International Journal of Medicine and Medical Sciences

Volume 5 Number 4 April 2013
ISSN 2006-9723

Academic Journals

ABOUT IJMMS

The **International Journal of Medicine and Medical Sciences** is published monthly (one volume per year) by Academic Journals.

The **International Journal of Medicine and Medical Sciences (IJMMS)** provides rapid publication (monthly) of articles in all areas of Medicine and Medical Sciences such as:
Clinical Medicine: Internal Medicine, Surgery, Clinical Cancer Research, Clinical Pharmacology, Dermatology, Gynaecology, Paediatrics, Neurology, Psychiatry, Otorhinolaryngology, Ophthalmology, Dentistry, Tropical Medicine, Biomedical Engineering, Clinical Cardiovascular Research, Clinical Endocrinology, Clinical Pathophysiology, Clinical Immunology and Immunopathology, Clinical Nutritional Research, Geriatrics and Sport Medicine

Basic Medical Sciences: Biochemistry, Molecular Biology, Cellular Biology, Cytology, Genetics, Embryology, Developmental Biology, Radiobiology, Experimental Microbiology, Biophysics, Structural Research, Neurophysiology and Brain Research, Cardiovascular Research, Endocrinology, Physiology, Medical Microbiology

Experimental Medicine: Experimental Cancer Research, Pathophysiology, Immunology, Immunopathology, Nutritional Research, Vitaminology and Ethiology

Preventive Medicine: Congenital Disorders, Mental Disorders, Psychosomatic Diseases, Addictive Diseases, Accidents, Cancer, Cardiovascular Diseases, Metabolic Disorders, Infectious Diseases, Diseases of Bones and Joints, Oral Preventive Medicine, Respiratory Diseases, Methods of Epidemiology and Other Preventive Medicine

Social Medicine: Group Medicine, Social Paediatrics, Medico-Social Problems of the Youth, Medico-Social Problems of the Elderly, Rehabilitation, Human Ecology, Environmental Toxicology, Dietetics, Occupational Medicine, Pharmacology, Ergonomy, Health Education, Public Health and Health Services and Medical Statistics
The Journal welcomes the submission of manuscripts that meet the general criteria of significance and scientific excellence. Papers will be published approximately one month after acceptance. All articles published in IJMMS are peer-reviewed.

Submission of Manuscript

Submit manuscripts as e-mail attachment to the Editorial Office at: ijmms@academicjournals.org. A manuscript number will be mailed to the corresponding author.

The International Journal of Medicine and Medical Sciences will only accept manuscripts submitted as e-mail attachments.

Please read the **Instructions for Authors** before submitting your manuscript. The manuscript files should be given the last name of the first author.

Editorial Board

Instructions for Author

Electronic submission of manuscripts is strongly encouraged, provided that the text, tables, and figures are included in a single Microsoft Word file (preferably in Arial font).

The **cover letter** should include the corresponding author's full address and telephone/fax numbers and should be in an e-mail message sent to the Editor, with the file, whose name should begin with the first author's surname, as an attachment.

Article Types
Three types of manuscripts may be submitted:

Regular articles: These should describe new and carefully confirmed findings, and experimental procedures should be given in sufficient detail for others to verify the work. The length of a full paper should be the minimum required to describe and interpret the work clearly.
Short Communications: A Short Communication is suitable for recording the results of complete small investigations or giving details of new models or hypotheses, innovative methods, techniques or apparatus. The style of main sections need not conform to that of full-length papers. Short communications are 2 to 4 printed pages (about 6 to 12 manuscript pages) in length.

Reviews: Submissions of reviews and perspectives covering topics of current interest are welcome and encouraged. Reviews should be concise and no longer than 4-6 printed pages (about 12 to 18 manuscript pages). Reviews are also peer-reviewed.

Review Process
All manuscripts are reviewed by an editor and members of the Editorial Board or qualified outside reviewers. Authors cannot nominate reviewers. Only reviewers randomly selected from our database with specialization in the subject area will be contacted to evaluate the manuscripts. The process will be blind review.
Decisions will be made as rapidly as possible, and the journal strives to return reviewers' comments to authors as fast as possible. The editorial board will re-review manuscripts that are accepted pending revision. It is the goal of the IJMMS to publish manuscripts within weeks after submission.

Regular articles

All portions of the manuscript must be typed **double-spaced** and all pages numbered starting from the title page.
The **Title** should be a brief phrase describing the contents of the paper. The Title Page should include the authors' full names and affiliations, the name of the corresponding author along with phone, fax and E-mail information. Present addresses of authors should appear as a footnote.

The **Abstract** should be informative and completely self-explanatory, briefly present the topic, state the scope of the experiments, indicate significant data, and point out major findings and conclusions. The Abstract should be 100 to 200 words in length.. Complete sentences, active verbs, and the third person should be used, and the abstract should be written in the past tense. Standard nomenclature should be used and abbreviations should be avoided. No literature should be cited.
Following the abstract, about 3 to 10 **key words** that will provide indexing references should be listed.

A list of non-standard **Abbreviations** should be added. In general, non-standard abbreviations should be used only when the full term is very long and used often. Each abbreviation should be spelled out and introduced in parentheses the first time it is used in the text. Only recommended SI units should be used. Authors should use the solidus presentation (mg/ml). Standard abbreviations (such as ATP and DNA) need not be defined.

The **Introduction** should provide a clear statement of the problem, the relevant literature on the subject, and the proposed approach or solution. It should be understandable to colleagues from a broad range of scientific disciplines.

Materials and methods should be complete enough to allow experiments to be reproduced. However, only truly new procedures should be described in detail; previously published procedures should be cited, and important modifications of published procedures should be mentioned briefly. Capitalize trade names and include the manufacturer's name and address. Subheadings should be used. Methods in general use need not be described in detail.

Results should be presented with clarity and precision. The results should be written in the past tense when describing findings in the authors' experiments. Previously published findings should be written in the present tense. Results should be explained, but largely without referring to the literature. Discussion, speculation and detailed interpretation of data should not be included in the Results but should be put into the Discussion section.

The **Discussion** should interpret the findings in view of the results obtained in this and in past studies on this topic. State the conclusions in a few sentences at the end of the paper. The Results and Discussion sections can include subheadings, and when appropriate, both sections can be combined.

The **Acknowledgments** of people, grants, funds, etc should be brief.
Tables should be kept to a minimum and be designed to be as simple as possible. Tables are to be typed double-spaced throughout, including headings and footnotes. Each table should be on a separate page, numbered consecutively in Arabic numerals and supplied with a heading and a legend. Tables should be self-explanatory without reference to the text. The details of the methods used in the experiments should preferably be described in the legend instead of
in the text. The same data should not be presented in both table and graph form or repeated in the text.

Figure legends should be typed in numerical order on a separate sheet. Graphics should be prepared using applications capable of generating high resolution GIF, TIFF, JPEG or Powerpoint before pasting in the Microsoft Word manuscript file. Tables should be prepared in Microsoft Word. Use Arabic numerals to designate figures and upper case letters for their parts (Figure 1). Begin each legend with a title and include sufficient description so that the figure is understandable without reading the text of the manuscript. Information given in legends should not be repeated in the text.

References: In the text, a reference identified by means of an author‘s name should be followed by the date of the reference in parentheses. When there are more than two authors, only the first author‘s name should be mentioned, followed by ’et al‘. In the event that an author cited has had two or more works published during the same year, the reference, both in the text and in the reference list, should be identified by a lower case letter like ’a‘ and ’b‘ after the date to distinguish the works.

Examples:

Nishimura (2000), Agindotan et al. (2003), (Kelebeni, 1983), (Usman and Smith, 2001), (Chege, 1998; Stein, 1987a,b; Tijani, 1993,1995), (Kumasi et al., 2001)
References should be listed at the end of the paper in alphabetical order. Articles in preparation or articles submitted for publication, unpublished observations, personal communications, etc. should not be included in the reference list but should only be mentioned in the article text (e.g., A. Kingori, University of Nairobi, Kenya, personal communication). Journal names are abbreviated according to Chemical Abstracts. Authors are fully responsible for the accuracy of the references.

Examples:

Giesielski SD, Seed TR, Ortiz JC, Melts J (2001). Intestinal parasites among North Carolina migrant farm workers. Am. J. Public Health. 82: 1258-1262

Stoy N, Mackay GM, Forrest CM, Christofides J, Egerton M, Stone TW, Darlington LG (2005). Tryptophan metabolism and oxidative stress in patients with Huntington’s disease. N. J. Neurochem. 93: 611–623.

Mussel RL, De Sa Silva E, Costa AM, Mandarim-De-Lacerda CA (2003). Mast cells in tissue response to dentistry materials: an adhesive resin, a calcium hydroxide and a glass ionomer cement. J. Cell. Mol. Med. 7:171-178.

Booth M, Bundy DA, Albonico P, Chwaya M, Alawi K (1998). Associations among multiple geohelminth infections in school children from Pemba Island. Parasitol. 116: 85-93.0.

Fransiscus RG, Long JC (1991). Variation in human nasal height and breath, Am. J. Phys. Anthropol. 85(4):419-427.

Stanislawski L, Lefeuvre M, Bourd K, Soheili-Majd E, Goldberg M, Perianin A (2003). TEGDMA-induced toxicity in human fibroblasts is associated with early and drastic glutathione depletion with subsequent production of oxygen reactive species. J. Biomed. Res. 66:476-82.

Case Studies

Case Studies include original case reports that will deepen the understanding of general medical knowledge

The **Title** should be a brief phrase describing the contents of the paper. The Title Page should include the authors' full names and affiliations, the name of the corresponding author along with phone, fax and E-mail information. Present addresses of authors should appear as a footnote.

The **Abstract** should be informative and completely self-explanatory, briefly present the topic, state the scope of the experiments, indicate significant data, and point out major findings and conclusions. The Abstract should be 100 to 200 words in length. Complete sentences, active verbs, and the third person should be used, and the abstract should be written in the past tense. Standard nomenclature should be used and abbreviations should be avoided. No literature should be cited.

Following the abstract, about 3 to 10 **key words** that will provide indexing references should be listed.

A list of non-standard **Abbreviations** should be added. In general, non-standard abbreviations should be used only when the full term is very long and used often. Each abbreviation should be spelled out and introduced in parentheses the first time it is used in the text. Only recommended SI units should be used. Authors should use the solidus presentation (mg/ml).

The **Introduction** should provide a clear statement of the problem, the relevant literature on the subject, and the proposed approach or solution. It should be understandable to colleagues from a broad range of scientific disciplines.
The presentation of the case study should include the important information regarding the case. This must include the medical history, demographics, symptoms, tests etc. Kindly note that all information that will lead to the identification of the particular patient(s) must be excluded

The conclusion should highlight the contribution of the study and its relevance in general medical knowledge

The **Acknowledgments** of people, grants, funds, etc should be brief.
References: Same as in regular articles

Short Communications
Short Communications are limited to a maximum of two figures and one table. They should present a complete study that is more limited in scope than is found in full-length papers. The items of manuscript preparation listed above apply to Short Communications with the following differences: (1) Abstracts are limited to 100 words; (2) instead of a separate Materials and Methods section, experimental procedures may be incorporated into Figure Legends and Table footnotes; (3) Results and Discussion should be combined into a single section.

Proofs and Reprints: Electronic proofs will be sent (e-mail attachment) to the corresponding author as a PDF file. Page proofs are considered to be the final version of the manuscript. With the exception of typographical or minor clerical errors, no changes will be made in the manuscript at the proof stage. Because IJMMS will be published freely online to attract a wide audience), authors will have free electronic access to the full text (in both HTML and PDF) of the article. Authors can freely download the PDF file from which they can print unlimited copies of their articles.

Copyright: Submission of a manuscript implies: that the work described has not been published before (except in the form of an abstract or as part of a published lecture, or thesis) that it is not under consideration for publication elsewhere; that if and when the
Manuscript is accepted for publication, the authors agree to automatic transfer of the copyright to the publisher.

International Journal of Medicine and Medical Sciences

Table of Contents: Volume 5 Number 4 April 2013

ARTICLES

International Journal of Medicine and Medical Sciences

Table of Contents: Volume 5 Number 4 April 2013

ARTICLES

Research Articles

academicJournals

Vol. 5(4), pp. 155-157, April 2013
DOI: 10.5897/IJMMS09.339
ISSN 2006-9723 ©2013 Academic Journals
http://www.academicjournals.org/IJMMS

International Journal of Medicine and Medical Sciences

Case Report

Successful pregnancy outcome in a woman with myasthenia gravis: Case report and literature review

Ritu Dalmia

Advanced Medicare Research Institute (AMRI), Kolkata, India.

Accepted 25 January, 2013

Myasthenia gravis (MG) is an acquired neuromuscular autoimmune condition that presents clinically with weakness and fatigue of the skeletal muscles. The case described here is a multidisciplinary management of a 28 yr old primigravida with myasthenia gravis. Although pregnancy with an uneventful course and a good outcome frequently is possible in women who have MG, there are many challenging therapeutic decisions unique to myasthenic women planning pregnancy.

Key words: Myasthenia gravis, neuromuscular autoimmune, skeletal muscles

INTRODUCTION

Myasthenia gravis (MG) is an acquired neuromuscular autoimmune condition that presents clinically with weakness and fatigue of the skeletal muscles. Women should be made aware of the issues and risks related to pregnancy based on the best current evidence available so that they can make an informed decision and successfully complete pregnancy. In the present paper management of pregnancy in a women with myasthenia gravis is discussed in light of the literature review

CASE REPORT

A 28-year-old primigravida who was known to have myasthenia gravis (MG) for the last 4 years attended the antenatal clinic at 9 weeks gestation. It was a planned pregnancy. She had a thymectomy in 2001 and had also had plasmapheresis in the past. She was taking pyridostigmine 60 mg three times a day. Previously, she had suffered from dysphagia, dysarthia and also decreased power in her arms and legs, mainly brought on by stress and tiredness. She was reviewed by the neurologist in the current pregnancy, no dose adjustment was required and apart from slight weakness in shoulder abduction, neck extension and the small muscles of both hands, she was quite well.

Her booking scan showed a live single intrauterine pregnancy. Viral serology and triple tests were normal. She had a detailed anatomy scan at 20 weeks at a tertiary referral centre during which fetal breathing movements were observed. She had regular growth scans at 28, 32, 36 and 38 weeks gestation which were all within normal limits. During the antenatal period, her case was reviewed by a multidisciplinary team comprising of a neurologist, an obstetrician, anaesthetist and a paediatrician. During the antenatal period, her condition remained stable and she was continued on drug therapy. By 38 week 3 days gestation, she was feeling very tired and ultrasound scan showed static growth and reduced amniotic fluid. As she was very keen to try vaginal delivery, the decision to induce labour at 39 weeks was made.

E-mail: rituritush@yahoo.co.uk. Tel: 00919831002250.

Epidural anaesthesia was sited early in labour to reduce stress and tiredness. She continued with the pyridostigmine during labour. During labour, she was monitored every half hour for ptosis, dysarthia, dysphagia, muscle strength of arms and legs and respiratory muscles (ability to cough). To shorten the second stage of labour, she had a ventouse delivery. A live baby weighing 2.92 kg was born with Apgar score of 9 at 1 min and 9 at 5 min. While on the postnatal ward, midwives were aware to look out for signs of poor feeding and difficulty in breathing for the baby. Both mother and baby made an uneventful postpartum recovery. Mother and baby were doing well at 6 weeks postnatal check up.

DISCUSSION

Myasthenia gravis (MG) is an acquired neuromuscular autoimmune condition that presents clinically with weakness and fatigue of the skeletal muscles. The disorder is characterized by a decrease in the number of acetylcholine receptors at the neuromuscular plates due to an autoimmune process mediated by antibodies directed against the alpha subunit of the nicotine receptor of the acetylcholine. This disease is twice as common in women as in men and frequently affects young women in second and third decades of life, overlapping with the childbearing years. The treatment of MG in women therefore poses unique and challenging issues to neurologists, obstetricians, anaesthetists and neonatologists, as the safety of both mother and fetus need to be carefully considered when choosing a therapeutic plan.

Batocchi et al. (1999) showed that MG relapsed in 17% of asymptomatic patients who were not on therapy before conception. In patients taking therapy, symptoms improved in 39% of pregnancies, remained unchanged in 42% and deteriorated in 19%. MG symptoms worsened after delivery in 28% of pregnancies. Djelmis et al. (2002) showed that worsening of symptoms is more likely during first trimester and first month postpartum. There is no increase in incidence of spontaneous abortion, growth restriction, pre-eclampsia or prematurity in women with MG though frequency of premature rupture of membranes is increased.

Ferrero et al. (2005) reported that anticholinesterase drugs are the mainstay of treatment when MG symptoms are not satisfactorily controlled, corticosteroids, azathioprine and in some cases, cyclosporine A can be used. Until information is available regarding safety, mycophenolate mofetil should be discontinued during pregnancy. Bermas and Hill, (1995) reported that cyclosporine can cause fetal myelosuppression, prematurity and spontaneous abortion. Fetus exposed to azathioprine has an increased risk of myelosuppression. Pyridostigmine is considered safe during pregnancy when used at the recommended dosage of less than 600 mg/day. An isolated case report by Nissen and Shah (2000) documented severe neonatal MG (NMG), growth retardation, microcephaly, joint contractures, and dysmorphic features in an infant born to a myasthenic mother who was taking four to eight times the recommended daily dosage of pyridostigmine during pregnancy (1,500 to 3,000 g/day). Watson et al. (1984) reported that plasmapheresis or Intravenous immunoglobulin (IVIG) can be used to manage severe MG symptoms or crisis during pregnancy or to avoid the use of immunosuppressant with potential teratogenic effects. Magnesium sulfate for the management of eclampsia should be used cautiously in myasthenic women, as it can precipitate weakness by interfering with neuromuscular transmission.

Regarding the mode of delivery, a vaginal birth should be preferred as the uterus is not affected by autoantibodies. Djelmis et al. (2002) reported that cesarean section should only be performed if there is an obstetric indication. MG usually does not change the course of the first phase of labour, as it does not affect smooth muscles. Striated muscle involved in the voluntary expulsive effort of the second phase of labor may be prone to fatigue, and the obstetrician should be prepared to assist in this stage, if needed, with forceps or vacuum extraction. Myasthenic fatigue occurring during labor can be helped by cholinesterase inhibitors. These should be administered parentally because of unpredictable gastric absorption. Neostigmine doses of 1.5 mg intramuscularly or 0.5 mg intravenously are equivalent to 60 mg of pyridostigmine taken orally.

Transient neonatal MG is a syndrome that affects 10 to 20% of newborns of myasthenic mothers and occurs shortly after birth (Plauche, 1991). Symptoms develop most commonly, 12 to 48 h after birth, and include generalized weakness and hypotonia, difficulty in feeding, feeble cry, ptosis, facial paresis, and respiratory distress. Placental transfer of antibodies against the fetal acetylcholine receptor can cause arthrogryposis multiplex congenita (AMC) in some infants born to myasthenic women. The syndrome consists of non-progressive multiple congenital joint contractures developing in uterus resulting from lack of fetal movements, preventing normal joint formation. Some infants born with AMC have survived, but AMC can lead to intrauterine fetal death or neonatal death because of pulmonary hypoplasia and polyhydramnios (Vincent et al., 1995; Polizzi et al., 2000). Ultrasound testing should be used to monitor fetal movements and to detect the development of joint contractures in uterus.

Breast feeding is not contraindicated in women with MG although serum antibodies versus acetylcholine receptors might reach the new born via breast milk, enhancing neonatal MG. Although pregnancy with an

uneventful course and a good outcome frequently is possible in women who have MG, there are many challenging therapeutic decisions unique to myasthenic women planning for pregnancy. Management should be aimed at optimizing muscle strength in the mother, minimizing maternal risk of bulbar and respiratory exacerbation, protecting the fetus, and maintaining integrity of the pregnancy. Women should be made aware of the issues and risks related to pregnancy based on the best current evidence available so that they can make an informed decision and successfully complete pregnancy.

REFERENCES

Batocchi AP, Marjolini L, Lino M, Minisci C, Tonali P (1999). Course and treatment of Myasthenia gravis during pregnancy. Neurol. 52:447-452.

Djelmis J, Sostarko M, Mayer D (2002). Ivanesevic M.Myasthenia gravis in pregnancy: report on 69 cases. Eur. J. Obstet. Gynaecol. Reprod. Biol. 104(1):21-25.

Bermas BL, Hill JA (1995). Effects of immunosuppressive drugs during pregnancy. Arthritis Rheum. 38(12):1722-1732.

Niesen C, Shah NS (2000) Pyridostigmine-induced microcephaly. Neurol. 54: 1873-1874.

Plauche WC (1991). Myasthenia gravis in mothers and their newborns. Clin. Obstet. Gynecol. 34:82-99.

Polizzi A, Huson S, Vincent A (2000). Teratogen update: Maternal myasthenia gravis as a cause of congenital arthrogryposis. Teratol. 62:332-341.

Vincent A, Newland C, Brueton L, Beeson D, Riemersma S, Huson SM (1995). Arthrogryposis multiplex congenita with maternal antibodies specific for a fetal antigen. Lancet 346:24-25.

Ferrero S, Pretta S, Nicoletti A, Petrera P, Ragni N (2005). Myasthenia gravis: management issues during pregnancy. Eur. J. Obstet. Gynaecol. Reprod. Biol. 121(2):129-38.

Watson WJ, Katz VL, Bowes WA (1984). Plasmapheresis during pregnancy. Obstet. Gynecol. 76: 451-457.

academicJournals

Vol. 5(4), pp. 158-162, April 2013
DOI: 10.5897/IJMMS11.117
ISSN 2006-9723 ©2013 Academic Journals
http://www.academicjournals.org/IJMMS

International Journal of Medicine and Medical Sciences

Full Length Research Paper

Spectrum of dysentery in children presenting to a tertiary level teaching hospital in New Delhi

Krishnan Rajeshwari[1]*, Madhumita Mishra[1], A. P. Dubey[1], Beena Uppal[2] and S. Anuradha[3]

[1]Department of Pediatrics, Maulana Azad Medical College and Associated Hospitals, New Delhi- 110002, India.
[2]Department of Microbiology, Maulana Azad Medical College and Associated Hospitals, New Delhi- 110002, India.
[3]Department of Medicine, Maulana Azad Medical College and associated hospitals, New Delhi

Accepted 18 February, 2013

Dysentery accounts for significant morbidity in pediatric population with a high case fatality rate, if left untreated. Further, the easy availability of antibiotics has led to widespread emergence of resistant strains. The aims of this study were: (1) to study the clinical spectrum of dysentery in children, and (2) to determine various enteropathogens causing dysentery in children. 60 children in the age group 1 month to 12 years, presenting with dysentery (defined as loose stools with visible blood), were enrolled. The stool samples were cultured to determine various enteropathogens and their antibiotic sensitivity pattern. About 61.7% of children were in the age group of 6 months to 2 years. 71.7% had no dehydration at presentation. No complication was documented in our study. 80% of stool samples were grossly bloody and 58.3% were grossly mucoid. Enteropathogens were identified in 44 cases (73.3%). Leading isolates were *Shigella* in 23 cases (38.3%), *Escherichia coli* in 18 (30%). *Salmonella* were seen in 2 patients, accounting for 3.3% and *Aeromonas* in one patient. Among the *Shigella*, *Shigella flexneri* was the most frequent isolate (73.9%). Majority of *Shigella* were resistant to nalidixic acid (95.7%), norfloxacin (87%), and amoxicillin (56.5%). Most isolates were sensitive to cefotaxime, gentamycin and amikacin (95.6% each). Among the *E. coli*, EHEC were seen in 9 out of 18 (50%) cases, followed by ETEC and EPEC in 22.2% patients each. EIEC were seen in 5.6% of cases. Majority of *E. coli* were resistant to amoxicillin (95%), nalidixic acid (88.9%), norfloxacin (66.7%), and cefotaxime (56%). However, most strains were sensitive to gentamycin (88.8%) and amikacin (100%). We conclude that enteropathogen resistance to commonly used antibiotics is rapidly rising however, resistance to extended spectrum cephalosporins is still rare. Thus, local susceptibility patterns should be assessed periodically to guide antimicrobial therapy.

