significant, there was a significant difference between atorvastatin and bupivacaine treatment groups in terms of serum levels for CK-MB and myoglobin but not for CK. Accordingly, a significant increase in CK-MB levels with atorvastatin compared to vehicle administration and a significant increase in myoglobin levels with bupivacaine compared to atorvastatin was noted in the present study.

Our findings related to lack of significant increase in serum CK levels between experimental groups and also between atorvastatin and bupivacaine treatment groups are in line with the statement that serum CK elevation is mostly not a common finding in and thereby not a reliable indicator of SIM,[32] while a 10-fold increase in upper limit of normal (ULN) of CK levels was considered in favor of statin-induced rhabdomyolysis. [32]

Whilst no toxicity was reported in an animal model through injecting very large volumes (1.5 ml) of bupivacaine, development of myotoxicity secondary to bupivacaine neurotoxicity has also been reported.[39] Nonetheless; bupivacaine myo-

toxicity has been considered to be related to the associated burst and duration of release kinetics. Even very low doses of bupivacaine were suggested to be myotoxic if the duration of exposure is prolonged enough, perhaps through the pro-inflammatory effects.[40]

Significantly higher scores for histopathological markers of myotoxicity including necrotic fibrillar focus and vacuolization were noted in rats received atorvastatin and/or bupivacaine when compared to rats in the control group in the present study. Nevertheless, it should be noted that muscle fiber degeneration is followed by complete regeneration and recovery of functional properties in rats within the next 60 days.[41]

Given the lack of myotoxic findings in atorvastatin, bupivacaine as well as atorvastatin plus bupivacaine treated rats compared to controls in the present study, subcellular pathogenic mechanisms of myotoxicity associated with bupivacaine and atorvastatin seem to be unrelated. However, tendency for higher values for CK-MB in all atorvastatin (A, AB) groups than the vehicle group and higher values for myoglobin in all bupivacaine (AB, B) groups than atorvastatin per se (A) are worth noting.

Consistent with the statement that increased intracellular calcium concentrations have a role in myocyte injury,[42] and demonstration of bupivacaine to lead to calcium release from the sarcoplasmic reticulum (SR) and inhibit calcium reuptake at the same time, thus yielding persistently increased calcium levels in skinned fiber preparations of skeletal muscle,[43] bupivacaine has been suggested to interfere with the mitochondrial energy metabolism in skeletal muscle, causing calcium dysregulation.[44] Besides, statins were also reported to trigger mitochondria-induced calcium signaling alteration in human skeletal muscle.[45]

Accordingly, given the lipophilic nature of bupivacaine and atorvastatin enhancing their accumulation in the mycoplasma[12] and the statement that increased intracellular calcium concentrations have a role in myocyte injury[5], disturbed intracellular calcium homeostasis seems to have a crucial role in myotoxicity induced by these drugs. Accordingly while, long term use of statin therapy should obviously be questioned in the preoperative period and the myotoxic effect should be monitored, our findings seem to emphasize that no need to cease or pause statin therapy may be needed in preoperative period under normal circumstances, i.e maintained calcium homeostasis.

SIM symptoms were reported to develop approximately 6.3 months after the onset of start of statin therapy, and continued up to 2.3 months after the discontinuation.[32] Given that a 12-day span in rat life corresponds to a year in human life,[17] administration of atorvastatin treatment for a 12-day long period in the present study seems to fulfil sufficient amount and period of atorvastatin in rats to create myopathy. Additionally, based on the fact that intramuscular injection of bupivacaine to a muscle's holding capacity leads to degeneration of muscle fibers within the first 2 days, we obtained the histopathological samples one day after the bupivacaine injection. Also, association of bupivacaine with a longer term effectiveness in sensory (up to 270 minutes) than motor (up to 150 minutes) block in both atorvastatin and atorvastatin plus bupivacaine groups is in agreement with the data on superiority of this LA in creating sensory than motor block.[8-12]In group M, methyl cellulose was used as atorvastatin solvent; in group C, we used the dry syringe to remove the trauma against the syringe while applying bupivacaine sciatic nerve block. There were no myotoxic findings in both groups, either.

Our study has several limitations. Firstly, while SIM is usually dose-related[31] higher doses of statins would be required in high levels of LDL-LDL-cholesterol, no data were collected in the present study regarding the lipid profile of the rats treated with atorvastatin. Secondly, we could not examine the interaction between atorvastatin and bupivacaine in terms of in vitro muscle contractile properties and conducted no electrophysiological evaluation. Thirdly, we used a myotoxicity classification method in the histopathological examination which is a well-known method by the pathologist of our hospital while we did not use electron microscopy. Fourthly, we could not measure the bupivacaine concentration in muscle tissue. And fifthly, we could not compare the first and second results of blood samples in the same rats but compared the average values of the groups.

In conclusion, to our knowledge, this is the first report on the relation between atorvastatin and bupivacaine in terms of myotoxicity in rats. Our fin-

dings revealed no significant effect of atorvastatin, bupivacaine or atorvastatin plus bupivacaine on biomarkers of muscle toxicity including CK, CK-MB and myoglobin as compared to controls and no difference between atorvastatin and bupivacaine treatment groups (A, AB, B) in terms of histopathological markers of myotoxicity. Accordingly, to be justified by further pharmacokinetic and pharmacodynamic studies considering the use of different doses of drugs for different treatment durations, our findings seems to indicate no significant increase in the risk for myotoxicity in statin treated rats during bupivacaine induced local anesthesia. However, tendency for higher values for CK-MB in all atorvastatin (A, AB) groups than the vehicle group and higher values for myoglobin in all bupivacaine (AB, B) groups than atorvastatin per se (A) are worth noting. Therefore, given the experimental design of the present study, limiting, unless proved otherwise via strong clinical evidence in a clinically relevant setting in humans, atorvastatin and bupivacaine combination seems to be worrisome for the anesthesiologists in terms associated risk of myopathy, muscular dysfunction or myotoxicity.

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Abstract

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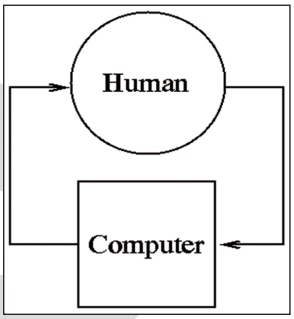

Figure 1. Text here

Conclusion

Be brief and give most important conclusion from your paper. Do not use equations and figures here.

Acknowledgements (If any)

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