Key words: Dysentery, enteropathogens, antibiotic resistance.

INTRODUCTION

Acute diarrheal diseases rank 2nd among all infectious diseases, as a killer in 0 to 5 years age group. India alone loses 0.6 million children each year due to diarrhea. Morbidity due to diarrheal diseases is also very high, amounting to 6 to 12 episodes of diarrhea/year/child (Sur and Bhattacharya, 2006). Dysentery accounts for a significant proportion of all diarrhea cases. It is characterised by the passage of loose stools mixed with blood and mucus, fever, abdominal cramps and tenesmus. Worldwide, the incidence of dysentery is

*Corresponding author. E-mail: rajeshwari.dr@gmail.com

estimated to be 164.7 million cases per year, of which163.2 million were in developing countries, where 1.1million deaths occurred. About 60% of all episodes and 61% of all deaths attributable to shigellosis involved children younger than 5 years (Ashkenazi, 2004).

There is increasing recognition of wide array of enteric pathogens associated with dysentery namely, *Shigellae*, EIEC, EHEC, *Salmonella* species, *Vibrio parahaemolyticus*, *Campylobacter*, *Yersinia* and *Entamoeba histolytica*. *Shigellae* alone cause 10 to 15% of acute diarrhea and more than 50% of all the dysentery cases in less than 5 years of age and a high case fatality rate if left untreated. For the Indian subcontinent, *Shigella flexneri* continues to be the most common serogroup isolated, in contrast to the developed world where *Shigella sonnei* is common (Kotloff et al., 1999; von Seidlin et al., 2006; Naik, 2006). *E. coli* and *Campylobacter jejuni* are responsible for 25% and 5 to 15% of the dysentery cases, respectively (Sur and Bhattacharya, 2006).

Effective antimicrobial therapy can reduce both the duration and severity of dysentery. Emergence of resistance to ampicillin and co-trimoxazole in the 1980s led to the use of nalidixic acid as the first line drug for shigellosis. However, increasing number of isolates are showing resistance to nalidixic acid and quinolones, leading to therapeutic problem which needs to be studied in detail (Pazhani et al., 2005; Mamatha et al., 2007; Taneja, 2007; Dhodapkar et al., 2008; Srinivasa et al., 2009).

There is a paucity of data from north India, especially Delhi, regarding exact incidence of various enteropathogens, causing dysentery. In this study, we present the clinico-etiologic spectrum of dysentery in children presenting to our hospital with an attempt to define the causative organisms and their sensitivity pattern to various antimicrobials.

MATERIALS AND METHODS

Sixty children were enrolled in a prospective observational study conducted in diarrhea ward of Department of Paediatrics, Maulana Azad Medical College and associated Lok Nayak Hospital, New Delhi.

Inclusion criteria

Children aged between 1 month to 12 years presenting to diarrhea ward of Department of Paediatrics, Maulana Azad Medical College and associated Lok Nayak Hospital presenting with dysentery (defined as loose stools with visible blood), during the years 2009 to 2010 were enrolled in the study. These children were assessed by a detailed history and complete physical examination using a pre designed proforma. The children were managed as per WHO protocol for management of dehydration and stool samples were cultured to determine various enteropathogens causing dysentery in children. The current antibiotic sensitivity pattern of isolated pathogens was studied.

Exclusion criteria

Children with known causes of blood in stools like rectal polyps, inflammatory bowel diseases, and bleeding diathesis were excluded.

Sample collection and transportation

Stool samples were collected directly in clean containers while the child passed stools. The sample collected was transported to microbiology lab within two hours of collection. In case of delay of more than two hours, samples were transported in Cary Blair medium/buffered glycerol saline and inoculated in selenite F broth medium for enrichment.

Stool examination

Stools were examined macroscopically for colour, consistency, presence of blood, mucus, worms, and microscopically by saline preparation, iodine and Gram staining for ova, cyst, and bacteria. Stool samples were cultured directly and after enrichment in selenite F broth and alkaline peptone water (APW) onto blood agar medium, MacConkey's agar, Xylose lysine desoxycholate agar, bile salt agar and sorbitol MacConkey agar. For *C. jejuni* isolation, stool samples were cultured on Skirrow's/Butzler's/charcoal cefoperazone desoxycholate agar (CCDA) media and incubated under microaerophilic conditions at 42°C for 48 h. For isolation of *Yersinia enterocolitica*, stool samples were inoculated onto *Yersinia* selective media. The organisms were identified on the basis of colony characteristics and biochemical tests and confirmed serologically by agglutination with specific antisera. All bacterial isolates were subjected to antibiotic sensitivity using disc diffusion method. Clostridium difficile toxin A was detected using commercially available Enzyme-linked immunosorbent assay (ELISA) kit. Other investigations wherever clinically indicated include:

(a) Complete blood counts;
(b) Serum electrolytes;
(c) Kidney function tests;
(d) Blood gas analysis;
(e) Blood culture and sensitivity;
(f) Chest X-ray

Data was analyzed using chi square, Fisher exact test and t-test using statistical software Statistical package for social sciences (SPSS) version 12.0.

RESULTS

Of the sixty children recruited, 21 (35%) were male and 39 (65%) were females. The male to female ratio is 0.53. Majority of cases (61.7%) were from 6 months to 2 years of age. Lowest incidence of dysentery was seen in age group 0 to 6 months [4 out of 60 (6.7%)]. Of the fathers of 53 children, 88.3% were educated till primary school, 10% were illiterates and only one father was educated till college. Similar trend was seen in mothers' education as well. 46 out of 60 (76.7%) of mothers were educated till primary school. A higher proportion (21.7%) was illiterate

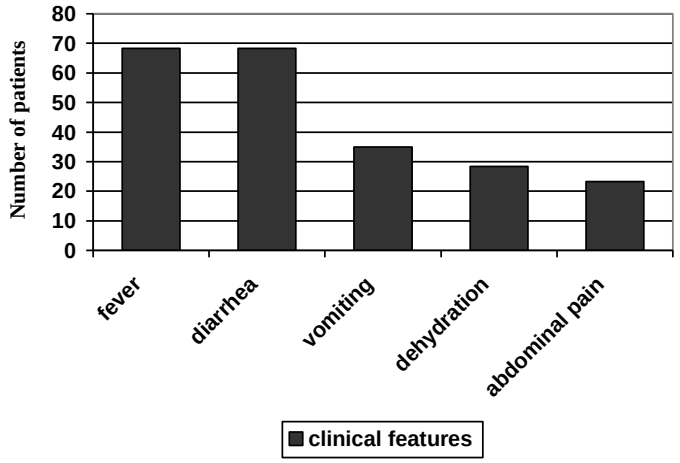


Figure 1. Clinical features of patients with dysentery.

and only a mother of only one child was educated till college.

The main presenting complaints were fever, diarrhea, vomiting and abdominal pain. Fever and diarrhea were observed in 68.3% of patients (41 out 60). Vomiting was observed in 21 (35%) children. Abdominal pain was noted in 23.3% of patients (Figure 1). In our study, majority of patients [43 out of 60 (71.7%)] had no dehydration at presentation. Some dehydration was observed in 28.3% of patients. Dehydrated patients required longer duration of hospital stay. The difference was statistically significant with p = 0.05. We found malnutrition in 44 patients (73.3%). However, severe malnutrition was only seen in 10% of children. Median time to recovery was 24 h; 51.7% (31 out of 60) recovered within 24 h. 58 patients out of 60 (96.7%) recovered within 72 h.

On gross examination of stools, blood was present in 80% (48 out of 60) of the samples. 35 out of 60 stools (58.3%) were mucoid. In microscopic examination, pus cells were seen in 90% of patients. Microscopically, RBCs were identified in 48 (80%) of cases. Presence of RBC correlated significantly with culture positive cases (p = 0.006). Cysts of *E. histolytica* were not isolated.

In hematological investigations, anemia was found in 12 (20%) patients of whom 5 (8.4%) had hemoglobin level of < 6 g/dl. Total leucocyte count was raised in 19 (31.7%) patients. All the patients had normal serum electrolytes and kidney function tests. Metabolic acidosis was not seen in anyone. 34 patients (56.7%) required only antibiotics, whereas 26 patients (43.3%) required intravenous fluids in addition to antibiotics. No complication was seen in any child.

Enteropathogens were identified in 44 cases (73.3%). Leading isolates were *Shigella* in 23 cases (38.3%), followed by *E. coli* in 18 (30%). *Salmonella* were seen in 2 patients, accounting for 3.3%. *Aeromonas* was isolated in one case (Table 1). *S. flexneri* was the most frequent isolate detected in 17 patients (73.9%) followed by *Shigella dysenteriae* in 3 (13.1%), *Shigella boydii* in 2 patients and *Shigella sonnei* was isolated in one patient. We documented a longer duration of diarrhea for cases of *Shigella* than that seen in patients of *E. coli*, p < 0.05. Majority (95.6%) of *Shigella* strains were sensitive to cefotaxime, gentamycin and amikacin. 56.5% of strains were resistant to amoxicillin, 87% were resistant to norfloxacin and 95.7% of strains were resistant to nalidixic acid (Table 2).

On serotypic analysis of *E. coli*, EHEC were seen in 9 out of 18 (50%) cases, followed by ETEC and EPEC in 22.2% patients each. EIEC were seen in 5.6% of cases. A greater degree of resistance to amoxycillin was noted (95%) and cefotaxime (56%). Majority of strains were sensitive to gentamycin and amikacin. 66.7% of strains were resistant to norfloxacin and 88.9% were resistant to nalidixic acid (Table 2).

Table 1. Enteropathogens isolated.

Parameter	N (%)
Aeromonas	1 (1.7)
E. coli	18 (30)
No growth	16 (26.7)
Salmonella	2 (3.3)
Shigella	23 (38.3)
Total	60 (100)

Table 2. Antibiotic sensitivity patterns of *Shigella* and *E. coli*.

Antibiotic	Shigella [N (%)]	E. coli [N (%)]
Amoxycillin	10 (43.5)	1 (5)
Cefotaxime	22 (95.6)	8 (44)
Gentamycin	22 (95.6)	16 (88.8)
Amikacin	22 (95.6)	18 (100)
Norfloxacin	3 (13)	6 (33.3)
Nalidixic acid	1 (4.3)	2 (11.1)

DISCUSSION

Sixty children with blood in stools were recruited. Majority of cases (61.7%) were 6 months to 2 years of age due to unhygienic introduction of complementary feeds. Lowest incidence of dysentery was seen in age group 0 to 6 months [4 out of 60 (6.7%)]. This is attributed to predominant breastfeeding practice in this age group. Male

preponderance was noted in shigellosis cases with M: F ratio being 2.2:1. Similar findings were reported by Naik (2006) from Africa, Taneja (2007) from India and Ghaemi et al. (2007) from Iran.

A relation was observed between educational status of parents and dysentery in children, with majority of parents being educated till primary school, a finding similar to that reported by Rustom et al. (2006). The main presenting complaints were fever, diarrhea, (68.3% each), vomiting (35%) and abdominal pain (23.3%). The findings are consistent with that observed by von Seidlin et al. (2006) who reported watery diarrhea in 65% of dysentery cases, fever in 45%, vomiting and abdominal pain in 20% cases. They also reported mucoid diarrhea and abdominal pain more frequently in culture positive cases, and vomiting and watery diarrhea were more often seen in culture negative cases. However no such association was seen in our study.

Dutta et al. (2003) also documented fever in 63.8% and abdominal pain in 20.4% of children presenting with bloody diarrhea. In our study, majority of patients [43 out of 60 (71.7%)] had no dehydration at presentation. Some dehydration was observed in 28.3% of patients. Dehydrated patients required longer duration of hospital stay. The difference was statistically significant, with $p = 0.05$. On the contrary, Dutta et al. (2003) documented moderate dehydration in 87.8% and severe dehydration in 10% of dysentery cases. We found malnutrition in 44 patients (73.3%). However, severe malnutrition was only seen in 10% of children. Malnutrition and dehydration have not been reported as significant problem in literature from the West.

On gross examination of stools, blood and mucus were found in 80% (48 out of 60) and 58.3% (35 out of 60) stool samples, respectively. In microscopic examination, pus cells were seen in 90% of patients. Rustam et al. (2006) documented mucoid stools in 96.3% of patients and pus cells in 100% of stool samples. Microscopically, WBCs were found in 73.3% and RBCs in 80% of samples. Presence of RBCs correlated significantly with culture positive cases, $p = 0.006$. Patwari et al. (1993) also observed the presence of RBC and WBC as significant predictor of dysentery. No complication was seen in any child. However, about 10 years back, Thapa et al. (1995) reported central nervous system (CNS) manifestations in 45% of patients, renal failure in 25% and subacute intestinal obstruction in 5% of cases. This difference might be due to change in health service seeking attitudes, leading to earlier treatment and better management of cases.

In our study, enteropathogens were identified in 44 cases (73.3%). Leading isolates were *Shigella* in 23 cases (38.3%), followed by *E. coli* in 18 (30%). Very few studies have documented spectrum of enteropathogens in dysentery cases. Our findings are similar to those of Dutta et al. (2003) who enrolled 100 patients with bloody diarrhea, among whom *Shigella* was isolated in 39% of cases. Among the cases due to *Shigella*, *S. flexneri* was the most frequent isolate detected in 17 patients (73.9%), followed by *S. dysenteriae* in 3 (13.1%). The findings are consistent with those of Pazhani et al. (2005) in Kolkata, who documented *S. flexneri* as the most common isolate (60%).

Mamatha et al. (2007) (Manipal) also reported *S. flexneri* in majority of cases (45%). Uppal et al. (2004) from New Delhi also isolated *S. flexneri* in 78.5% of cases. This is in accordance with the fact that *S. flexneri* is more common in developing countries in contrast to developed countries where *S. sonnei* is most common (Kotloff et al., 1999).

Among the *Shigella* spp., majority were resistant to nalidixic acid (95.7%), norfloxacin (87%), and amoxicillin (56.5%). Most isolates were sensitive to cefotaxime, gentamycin and amikacin (95.6% each). Similar resistance patterns have been reported by Pazhani et al. (2005) and Dutta et al. (2003) from east, Dhodapkar et al. (2008) and Srinivasa et al. (2009) from south and Taneja (2007) from north India. None of the strains were resistant to ceftriaxone. Our study confirms that amoxicillin and nalidixic acid have no role in dysentery now. Nalidixic acid was recommended by the WHO as the first-line treatment against shigellosis until 2004, when it was replaced by ciprofloxacin (von Seidlin et al., 2006). However there is growing concern regarding resistance to quinolones as documented by this study and by other authors (Pazhani et al., 2005; Mamatha et al., 2007; Taneja, 2007; Uppal and Arora, 2004).

A total of 18 isolates of *E. coli* were documented (30% of total dysentery cases) which was higher that that reported from other parts of India (Das et al., 2007). On serotypic analysis, EHEC were seen in 9 out of 18 (50%) cases, followed by ETEC and EPEC in 22.2% patients each. EIEC were seen in 5.6% of cases. Serotypic prevalence of *E. coli* has not been studied extensively in bloody diarrhea. A study in India by Bhan et al. (1989) found no STEC in children with diarrhoea in Delhi. In low and middle income countries, prevalence of STEC in childhood diarrhea has been reported to be lower than that of ETEC, EPEC, or EAEC by Presterl et al. (2003), however our study documents the growing significance of STEC in childhood dysentery. There is paucity of studies reporting antibiotic susceptibility of *E. coli* strains from childhood dysentery.

In our study, 95% of isolates were resistant to commonly used drug amoxicillin, 88.9% of strains were resistant to nalidixic acid, resistance to norfloxacin was observed in 66.7% cases. 56% isolates were resistant to cefotaxime. However, majority of strains were susceptible to aminoglycosides, gentamycin (88.8%) and Amikacin (100%). High degree of resistance to commonly used antibiotics was also noted by Das et al. (2007) who reported

33% of *E. coli* strains to be resistant to norfloxacin, 33% were resistant to gentamicin, 44% of the strains were resistant to cefotaxime and 77% were resistant to nalidixic acid. Thus local susceptibility patterns should be assessed periodically to guide antimicrobial therapy.

Abbreviations: EAEC, Enteroaggregative *Escherichia coli*; **EHEC,** enterohemorrhagic *Escherichia coli*; **EIEC,** Enteroinvasive *Escherichia coli*; **EPEC,** Enteropathogenic *Escherichia coli*; **ETEC,** Enterotoxigenic *Escherichia coli*; **RBC,** red blood cell; **STEC,** Shiga toxin producing *E. coli*; **WBC,** white blood cells; **WHO,** World Health Organization.

REFERENCES

Sur D, Bhattacharya SK (2006). Acute diarrheal diseases- An approach to management. J. India Med. Assoc. 104(5):220-223.

Ashkenazi S (2004). Shigella infections in children: New insights. Semin. Pediatr. Infect. Dis. 5:246-52.

Kotloff KL, Winickoff JP, Ivanoff B, Clemens JD, Swerdlow DL, Sansonetti PJ, Adak GK, Levine MM (1999). Global burden of *Shigella* infections: implications for vaccine development and implementation of control strategies. Bull. World Health Organ. 77 (8):651-66.

von Seidlein L, Deok RK, Mohammad A, Hyejon L, XuanYi W, Vu Dinh T, Do Gia C, Wanpen C, Magdarina DA, Anowar H, ulfiqar AB, Carl M, Ornthipa S, Kaisar T, Nair GB, Jacqueline LD, Karen K, John C (2006). A Multicentric study of shigella diarrhea in six Asian countries: Disease burden, clinical manifestation and microbiology. PLoS Med. 3(9):e353.

Naik DG (2006). Prevalence and Antimicrobial susceptibility patterns of shigella species in Asmara, Eritrea, North East Africa. J. Microbiol. Immunol. Infect. 39:392-5.

Pazhani GP, Ramamurthy T, Mitra U, Bhattacharya SK, Niyogi SK (2005). Species diversity and antimicrobial resistance of shigella spp. Isolated between 2001 and 2004 from hospitalised children with diarrhea in Kolkata, India. Epidemiol. Infect. 133(6):1089-95.

Mamatha B, Pusapati BR, Rituparna C (2007). Changing patterns of antimicrobial susceptibility of shigella serotypes isolated from children with acute diarrhea in Manipal, South India, a 5 year study. Southeast Asian J. Trop. Med. Pub. Health 38(5):863-6.

Taneja N (2007). Changing Epidemiology of Shigellosis and Emergence of Ciprofloxacin Resistant Shigellae in India. J. Clin. Microbiol. 45(2):678–679.

Dhodapkar R, Acharya NS, Harish BN, Parija SC (2008). Shigellosis in Puducherry. Indian J. Med. Res. 127:621-622.

Srinivasa H, Baijayanti M, Raksha Y (2009). Magnitude of drug resistant Shigellosis: A report from Bangalore. Ind. J. Med. Microbiol. 27(4): 358-360.

Ghaemi EO, Aslani MM, Moradi AV, Dadgar T, Livani S, Mansourian AR, Nosrat SB, Ahmadi AR (2007). Epidemiology of *Shigella*-associated diarrhea in Gorgan, north of Iran. Saudi J. Gastroenterol. 13:129-32.

Rustam S, Noor-us-Saba, Qayyum M, Islam B, Qazilbash A (2006). J. Med. Sci. 6(2):149-54.

Dutta D, Bhattacharya MK, Dutta S, Datta A, Sarkar D, Bhandari B, Bhattacharya SK (2003). Emergence of Multidrug resistant Shigella dysenteriae type 1 Causing Sporadic Outbreak In and Around Kolkata, India. J. Health Popul. Nutr. 21(1):79-80.

Patwari AK, Deb M, Dudeja M, Jayasheela M, Aggarwal A, Singh P (1993). Clinical and Laboratory Predictors of invasive diarrhea in children less than five years old. J. Diarrheal Dis. Res. 11(4):211-6.

Thapa BR, Venkateswarlu K, Malik AK, Panigrahi D (1995). Shigellosis in children from north India-clinico pathological study. J. Trop. Pediatr. 41(5):303-7.

Uppal B, Arora VM (2004). Changing resistance pattern of shigella isolates in a Delhi hospital: An alarming trend. Indian J. Med. Microbiol. 22:199-200.

Das S, Saha R, Singhal S (2007). Enteric Pathogens in North India Patients with Diarrhea. Indian J. Community Med. 32(1):27-31.

Bhan MK, Raj P, Levine MM, Kaper JB, Bhandari N, Srivastava R, Kuman R, Sazawal S (1989). Enteroaggregative *Escherichia coli* associated with persistent diarrhea in a cohort of rural children in India. J. Infect. Dis. 159:1061-4.

Presterl E, Zwick RH, Reichmann S, Aichelburg A, Kremsner PG, Winkler S, Kremsner PG, Graninger W (2003). Frequency and virulence properties of diarrheagenic *Escherichia coli* in children with diarrhea in Gabon. Am. J. Trop. Med. Hyg. 69:406-410.

academicJournals

Vol. 5(4), pp. 163-167, April 2013
DOI: 10.5897/IJMMS11.131
ISSN 2006-9723 ©2013 Academic Journals
http://www.academicjournals.org/IJMMS

International Journal of Medicine and Medical Sciences

Full Length Research Paper

A community trial of *Pinak*® medicine in the management of snakebite cases in a rural setting of Western Maharashtra, India

R. G. Raut[1], P. S. Pawar[2] and H. H. Chavan[3]

[1]Primary Health Centre Dimbhe, Maharashtra, India
[2]Sassoon General Hospital, Pune, India.
[3]Zilla Parishad, Pune, India.

Accepted 18 February, 2013

Narodi is a hilly village which has a small river also. Hence, snakebite incidence is more. In 2006 and 2007, there were 17 deaths in a population of 6,000. By cleaning the village (cutting grass in and around the village), and health education, gradually snakebite incidence reduced. But we introduced '*Pinak* tablet' with the hope of reducing deaths due to snakebite. Records of snakebite events also improved. Other factors like transport, type of snakes, toxicity of snake's venom, time to take patients to nearby hospital, and tying ligature were same as before. The only change was that many victims received *Pinak* tablet earlier, usually within 15 to 20 min before transporting victim to hospital. Those who did not take *Pinak* were also rushed to the hospital within same time, but some deaths were noted in those patients by analyzing the two groups of patients; treated with *Pinak* and not treated with *Pinak*. Two groups like 'after' and 'before' *Pinak* were not made because after *Pinak* was started, there were some patients who did not take *Pinak*. Hence, results obtained by are solely due to *Pinak*.

Key words: Narodi, *Pinak* tablet, snakebite, patients.

INTRODUCTION

Snakebite is the most important killing accident and poisoning affecting mankind worldwide. Snake bite is a major health hazard that leads to high mortality and great suffering in victims. Conservative sources estimate that the number of accidents globally reach one million, resulting in 600,000 envenomations and more than 20,000 deaths annually (Chippaux, 1998). In India alone, more than 200,000 cases are reported, and an estimated 35,000 to 50,000 people die each year (Bawaskar, 2004). A community-based retrospective survey in Kenya estimated that only 19% of the annual 151 snake bites per 100,000 people were potentially of venomous snakes (Snow et al., 1994). It is more predominant in hilly and wild areas, though snakebite cases are seen in all districts. Most of the victims of snakebites are rural farmers.

Antiserum is the only therapeutic agent available throughout the world. A major drawback of serum therapy is its prohibitive cost, and chance that victims are often some distance away from medical care when bitten. Serum sickness is a possible side effect of serum therapy that results in inflammation of certain tissues, and hypersensitivity is quite frequent, which may sometimes be lethal. Generally, anti-venom serum is a scarce commodity, and in the world market sometimes, even governments with money to purchase large quantities

*Corresponding author. E-mail: pawarps@gmail.com.

cannot obtain it. There is a crisis in the quality and supply of anti-venom serum in the sub-Saharan Africa due to fallen production and business pressures resulting from privatization of production plants (Theakston and Warrell, 2000). Experts and trained persons are required to administer the anti-snake venom (ASV).

In a study carried out on 119 cases of snakebite in a north Indian hospital, the average dose of anti-venom was 51.2 vials for elapid bites and 31 vials for viper bites, and an overall mortality rate of 3.5% was observed (Sharma et al., 2005).

Ayurveda, the ancient medical discipline of India offers several medicinal plants which are useful in the treatment of snake venom poisoning. Manufactured by Shree Bharadi Ayurvedic Pharmaceuticals, Karad, *Pinak*® is a unique combination prepared from four Indian medicinal plants that is, *Erythrina indica (Pangara mool), Magnefera indica (Amba Chhal), Eugenia jambolana (Jambhul Chhal),* and *Jusminum sambac (Mogra mool*) found to be useful in Ayurveda in the treatment of snake venom poisoning (Nadkarni and Nadkarni, 1989; Satyavati et al., 1987).

In a clinical study conducted in 75 patients of poisonous snake bite, 37 patients treated with Tab. *Pinak*®, given sublingually, were found to benefit significantly, with reduction in signs and symptoms, and there was no mortality (Pawar and Pawar, 2008). Narodi, a small village inhabited by 6,000 people, is situated at a distance of 72 km from Pune in western Maharashtra. The nearest primary health centre, Mahalunge Padwal, is situated at a distance of 23 km. It is a subcentre which consists of Narodi gaothan, Landewadi pinglewadi, Landewadi and Chichodi. Ghodegaon rural hospital and Manchar rural hospitals are functioning at a distance of 15 and 9 km, respectively. Narodi is surrounded by water bodies on three sides; hence snakes are a common site here. Every year particularly during rainy season, many incidences of snake bites and subsequent deaths are reported. With no medical aid for snakebite in the near vicinity and more deaths before reaching hospital, the problem of snake bite was acutely felt. There was no easy solution to this problem. Hence, the present study was taken up to demonstrate the feasibility trial of *Pinak* tablets in the management of snake bite cases in Narodi, a rural community of Maharashtra.

The objectives of this study are: (1) to know the death rate among snakebite victims treated with *Pinak*; (2) to know the death rate among snakebite victims treated without *Pinak* and; (3) to demonstrate feasibility of using *Pinak* tablet in the management of snakebite cases in a community setting.

Ethical permission

Ethical permission of Ethical Committee of B. J. Medical college and Sasoon hospital was obtained before study was started.

METHODOLOGY

The data of snakebite cases and resultant deaths before and after the *Pinak* tablet usage by the community is compared here. The data collection was done by the investigators by:

1. Review of records at the Narodi gram panchayat;
2. Review of records at the Narodi Primary health unit/subcentre;
3. Review of records at the Ghodegaon rural hospital;
4. Interview of village community leaders, surviving patients and their relatives, doctors, auxiliary nurses and midwives of the subcentre;
5. Review of the news/report items from the local newspapers specially for cases prior to introduction of *Pinak*.

The villagers were given health education in the form of lectures and demonstrations on how to put ligature (which is found useful in our experience) to the limb after snakebite, administration of sublingual route of *Pinak* tablets, and identification of snake's species. A booklet on these guidelines was printed and distributed free of charge to the people in the region. Eleven (11) boxes containing *Pinak* tablets were kept at various places such as schools, village-grampanchayat office, anganwadi, post office and some houses to ensure that every snakebite victim gets *Pinak* tablet easily. A meeting of owners of all the vehicles available in the region was conducted and an action plan in the event of snakebite for transport of the victims was chalked out.

Medical care

Procedures for field care include the following:

1. Immobilization of the limb.
2. Apply the tourniquet, one joint above the site.
3. Do not give anything to eat and drink.
4. Remove anything that may constrict the limb.
5. Arrange early transportation to a good hospital.
6. Give ABC if required.

Victims with definite history of snake bite were included in this study. Any case of unknown bite was noted separately. Transport was made available to each victim. Every victim was provided the same set of facilities. *Pinak* tablet was given sub-lingually to victims. A single tablet was given as soon as victim arrived at the depot holding *Pinak*, and victim was sent to hospital for further treatment. No victim required ABC on route to hospital. No victim died on spot or before receiving *Pinak*.

RESULTS

From Table 1, it is seen that there was a total of 53 cases of snake bite out of which 10 people died. The youngest victim was a 12 year old boy and the oldest was 60 years old. Median age of these deaths was 15 years and two of these deaths occurred in Ghodegaon rural hospital, one in Manchar rural hospital, two in a private nursing home and five occurred on the way to Sassoon General Hospital,

Table 1. Yearwise snakebite cases at Narodi subcentre.

Year	Snake bite cases		Unknown bite cases	
	No.	Death (%)	No.	Death (%)
2004	4	2 (50)	2	1 (50)
2005	9	2 (22.2)	9	5 (55.5)
2006	4	1 (25)	7	4 (57.1)
2007	29	4 (13.8)	11	5 (45.5)
2008	7	1 (14.3)	2	0 (0.0)
All	53	10 (18.9)	31	15 (48.4)

Table 2. Yearwise deaths among snakebite patients according to *Pinak* treatment.

Year	Treatment for snakebite				No. of snake bite cases	
	***PINAK* given**		***PINAK* not given**			
	No.	Death	No.	Death	No.	Death
2004	0 (0.0)	0 (0.0)	4	2 (50.0)	4	2 (50.0)
2005	0 (0.0)	0 (0.0)	9	2 (22.2)	9	2 (22.2)
2006	0 (0.0)	0 (0.0)	4	1 (25.0)	4	1 (25.0)
Up to 1.8.07	0 (0.0)	0 (0.0)	9	3 (33.3)	9	3 (33.3)
After 1.8.2007	17	0 (0.0)	3	1 (33.3)	20	1 (5.0)
2008	5	0 (0.0)	2	1 (50.0)	7	1 (14.3)
Total	22	0 (0.0)	31	10 (32.3)	53	10 (18.9)

Pinak was made available from 2nd August, 2007.

Table 3. Association between *Pinak* administration and deaths due to snakebite (N = 53).

PINAK	**Death**	**Survived**	**Total No. of snake bite cases**
Given	0 (0.0)	22 (100.0)	22 (100.0)
Not given	10 (32.3)	21 (67.7)	31 (100.0)

Figures in parentheses indicate percentages. $P < 0.001$, highly significant.

Pune. From August 2007, *Pinak* tablet was made available to the local population as described in the study methodology.

As it can be seen from Table 2 that There were a total of 53 cases of snake bites, out of which 10 people died. As at 1st August, 2007, *Pinak* tablet was not available and deaths were observed due to snake bite (up to 50.0%). Once *Pinak* tablet was made available, the death rate dropped. When the death rate was analysed as per the *Pinak* tablet administration, it was observed that there was not a single case of death among the snakebite group that was given *Pinak* tablet. There was one case of death of a patient due to snakebite observed after 1st August, 2007, who was not given *Pinak* tablet due to logistic problems.

As it can be seen from Table 3, out of the 53 cases of snakebite studied from 2004 to 2008, 22 patients were given *Pinak* tablets. When the incidence of deaths among these total snakebite cases was studies according to administration of *Pinak* tablets, it was evident that out of the 31 patients, 10 (32.3%) had died; while none of the patients who were administered *Pinak* died, which was highly significant. So, it can be seen that *Pinak* was acting as a protective factor in cases of snakebite. Among the admitted patients, it was observed that administration of *Pinak* tablets not only prevents death but also the following:

1. As it can be seen from Table 4 that; all patients on *Pinak* tablets were relieved of their signs and symptoms

Table 4. ASV required and duration of hospital stay.

Group	No. of patients	ASV vials required	Hospital stay
Pinak given	1*	0	2
	5	2	2
	5	5	2
	6	10	3
Pinak not given	1	31	5
	1	48	5
	1	61	8

*60 year old patient was allergic to ASV, treated with *Pinak*, recovered in two days. Most of the snakes observed in the area are Kraits and cobras.No adverse reaction was noted in *Pinak* given group. No gangrene was observed in any patient.

earlier than patients on ASV, hence recovery time also reduced significantly.
2. Patients with ASV reaction were successfully treated with *Pinak* tablet.

In the present study, one patient in Neuroparalysis group had allergic reaction to ASV, so he was given only *Pinak* tablets and recovered within 2 days.

DISCUSSION

Snake venom, mostly liquid, is secreted as specialized saliva from modified parotid glands. Its main function is to immobilize the prey and assist in digestion. Active agents in venom are enzymes which are antigenic. Enzymatic proteins in venom impart its destructive properties. The amount of venom injected depends upon various factors of the bite on the naked skin, elapsed time since the last bite, the degree of threat the snake feels, and the size of the prey.

Traditional healers have reputation of treating difficult snake bite cases and are trusted by their patients. In both study areas, cases of deaths in victims attended by traditional healers were very rare (less than 3%). In a Colombian study, healers interviewed reported only 4.4% death in cases they handled (Mebs, 2000). Snake bites in rural areas are commonly treated with plant extracts (Asuzu and Harvey, 2003; Houghton and Osibogun, 1993; Mebs, 2000; Otero et al., 2000; Pawar and Pawar, 2008; Yang et al., 1998).

Though medicinal plants remain largely unnoticed and neglected, protective activity of plant extracts have been confirmed in biological assays: resverotrol (3,4'5-trihydroxy trans-stilbene) from a snake bite herbal Cissus assamica (Asuzu and Harvey, 2003); reduction of venom-induced effects of *Naja nigricollis* in rats by pre-incubation with *Parkia biglobosa* extracts (Houghton and Osibogun, 1993) and activation of coagulative (prothrombin) activity by *Mucuna pruriens* seed extract (Mors, 1991). Anti-inflammatory activity in *Bidens pilosa* is recorded (Geissberger and Sequin, 1996; Guerranti et al., 2001; Geissberger and Séquin, 1991).

There was an acute and serious problem of fatality due to snake bite faced by the Narodi village people in the past. The geographic conditions were very difficult to deal with. Under those conditions, many deaths occurred. We took this venture because till date also, hundreds die due to snakebite inspite of having ASV. There is no safe, polyvalent, oral medicine like *Pinak* tablet readily available, immediately after snakebite anywhere. But once *Pinak* tablet was made available, it was observed that the death rate dropped significantly.

Hypothesis for venom neutralization

This includes the following:

1. Protein precipitation
2. Enzyme inactivation
3. Chelation action
4. Adjuvant action
5. Anti-oxidant action
6. Protein folding
7. Combination of the above which is observed with herbal combination like *Pinak*.

More studies are required to know the exact mechanism of action.

Limitations

1. Snake is not seen many times after bite; hence it is labelled as unknown bite.

2. Some deaths occurred before *Pinak* started, and were not properly certified and reported as snake bite deaths.

Conclusion

The death rate among snakebite victims treated with *Pinak* was significantly lower than observed among those treated without *Pinak*. The use of *Pinak* tablets in management of snake bite cases in a community setting is feasible.

REFERENCES

Asuzu IU, Harvey AL (2003). The antisnake venom activities of *Parkia biglobosa* (Mimosaceae) stem bark extract. Toxicon 42(7):763-8.

Bawaskar HS (2004). Snake venoms and antivenoms: critical supply issues. J. Assoc. Physicians India 52:11-13.

Chippaux JP (1998) Snake-bites: Appraisal of the global situation. Bull. WHO 76:515-524.

Geissberger P, Sequin U (1996). Constituents of *Bidens pilosa* L.: do the components found so far explain the use of this plant in of Zulu medicinal plants used for medicinal purposes. J. Ethnopharmacol. 52(2):95-100.

Geissberger P, Séquin U (1991). Constituents of Bidens pilosa L.: Do the components found so far explain the use of this plant in traditional medicine? Acta Trop. 48(4):251–261.

Guerranti R, Aguiyi JC, Errico E, Pagani R, Marinello E (2001). Effects of *Mucuna pruriens* extract on activation of prothrombin by *Echis carinatus* venom. J. Ethnopharmacol. 75(2– 3):175-80.

Houghton PJ, Osibogun IM (1993). Flowering plants used against snakebite. J. Ethnopharmacol. 39(1):1-29.

Mebs D (2000). Notes on the traditional use of plants to treat snake bite in northern Papua New Guinea. Toxicon. 38(2):299-302.

Mors WB (1991). Plants against snake-bites. Mem. Inst. Oswaldo Cruz 86(suppl 2):193.

Nadkarni KM, Nadkarni AK (1989). In: Nadkarni KM, Nadkarni AK (Eds.), Dr. KM Nadkarni's Indian material medica. Bombay popular Prakashan, 3rd ed. pp. 509-17.

Sharma N, Chauhan S, Faruqi S, Bhat P, Varma S (2005). Snake envenomation in a North Indian hospital. Emerg. Med. J. 22:118-120.

Otero R, Fonnegra R, Jimenez SL, Nunez V, Evans N, Alzate SP, Garcia ME, Saldarriaga M, Del Valle G, Osorio RG, Diaz A, Valderrama R, Duque A, Velez HN (2000). Snakebites and ethnobotany in the Northwest region of Colombia: Part I: traditional use of plants. J. Ethnopharmacol. 71(3):493-504.

Pawar GP, Pawar PS (2008). *Pinak* - the Ayurvedic antivenin. Global Ayurveda, April-May.

Satyavati GV, Gupta AK, Tandon N (1987) "Medicinal Plants of India Vol. II" (ICMR, New Delhi)

Snow RW, Bronzan R, Roques T, Nyamawi C, Murphy S, Marsh K (1994). The prevalence and morbidity of snake bite and treatment seeking behaviour among a rural Kenyan population. Ann. Trop. Med. Parasitol. 88(6):665-671.

Theakston RDG, Warrell DA (2000). Crisis in snake antivenom supply for Africa. Lancet 356:2104.

Yang LC, Wang F, Liu M (1998). A study of an endothelin antagonist from a Chinese anti- snake venom medicinal herb. J. Cardiovasc. Pharmacol. 31:S249-50.

academicJournals

Vol. 5(4), pp. 168-170, April 2013
DOI: 10.5897/IJMMS12.123
ISSN 2006-9723 ©2013 Academic Journals
http://www.academicjournals.org/IJMMS

International Journal of Medicine and Medical Sciences

Full Length Research Paper

Efficacy of digital remote tel-system for stroke patients in Taiwan

Wen-Hui Tsai[1], Heng-Hsin Tung[2]*, Shu-Yuan Liang[2], Tsae-Jyy Wang[2], Shu-Fang Wu[2] and Chieh-Yu Liu[2]

[1]Min-Sheng General Hospital, 168, Ching- Kuo Rd, 330 Taoyuan City, Taoyuan County, Taiwan R. O. C.
[2]College of Nursing, National Taipei University of Nursing and Health Science, 365 Ming Te Road PeiTou, Taipei 112, Taiwan, R. O. C.
[3]ChangHua Christian Hospital, Taiwan.

Accepted 25 March, 2013

The purpose of this study is to examine the efficacy of digital remote tel-system program on stroke patients in Taiwan. Pre-experimental design with convenience sampling was used in this study. A total of 91 participants were recruited with two questionnaires (demographic and SF-36) used. The results showed the quality of life improved significantly after the digital remote tel-system program. The need of home based care for stroke patients after discharge is reported and a digital remote tel-system program is a potentially effective way to fill the need of the patients.

Key words: Stroke, Taiwan, digital remote tel-system.

INTRODUCTION

In Taiwan, stroke is the most common cause of various disabilities (Lin et al., 2008). Stroke is a very complicated disease and many stoke patients have multiple chronic diseases such as cardiovascular diseases or diabetes. Recovery from stroke is a long term process, thus, the scope of health care services of this population includes not only managing their symptoms but also to maintain the best health status and prevent complications (Lai and Wang, 2008). Nevertheless, stroke management is very challenging. The outcome measure of successful treatment of chronic diseases is changed from physical or functional indicators to quality of life which include not only physical aspects but also mental aspects (Kauhanen et al., 2000).

Digital remote tel-system programs can provide physical indicator monitoring where health care providers can provide consultation efficiently and as needed based on the data collected from this program to potentially improve patient outcome (Lin et al., 2009). A previous study reported that chronic illness patients were satisfied with this new method of care (Borsis and Harvigesen, 2008).

Thus, this study aims to examine the efficacy of digital remote tel-system program on stroke patients in Taiwan.

METHODS

Pre-experimental design with convenience sampling was used in this study. A total of 91 participants were recruited from September 2010 to January 2011 in a Taiwan hospital. All of the participants were asked to complete the demographic questionnaire at the first meeting and to additionally complete a SF-36 at three different points in time, prior to intervention, one month after intervention and three months after intervention. The inclusion criteria were: (1) diagnosed with stroke within the last three months (2) length of stay more than three days (3) The NIHH score below 15 (4) have a clear conscience (5) able to communicate, and (6) Above 18 years of age. Institutional Review Board approval at the Ming-Che Hospital was obtained before the study was conducted.

*Corresponding author. E-mail: shannontung719@gmail.com. Tel: 886+ 953-005-831.

Table 1. Repeated-measure ANOVAs.

Variance	1			2			3			SS	df	MS	F	*p*	Post-Hosc
	Mean	SD	95% CI	Mean	SD	95% CI	Mean	SD	95% CI						
PCS	46.32	2.48	45.80~46.84	48.08	2.69	47.53~48.64	49.51	2.47	49.00~50.03	2505.80	1	2505.80	224.88	<0.001	3>2 ; 2>1
MCS	44.83	7.14	43.34~46.32	48.19	5.87	46.97~49.41	51.08	4.50	50.14~52.02	2093.61	2	2093.61	30.47	<0.001	3>2 ; 2>1

ps:1:pre-test, 2:post-test1, 3:post-test2; Covariates: age, employee status, marital status, family support, education, stroke type, BI, MRS.

All of the eligible patients were given written consent forms and an explanation of the study content was provided. Two instruments used to collect data are as follows:

1. A demographic questionnaire which includes two sections: (1) patient demographic information: age, gender, education, marital status, employee status, family support and economic status (2) patient medical related information: location of stroke, type of stroke, severity of stroke, comorbidity disease, the National Institute of Health Stroke scale (NIHSS), Barthel Index (BI) and the Modified Rankin Scale (MRS) (Quinn et al., 2007, 2009).
2. Medical Outcomes Short Form 36-Health Survey (SF-36). The SF-36 was used to measure outcomes of the study intervention, which is quality of life. The respondents were asked to fill in the questionnaire based on the past month experience. There are 36 questions divided into eight subscales, which are physical function, role limitation caused by physical problems, role limitation caused by emotional problems; social function, bodily pain, mental health, vitality, energy/fatigue, and general health. The eight subscales were divided into two categories, which are the physical component summary (PCS) and the mental component summary (MCS). The score ranges from 0 - 100 with the higher score indicating a better quality of life. The psychometric properties of the Taiwanese version of SF-36 were confirmed by Tseng et al. (2003).

Regarding one–month digital remote tel-system program (DRTSP), two designated nurses took responsibility for consultations in the call center and the hardware patients will bring home including the portable tele-system and the physiological measurement devices. Patients receive consultation calls twice a week (8 total sessions). The content of consultation was focused on follow up of patients' health related status after discharge, which include physiological measurements such as blood pressure twice a day, daily physical activity, healthy lifestyle, medications use, disease related health education and arrangement of next appointment. In addition, the participants could use the telephone or internet to contact call centers after discharge when necessary, and these two designated nurses respond and locate related resources.

RESULTS

The data were analyzed using SPSS for Windows, Version 17 software. Demographic data was analyzed by descriptive statistic method such as mean and percentage. A repeat measure ANOVA was used to examine the efficacy of the intervention with PCS and MCS of the quality of life as outcome measure. Pearson correlation and ANOVA were used to identify the demographic factors that are associated with the quality of life. Those factors were put into covariance with a Repeat measure ANOVA.

The majority (73.6%) of the participants were males with an average age of 61.08 years. The mean of NIHSS was 5.89 and 60.4% of patients were considered mildly severe. Mean of Barthel index was 73.40 and MRS was 2.62.

As shown in Table 1, patients achieved better PCS and MCS three month post intervention (mean=49.5±2.47, 51.08±4.50; P<0.001), compared to first month after intervention (mean=48.08±2.69; 48.19±5.87) and prior to intervention (mean=46.32±2.48; 44.83±7.14). Demographic patient information including age, employee status, marital status, family support, education as well as patient medical information including stroke types, BI, and MRS were put into covariance.

DISCUSSION AND RELEVANCE TO PRACTICE

The results showed the quality of life improved significantly after the digital remote tel-system program was used in stroke patients in Taiwan. This finding was consistent with previous research focusing on remote tel-system intervention. Hordam et al. (2010) reported tel-system patient education is an effective way to improve quality of life in total knee replacement patient after discharge. A pre-experimental design with only one group is considered a limitation for this study, but it still provide valuable information for the clinician to know the importance of utilization of electronic technology in patients who were at home after discharge. An experimental design with a random control trail is recommended in future studies to ensure the efficacy of the program.

The need of home based care for stroke patients after discharge is reported and a digital remote tel-system program is a potentially effective way to fill the need of the patients (Hu, 2007). Hospital managers and clinicians should be aware of the importance of this program in order to perform an appropriate referral and utilize this method to

benefit not only the stroke patients but also their families potentially. In addition, this digital remote tel-system program can be applied to different chronic disease patients in the community who need monitoring of their health condition regularly. There were no conflicts of interests in this study.

REFERENCES

Hu JW (2007). A Service Oriented Home Care System. Electronic and Ubiquitous Technology for Excellent Healthcare. pp. 237-250.

Lin CJ, Lord YZ, Yu YJ, Yeh HC (2009). Application of Telehealth in Case Management. J. Nurs. 56:5-10.

Lin TH, Huang LY, Hung LC, Fan SH (2008). The First Probe for Stroke Patient's Health-related Quality of Life. J. Health Management 6:121-134.

Lai TY, Wang CY (2008). Chronic Care Model and Potential of Informatics Applications. J. Long term care 12:413-421.

Botsis T, Hartvigesen G (2008). Current status and future perspectives in telecare for elderly people suffering from chronic disease. J. Telemed. Telecare 14:195-203.

Kauhanen ML, Korpelainen JT, Hiltunen P, Nieminen P, Sotaniemi KA, Myllyla VV (2000). Domains and determinants of quality of life after stroke caused by brain infarction. Arch. Phys. Med. Rehabil. 81:154-1546.

Quinn TJ, Lees KR, Hardemark HG, Dawson J, Walters MR (2007). Initial experience of a digital training resource for modified Rankin scale assessment in clinical trials. Stroke 38(8):2257–2261.

Quinn TJ, Dawson J, Walters MR, Lees KR (2009). Functional outcome measures in contemporary stroke trials. World Stroke Organ. Int. J. Stroke 4:200-205.

Tseng HM, Lu JF, Tsai YJ (2003). Assessment of health-related quality of life, II: Norming and validation of SF-36 Taiwan version. Taiwan J. Public Health 22(26):512-518.

academicJournals

Vol. 5(4), pp. 171-177, April 2013
DOI: 10.5897/IJMMS12.135
ISSN 2006-9723 ©2013 Academic Journals
http://www.academicjournals.org/IJMMS

International Journal of Medicine and Medical Sciences

Full Length Research Paper

Autism: An epigenomic side-effect of excessive exposure to electromagnetic fields

Yog Raj Ahuja[1]*, Sanjeev Sharma[2] and Bir Bahadur[3]

[1]Department of Genetics and Molecular Medicine, Vasavi Medical and Research Centre, 6-1-91, Khairatabad, Hyderabad 500004, India.
[2]Department of Clinical Pharmacology, Apollo Hospital, Jubillee Hills, Hyderabad, 500033, India.
[3]Department of Genetics, Shadan College, Khairatabad, Hyderabad 500004, India.

Accepted 27 February, 2013

Autism is a disorder which mainly involves the nervous system. It is characterized by lack of communication, incoherent language and meaningless repetitive movements. Its onset is in early childhood and its incidence has been reported to be increasing. Several genes and environmental factors have been implicated in the causation of autism, and electromagnetic fields may be one of those environmental factors. Industrialization has added a large number of electronic gadgets around us. Indiscriminate use of these gadgets, particularly mobile phones, has raised the question of electropollution and health hazard caused by their usage. Electromagnetic fields emitted during their operation do not have enough energy to cause DNA alterations directly; however, ample evidence is available from *in vitro* and *in vivo* studies to demonstrate their ability to cause DNA alterations indirectly as well as epigenetic modifications. In addition to genetic alterations, the epigenetic modifications may have an important role in causing disruption of the nervous system leading to neurodegenerative disorders, including autism.

Key words: Autism, neurodegenerative disorders, epigenome, electromagnetic fields, electronic gadgets, mobile phones.

INTRODUCTION

Autism is an example of a disorder caused by disruption of the nervous system. It is a severe neurodevelopmental disorder with an onset in infancy (Ploeger et al., 2010), and occurs more often in males than in females with a ratio of 4:1. It is characterized by social and communicative impairment accompanied by hyperactivity, repetitive and stereotype behavior (Fatima et al., 2006). IQ varies from normal (or even above normal) in more than 50% of the cases and mental retardation occurring in 30 to 50% of them. Various parts of the brain have been identified to be involved leading to neuronal disconnection or circuit disruption. Using diffusion tensor imaging, Weinstein et al. (2011) observed differences in white matter of the brain between autistic children and controls. They suggested that abnormal white matter integrity in young children with autism may adversely affect connectivity between different brain regions. Voineagu et al. (2011) carried out microarray analysis on RNA of more than 30,000 genes in three regions of post-mortem brains among 19 autistic individuals and 17 controls. They identified 444 genes in cerebral cortices of the autistic patients that showed significant expression changes as compared to the controls. Of these 444 genes, downregulation was seen in 209 genes which were related to synaptic and neuronal signaling functions, and upregulation was observed in

*Corresponding author. E-mail: yrahuja@yahoo.com. Tel: +91-40-23210251(O), +91-40-23323235(R). Fax: +91-40-66107930.

235 genes which were connected with immune and inflammatory response. The authors strongly suggested convergent molecular abnormalities involving transcriptional and splicing disregulation in the temporal and the frontal cortical regions in autism. This study was supported by an earlier study by Zikopoulos and Barbas (2010), who carried out anatomical studies on 5 cases with adult autism, and observed typical ultrastructure abnormalities in their frontal regions of the cortex.

RELATIVE ROLE OF GENETIC AND ENVIRONMENTAL FACTORS IN AUTISM

Autism is a familial disorder with high heritability. Genome-wide association studies as well as studies on submicroscopic and chromosomal structural variations (polymorphisms and copy number variants) suggest the involvement of a large number of genes in the causation of autism (Veenstra-Vanderweele et al., 2004). Being a polygenic disorder, autism is attributable to the effect of an unknown number of mutations and their possible interactions. These mutations and their interactions, *inter se,* as well as with the environmental factors, display a wide phenotypic spectrum, known as the autism spectrum disorder (ASD) (Currenti, 2010). The recurrence rate of an autistic child in a family is about 5% if one child is already affected with autism. The concordance rate of autism in monozygotic twins has been reported to range between 50 and 90% (Abrahams and Geschwind, 2008). The very fact that the concordance in monozygotic twins is short of 100% and autism has a wide phenotypic spectrum, it is reasonable to assume that environmental factors have a significant role to play in the causation of this disorder. Interestingly, according to Hallmayer et al. (2011) environmental factors play a larger role than genetic factors in the pathogenesis of autism. Various environmental factors like copper, lead, mercury, arsenic, fertilizers and pesticides, at toxic exposure levels have been implicated in autism. Not only are the higher levels implicated, but even the lower levels of some of the essential elements may also be involved in autism. For example, lower concentrations of magnesium, selenium, iodine and lithium have been observed in autistic individuals (Herbert et al., 2006; Adams et al., 2006, 2007; Lakshmi Priya and Geetha, 2011).

ELECTROMAGNETIC FIELDS AND THEIR BIOLOGICAL EFFECTS

We are surrounded by electrical/electronic gadgets at home as well as in the work place. These gadgets operate at a broad-range of electromagnetic frequencies, e.g., extremely low frequencies (AC:10 to 60 Hz) for domestic and power lines; medium-range radio-frequencies and microwave radiations (AC:1 to 900 MHz) for FM radio, television and mobile communication; and very high frequencies (AC:2 to 10 GHz) for microwave ovens and satellite communications. Biological systems have been shown to be sensitive to external magnetic fields in several investigations. Magnetic fields affect the basic life processes, like growth and development, orientation, structure and function of proteins, lipids, metabolic pathways, membranes, antioxidant defense and genetic material (Todorovic et al., 2012). Experimental studies on invertebrates and vertebrates alike confirm a higher sensitivity to low frequency magnetic fields (LF-MFs) during embryonic stages and development (Graham et al., 2000; Saunders et al., 2005). For an adult stable nervous system, such a response may amount to little more than transitory and barely detectable perturbation. On the other hand, for a developing fetus or an infant in which the nervous system is still in their formative stages, these very same mild perturbations could be catastrophic. For example, Ravera et al. (2006) exposed fertilized eggs of the sea urchin (*Paracentrotus lividus*) to extremely low frequency electromagnetic fields (ELF-EMFs) and observed a dramatic loss of synchronization of the first cell cycle accompanied by irregular separation of chromatids, resulting in the formation of anomalous embryos.

Endogenous direct current (DC) electric fields play an important role in embryonic development, particularly in the development of the nervous system. Precise interconnections need to be made between neurons migrating to the proper place at a particular time. Any exogenous electric fields that have a tendency to modulate the endogenous fields could conceivably modify the synaptogenesis (Saunders and McCaig, 2005). Disruption of endogenous electric fields in amphibians has been seen to result in aberrant development of the nervous system. Electromagnetic radiations emitted by electronic gadgets in the environment during their operation are not strong enough to cause DNA alterations directly, though indirect DNA alterations have been shown through the production of reactive oxygen species (Lai and Singh, 1997). Moreover, *in vitro* and *in vivo* studies have demonstrated the ability of electromagnetic fields (EMFs) to cause epigenetic modifications (Ahuja et al., 2005). Recently, Sarimov et al. (2011) have demonstrated the mechanism of transcriptional activation/silencing at the chromatin level during exposure to EMFs. They exposed lymphocytes from two healthy men to extremely low frequency EMFs, and observed significant differences in chromatin conformation in them depending on the initial state of chromatin and temperature during exposure. In general, the magnetic field of 50 Hz at peak amplitude within the range of 5 to 20 μT condensed the relaxed chromatin and relaxed the condensed chromatin. In this report, focus has been laid on the epigenomic disturbances induced by the excessive exposure to EMFs leading to neurodegenerative disorders, including autism.

ELECTROMAGNETIC FIELDS AND THEIR EFFECTS ON HEALTH

The health effects of modulated EMFs have been a subject of debate. There are reports showing beneficial as well as deleterious effects. For example, magnetic fields (MFs) are used in medicine for diagnostic and therapeutic purposes. MFs have shown promise in improving the management of osteoarthritis as well as the pain associated with it (Vavken et al., 2009) and they have also been used in enhancing the rate of healing of fractured long bones (Strauch et al., 2006; Gao et al., 2004), nerve regeneration (Walker et al., 2007) and spinal fusion (Gan and Glazer, 2006). Recently, Costa et al. (2011) and Zimmerman et al. (2012) have reported anticancer properties of EMFs. Magnetic fields used in magnetotherapy are effective at specific frequencies as well as densities at regulated durations of exposure. On the other hand, variable and chronic exposure to magnetic fields has been implicated in the induction of cancer and neurodegenerative disorders. Ever since the report made by Wertheimer and Leeper (1979) on increased cancer mortality among children living in homes located near power lines, there have been a large number of studies on the health effects of elevated exposure to magnetic fields. Although, the results of these studies are controversial, the International Association on Research (IARC) in Cancer Working Group classified ELF-MFs in category 2B (that is, possible human carcinogen) (IARC, 2002). A comprehensive document of the World Health Organization confirmed the IARC evaluation (WHO, 2007). Several studies have also reported an association between exposure to ELF-EMFs and neurodegenerative disorders. Recently, in a comprehensive review, Maes and Verschaeve (2012) highlighted that *in vivo* as well as *in vitro* exposure to ELF-EMFs is associated with cytogenetic aberrations, some of which in turn may be related to genetic abnormalities seen in Alzheimer's disease.

ELECTROMAGNETIC FIELDS AND AUTISM

Among the natural sources, sun is a major source of radiofrequency radiations (RFRs). Lately, telecommunication systems and gadgets using microwaves, like microwave ovens, have added RFRs into the environment, thousands of times higher than those received from the sun alone. While operating an RFR gadget, not only will the user, but also those in the surrounding vicinity will be exposed to similar biologically interactive levels of EMF intensity. Some of the RFR-producing gadgets are routinely utilized in monitoring embryonic, fetal and neonatal wellbeing. Ultrasonography is commonly used in pelvic examination at regular intervals during pregnancy, and this radiation is used to observe the developing embryo or fetus during pregnancy.

Modulation is used in all wireless communication systems to enable the signal to carry information. Some studies have shown that there may be specific effects from amplitude modulated radiofrequency fields on the human central nervous system (Juutilainen et al., 2011). The range of RFR exposure from mobile phones is 0.1 to 10 mW/cm^2. Philips et al. (1998) have reported that RFRs as low as 0.1 $\mu W/cm^2$ (< 1000 times the RFR of cell-phone range) can induce significant changes in the biological processes or molecular repair mechanisms. Some of the observed effects of exposure to RFRs include cognitive impairment (Chiang et al., 1989) and memory deficit (Lai et al., 1994), both of which are seen in autism.

The incidence of autism before 1980 was reported to be 1/2000, while the present incidence has increased to about 1/100 (Toro et al., 2010). Although, the question of actual increase in incidence is debatable (Newschaffer et al., 2007) a review of epidemiological surveys by Fambonne (2003) support the proposition that the increased incidence of autism has an origin around the 1980s, the very same time that telecommunication devices, particularly mobile phones that emit RFRs came into popular use. On the basis of these observations, Kane (2004) suggested that fetal or neonatal exposure to RFRs associated with the use of these devices may be associated with autism.

In the grey matter of brain of humans, there is a subset of neurons called the mirror neurons which respond when an individual performs certain actions and also when one observes others performing the same movements. Mirror neurons may also underlie the ability to imitate and learn the action of others making the mirror mechanism a bridge for communication and connection on multiple levels. As mirror neurons appear to be involved in social interaction, dysfunctions of this neural system could explain some of the primary symptoms of autism, including isolation and absence of empathy. Studies of people with autism show a lack of mirror neuron activity in several regions of the brain (Ramachandran and Oberman, 2006). Decrease of gray matter in the area belonging to the mirror neuron system has also been observed in autism. Developing nervous system network of an infant may be particularly prone to environmental factors like temporal noise. According to Thornton (2006), the most likely source of temporal noise in the environment is due to artificially generated electromagnetic radiations, which may be involved in disturbing the development/function of mirror neurons. This disturbance in mirror neurons, due to temporal noise from EMFs may be involved in the causation of autism.

ENVIRONMENTAL VERSUS EPIGENOME FACTORS

Prenatal and postnatal environmental factors have the potential to modify epigenetic programming and bring about subsequent changes which may have relevance in

health and disease. In complex diseases like cancer, diabetes and neurodegenerative disorders environmental factors, in addition to genetic factors, have an important role to play, and the contribution made by environmental factors may be mediated through epigenetics (Herceg, 2007).

Epigenetics may be defined as the heritable/transient changes in phenotypes that cannot be explained by changes in DNA sequence. Epigenetic mechanisms provide an extra layer of transcriptional/translational/post-translational controls that regulate how genes are expressed. The epigenetic changes are mostly the result of altered DNA methylation, histone modifications, non-coding RNAs and protein interactions (Richards, 2006; Ahuja et al., 2009).

ELECTROMAGNETIC FIELDS, EPIGENOMIC DISTURBANCES AND AUTISM

With the increasing number of electronic gadgets being used worldwide today, EMFs have become an important environmental source of electropollution. The central nervous system is sensitive to the action of EMFs, which can alter the bioelectric activity of the brain (Tattersal et al., 2001; Sidorenko and Tasaryuk, 2002). At non-thermal levels (with no increase in temperature), EMFs have been seen to bring about changes in biogenic amines involved in neurotransmission, like acetylcholine in the hypothalamus (Inaba et al., 1992; Lai et al., 1998). JorgeMora et al. (2011) exposed the paraventricular nucleus (PVN) of rat hypothalamus to 2.45 GHz microwave radiation at non-thermal specific absorbance rate (SAR) levels and observed its reactivity through c-Fos expression. PVN is a regulatory center for homeostasis (Sawchenko and Swanson, 1981) and the most important nucleus in relation to neurocircuitry stress (Herman and Cullinan, 1997).

EMFs of mobile phones (890 to 915 MHz) with (SAR 0.95 W/kg) were seen to be associated with increased free radical production and lipid peroxidation levels in both brain tissue and blood of guinea pigs (Meral et al., 2007). The brain seems to be especially sensitive to the influence of high frequency EMFs (Sidorenko 1999) causing oxidative stress in brain cells, which may lead to neurodegenerative diseases (Polydoro et al., 2004; Lima et al., 2005). Zhao et al. (2007) studied gene expression profile of rat neurons exposed to mobile phone radiofrequency (1800 MHz) electromagnetic fields with cDNA microassay. Among 1200 candidate genes, 24 up-regulated genes and 10 down-regulated genes were identified. From these studies, they concluded that RF exposure might alter the cytoskeleton and central nervous system functions by activating signal transduction pathways, thus leading to the abnormal neural growth and metabolism. Some effects of radiofrequency and power fields that have been observed affecting the brain functions by other investigators, include increased blood brain barrier permeability and neuronal damage in cortex, hippocampus, and basal ganglia (Salford et al., 2003), memory impairment (Krylov et al., 1993; Lai et al., 1994, 1998) and changes in choline uptake (Lai et al., 1989, 1990). Excessive exposure to EMFs has also been implicated in disturbing the epigenetic patterns as well as the rise of neurodegenerative disorders (Levin, 2003; Ahuja et al., 2005).

During early growth and development, epigenetic mechanisms like DNA methylation, histone modifications, non-coding RNAs and regulatory proteins are involved. During further growth and differentiation of the embryo and fetus, switching on and switching off of specific genes at specific times, is also governed by epigenetic mechanisms. Disturbance of epigenetic imprints due to endogenous or exogenous factors may lead to death or a diseased condition. There is ample evidence to support the contention that EMFs affect the gene expression and differentiation process through epigenetic mechanisms (Ahuja et al., 2005; Akan et al., 2010; Munshi et al., 2011). This contention is further supported by the following studies. Chung et al. (2010) compared the gene expression pattern in the thymus taken from 3 mice exposed to 83.3 μT polarized magnetic field and controls; the expression change was over 1.5 times in 100 preselected genes in the thymus of exposed mice as compared to the controls. Frahm et al. (2010) exposed mouse macrophages to ELF-MFs (50 Hz, 0.1 mT) and observed immune cell activation, in response to an increased production of reactive oxygen species, which in turn was due to modulation of the expression level of important proteins acting in redox regulatory processes. Collard et al. (2011) applied extremely low frequency electrical fields to human epidermal cells and carried out microarray analysis of 38500 human gene expression. Among the deviant genes identified by the authors were 4 up-regulated genes (DKK1, TXNRD1, ATF3 and MME) and one down-regulated gene (MACF1), all the 5 genes being involved in the regulation of cell proliferation and differentiation. Bisceglia et al. (2011) exposed human bone cell line (SaOS-2) to a low frequency electric field from apparatuses used in clinical therapies, and observed a significantly increased alkaline phosphatase enzymatic activity in the exposed cells as compared to the controls. Enhanced bone repair has been seen after exposure to low frequency electromagnetic fields, and the authors have demonstrated the molecular mechanism for this enhancement, that is, through an elevated level of alkaline phosphatase, which was previously demonstrated to be involved in bone mineralization (Anderson, 1989). Aydin and Akar (2011) exposed immature and mature rats to 900 MHz for 2 h/day for 45 days and observed oxidative stress metabolism in all the three lymphoid tissues studied (spleen, thymus and bone marrow). As compared to the mature rats, damage found in the immature animals was greater and there was less

recovery from oxidative stress injury after the specified recovery period study. Exposure to EMFs conspires to threaten epigenomic stability in the nervous system, because neurons are particularly susceptible to oxidative stress,

Evidence is accumulating that, in addition to deleterious mutations in genes of pivotal importance, epigenetic dis-regulation of DNA methylation and histone modification, which are important for regulation of chromatin structure and function, could play a prominent role in the patholo-physiology of autism and related neurodegenerative disorders (Thatcher and LaSalle, 2006; Schanen, 2006; Petronis, 2010). Using deep sequencing of DNA, Shulha et al. (2012) observed that there was loss or excess of a histone mark, trimethylated H3K4 (H3K4me3), at hun-dreds of loci in prefrontal cortex but not in other parts of the brain, in a subset of autistic individuals. The affected loci were associated with disregulated expression of transcripts implicated in neuronal communication and other higher order communication. Since, it has been suggested by various investigators that epigenetic distur-bances appear to play an important role in the causation of autism (Migliore and Coppede, 2009; Dufour-Rainfray et al., 2011), role of excessive exposure to EMFs emitted by electronic gadgets in causing these disturbances seems likely.

CONCLUSION

Different approaches have been considered to identify susceptible loci or genes (having mutations) for the causation of autism. Using various models, researchers have now begun to link novel molecular mechanisms of transcriptional regulation to intellectual and cognitive dys-functions in autism. Such studies highlight an increasing recognition of the key role epigenetic regulation plays in silencing and induction of the genes linked with distinct genotypes and phenotypes contributing to autism. Besides other environmental factors, electrical and mag-netic fields also have the ability to modify the epigenome. Epigenetic aberrations of certain loci, which are possibly triggered by undesirable doses of electromagnetic radiations from electronic gadgets during early develop-ment (particularly first trimester), may lead to autism in a certain set of infants carrying abnormal genetic and epigenetic modifications.

In future, it would be interesting to evaluate *in vivo* and *in vitro* epigenetic modifications in some of the major genes associated with autism, e.g. gaba-amino-butyric acid (GABA) receptor, serotonin transporter (SLC6A4) and neuroligin (NLGN), in normal cells after exposure to EMFs at different doses and durations.

ACKNOWLEDGEMENTS

The authors are thankful to Mr. Divakar, Samba and Shivani Shivani for their help in the reference work, and to Srinivas and Khaliq for secretarial assistance. They are also grateful to the anonymous referees for suggesting improvements in the manuscript.

Abbreviations: AC, Alternate current, **DC,** direct current; **cDNA,** complementary DNA; **DNA,** deoxyribonucleic acid; **ELF,** extremely low frequency; **EMF,** electromagnetic field; **FM,** frequency mode; **GHz,** Gega Hertz; **Genotype,** genetic composition; **Hz,** Hertz (cycles/second; named after German scientist Henrik Hertz); **LMF,** low magnetic field; **MF,** magnetic field; **MHz,** Mega Hertz; **µW,** Micro Watt; **mW,** Milli Watt; **Phenotype,** result of interaction between genotype and environment (end product due to epigenetic modifications of gene expression); **RFR,** radiofrequency rays; **RNA,** ribonucleic acid; **SAR,** specific absorption rate; **T,** Tesla (unit of magnetic field; named after an Italian scientist); **W,** Watt (unit of power; named after a British scientist); **W/Kg,** Watts/kilogram.

REFERENCES

Abrahams BS, Geschwind DH (2008). Advances in autism genetics: on the threshold of a new neurobiology. Nat. Rev. Genet. 9:341-355.

Adams JB, Holloway CE, George F, Quig D (2006). Analyses of toxic metals and essential minerals in the hair of Arizona children with autism and associated conditions, and their mothers. Biol. Trace Elem. Res. 110:193-209.

Adams JB, Romdalvik J, Ramanujam VM, Legator MS (2007). Mercury, lead, and zinc in baby teeth of children with autism versus controls. J. Toxicol. Environ. Health A. 70:1046-51.

Ahuja YR, Bhargava SC, Ratnakar kS (2005). Electrical and magnetic fields in stem cell research. Electromagn. Biol. Med. 24:121-124.

Ahuja YR, Munshi AD, Jehan P (2009). Environmental modifications of epigenetics and its consiquences. Proc. Andhra Pradesh Acad. Sci. 13:87-92.

Akan Z, Aksu B, Tulunay A, Bilsel S, Inhan-Garip A (2010). Extremely low-frequency electromagnetic fields affect the immune response of monocyte-derived macrophages to pathogens. Bioelectromagnetics 31:603-12.

Anderson HC (1989). Mechanism of mineral formation in bone. Lab. Investig. 60:320-330.

Aydin B, Akar A (2011). Effects of a 900-MHz electromagnetic field on oxidative stress parameters in rat lymphoid organs, polymorphonuclear leukocytes and plasma. Arch. Med. Res. 42:261-267.

Bisceglia B, Zirpoli H, Caputo M, Chiadini F, Scaglione A, Tecce MF (2011). Induction of alkaline phosphatase activity by exposure of human cell lines to a low-frequency electric field from apparatuses used in clinical therapies. Bioelectromagnetics 32:113-119.

Chiang H, Yao GD, Fang QS, Wang KQ, Lu DZ, Zhou YK (1989). Health effects of environmental electromagnetic fields. J. Bioelectricity 8:127-131.

Chung MK, Yu WJ, Kim YB, Myung SH (2010). Lack of a co-promotion effect of 60 Hz circularly polarized magnetic fields on spontaneous development of lymphoma in AKR mice. Bioelectromagnetics 31:130-139.

Collard JF, Mertens B, Hinsenkamp M (2011). *In vitro* study of the effects of ELF electric fields on gene expression in human epidermal cells. Bioelectromagnetics 32:28-36.

Costa FP, de Oliveira AC, Meirelles R, Machado MC, Zanesco T, Surjan R, Chammas MC, de Souza Rocha M, Morgan D, Cantor A,Zimmerman J, Brezovich I, Kuster N, Barbault A, Pasche B (2011). Treatment of advanced hepatocellular carcinoma with very low levels

of amplitude-modulated electromagnetic fields. Br. J. Cancer 105:640-648.
Currenti SA (2010). Understanding and determining the etiology of autism. Cell Mol. Neurobiol. 30:161-71.
Dufour-Rainfray D, Vourc'h P, Tourlet S, Guilloteau D, Chalon S, Andres CR (2011). Fetal exposure to teratogens: Evidence of genes involved in autism. Neurosci. Biobehav. Rev. 35:1254-1265.
Fambonne E (2003). Epidemiological surveys of autism and other pervasive developmental disorders: an update. J. Autism Dev. Disorder 33:365-382.
Fatima W, Banu H, Ahuja YR (2006). Autism: An overview. Proc. Andhra Pradesh Acad. Sci. 10: 215-221.
Frahm J, Mattsson MO, Simkó M (2010). Exposure to ELF magnetic fields modulates redox related protein expression in mouse macrophages. Toxicol. Lett. 192: 330-336.
Gan JC, Glazer PA (2006). Electrical stimulation therapies for spinal fusions: current concepts. Eur. Spine. J. 15:1301-1311.
Gao KD, Yu YL, Qi DY, Zhou JW, Lu Y (2004). Analysis of curative effect of pulsed electromagnetic fields on pain of primary osteoporosis. Chin. J. Phys. Med. Rehabil. 26: 669-670.
Graham JH, Fletcher D, Tigue J, McDonald M (2000). Growth and developmental stability of *Drosophila melanogaster* in low frequency magnetic fields. Bioelectromagnetics 21:465-472.
Hallmayer J, Cleveland S, Torres A, Phillips J, Cohen B, Torigoe T, Miller J, Fedele A, Collins J, Smith K, Lotspeich L, Croen LA,Ozonoff S, Lajonchere C, Grether JK, Risch N (2011). Genetic heritability and shared environmental factors among twin pairs with autism. Arch. Gen. Psychiatry 68:1095-1102.
Herbert MR, Russo JP, Yang S, Roohi J, Blaxill M, Kahler SG, Cremer L, Hatchwell E (2006). Autism and environmental genomics. Neurotoxicology 27:671-684.
Herceg Z (2007). Epigenetics and cancer: towards an evaluation of the impact of environmental and dietary factors. Mutagenesis 22:91-103.
Herman JP, Cullinan WE (1997). Neurocircuitry of stress: central control of the hypothalamo-pituitary-adrenocortical axis. Trends Neurosci. 20:78-84.
IARC (2002). Non-ionizing radiation, Part 1: static and extremely low-frequency (ELF) electric and magnetic fields. IARC Monograph Evaluation of Carcinogenic Risks on Humans, Vol 49. International Agency for Research on Cancer, Lyon, France.
Inaba R, Shishido K, Okada A, Moroji T (1992). Effects of whole body microwave exposure on the rat brain contents of biogenic amines. Eur. J. Appl. Physiol. Occup. Physiol. 65:124-128.
JorgeMora T, Misa-Agustiño MJ, Rodríguez-González JA, Jorge-Barreiro FJ, Ares- Pena FJ, López-Martín E (2011). The effects of single and repeated exposure to 2.45 GHz radiofrequency fields on c-Fos protein expression in the paraventricular nucleus of rat hypothalamus. Neurochem. Res. 36:2322-2332.
Juutilainen J, Höytö A, Kumlin T, Naarala J (2011). Review of possible modulation-dependent biological effects of radiofrequency fields. Bioelectromagnetics 32:511-534.
Kane RC (2004). A possible association between fetal/neonatal exposure to radiofrequency electromagnetic radiation and the increased incidence of autism spectrum disorders (ASD). Med. Hypotheses 62:195-197.
Krylov IN, Iasnetsov VV, Dukhanin AS, Pal'tsev IUP (1993). Pharmacologic correction of learning and memory disorders induced by exposure to high-frequency electromagnetic radiation. Biull. Eksp. Biol. Med. 115:260-262.
Lai H, Carino MA, Horita A, Guy AW (1989). Low-level microwave irradiation and central cholinergic systems. Pharmacol. Biochem. Behav. 33:131-138.
Lai H, Carino MA, Horita A, Guy AW (1990). Corticotropin-releasing factor antagonist blocks microwave-induced decreases in high-affinity choline uptake in the rat brain. Brain Res. Bull. 25:609-612.
Lai H, Carino MA, Ushijima I (1998). Acute exposure to a 60 Hz magnetic field affects rats' water-maze performance. Bioelectromagnetics 19:117-122.
Lai H, Horita A, Guy AW (1994). Microwave irradiation affects radial-arm maze performance in the rat. Bioelectromagnetics 15:95-104.
Lai H, Singh NP (1997). Melatonin and a spin-trap compound block radiofrequency electromagnetic radiation-induced DNA strand breaks in rat brain cells. Bioelectromagnetics 18:446-54.
Lakshmi Priya MD, Geetha A (2011). Level of trace elements (copper, zinc, magnesium and selenium) and toxic elements (lead and mercury) in the hair and nail of children with autism. Biol. Trace Elem. Res. 142:148-58.
Levin M (2003). Bioelectromagnetics in morphogenesis. Bioelectromagnetics 24:295-315.
Lima MN, Polydoro M, Laranja DC, Bonatto F, Bromberg E, Moreira JC, Dal-Pizzol F, Schröder N (2005). Recognition memory impairment and brain oxidative stress induced by postnatal iron administration. Eur. J. Neurosci. 21:2521-2528.
Maes A, Verschaeve L (2012). Can cytogenetics explain the possible association between exposure to extreme low-frequency magnetic fields and Alzheimer's disease? J. Appl. Toxicol. 32:81-87.
Meral I, Mert H, Mert N, Deger Y, Yoruk I, Yetkin A, Keskin S (2007). Effects of 900-MHz electromagnetic field emitted from cellular phone on brain oxidative stress and some vitamin levels of guinea pigs. Brain Res. 1169:120-124.
Migliore L, Coppedè F (2009). Genetics, environmental factors and the emerging role of epigenetics in neurodegenerative diseases. Mutat. Res. 667:82-97.
Munshi A, Krupanidhi S, Ahuja YR (2011). Epigenome: genomic response to environmental eccentricities. In: Barh D, Zambare V, Raja R (Eds), OMICS: Applications in Biomedical, Agricultural and Environmental Sciences. CRC Press, Taylor & Francis group.
Newschaffer CJ, Croen LA, Daniels J, Giarelli E, Grether JK, Levy SE, Mandell DS, Miller LA, Pinto-Martin J, Reaven J, Reynolds AM, Rice CE, Schendel D, Windham GC (2007). The epidemiology of autism spectrum disorders. Ann. Rev. Public health. 28:235-258.
Petronis A (2010). Epigenetics as a unifying principle in the aetiology of complex traits and diseases. Nature 465:721-727.
Phillips JL, Ivaschuk O, Ishida-Jones T, Jones RA, Campbell-Beachler M, Haggren W (1998). DNA damage in Molt-4 T- lymphobastoid cells exposed to cellular telephone radio frequency fields in vitro. Bioelectrochem. Bioenerg. 45:103-110.
Ploeger A, Raijmakers ME, van der Maas HL, Galis F (2010). The association between autism and errors in early embryogenesis: what is the causal mechanism? Biol. Psychiatry 67:602-7.
Polydoro M, Schröder N, Lima MN, Caldana F, Laranja DC, Bromberg E, Roesler R, Quevedo J, Moreira JC, Dal-Pizzol F (2004). Haloperidol- and clozapine-induced oxidative stress in the rat brain. Pharmacol. Biochem. Behav. 78:751-756.
Ramachandran VS, Oberman LM (2006). Broken mirrors: a theory of autism. Sci. Am. 295:62-69.
Ravera S, Falugi C, Calzia D, Pepe IM, Panfoli I, Morelli A (2006). First cell cycles of sea urchin *Paracentrotus lividus* are dramatically impaired by exposure to extremely low-frequency electromagnetic field. Biol. Reprod. 75:948-953.
Richards EJ (2006). Inherited epigenetic variation--revisiting soft inheritance. Nat. Rev. Genet. 7:395-401.
Salford LG, Brun AE, Eherhardt JL, Malmgren L, Persson BR (2003). Nerve cell damage in mammalian brain after exposure to microwaves from GSM mobile phones. Environ. Health. Perspect. 111: 881-893.
Sarimov R, Alipov ED, Belyaev IY (2011). Fifty Hertz magnetic fields individually affect chromatin conformation in human lymphocytes: dependence on amplitude, temperature, and initial chromatin state. Bioelectromagnetics 32:570-579.
Saunders RD, McCaig CD (2005). Developmental effects of physiologically weak electric fields and heat: an overview. Bioelectromagnetics. Suppl. 7:S127-32.
Sawchenko PE, Swanson LW (1981). Central noradrenergic pathways for the integration of hypothalamic neuroendocrine and autonomic responses. Science 214:685-687.
Schanen NC (2006). Epigenetics of autism spectrum disorders. Hum. Mol. Genet. 15(2):R138-50.
Shulha HP, Cheung I, Whittle C, Wang J, Virgil D, Lin CL, Guo Y, Lessard A, Akbarian S, Weng Z (2012). Epigenetic signatures of autism: trimethylated H3K4 landscapes in prefrontal neurons. Arch. Gen. Psychiatry 69:314-324.
Sidorenko AV (1999). The analysis of animal bioelectric brain activity influenced by microwaves or by the introduction of strychnine. Bioelectrochem. Bioenerg. 48:223-226.

Sidorenko AV, Tasaryuk VV (2002). The effect of electromagnetic radiation in the millimeter range on the brain bioelectrical activity. Radiat. Biol. Radioecol. 42:546-550.
Strauch B, Patel MK, Rosen DJ, Mahadevia S, Brindzei N, Pilla AA (2006). Pulsed magnetic field therapy increases tensile strength in a rat Achilles' tendon repair model. J. Hand Surg. Am. 31:1131-1135.
Tattersall JE, Scott IR, Wood SJ, Nettell JJ, Bevir MK, Wang Z, Somasiri NP, Chen X (2001). Effects of low intensity radiofrequency electromagnetic fields on electrical activity in rat hippocampal slices. Brain Res. 904:43-53.
Thatcher KN, LaSalle JM (2006). Dynamic changes in histone H3 lysine 9 acetylation localization patterns during neuronal maturation require MeCP2. Epigenetics 1:24-31.
Thornton IM (2006). Out of time: a possible link between mirror neurons, autism and electromagnetic radiation. Med. Hypotheses 67:378-382.
Todorović D, Mirčić D, Ilijin L, Mrdaković M, Vlahović M, Prolić Z, Mataruga VP (2012). Effect of magnetic fields on antioxidative defense and fitness-related traits of *Baculum extradentatum* (insecta, phasmatodea). Bioelectromagnetics 33:265-273.
Toro R, Konyukh M, Delorme R, Leblond C, Chaste P, Fauchereau F, Coleman M, Leboyer M, Gillberg C, Bourgeron T (2010). Key role for gene dosage and synaptic homeostasis in autism spectrum disorders. Trends Genet. 26:363-372.
Vavken P, Arrich F, Schuhfried O, Dorotka R (2009). Effectiveness of pulsed electromagnetic
field therapy in the management of osteoarthritis of the knee: a meta-analysis of randomized controlled trials. J. Rehabil. Med. 41:406-411.
Voineagu I, Wang X, Johnston P, Lowe JK, Tian Y, Horvath S, Mill J, Cantor RM, Blencowe BJ, Geschwind DH (2011). Transcriptomic analysis of autistic brain reveals convergent molecular pathology. Nature 474:380-384.
Walker JL, Kryscio R, Smith J, Pilla A, Sisken BF (2007). Electromagnetic field treatment of nerve crush injury in a rat model: effect of signal configuration on functional recovery. Bioelectromagnetics 28:256-263.
Veenstra-Vanderweele J, Christian SL, Cook EH Jr (2004). Autism as a paradigmatic complex genetic disorder. Annu. Rev. Genomics Hum. Genet. 5:379-405.
Weinstein M, Ben-Sira L, Levy Y, Zachor DA, Ben Itzhak E, Artzi M, Tarrasch R, Eksteine PM, Hendler T, Ben Bashat D (2011). Abnormal white matter integrity in young children with autism. Hum. Brain Mapp. 32:534-543.
Wertheimer N, Leeper E (1979). Electrical wiring configurations and childhood cancer. Am. J. Epidemiol. 109:273-284.
World Health Organization (2007). Extremely low fields. Geneva, WHO.
Zhao R, Zhang S, Xu Z, Ju L, Lu D, Yao G (2007) Studying gene expression profile of rat neuron exposed to 1800MHz radiofrequency electromagnetic fields with cDNA microassay. Toxicology 235:167-175.
Zikopoulos B, Barbas H (2010). Changes in prefrontal axons may disrupt the network in autism. J. Neurosci. 30:14595-4609.
Zimmerman JW, Pennison MJ, Brezovich I, Yi N, Yang CT, Ramaker R, Absher D, Myers RM, Kuster N, Costa FP, Barbault A,Pasche B (2012) Cancer cell proliferation is inhibited by specific modulation frequencies. Br. J. Cancer 106:307-313.

academicJournals

Vol. 5(4), pp. 178-183, April 2013
DOI: 10.5897/IJMMS12.140
ISSN 2006-9723 ©2013 Academic Journals
http://www.academicjournals.org/IJMMS

International Journal of Medicine and Medical Sciences

Full Length Research Paper

Oral cancer: The Nigerian experience

Ahmed Oluwatoyin LAWAL*, Bamidele KOLUDE and Bukola Folasade ADEYEMI

Department of Oral Pathology, College of Medicine, University of Ibadan, Nigeria.

Accepted 25 March, 2013

Oral cancer is one of the ten most common cancers worldwide and the five years survival rate is still disappointingly low. The incidence of oral cancer varies from different regions of the world with highest rates reported in the South-east Asia especially in India where oral cancer accounts for 15 - 40% of cancers and is the most common cancer in men. Although, tobacco and alcohol use are the most important risk factors in the development of oral cancer, some reports from Nigeria suggests otherwise. Tobacco and chronic alcohol use have been found to be low in cancer patients in various studies from Nigeria, whereas most of these patients are from the lower income, poorly educated class in the society. More so, oral cancer patients from a study in Nigeria were found to consume less fruits than controls and had statistically significant lower serum antioxidant vitamins levels when compared with a non-cancer group. We suggest that poverty, illiteracy, malnutrition and possibly a yet to be determined chronic infection may be more important risk factors than the well-established risk factors (tobacco and alcohol use) in the development of oral cancer in Nigerian cases.

Key words: Oral cancer, tobacco, alcohol, risk factors.

INTRODUCTION

Cancers of the oral cavity, pharynx and salivary glands are responsible for an estimated 390,000 (3.9% of total) new cases of cancer worldwide in 2000 and is still a major health challenge because the five years survival rate remains disappointingly low at about 50% (Macfarlane et al., 1994; Otoh et al., 2004). The highest rates of oral cancer have been reported in countries such as India, Sri-Lanka, South Vietnam, Papua New Guinea, the Philippines, Hong Kong and Taiwan and mainly attributable to use of tobacco in various forms (Garewal, 1991). Oral malignant neoplasms represent 2 - 4% of all malignant lesions in the United States of America (Krutchkoff et al., 1990), 2% of cancers in Britain (Binnie, 1976) and 1% in Australia (Sugerman and Savage, 1999). Oral cancers are the commonest cancers amongst men in India and the number three amongst women after breast and cervix uteri tumours (Nair et al., 1988; Rudolph and Atvis, 2003) accounting for between 15 - 50% of all cancer cases in India.

Oral cancer is believed to be relatively rare amongst Africans (Davies and Wilson, 1954). Arotiba et al. (1999) in a review of 246 oral squamous cell carcinoma patients in University College Hospital Ibadan reported that 1.2% of all malignant lesions are oral squamous cell carcinoma. Oji et al. (2007) in Eastern Nigeria reported that oral cancer accounted for 2.7% of all cancer cases seen at the University of Nigeria Teaching Hospital (UNTH) Enugu over a six-year period while Otoh et al. (2005) in Maiduguri, North-eastern Nigeria, reported an average rate of 20 cases per annum over a six-year period Though, it has been suggested that these rates may be under reported in Nigeria because of the low dentist: population ratio, poor and inadequate hospital services and a poor (and almost non-existent) cancer registry in

*Corresponding author. E-mail: lawaloluwatoyin@gmail.com. Tel: +2348055133964. Fax: +23422411768.

Nigeria (Otoh et al., 2004).

Age distribution

Though oral cancer may occur at any age, it is essentially a disease of the elderly; and it has been reported that more than 95% of oral cancers occur in persons older than 40 years of age in most regions of the world (Johnson, 1991). The increased incidence of cancer with advancing age may be partly due to the increasing level of free radical reactions with age. Also, there is said to be a diminishing ability of the immune system to eliminate altered cells because of immune tolerance, thus the effectiveness of cancer surveillance by immune cells is reduced with advancing age (Enwonwu and Meeks, 1995).

However, some reports from Africa found less proportion of oral cancer cases occurring in persons older than 40 years with Chidzonga in Zimbabwe and Ajayi in Lagos reporting 70.8 and 75% of oral carncinomas occurring above 40 years of age respectively (Chidzonga, 2006; Ajayi et al., 2007). The lower percentage of oral cancer occurring above 40 years of age in African studies compared to Caucasian studies, may be due to the lower life expectancy and or early exposure to risk factors in Africans. It is known that the percentage of people under 40 years is much higher and life expectancy (47 years) is much lower in the Nigerian population than in the developed nations (Ajayi et al., 2007). A trend towards increased incidence of oral cancers in relatively younger age groups has been reported in United Kingdom and United States. Though the reason(s) for this trend is uncertain (Effiom et al., 2008), infection by human papilloma virus (HPV) is thought to be more plausible than the more established aetiologic factors such as tobacco and alcohol, mainly because HPV positivity was found chiefly in young oral cancer patients who consume less alcohol and tobacco (Monsjou et al., 2012). Other researchers are of the opinion that inherited inability to metabolize pro-carcinogens and carcinogens or defects in a patient's ability to repair DNA damage may be important in the aetiogenesis of squamous cell carcinoma in patients below 40 years of age (Adeyemi et al., 2008).

Sex distribution

Oral cancer affects males about twice as often as females in the industrialized world, possibly due to a greater exposure of males to established risk factors like tobacco and alcohol use. Also, most studies from Africa have shown oral cancer to be more prevalent in males than females (Arotiba et al., 1999; Odukoya et al., 1986; Ajayi et al., 2007). However, Otoh et al. (2005) in Maiduguri North-east Nigeria found a male to female ratio of 3:4 and suggested that the relative higher preponderance of females in their study may be attributed to the increasing exposure of females in North-eastern Nigeria to carcinogens such as tobacco and alcohol. Similarly, some other reports from India, Singapore, Hawaii and in Denmark (Johnson, 1991) found no marked difference in incidence of oral cancer in males and females whilst Van Wyk et al. (1993) found a higher female preponderance in South African Indians with male to female ratio of 1 to 1.6. They attributed this to the fact that areca nut chewing was more common in South African Indian women than men.

Site distribution

The lip is the most common site of oral cancer in fair skinned races particularly in men who work out of doors due their exposure to ultra-violet radiation from the sun (Spitzer et al., 1975). Intraoral cancer in western countries most commonly affects the lateral border of tongue and the floor of the mouth; the buccal mucosa is the next most common site of occurrence followed by mandibular alveolus, retro-molar region and soft palate with the hard palate and dorsum of tongue having the lowest risk (Johnson, 1991). In South-east Asia, the buccal, retro-molar, and commissural mucosa are the most prone sites (Johnson, 1991). Some Nigerian studies from Ibadan, however, show that the tongue, palate and the mandibular alveolus are the sites most commonly affected with the floor of mouth and buccal mucosa being the least affected (Daramola et al., 1979; Arotiba et al., 1999). This is in contrast with studies from Lagos which showed the mandibular gingival to be the commonest site of occurrence followed by the maxillary gingiva and tongue with the floor of mouth having the lowest occurrence (Effiom et al., 2008).

AETIOLOGY

Cancers of the head and neck have been associated with known aetiological and predisposing factors such as tobacco and chronic alcohol use, ingestion of smoked fish, infections especially by viruses, dietary deficiencies and industrial pollution. The association of these predisposing factors are important since their control makes the prevention of cancer possible (Otoh et al., 2005).

Tobacco

The use of tobacco in whatever form is associated with increased risk of intra oral cancer worldwide (Johnson, 1991). It is commonly consumed in betel quid or pan consisting of tobacco mixed with chopped areca nut, slaked lime and catechu, wrapped in a leaf of piper betel

vine. In Indians, spices such as cardamom, cloves and aniseed may be added. In North Africa and the Middle East, a mixture of tobacco, ash and lime in water or oil called nass or nasswar, is commonly held in the mouth (Johnson, 1991). Many different forms of snuff are placed in contact with oral mucosa in northern Europe, France, the USA and parts of Africa including Sudan, Southern Egypt and Saudi Arabia (Johnson, 1991). Users of tobacco quid especially if associated with smoking, have a 10 to 20 times greater risk of developing oral cancer than those who neither chew nor smoke (Enwonwu and Meeks, 1995; Davis and Severson, 1987).

All forms of tobacco use have been strongly linked to the development of oral cancer. Cigar and pipe smoking are associated with a greater risk of the development of oral cancer when compared with cigarette smoking, probably because most cigarettes have filters that reduce the load of carcinogen that will come in contact with the oral mucosa (Rudolph and Atvis, 2003). Carcinogens in tobacco (mainly polycyclic aromatic hydrocarbons), can cause an accumulation of genetic mutations in oral epithelial cells including p53 mutation, mutation and loss of heterozygosity (H-RAS) and amplification (K-RAS and N-RAS) of the RAS oncogenes leading to abnormal and uncontrollable cell division and growth (Scully and Bedi, 2000).

Although, tobacco use is regarded as the most significant factor in aetiology of oral cancer, some studies from Nigeria seem to dispute this notion. Oji and Chukwuneke (2007) in Enugu, found that patients with oral cancer in their tertiary health centre gave no history of tobacco or alcohol misuse. They postulated that poverty, malnutrition, lack of education, poor oral hygiene and chronic malaria may be more important in the aetiology and severity of oral cancer in their series (Oji and Chukwuneke, 2007). A report by Lawoyin et al. (2003) from Ibadan, South-west Nigeria also reported low prevalence of recognised risk factors (tobacco and alcohol use) for oral cancer in their patients.

Furthermore, we previously reported in a study in South-west Nigeria that only 26.1% of oral cancer patients in our study gave history of tobacco use (Lawal et al., 2011). Most other studies have shown higher percentages of tobacco use. Blot et al. (1988) in a study in the United States found 75% of oral cancer cases are associated with tobacco smoking and heavy alcohol use (1988). Lissowaka et al. (2003) reported that 82% of oral cancer cases in Poland reported that they use tobacco compared to 65% of those in their control group. Other studies in Denmark and Brazil reported 86 and 63.9% of oral cancer cases in their studies use alcohol and tobacco (Gervasio et al., 2001; Pinhort et al., 1997).

Alcohol

Alcohol as a risk factor in development of cancer was previously thought to act indirectly and through its synergistic effect with tobacco. The effect of alcohol has been thought to occur through its ability to irritate the oral mucosa and to act as a solvent for carcinogens (especially in tobacco). Contaminants and additives with carcinogenic potentials that are found in alcoholic drinks have also been thought to have a role in oral cancer development (Rudolph and Atvis, 2003). Also, acetyl aldehyde a metabolite of alcohol is known to be a direct carcinogen by causing alteration of the p53 gene and Ras oncogenes (Rudolph and Atvis, 2003). Acetaldehyde is also known to be cytotoxic and causes production of free radicals (Harty et al., 1997). Garro et al., 1992; Mufti et al., 1993 also demonstrated that chronic alcohol consumption interferes with repair of alkylated DNA (Ogden and Wight, 1998). Alcohol consumption has consistently being found to be low amongst oral cancer patients in Nigeria (Lawoyin et al., 2003; Oji and Chukwuneke, 2007; Adeyemi et al., 2008) and may not be an important factor in the aetiology of oral cancer in Nigerians. Lawal et al. (2011) had recently shown that only 25.8% of oral cancer patients seen in our study were exposed to alcohol use.

Human Papilloma Virus (HPV) infection

The increasing incidence of oral squamous cell carcinoma in young people and especially in those who do not smoke or use alcohol has indicated a possible aetiologic role for infections such as HPV. High-risk types of HPV include HPV16 and HPV18, both of which are well-established initiators of cervical and anogenital carcinogenesis (Kreimer et al., 2005; Monsjou et al., 2012). The oncogenic potential of HPV is attributable to its ability to insert specific viral DNA fragments (early genes E5, E6 and E7) into the host cellular genome. As a result of this integration, some key functions of tumour suppressor factors are abrogated (p21, p53 and pRb pathways, respectively), leading to defects in apoptosis, DNA repair mechanisms, cell cycle regulation and, finally, to cellular immortalization (Ragin et al., 2007). High-risk HPV DNA was found in nearly 100% of cervical carcinomas and 84% of anal carcinomas, 70% of lower vaginal carcinoma and 40% in vulvar carcinomas. Although, HPV genomic sequences have been identified in head and neck squamous cell carcinoma, a wide range of viral detection rate of between 0 - 100% have been reported. This great disparity in prevalence rate has been attributed to ethno-geographical differences, site of lesions studied (oral, pharyngeal or tonsillar), the differences in specimen type (blood, paraffin embedded tissue, frozen sections) and HPV detection methods.

The risk factors for HPV positive head and neck squamous cell carcinoma are mainly related to sexual habits rather than tobacco and alcohol use in HPV negative cancers (Hennessey et al., 2009). The risk for HPV-positive HNSCC increases with increasing numbers

of both oral and vaginal sexual partners, a history of genital warts, and a younger age at first intercourse (Hennessey et al., 2009). Previous studies have consistently shown that HPV infection conferred a higher risk of oropharyngeal cancer when compared with oral cavity cancer (Hennessey et al., 2009). A meta-analysis of 17 studies found that HPV is most strongly associated with tonsillar cancer (OR 15.1, 95% CI 6.8-33.7), is intermediate for oropharyngeal cancer in general (OR 4.3, 95% CI 2.1-8.9), and is weakest for oral cancer (OR 2.0, 95% CI 1.0-4.2) (Hobbs et al., 2006). Similarly, a study found HPV in 3.9% (95% CI, 2.5-5.3) of oral cavity tumours and 18.3% of oropharyngeal squamous cell carcinomas (Herrero et al., 2003).

Additionally, many studies have shown that persons with HPV-positive oropharyngeal cancers are more responsive to treatment and have better rates of disease-specific survival than those with HPV-negative oropharyngeal cancers (Hennessey et al., 2009). Preliminary reports from a study in our centre using PCR to detect HPV (unpublished), showed that all the oral cancer cases studied were HPV negative and HPV may not be important in the aetiogenesis of oral cancer in Nigerians.

Socio-economic factors

Studies on the association of socio-economic status and oral cancer have been somewhat conflicting (Hashibe et al., 2003). Some studies reported no association between oral cancer and education and occupation, while others showed a decreased risk of oral cancer with higher socio economic status based on occupation and higher levels of education (Greenberg et al., 1991; Elwood et al., 1984; Williams and Horm, 1977).

The reports of studies from Nigeria, however, have consistently shown oral cancer to be more prevalent in the low socio-economic groups (Oji and Chukwuneke, 2007; Adeyemi et al., 2008; Lawal et al., 2011). We previously reported that income of less than ₦50,000 a month (approximately $1 a day) and lack of a secondary education were associated with increased risk (odds ratio 5.75 and 1.04 respectively) of developing oral cancer in a South-west Nigerian population (Lawal et al., 2011). These findings have been previously corroborated by studies from India and United States of America. Kerr et al. (2004) in a study in the United States reported that in addition to higher prevalence of alcohol and tobacco use, people of lower socio economic groups were more likely to consume less fruits and vegetables and this was similar to the findings of Hashibe in India (Greenberg et al., 1991).

Furthermore, people in low socio-economic groups were less likely to have access to proper health services and health education that would empower them to make informed decisions that would protect and improve their health (Poul, 2008). A study in Canada found that people in lower socio economic class were less likely to visit their dentist regularly and suggested that increased incidence of oral cancer in this group may also be related to their poor oral hygiene (Johnson et al., 2010).

DIET AND NUTRITION

One of the earliest suggestions that nutrition may play a role in aetiology of oral cancer comes from studies in Sweden that found a link between Iron deficiency anaemia (Plummer- Vinson syndrome) and pharyngeal cancer in women (Winn, 1995). People whose diets are deficient in fruits and vegetables have been found to be at a higher risk of developing oral cancer (Winn, 1995). Winn (1995) in a study in North Carolina USA showed that people who consumed 0 - 1 servings of fruits per week were significantly more likely to have oral cancer than those who consumed 7 or more servings per week. Other studies from India and Brazil have shown that regular consumption of fruits and vegetables are protective against oral cancer (Franco et al., 1989).

Franceschi et al. 1992, suggested that high intake of particular dietary staples may be an indication of poor diet in general and that inadequate nutrition enhances cancer risk. They opined that this may be due to the fact that dietary deficiencies are linked to high consumption of certain foods (for example maize is low in riboflavin) (Winn, 1995). Martinez in Puerto-Rico (Winn, 1995) found that patients with oral, pharyngeal and esophageal cancers ate less food than did control subjects, were more likely to eat only one meal a day and were more likely to eat irregularly. However, it was reported that subjects with head and neck cancer were not more likely than control subjects to take inadequate diet (Winn, 1995).

In a study conducted in South-west Nigeria, we discovered that not consuming fruits and vegetables regularly was associated with an increased risk of developing oral cancer (OR 3.0 and 1.32) (Lawal et al., 2011). Also, we found that the serum levels of antioxidant vitamins A, C and E were significantly lower in oral cancer patient compared with those of normal patients ($p=0.001$, $p=0.013$ and $p=0.015$ respectively). In the same vein, we reported that the risk of developing oral cancer was much higher in people with lower serum vitamins A, C and E (OR= 10.89, 11.35 and 5.6) when compared with the risk in people who took tobacco or alcohol (OR= 4.05 and 1.09) (Lawal et al., 2012).

It is suggested that protective effects of the antioxidant vitamins against cancers may be attributable to their free radical mopping effects and their ability to boost immune response. Free radicals are highly unstable and if unchecked by antioxidants, are capable of damaging cell constituents, including DNA, as well as other opportune targets, particularly those containing polyunsaturated fatty acids (Lippman et al., 1994).

Kola nut

Smoking cigarette and habitually chewing kola nut with it may be a social habit that is peculiar to some Nigerian smokers. Some people, however, chew kola nut to induce sleeplessness, prolong their capacity for work and increase their mental efficiency probably because of its caffeine content (Odukoya et al., 1990). *Cola acuminata* and *Cola nitida rubra* are two types of kola nut commonly eaten by Nigerians but *C. acuminata* is preferred because of its perceived greater stimulating effect as confirmed by Somorin who reported higher caffeine content for *C. acuminata* (Odukoya et al., 1990). Although, studies linking the use of kola nut with oral cancer are rare, Odukoya et al. (1990) in a study in South-west Nigeria, observed that smoking and kola nut chewing had a statistically significant ($p<0.005$) influence on the karyopyknotic index scores of palatal mucosa of volunteers with people that smoke and chew kola nut having a higher karyopyknotic index score than those that smoke alone and in non-smokers (Odukoya et al., 1990).

Furthermore, people who chew kola nut without smoking had a higher karyopyknotic index score than in the non-kolanut, non-smoking group. They suggested a potentiation of the cigarette smoking-induced palatal keratinization by kola nut (Odukoya et al., 1990). The carcinogenic potential of kola nut has been linked with the fact that it contains up to 5 - 10% of tanins which previously has been listed among compounds with known and suspected carcinogenic potentials (Odukoya et al., 1990).

FUTURE RESEARCH OPPORTUNITIES

The investigation of the molecular events associated with oral cancer in Nigeria is desirable, since it has previously been shown that there are racial differences in molecular events that lead to oral cancer. The molecular changes found to be associated with oral carcinomas in western countries (UK, USA and Australia) are mainly p53 mutations but these are rare in the east (India and South east Asia) where more of RAS oncogenes abnormalities are common (Scully and Bedi, 2000). Also, the concept of the role of tumour microenvironment in the pathogenesis and prognosis of cancers have generated keen interest amongst many researchers, and studies of tumour microenvironment in Nigerian oral cancer cases will add to the knowledge in this novel area of cancer research which might in future be a potential target for more effective cancer treatment.

Conclusion

Though considered to be the most important aetiologic factors, the experiences from Nigeria suggests that tobacco and alcohol play a less important role in the aetiology of oral cancer compared to reports from other parts of the world. We opine that oral cancer in Nigeria may be an interplay of poverty, malnutrition, immune suppression and possibly a yet to be determined infective agent which act as modifiers to genetic predisposition. The efforts by the Nigerian government to alleviate poverty and improve both adult and general education needs to be strengthened as we believe these will go a long way in reducing the burden of oral cancers in the Nigerian population. In addition, concerted efforts is needed by both government and non-governmental organisations (NGOs) in improving oral health education and advocacy, and the importance of proper diet and nutrition in preventing oral cancer should be emphasised in cancer awareness campaigns.

REFERENCES

Adeyemi BF, Adekunle LV, Kolude BM, Akang EEU, Lawoyin JO (2008). Head and neck cancer - a clinicopathological study in a tertiary care centre. J. Natl. Med. Assoc. 100:690-697.

Ajayi OF, Adeyemo WL, Ladeinde MO, Ogunlewe MO, Effiom OA, Omitola OG, Arotiba GT (2007). Primary malignant neoplasms of orofacial origin: a retrospective review of 256 cases in a Nigerian tertiary hospital. Int. J. Maxillofac. Surg. 36:403-408.

Arotiba JT, Obiechina AE, Fasola OA, Ajagbe HA (1999). Oral Squamous Cell Carcinoma: A review of 246 Nigerian cases. Afr. J. Med. Med. Sci. 28:141-144.

Binnie WH (1976). A perspective of oral cancer Proc. Roy. Soc. Med. 69: 737-740.

Blot WJ, McLaughlin JK, Winn DM (1988). Smoking and drinking in relation to oral and pharyngeal cancer. Cancer Res. 48:3282-3287.

Chidzonga MM (2006). Oral malignant neoplasia: a survey of 428 cases in two Zimbabwean hospitals. Oral Oncol. 42:177-183.

Daramola JO, Ajagbe HA, Oluwasanmi JO (1979). Pattern of oral cancer in a Nigerian population. Br. J. Oral. Surg. 17:123-28.

Davies JN, Wilson BA (1954). Cancer in Kampala, 1952-1953. East Afr. Med. J. 31:395-401

Davis S, Severson RK (1987). Increasing incidence of oral cancer of tongue in United States among young adults. Lancet 11:910-911.

Effiom OA, Adeyemo WL, Omitola OG, Ajayi OF, Emmanuel MM, Gbotolorun OM (2008). Oral squamous cell carcinoma: a clinicopathologic review of 233 cases in Lagos, Nigeria. J. Oral. Maxillofac. Surg. 66:1595-9.

Elwood JM, Pearson JC, Skippen DH, Jackson SM (1984). Alcohol, smoking, social and occupational factors in the aetiology of cancer of the oral cavity, pharynx and larynx. Int. J. Cancer 34:603-612.

Enwonwu CO, Meeks VI (1995). Bionutrition and oral cancer in humans. Critical Rev. Oral. Biol. Med. 6:5-17.

Franco EL, Kowalski LP, Oliveira BV, Curado MP, Pereira RN, Silva ME, Fava AS, Torloni H (1989). Risk factors for oral cancer in Brazil: a case-control study. Int. J. Cancer 43:992-1000.

Garewal S (1991). Potential role of β-carotene in prevention of Oral Cancer. Am. J. Clin. Nutr. 53: 2948-2954.

Garro AJ, Espina N, McBeth D, Wang SL, Wu-Wang CY (1992). Effects of alcohol consumption on DNA methylation reactions and gene expression: implications for increased cancer risk. Eur. J Cancer Prev. 3:19-23.

Gervasio OL, Dutra RA, Tartagha SM, Vascon WA (2001). Oral squamous cell carcinoma: A retrospective study of 740 cases in a Brazilian population. Braz. Dent. J. 12:57-61.

Greenberg RS, Haber MJ, Clark WS (1991). The relationship of socio-economic status to oral and pharyngeal cancer. Epidemiology 2:94-200.

Harty LC, Capraso NE, Hayes RB, Winn DM, Bravo-Otero E, Blot WJ, Kleinman DV, Brown LM, Armenian HK, Fraumeni JF Jr, Shields PG (1997). Alcohol dehodrogenase 3 genotype and the risk of oral cavity

and pharyngeal cancer. J. Natl. Cancer Inst. 89:1698-705.
Hashibe M, Jacob BJ, Thomas G, Ramadas K, Mathew B, Sankaranarayanan R, Zhang ZF (2003). Socio-economic status, lifestyle factors and oral premalignant lesions. Oral Oncol. 39:664-671.
Hennessey PT, Westra WH, Califano JA (2009). Human Papillomavirus and Head and Neck squamous Cell Carcinoma: Recent Evidence and Clinical Implications. J. Dent. Res. 88:300-306.
Herrero R, Castellsague X, Pawlita M, Lissowska J, Kee F, Balaram P, Rajkumar T, Sridhar H, Rose B, Pintos J, Fernández L, Idris A, Sánchez MJ, Nieto A, Talamini R, Tavani A, Bosch FX, Reidel U, Snijders PJ, Meijer CJ, Viscidi R, Muñoz N, Franceschi S, IARC Multicenter Oral Cancer Study Group (2003). Human papilloma virus and oral cancer: the International Agency for Research on Cancer multicentre study. J. Natl. Cancer Inst. 95:1772-178.
Hobbs CG, Sterne JA, Bailey M, Heyderman RS, Birchall MA, Thomas SJ (2006). Human papillomavirus and head and neck cancer: a systematic review and meta-analysis. Clin. Otolaryngol. 31:259-266.
Johnson NW (1991). Orofacial neoplasms: Global epidemiology, risk factors and recommendations for research. Int. Dent. J. 41:365-375.
Johnson S, McDonald JT, Corsten M, Rourke R (2010). Socio-economic status and head and neck cancer incidence in Canada: a case-control study. Oral Oncol. 46:200-203.
Kerr RA, Changrani JG, Granny FM (2004). An academic dental centre grapples with oral cancer disparities: current collaboration and future opportunities. J. Dent. Educ. 68:531-541.
Kreimer AR, Clifford GM, Boyle P, Franceschi S (2005). Human papillomavirus types in head and neck squamous cell carcinomas worldwide: a systematic review. Cancer Epidemiol. Biomarkers Prev. 14:467–75.
Mufti SI, Eskelson CD, Odeleye OE, Nachiappan V (1993). Alcohol-associated generation of oxygen free radicals and tumor promotion. Alcohol 28:621-8.
Nair M, Sankaranarayanan R, Padmanaabhan TR (2005). Clinical profile of 2001 oral cancers in Kerala, India. Ann. Dent Summar. 47:23-26.
Krutchkoff DJ, Chen J, Eissenberg E, Katz RV (1990). Oral Cancer: A Survey of 556 cases from the University of Connecticut. Oral Pathology Biopsy Service 1975-86. Oral Surg. Oral Med. Oral. Pathol. 70: 192-198.
Lawal A, Kolude B, Adeyemi BF, Lawoyin J, Akang E (2011). Social profile and habits of oral cancer patients in Ibadan. Afr. J. Med. Med. Sci. 40:247-51.
Lawal AO, Kolude B, Adeyemi BF, Lawoyin JO, Akang EE (2012). Serum antioxidant vitamins and the risk of oral cancer in patients seen at a tertiary institution in Nigeria. Niger. J. Clin. Pract. 15:30-3.
Lawoyin JO, Aderinokun GA, Kolude B, Adekoya SM, Ogundipe BF (2003). Oral cancer awareness and prevalence of risk behaviours among dental patients in South-western Nigeria. Afr. J. Med. Med. Sci. 32:203-207.
Lippman SM, Benner SE, Hong WK (1994). Cancer chemoprevention. J. Clin. Oncol.12:851-73.
Lissowska J, Pilarska A, Samolczyk- Wanyura (2003). Smoking, alcohol, diet, dentition and sexual practices in epidemiology of oral cancer in Poland. Eur. J. Cancer Prevent. 12:25-33.
Macfarlane GJ, Boyle P, Evstifeeva TV, Robertson C, Scully C (1994). Rising trends of oral cancer mortality among males worldwide: the return of an old public health Cancer Causes Control 5: 259-65.
Monsjou HS, Velthuysen MLS, Brekel MWM, Jordanova ES, Melief CJM, Balm AJM (2012). Human papillomavirus status in young patients with head and neck squamous cell carcinoma Int. J. Cancer 130:1806–1812
Odukoya O, Mosadomi A, Sawyer DR, Orejobi A, Kekere-Ekun A (1986). Squamous cell carcinoma of the oral cavity- A clinico-pathological study of 106 Nigerian cases. J. Maxillofac. Surg. 14:267-9
Odukoya O, Roberts T, Aroll G (1990). A cytologic study of the effect of Kola nut on the keratinization of the palatal mucosal of Nigerian smokers. Afr. Dent. J. 4:1-5.
Ogden GR, Wight AJ (1998). Aetiology of oral cancer: alcohol. Br. J. Oral. Maxillofac. Surg. 36:247-51.
Oji C, Chukwuneke F (2007). Oral cancer in Enugu, Nigeria, 1998-2003. Br. J. Oral. Maxillofac. Surg. 45: 298-301.
Otoh EC, Johnson NW, Olasoji HO, Danfillo IS, Adeleke OA (2005). Intra-oral carcinoma in Maiduguri, North- eastern Nigeria. Oral Dis. 11: 379-85.
Otoh EC, Johnson NW, Mandong BM, Danfillo IS (2004). Pattern of oral cancers in the North central zone of Nigeria. Afr. J. Oral. Health 1:47-53.
Pinhort EM, Rindum J, Pindborg JJ (1997). Oral cancer: a retrospective study of 100 Danish cases. Br. J. Oral. Max. Surg. 35:77-80.
Poul EP (2008). Oral cancer prevention and control-- The approach of the World Health organization. DOI:10.1016/j.oraloncology.05.023.
Ragin CCR, Modugno F, Gollin SM (2007). The epidemiology and risk factors of Head and neck Cancer: a focus on Human Papillomavirus. J. Dent. Res. 86:104–114.
Rudolph P, Atvis K (2003). Ulcerative conditions. In: Regezi J, Sciubba J, Jordan R (Eds.), Oral Pathology Clinical Pathologic Correlations. Saunders, Missouri. pp. 52-55.
Scully C, Bedi R (2000). Ethnicity and oral cancer. Lancet Oncol. 1:37-42.
Spitzer WO, Hill GB, Chamber LW, Helliwell BE, Murphy HB (1975). The occupation of fishing as a risk factor in cancer of the lip. N. Engl. J. Med. 293:419-424.
Sugerman PB, Savage NW (1999). Current concepts in oral cancer. Aust. Dent. J.44:147-156.
van Wyk CW, Stander I, Padayachee A, Grobler-Rabie AF (1993). The areca nut chewing habit and oral squamous cell carcinoma in South African Indians. A retrospective study. S. Afr. Med. J. 83:425-9.
Williams RR, Horm JW (1977). Association of cancer sites with tobacco and alcohol consumption and socio-economic status of patients, interview study from the third National cancer survey. J. Natl. Cancer Inst. 58:525-547.
Winn DM (1995). Diet and Nutrition in the etiology of oral cancer. Am. J. Clin. Nutr. 61:437S-445S.

academicJournals

Vol. 5(4), pp. 184-190, April 2013
DOI: 10.5897/IJMMS2013.0886
ISSN 2006-9723 ©2013 Academic Journals
http://www.academicjournals.org/IJMMS

International Journal of Medicine and Medical Sciences

Full Length Research Paper

Cancer of the cervix and cervical screening: Current knowledge, attitude and practices of female health workers in Sokoto, Nigeria

Oche M. O.*., Kaoje A. U., Gana G and Ango J. T.

Department of Community Health, Usmanu Danfodiyo University Teaching Hospital, Sokoto, Sokoto State, Nigeria.

Accepted 26 February, 2013

This descriptive cross sectional study assessed the knowledge, attitude and practices of female health workers at Usmanu Danfodiyo University Teaching Hospital, Sokoto with respect to cervical cancer Pap smear screening. A multistage sampling method was used to select a total of 240 subjects. Data was collected using a set of structured, self administered questionnaire which sought information on socio-demographic characteristics, knowledge of cervical cancer, knowledge of cervical cancer screening and uptake of screening services amongst the respondents. Data was analysed using Epi-info statistical software with level of statistical significance set at P < 0.05. Almost all [217 (98.6%)] of the respondents had ever heard of cervical cancer, 217 (98.6%) of the respondents had good knowledge (≥ 50%) about cancer of the cervix, while 199 (90.5%) knew that it can be detected by cytological screening. The mean knowledge score was 82.2 ± 13.8. Of the 220 study subjects, only 22 (10%) had ever done the screening test. The most common reason for not assessing Pap smear screening services was the perception that the subjects were not at risk of the disease. Education of female health workers on the dangers posed by the disease, and reassurance to overcome all possible barriers towards acceptance of the screening test are recommended.

Key words: Cervical cancer, Pap smear, female health workers, knowledge.

INTRODUCTION

Cervical cancer poses a major public health threat to women in many low and medium resourced countries in South and Central America, Sub-Saharan Africa, South and Southeast Asia, where it is still the leading type of cancer among women (Ferlay et al., 2001; Parkin et al., 2005). With about 500,000 new cases and 250,000 deaths each year worldwide, it is the second most common cancer among women (World Health Organization (WHO), 2006) with incidence in Sub-Saharan countries ranging from 30 to 40 per 100,000 women (Kahesa et al., 2008). In Nigeria, the national incidence of cervical cancer is 250/100,000 (Adewole et al., 1997). Cancer is responsible for about 51 million deaths yearly, out of which cervical cancer accounts for 8.5%, most of which occur in the developing countries (Hakulinen et al., 1986).

There are many factors related to the development of cervical cancer. These include infection with high-risk human papiloma virus (HPV), early sexual debut, high

*Corresponding author. E-mail: ochedr@hotmail.com. Tel: +2348035045144.

parity, multiple sexual partners and co-infection with human immunodeficiency virus (HIV). Chlamydia trachomatis, herpes simplex virus type-2, immunosuppressants, and certain dietary deficiencies are also known to be associated risk factors for HPV infection (WHO, 2002). Moreover, HIV increases the incidence rates of cervical cancer, where several studies have shown a strong association between HIV-1 and invasive cancer of the cervix (Adjorlolo-Johnson et al., 2010). As many as 80% of diagnosed cases are detected in the advanced stages in which treatment, even when available, has a markedly reduced likelihood of success (Luthra et al., 1988).

The Papanicolaou (Pap) smear is one of the most essential screening tools for the early diagnosis of cervical cancer and has been found to be the most effective preventive measure (WHO, 2006b). The value of cervical cancer screening in reducing the risk of cervical cancer and mortality has been established, and the risk of developing cervical cancer can be reduced by 80% through regular screening (Stewart and Kleihues, 2003; Özgül, 2007). The benefits of Pap smear's wide availability and usage have been documented, resulting in lowering of mortality rates by up to 60 to 90% in some developed countries (Sankaranarayanan et al., 2001; Wong et al., 2009).

Findings from studies have suggested that unscreened women were at high risk of cervical cancer which had necessitated researchers to continue to investigate different reasons for non-screening among women (Ponten et al., 1995; Oscarsson, 2008). The American Cancer Society recommends that all women should begin cervical screening at age 21 years; a 3 year interval can be considered in the age group 21 to 29 years while women who have had the HPV vaccine should follow the screening recommendations for their age group (American Cancer Society, 2012).

Several reasons for low rates of Pap testing have been observed including low education, lower cognitive scores, low acculturation, and other demographic, social and psychological factors (Wolff et al., 2003; Wu et al., 2001; Coronado et al., 2004). A previous study from Zaria noted that only 270 patients were screened as part of routine screening in 5 years (Oguntayo and Samaila, 2010).

Several studies have shown the importance of health care professionals as predictors of the use of cervical cancer screening. Women's knowledge is also implicated in screening uptake. Women with low levels of knowledge about cervical cancer and its prevention are unlikely to access screening services (Abotchie and Shokar, 2009; Liao et al., 2006; Hummeida et al., 2009).

Previous studies done among female health workers have shown good knowledge of cervical cancer; however, cervical screening attendance rates are still far from satisfactory in most countries (Udigwe, 2006; Anya et al., 2005; Mutyaba et al., 2006). For example, only 18% of female health workers (who were aware of the Pap smear) had actually accessed it (Cyril et al., 2009). This study was therefore carried out to investigate the current knowledge of cervical cancer and cervical screening among female health workers at the Usmanu Danfodiyo University Teaching Hospital, Sokoto. It is hoped that data obtained from this study will form the ba-sis for further interventions on cervical cancer prevention.

METHODOLOGY

Setting and study population

This study was carried out among female health workers of the Usmanu Danfodiyo University Teaching Hospital (UDUTH), Sokoto. The hospital is located in Wamakko Local Government Area within Sokoto metropolis, and serves the host state Sokoto and neighboring Kebbi and Zamfara states, including patients from Niger Republic. It serves as a regional center for Neurosurgery and carries out services such as dialysis, computerized tomography (CAT scan), magnetic resonance imaging (MRI), radiotherapy among others. The hospital has a total of 700 health workers comprising of doctors, nurses, midwives, laboratory technologists/ technicians, pharmacists radiologists/radiographers and physiotherapists. The teaching hospital has about 450 bed spaces and serves both the host state and the surrounding states of Kebbi and Zamfara. It records over 5,000 patients visits in a month.

Study design

This was a descriptive cross sectional study conducted among female health workers comprising doctors, nurses, pharmacists and medical laboratory scientists at the Usmanu Danfodiyo University Teaching Hospital, Sokoto. Using an appropriate sample size formula for estimating minimum sample size for a descriptive study in a population less than 10,000 (Kirkwood, 1998), and 69.8% prevalence of knowledge of cervical screening from a previous study in Nigeria (Aboyeji et al., 2006), a sample size of 216 was determined. The estimated sample size was adjusted to accommodate for non-response and wrong/incomplete responses to 240.

Sampling method

A multistage sampling method was used to select the study subjects. First, the four professional areas with female health workers (FHW) were identified namely doctors, nurses, pharmacists and medical laboratory technologists. At stage 2, the populations of each group of FHW were obtained and samples proportionately allocated based on the population of each group of FHW. There were a total of 356 FHWs in the study centre comprising of 30 doctors, 306 nurses, 12 laboratory technologist and 8 pharmacists. In the final stage, the FHWs were randomly selected until the calculated sample size of 240 was obtained and made up of the following; doctors (20), nurses (206), laboratory scientists (8) and pharmacists (6).

Data collection

Data was collected using a set of structured, self administered

Table 1. Socio-demographic data.

Age (years)	N (%)
20-29	101 (45.9)
30-39	67 (30.5)
40-49	34 (15.5)
50-59	17 (7.7)
≥60	1 (0.4)
Religion	
Christianity	116 (52.7)
Islam	102 (46.4)
Others	2 (0.9)
Marital status	
Single	81 (36.8)
Married	131 (59.5)
Divorced	1 (0.4)
Widowed	7 (3.2)
Educational training	
Basic	51 (23.2)
Post – basic	145 (65.9)
Others	24 (10.9)
Tribe	
Hausa	79 (35.9)
Ibo	48 (21.8)
Yoruba	48 (21.8)
Others	45 (20.5)

Table 2. Awareness and source of information about cervical cancer.

Awareness	N (%)
Yes	217 (98.6)
No	3 (1.4)
Source of information	
From school	165 (76)
Public lectures	44 (20.3)
Radio/TV	7 (3.2)
News papers	1 (0.46)

questionnaire which sought information on socio-demographic characteristics, knowledge of cervical cancer, knowledge of cervical cancer screening and uptake of screening services amongst the respondents. The questionnaire was pretested on 20 FHWs in the state specialist hospital and there after administered to the study subjects by three resident doctors trained on the objectives of the study, interpersonal communication skills and administration of the study tool. Informed consent was obtained from the respondents after explaining the objectives of the study to them and with a pledge to keep all information volunteered confidential. Ethical clearance was obtained from the ethical committee of the Teaching hospital.

Data analysis

Incomplete entries and none responses were excluded and 220 questionnaires were processed using the EPI-INFO statistical soft ware version 3.3.2 program. All quantitative variables were summarized using appropriate measures of location and variability, while categorical variables were presented as percentages and frequencies. Each correct answer to knowledge question attracted one mark, with no marks awarded wrong answers. Scores < 50 and ≥ 50 were graded as inadequate and adequate knowledge, respectively. The chi-square test was used to test for associations between variables, with level of statistical significance set at $P \leq 0.05$.

RESULTS

A total of 220 female health workers filled and returned the questionnaires (92% response rate). The ages of the respondents ranged from 20 to 60 years, with a mean age of 33 ± 9 years. A total of 52.7% of the respondents were Christians while 46.4% were Muslims, 36.8% were single and 59.5% were married; 23.2% had only basic training while 65.9% had some form of post basic training. The tribal distribution showed that 35.9, 21.8 and 21.8% were Hausa, Igbo and Yoruba, respectively (Table 1). Almost all [217 (98.6%)] the respondents had ever heard of cervical cancer. About three quarters 165 (76%) heard of it from lectures and seminars in schools while most, 44 (20.3%) got information about it through public lecture (Table 2). Of the 220 respondents, 188 (85.5%) and 193 (87.7%) knew that cervical cancer is associated with HPV and multiple sexual partners, respectively. Majority [199 (90.5%)] knew that the disease can be detected at the precancerous stage through the Pap smear. A total of 217 (98.6%) of the respondents had good knowledge (≥ 50%) about cancer of the cervix and its associated risk factors while 199 (90.5%) knew that it can be detected by cytological screening. The mean knowledge score was 82.2 ± 13.8 (Table 3).

A total of 176 (79.6%) respondents were aware of the presence of cervical cancer screening service in the study centre.

A total of 191 (86.8%) respondents were of the opinion that cervical cancer screening is for all women of child bearing age, 15 (6.8%) believed that only women with symptoms suggestive of the disease should go for the screening test while 10 (4.5%) opined that only women who have had promiscuous life style should undergo the test (Table 4). Of the 220 study subjects, only 22 (10%) had ever done the screening test. Of these, 19 (86.4%)

Table 3. Knowledge of cervical cancer (n = 220).

Variable	N (%)
Sexual transmission	67 (30.5)
Early marriage	55 (25.0)
Runs in the family	85 (38.6)
Caused by HPV	188 (85.5)
Caused by bacteria	33 (15.0)
Caused by local insertions into the vagina	186 (84.5)
Onset of old age	55 (25.0)
Caused by multiple sexual partners	193 (87.7)
Post coital bleeding is a sign	209 (95.0)
Offensive discharge is a sign	209 (95.0)
Prevention with antibiotics	23 (10.5)
Can be detected by Pap smear screening	199 (90.5)

Mean knowledge score = 82.2 ± 13.8.

Table 4. Respondents' perception of those to undergo cervical screening test.

Those expected to do Pap smear	N (%)
All women of child bearing age	191 (86.8)
Only women with symptoms suggestive of cancerous cervix	15 (6.8)
Only women with promiscuous life style	10 (4.5)
Don't know	4 (1.8)

had done it only once while only one person (4.5%) had done the test thrice. Of the 22 respondents who had undergone the screening test, 15 (68.2%) of them did it within the last 3 years. Seventeen (77.3%) of the study subjects did the test voluntarily without anybody prompting them or having any signs or symptoms of the disease while the rest carried it out on the request of their physicians. The most common reason adduced by the respondents for not undergoing the screening test was that they believed they were not at risk (34.4%). Other reasons included fear of experiencing pain during the procedure (24.7%) and also fear of the outcome of the test (21.1%) (Table 5).

A total of 171 (77.7%) of respondents who were yet to be screened for cervical cancer opined that they intend to avail themselves of the opportunity as soon as they can while 196 (81.9%) of the study subjects would recommend the screening to others. There was a spurious relationship between knowledge score and uptake of Pap smear. Poor knowledge being associated with a higher uptake rate of Pap smear. There was a statistically significant association between increasing age of respondents and marital status of the respondents with uptake of Pap smear test. There was however no statistically significant relationship between ethnicity, religion, educational qualification and uptake of Pap smear (Table 6).

DISCUSSION

At every stage in life, a woman in the third world risks some serious health problems including human immunodeficiency virus/acquired immune deficiency syndrome (HIV/AIDS), high maternal mortality ratios and cancer of the cervix later in life (Ezem, 2007). In recent times, there appears to be a surge in non-communicable diseases as has been seen in the last few decades in the developed world. Cervical cancer an easily preventable disease is prominent amongst them.

This study examined the knowledge of cervical cancer among female health workers and their attitude and practices of Pap smear screening. The knowledge of cervical cancer and the detection of premalignant form by screening is fundamental to the prevention of the disease. Our study showed a very high level of awareness of cancer of the cervix among our respondents; similar high levels of awareness was observed in the study carried out among nurses at the Lagos University Teaching Hospital (LUTH) Nigeria, where 99% of their respondents were aware of the disease (Awodele et al., 2011). This high level of awareness observed in our study is not unexpected considering the educational background of the subjects. However, the high level of awareness observed in this study is in contrast to the findings from similar studies carried out in Maiduguri and Abuja, Nigeria where less than 10% of their respondents were aware of the disease (Audu et al., 1999; Nnodu et al., 2010).

A greater proportion of our respondents got information about cervical cancer through lectures and seminars (76%) while in school and the work place. This is not in agreement with findings from Lagos, Nigeria, where most of their subjects got information regarding the disease from the electronic media (Awodele et al., 2011).

Findings from our study showed that majority (87.7%) of our study subjects correctly identified HPV as the primary cause of cervical cancer. This collaborates with the findings of Awodele in Lagos and Oyedunni in Ibadan, Nigeria (Awodele et al., 2011; Oyedunni and Opemibo, 2012). The respondents in our study recorded a mean knowledge score of 82.2 ± 13.8 for cervical cancer. This is high when compared with findings from other parts of Nigeria (Awodele et al., 2011; Nnodu et al., 2010; Oyedunni and Opemibo, 2012).

Majority (90.5%) of the subjects were aware of Pap smear as one of the screening procedures for cervical cancer. This is in consonance with findings from a similar study by Awodele in Lagos Nigeria (Awodele et al., 2011). Several other studies in Nigeria showed lower levels

Table 5. Reasons for not undergoing cervical cancer screening test.

Reason	N (%)
Not at risk	68 (34.4)
Afraid of experiencing pain	49 (24.7)
Not aware of the service	4 (2.0)
Does not want to be exposed to male doctors	34 (17.2)
Afraid of the outcome of the screening test if it comes out to be positive	43 (21.1)

Table 6. Association of some variables with uptake of cervical cancer screening.

Knowledge of cervical cancer	Uptake of cervical cancer screening test		Test statistics
	Yes	No	
Poor (<50%)	2	1	P=0.027
Good (≥50%)	20	197	
Age (years)			
<40	6	163	X^2=33.70; df=1; P<0.0001
≥40	16	35	
Marital status			
Married	18	113	P=0.037
Unmarried	4	85	
Religion			
Christianity	15	101	P=0.177
Islam	7	95	
Educational attainment			
Basic	2	49	X^2=3.45; df=2; P=0.178
Post basic	16	129	
Others	4	20	

levels of cervical cancer knowledge (Audu et al., 1999; Ojiyi and Dike, 2008; Ayinde et al., 2004; Ogunbode, 2005). The high level of awareness and knowledge of cervical cancer and Pap smear demonstrated by our subjects did not translate to proper utilization of the screening procedure. Although a greater proportion of our subjects (79.6%) were aware of the presence of the screening services in the study center, only 22 (10%) of the respondents had undergone the screening for cervical cancer. This poor utilization of the screening services observed in our study affirms similar findings among nurses in Nnewi (7.1%), Ibadan (34.6%), and Owerri (7.1%), all in Nigeria (Udigwe, 2006; Ayinde et al., 2004; Ezem, 2007).

Similar low utilization of Pap smear was recorded in other countries (Mutyaba et al., 2006; Lyimo and Beran, 2012). The observed low uptake of Pap smear in our study portends a dangerous sign as a low uptake among the predictors of its use (female health workers) might have a negative effect on the attitude of the general population towards utilization of the screening procedure. Female health workers must be proactive and in the vanguard for the fight against cervical cancer and anything short of this will erode the confidence the women folk have on them.

Although majority (89.2%) of the respondents were of the opinion that Pap smear should be done by all women, only 10% of our subjects had undergone the procedure. This may not be unrelated to the widely held view that cervical cancer increases with age, as findings from this study showed a statistically significant association between increasing age of respondents and uptake of

Pap smear ($P < 0.05$). This is further buttressed by the fact that 77.7% of the subjects intend going for the procedure in future when they are older and at greater risk. However, the marital status, ethnicity, religion and educational qualification did not have any statistically significant association with uptake of Pap smear.

The most common reason for not assessing Pap smear among the respondents was the perception that they were not at risk of contracting the disease (34.4%). This widely held view has also been expressed by female health workers from other centers in Nigeria (Udigwe, 2006; Ezem, 2007). Despite the high awareness by our respondents of the link between cervical cancer and sexual activity, as well as the place of sexually transmitted diseases, a large proportion of study subjects still believed they were not at risk. The perception of one's susceptibility can on the long run affect screening behavior. A similar finding was also observed from the study in Ghana where 47% of their subjects felt they were not at risk of the disease (Abotchie and Shokar, 2009).

Other reasons given for not undergoing the screening test include fear of pain, fear of outcome of the test and lack of awareness. These findings are in consonance with other studies in Nigeria (Udigwe, 2006; Oyedunni and Opemibo, 2012). With the National Health Insurance scheme (NHIS) in place, it is hoped that all women covered by the scheme will avail themselves the opportunity of being screened for cervical cancer.

Conclusion

This study revealed high knowledge of cervical cancer and low Pap smear uptake. Several barriers have been identified to have contributed to the low uptake of the screening for cervical cancer. If the fight against the disease is to be won, concerted efforts should be made to educate female health workers who are involved in health education of the general population on the dangers posed by the disease and reassurance to overcome all possible barriers towards acceptance of the screening test. The NHIS should be strengthened to increase access of the entire women folk to screening; this will go a long way in reducing the burden of cervical cancer in the country.

Reliability and validity of the responses were not verified although the instruments for the study were pretested.

REFERENCES

Abotchie PN, Shokar NK (2009). Cervical cancer screening among college students in Ghana: knowledge and health beliefs. Int. J. Gynecol. Cancer 19:412-416

Aboyeji PA, Ijaiya MA, Jimoh AA (2006). Knowledge, attitude and practice of Cervical smear as a screening procedure for cervical acancer in Ilorin, Nigeria. Trop. J. Obstet. Gynecol. 21:114-117

Audu BM, El-Nafaty AU, Khalil M, Otubu JA (1999). Knowledge and attitude to cervical cancer screening among women in Maiduguri, Nigeria. J. Obstet. Gynaecol. 19(3):295-7.

Adewole IF, Edozien EC, Babarinsa IA, Akang CE (1997). Invasive and in situ carcinoma of the cervix in young Nigerians. A clinico-pathologic study of 27 cases. Afr. J. Med. Sci. 26:191-193.

Adjorlolo-Johnson G, Unger ER, Boni-Ouattara E, Coulibaly KT, Maurice C, Vernon SD, Sissoko M, Greenberg AE, Wiktor SZ, Chorba TL (2010). Assessing the relationship between HIV infection and cervical cancer in Côte d'Ivoire: A case–control study. BMC Infect. Dis. 10:242

American Cancer Society (2012). Screening Guidelines for the Prevention and Early Detection of Cervical Cancer. American Cancer Society, Atlanta, Ga.

Anya SE, Oshi DC, Nwosu SO, Anya AE (2005). Knowledge, attitude, and practice of female health professionals regarding cervical cancer and Pap smear. Niger J. Med. 14(3):283-6.

Awodele O, Adeyomoye AAA, Awodele DF, Awodele IO, Dolapo DC (2011). A Study on Cervical Cancer Screening Amongst Nurses in Lagos University Teaching Hospital, Lagos, Nigeria. J. Cancer Educ. 26(3):497–504 doi: 10.1007/s13187-010-0187-6

Ayinde OA, Omigbodun AO, Ilesanmi AO (2004). Awareness of cervical cancer, Papanicolaou's smear and it's utilisation among female undergraduates in Ibadan. Afri. J. Reproductive Health 8(3):68-80.

Coronado GD, Thompson B, Koepsell TD, Schwartz SM, McLerran D (2004). Use of Pap test among Hispanics and Non-Hispanic whites in a rural setting. Prev. Med. 38:713-2.

Cyril CD, Esther E, Madubuko T, Ngozi R, Ezegwui HU (2009). Improved awareness of Pap smear may not affect its use in Nigeria: a case study of female medical practitioners in Enugu, south eastern Nigeria. Transact. Royal Society Trop. Med. Hyg. 103:852-854.

Ezem BU (2007). Awareness and uptake of cervical cancer screening in Owerri, South-Eastern Nigeria. Ann. Afr. Med. 6:94-8.

Ferlay BF, Bray F, Pisani P, Parkin DM (2001). GLOBOCAN 2002: Cancer Incidence, Mortality and Prevalence Worldwide. IARC Cancer Base No. 5 Version 2 .O. IARC Press, Lyon. Available at: http:www-dep.iarc.fr Accessed February 11, 2012

Hakulinen T, Hanshuwka H, Lopez AD, Nakada T (1986).Global and regional mortality pattern by cause of death in 1980. Int. J. Epidemiol.15:227.

Hummeida M, Elrasheed T, Burhan A (2009). Cervical cancer prevention in Sudan. Barriers and missed opportunities. Free communication (oral) presentations. Int. J. Gynecol. Obstet. 107(2):93-396

Kahesa C, Mwaiselage J, Wabinga HR, Ngoma T, Kalyango JN, Karamagi C (2008). Association between invasive cancer of the cervix and HIV-1 infection in Tanzania: the need for dual screening. BMC Public Health 8(1):262.

Kirkwood B (1998). Essentials of Medical Statistics, 2nd edition. Blackwell Scientific Publications Ltd., Oxford. P 197.

Liao CC, Wang HY, Lin RS, Hsieh CY, Sunga FC (2006). Addressing Taiwan's high incidence of cervical cancer: Factors associated with the Nation's low compliance with Papanicolaou screening in Taiwan. Public Health 120:1170-1176

Luthra UK, Ray M, Sehgal A (1988). Clinical down staging of uterine cervix by paramedical personnel. Lancet 1:1401

Lyimo FS, Beran TN (2012). Demographic, knowledge, attitudinal and accessibility factors associated with uptake of cervical cancer screening among women in a rural district of Tanzania: Three public policy implications. BMC Public Health 12:22 doi: 10.1186/1471-2458-12-22

Mutyaba T, Mmiro FA, Weiderpass E (2006). Knowledge, attitudes and practices on cervical cancer screening among the medical workers of Mulago Hospital, Uganda. BMC Medical Education; 6:13 Assessed at: http://www.biomedcentral.com/1472-6920/6/13

Nnodu O, Erinosho L, Jamda M, Olaniyi O, Adelaiye R, Lawson L,

Odedina F, Shuaibu F, Odumuh T, Isu N, Imam H, Owolabi O, Yaqub N, Zamani A (2010). Knowledge and Attitudes towards Cervical Cancer and Human Papillomavirus: A Nigerian Pilot Study. Afr. J. Reprod. Health 14(1):95

Ogunbode OO (2005). Awareness of cervical cancer and screening in a Nigerian female market Popul. Ann. Afr. Med. 4(4):160-3.

Oguntayo OA, Samaila MO (2010). Prevalence of cervical intraepithelial neoplasia in Zaria. Ann. Afr. Med. 9:194–5.

Ojiyi EO, Dike EI (2008). Knowledge and practice of cervical cancer screening at the Imo State University Teaching Hospital, Orlu. PHMJ 2(2):145-51.

Oscarsson MG, Benzein EG, Wijma BE (2008). Reasons for non-attendance at cervical screening as reported by non-attendees in Sweden. J. Psychosom. Obstet. Gynaecol. 29(1):23–31.

Oyedunni SA, Opemipo OM (2012). Perception and utilization of cervical cancer screening services among female nurses in University College Hospital, Ibadan, Nigeria. Pan Afr. Med. J. 11:69.

Özgül N (2007). The Condition of cervix cancer in Turkey and cervical cancer screening programs, In: Tuncer AM (ed.), Türkiye'de Kanser Kontrolü (Cancer Control in Turkey) , T.C. Sa_lık Bakanlı_ı, (Ministry of Health), Sayfa. pp. 349-58.

Parkin DM, Whelan SL, Ferlay J, Storm H (2005). Cancer Incidence in Five Continents, Volumes I to VIII IARC Cancer Base No. 7, Lyon, 2005. http://www.iacr.com.fr/statist.htm, accessed February 11.

Ponten J, Adami HO, Bergstrom R, Dillner J, Friberg LG, Gustafsson L, Miller AB, Parkin DM, Sparen P and Trichopoulos D (1995). Strategies for global control of cervical cancer. Int. J. Cancer 60:1–26.

Sankaranarayanan R, Budukh AM, Rajkumar R (2001). Effective screening programmes for cervical cancer in low- and middle-income developing countries. Bull. World Health Organ. 79:954-962.

Stewart BW, Kleihues P (Eds) (2003). Cancers of the female reproductive tract. In: World Cancer Report, International Agency for Research on Cancer. IARC Press, Lyon, France.

Udigwe GO (2006). Knowledge, attitude and practice of cervical cancer screening (pap smear) among female nurses in Nnewi, South Eastern Nigeria. Niger. J. Clin. Pract. 9(1):40-3.

World Health Organization (2002). National cancer control programmes: policies and managerial guidelines, 2nd edition. WHO, Geneva.

World Health Organization (2002). Comprehensive cervical cancer control: A guide to essential practice. Screening for cervical cancer. Geneva, WHO Press.

World Health Organization (2006a). Preparing for the Introduction of HPV Vaccines: Policy and Programme Guidance for Countries. World Health Organization, Geneva.

World Health Organization (2006b). Comprehensive cervical cancer control: A guide to essential practice. Geneva, Switzerland. 272 p.

Wolff M, Bates T, Beck B, Young S, Ahmed S, Maurana C (2003). Cancer prevention in underserved African American communities: barriers and effective strategies—a review of the literature. WMJ 10:36-40.

Wong LP, Wong YL, Low WY, Khoo EM, Shuib R (2009). Knowledge and awareness of cervical cancer and screening among Malaysian women who have never had a Pap smear: a qualitative study. Singapore Med. J. 50(1): 49-53

Wu Z, Black S, Markides K (2001). Prevalence and associated factors of cancer screening: Why are so many older Mexican-American women never screened? Prev. Med. 33:268-73.

academicJournals

Vol. 5(4), pp. 191-197, April 2013
DOI: 10.5897/IJMMS2012.0873
ISSN 2006-9723 ©2013 Academic Journals
http://www.academicjournals.org/IJMMS

International Journal of Medicine and Medical Sciences

Full Length Research Paper

Increase of Bcl-2/Bax ratio corelated with decrease of lymphocyte apoptosis: A study in the bronchiolus and lung of asthmatic mice

Taufiq Hidayat[1]*, Saptadi Yuliarto[2], Wisnu Barlianto[2], Hidayat Sujuti[3] and H. M. S. Chandra Kusuma[2]

[1]Department of Paediatrics, Kulliyyah of Medicine, International Islamic University, Malaysia.
[2]Department of Child Health, Faculty of Medicine, Brawijaya University, Malang, Indonesia.
[3]Laboratory of Biochemistry and Biomolecular, Faculty of Medicine, Brawijaya University, Malang, Indonesia.

Accepted 11 March, 2013

The failure of lymphocyte apoptosis is one of the factors causing chronic airway inflammation in asthma. Some studies indicate the role of Bcl-2 in inhibition of lymphocyte apoptosis, but still little research is on the role of Bax and its relationship to Bcl-2 in asthma. The purpose of this study was to prove the role of Bcl-2-lymphocyte in inhibition of lymphocyte apoptosis and decrease in Bax-lymphocytes in bronchiolus and lung of asthmatic mice. This study was a randomized control group design. Subjects were Balb/c mice which divided into 2 groups: non-asthma and asthma. Asthma group were sensitized with ovalbumin intraperitoneally on day 0 and 14, followed by inhalation every 2 to 3 days for 6 weeks. At week 8, all subjects terminated. Bcl-2 and Bax-lymphocytes expression were examined with immunohistochemical method, whereas apoptotic lymphocytes by Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) method. Statistical analysis used was the independent sample t-test and regression analysis, with 95% confidence interval. Bronchial and lung specimens were obtained from 18 subjects (9 from each group). The ratio of apoptotic lymphocytes decreased in the asthma group ($p = 0.003$), Bcl-2-lymphocytes increased in the asthma group ($p < 0.001$), and Bax-lymphocytes decreased in the asthma group ($p = 0.003$). There was a strong negative correlation ($r = -0.66$, $r^2 = 0.43$, $p = 0.003$) between the Bcl-2 and lymphocyte apoptosis. There was also a strong negative correlation ($r = -0.56$, $r^2 = 0.35$, $p = 0.009$) between the ratio Bcl-2/Bax and lymphocyte apoptosis. However, no significant relationship was found between Bax-lymphocytes and lymphocyte apoptosis ($r = 0.36$, $r^2 = 0.13$, $p = 0.15$). Increasing the ratio of Bcl-2/Bax inhibit lymphocyte apoptosis where Bcl-2 plays more role than Bax.

Key words: Asthma, ovalbumin, lymphocyte apoptosis, Bcl-2, Bax.

INTRODUCTION

Asthma is a chronic inflammatory disorder of respiratory tract with many cells that play a role, especially mast cells, basophils, and lymphocytes T. Asthma is a problem throughout the world with a prevalence estimated at 300 million people (1 to 18% of the population in various countries and 10% in children), with mortality reaching 250,000 people per year. Chronic inflammation is associated with respiratory tract hyperresponsiveness that causes recurrent episodes of wheezing, shortness of breath, feeling heavy in the chest (chest tightness), and

*Corresponding author. E-mail: dr_taufiq@iium.edu.my. Tel: +60 95704539. Fax: +60 9 571 6545.

cough particularly at night or early morning (GINA Executive Committee, 2009; UKK Pulmonologi, 2004).

Failure to ease the inflammatory process after the occurrence of exacerbations is a major problem in asthma. Chronic or persistent inflammation has been proved as one of the main factors that influence the severity and frequency of exacerbations, and has a role in airway remodeling, smooth muscle hypertrophy, and airway hyperreactivity (Busse and Lemanske, 2001; Tong et al., 2006). One of the mechanisms that suspected playing a role in the chronicity of asthma is the failure of inflammatory cells apoptosis, such as: eosinophil, neutrophil, T lymphocytes, and macrophages (Lamb et al., 2005; Muller et al., 2006; Spinozzi et al., 2008). Physiologically, apoptosis is a programmed cell death which is a form of cell death to maintain the balance of the development of body cells. Apoptosis occurs only in some cells in small quantities, and selectively (Guicciardi and Gores, 2005).

In the inflammation process, apoptosis serves to control the "excess" of inflammatory cells, limiting tissue damage, and ease the inflammation process (Spinozzi et al., 2008). Research on several diseases, for example nasal polyps and rheumatoid arthritis, suggest that the chronicity of the disease is associated with failure or delay apoptosis in inflammatory cells and that these cells survive in inflammation places (Salmon et al., 1997; Simon et al., 1997). T lymphocytes, especially Th2 cells, have a central role in the regulation of the immune system.

One of the factors that play a role in apoptosis of T lymphocytes is pro-and anti-apoptotic proteins. Bax is a pro-apoptotic proteins that act as an executor protein in mitochondrial channels of apoptosis (intrinsic pathway) so that its expression can be used as an apoptotic marker, whereas expression of Bcl-2 showed the existence of apoptosis inhibitors associated with the remodelling process (Akbar et al., 1996; Kim et al., 2006). This event was reinforced by the evidence of increased Bcl-2 expressions which was proportional to the number of T lymphocytes that infiltrate into the bronchial submucous, and also comparable with the severity of asthma. In addition, Bcl-2 mRNA in asthma were found in T lymphocytes (Hamzaoui et al., 1999; Vignola et al., 1999). There was another evidence which states that Bcl-2 can inactivate Bax (Kim et al., 2006). Therefore, we perform an *in vivo* study to examine the influence of pro- and anti-apoptosis proteins in lymphocyte apoptosis.

MATERIALS AND METHODS

Experimental animals

Experimental animals were Mus musculus mice (Balb/c) obtained from Veterinaria Farma experimental animal cages center at Ahmad Yani Street Surabaya. The sex of mice selected was female because it has a better response to allergens than male mice (Epstein, 2004). Mice inclusion criteria were: age 6 to 12 weeks, weight 20 to 30 g, and healthy (good appetite and activity, fur did not fall out). Experimental animals were excluded if dignosed to be ill during observations, which appear from the decrease in activity and other important clinical signs (weight loss, breath pattern, diarrhea and vomiting), or died.

Study design

This research was a true experimental study design with randomized control group, performed at the Laboratory of Pharmacology and Biomedics, Faculty of Medicine, Brawijaya University for 7 months, in February, 2009 to September, 2009. The large size of the sample was determined by: $p (n - 1) \geq 15$; where n = number of subjects per group, p = number of research group (Hanafiah and Ali, 1991). Based on this formula, by using 2 groups of studies, research samples required were ≥ 8.5 samples per group. Samples that meet the inclusion criteria will be divided into 2 groups: treatment group (asthma) and control groups (non-asthma) with simple random sampling technique.

Initial sensitization was injected intraperitoneally by injecting a mixture of 10 µg ovalbumin (OVA) + 1 mg $Al(OH)_3$ dissolved in 0.5 ml of normal saline (NaCl 0.9%) on day 0 and day 14.

Inhalation sensitization tests was conducted by giving ovalbumin (OVA) 1% in 8 ml normal saline (NaCl 0.9%) per treatment using OMRON Nebulizer type NU-017 for 20 min, with air flow volume and nebulization volume on a scale of 1. Sensitization by inhalation was repeated for 6 weeks. After 8 weeks, all experimental animals in both groups were terminated. The pulmonary organs placed in the organs storage device and fixed in formalin 10%, and then made for histopathology preparations.

Ovalbumin (OVA)

OVA used in this study were ovalbumin with SERVA brand. This OVA was an albumin egg lyophil salt-free and lysozyme-free produced by SERVA electrophoresis GmbH. Aluminium hydroxide (alum) was used as an adjuvant (Zosky at al., 2007).

Microscopic observation

Each tissue sample was made for slide preparation. There were 18 slides, consisting of 9 slides from each group. Slide examination was conducted covertly (blinding) by 2 separate biomedical analyst. Examination and calculation of apoptotic lymphocytes, Bcl-2 expression, and Bax expression were performed on each slide using 1000× magnification, and calculated per mm^2 and the average value taken.

Lymphocyte apoptosis observation

Staining technique of lymphocyte apoptosis was done using TUNEL labeling of fragmented DNA (*Apo-BrdU-IWC CatK403-50:Lot P10013*). The number of apoptotic lymphocytes is the number of lymphocytes per mm^2 (single core cell, nucleus and cytoplasm ratio 3:4, with a red cytoplasm), with dark brown cell nuclei after staining with TUNEL (Lamb et al., 2005).

Bcl-2 dan Bax-limfosit expression

Measurement of Bcl-2 and Bax expression was done using rabbit polyclonal anti-Bcl-2 (Cat. # MS-598 - P0 LabVision) and anti-Bax

Table 1. Experimental animal characteristic.

Characteristic	Treatment group	Control group	p value
Type	*Mus musculus* (*balb/c mice*)	*Mus musculus* (*balb/c mice*)	
Number	9	9	
Age (week)	6-12	6-12	p = 0.751
Sex	Female	Female	
Weight (g)	26.82±2.19	26.36±3.36	

On the characteristics of experimental animals, there were no differences in animal species, number, age, gender, and body weight.

(Cat. # MS-711 - P1ABX LabVision). The last step was counterstaining using Mayer Hematoxilen. The number of lymphocytes which express Bcl-2 and Bax were the number of lymphocytes that showed a brown color in cytoplasm.

IgE OVA examination

Measurement of IgE OVA was by ELISA method with anti IgE biotin conjugate (S*anta Cruz cat# sc-66169*). The result was read using ELISA reader at a 450 nm wavelength.

Statistical analysis

The average (mean) ratio of lymphocyte apoptosis, expression of Bcl-2 and Bax lymphocytes between two groups (treatment and control) were analyzed using independent-sample T-test. The relationship between expression of Bcl-2 lymphocytes and ratio of lymphocyte apoptosis, Bax expression ratio of lymphocytes and lymphocyte apoptosis and Bcl-2/Bax expression ratio and ratio of apoptotic lymphocytes were analyzed using correlation and linear regression test, with the value of confidence interval at 95%. All data were processed using statistical package for social sciences (SPSS) 17.0 for windows.

Research approval

Research was conducted after getting approval by the Commission on Health Research Ethics of Saiful Anwar Hospital Malang.

RESULTS

Samples characteristic

This study used 6 to 12 week-old female Balb/c mice. Based on the calculation, the number of samples needed was ≥ 8.5 samples per group, so we used 9 mice in each group (total of 18 mice). The mean of mice body weight was 26.82 g in the treatment group (asthma), and 26.36 g in the control group (non-asthma) (Table 1).

Comparison of Bcl-2 lymphocyte expression, Bax lymphocyte ratio, lymphocyte apoptosis and IgE OVA between experimental and control group

From the result, it was found that the expression of Bcl-2 was higher in the treatment group (asthma) than the control group (Figure 2). Conversely, the Bax expression was significantly lower in the treatment group than the control group (Figure 3). In observation of apoptotic lymphocytes, it was found that the ratio of apoptotic lymphocytes was lower in the treatment group than the control group (Figure 1). On the measurement of OVA and total IgE levels, a high level was found in the treatment group. There was a negative relationship between expression of Bcl-2 and the ratio of apoptotic lymphocytes, where the ratio of lymphocyte apoptosis is influenced by Bcl-2 expression by 43%. The ratio of apoptotic lymphocytes could be predicted by the formula: The ratio of apoptotic lymphocytes = 0.96 to 0.06 (expression of Bcl-2) (Figure 3). On the other hand, there was no significant correlation between Bax expression ratio of lymphocytes and lymphocyte apoptosis. There was a negative relationship between the ratio of Bcl-2/Bax expression and lymphocyte apoptotic ratio, where the ratio of lymphocyte apoptosis is influenced by the ratio of Bcl-2/Bax expression by 31%. The ratio of apoptotic lymphocytes could be predicted by the formula: The ratio of apoptotic lymphocytes = 0.66 to 0.21 (the ratio of Bcl-2/Bax expression) (Figure 3).

DISCUSSION

Provision of ovalbumin (OVA) in experimental animals (rats) will trigger a systemic allergic response with elevated levels of total IgE and specific IgE OVA (Tumes et al., 2008). In this study, a significant difference was found between the two groups: OVA IgE levels were higher in the asthma group (p = 0.001) (Table 2). Increased expression of IgE OVA proved that true asthma occured in the treatment group. Sensitizing antigent on normal airway immune response will cause proliferation or clonal expansion of lymphocytes and will be followed by apoptosis of lymphocytes immediately after peak phase of clonal expansion, a mechanism called activation induced cell death (AICD) (Krammer, 2000; Spinozzi et al., 2008). In asthma, there was an apoptotic T cell dysfunction. Selective resistance to apoptosis of CD4 + T cells in patients with asthma led to the occurrence of T-cell-dependent immunoinflammation

Table 2. Level of IgE OVA, lymphocyte apoptotic ratio, Bcl-2 and Bax expression in treatment and control group.

Variable	Treatment group (Mean ± SD)	Control group (Mean ± SD)	p value
Bcl-2	13.06±1.67	7.22±1.72	p<0.001
Bax	6.11±1.08	8.94±2.20	p =0.003
Apoptosis ratio	0.14±0.08	0.54±0.34	p =0.003
IgE OVA	11.22±2.30	7.60±1.13	p =0.001

Table 3. Corelation between Bcl-2, Bax, Bcl-2/Bax expression ratio and lymphocyte apoptotic ratio.

Parameter	Lymphocytes apoptosis ratio (cell/mm^2)	
Bcl-2 expression (cell/mm^2)	r=-0.66 r^2=0.43 y=0.96-0.06x	p = 0.003
Bax expression (cell/mm^2)	r=0.36 r^2=0.13	p = 0.15
Bcl2/Bax expression ratio	r=-0.56 r^2=0.31 y=0.66-0.21x	p = 0.017

in asthma. It was suspected that asthma could be due to decreased elimination of activated T cells and increased recruitment and activation of T cells. Thus, the phenotype of T cells was found consistently in asthma patients (Pierce et al., 2007).

In this study, there was a difference in the ratio of lymphocyte apoptosis in the group who experienced asthma (allergy) and non-asthma (p = 0.003) (Table 2). Cell death is necessary to maintain T lymphocyte homeostasis. Failure in cleaning the activated cells will prolong immune response and cause chronic inflammation (Abdulamir et al., 2009; Hildeman et al., 2002). Asthma is a chronic inflammation with characteri-zation of activated T cell in peripheral blood vessels and airway. There was a direct correlation between numbers of activated T lymphocyte in bronchial mucous and severity of asthma. In allergen induction, T lymphocyte CD4+ was recruited selectively to bronchial mucous. This recruitment was accompanied by decrease number of T lymphocyte in circulation (Abdulamir et al., 2009; Darveau et al., 2008).

Apoptosis is regulated by intrinsic point of several proteins, including Bcl-2 and Bcl-XL. Apoptosis is triggered by activation of caspase-9 and caspase-3, and can be inhibited by the expression of Bcl-2 (Adams, 1998; Darveau et al., 2008; White and Dorscheid, 2002). In this study, proved levels of Bcl-2 lymphocytes was higher in asthma group (p < 0.001) (Table 2). In addition, beside the increased expression of anti-apoptotic protein, apoptosis failure in asthma is also caused by decreased expression of pro-apoptotic proteins such as Bcl-2-associated X protein (Bax) and Fas/FasL (Abdulamir et al., 2009; Darveau et al., 2008), whereas Bax is an executor of apoptosis proteins and can be used as a marker apoptosis (Kim et al., 2006). This study proved that lymphocytes Bax expression was lower in the asthma group (p = 0.003) (Table 2).

Although there was a decrease Bax apoptotic lymphocytes and lymphocytes in asthma group, however our study found no significant correlation between Bax expression lymphocytes with lymphocyte apoptosis (r = 0.36, r^2 = 0.13, p = 0.15) (Table 3). Possible causes of the existence of a weak association was due to experimental animals used in this study which are strains of Balb/c mice. Previous studies using this strain of mice also found increased antiapoptosis protein Bcl-2 and Bcl-XL without a decrease in antiapoptosis protein Bax after sensitization with ovalbumin, unlike the case of used mice strains CBA/Ca where apoptosis is obtained more quickly because the protein expression proapotosis Bax (Tumes et al., 2008). Second possibility is the presence of other factors that play a role in lymphocyte apoptosis rather than Bax lymphocytes. This suggests that inhibition of apoptosis of lymphocytes in asthma not only played by Bax, but probably by a complex interaction of many factors.

Increased expression of protein-anti-apoptosis proteins, especially Bcl-2 as it has been proven in this study, is one

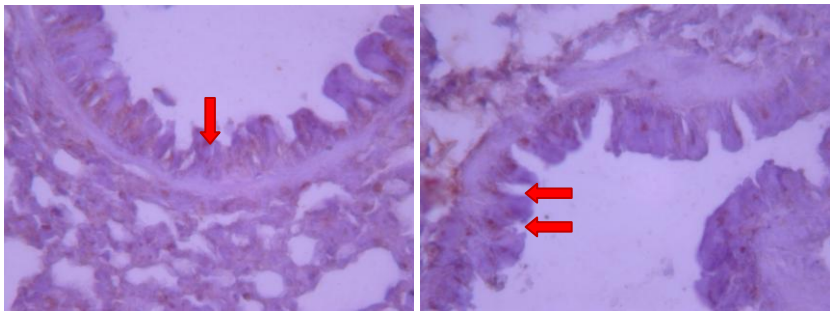


Figure 1. Apoptosis in mice bronchioles by TUNEL. At 1000× magnification, there are less lymphocytes with dark brown cell nucleus (red arrow) in the bronchioles and lungs of asthmatic mice (left) than non-asthmatic one (right).

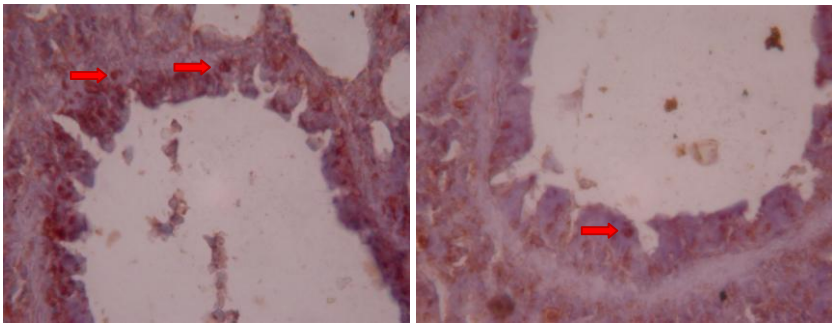


Figure 2. Bcl-2 lymphocyte expression in mice bronchioles and lung by Immunohistochemistry. At 1000× magnification, there are more lymphocytes with a brown cytoplasm (red arrow) in the bronchioles and lungs of asthmatic mice (left) than non-asthmatic one (right).

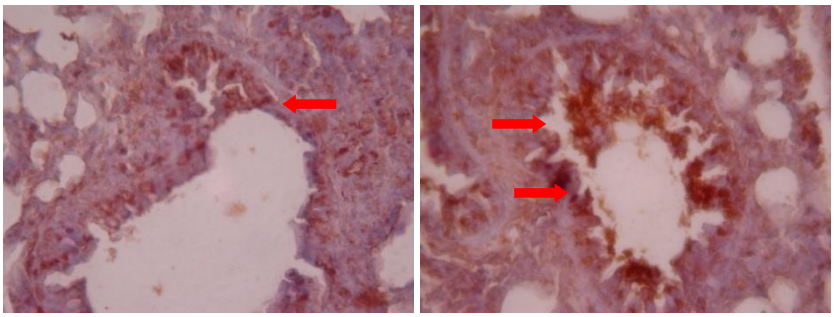


Figure 3. Bax lymphocyte expression in mice's bronchiolus and lung by Immunohistochemistry. At 1000× magnification, there are less lymphocytes with a brown cytoplasm (red arrow) in the bronchioles and lungs of asthmatic mice (left) than non-asthmatic one (right).

of the factors that cause barriers to lymphocyte apoptosis. Other factors were not examined in this study, including expression of anti-apoptotic protein Bcl-XL, expression of Fas/Fas-L, Bak, and the proteins pro-apoptotic subclass III (BH3-only proteins).

Bcl-XL is a Bcl-2 homologue. Experiments *in vivo* in rats showed that during the onset of inflammation, there was increased expression of Bcl-XL along with significant decrease in Bax expression. In the same experiment, inhibition of Bax by V5 (Bax inhibitory peptide) results in prolonged inflammation, supporting the hypothesis that Bax has an important role in the induction of apoptosis of inflammatory cells, potentially accelerating the resolution of inflammation (Hallett et al., 2008).

The role of Fas/Fas-L in regulating lymphocyte apoptosis has been demonstrated by Tong et al. (2006) through studies *in vivo* in Fas deficient mice, in which the number of lymphocytes was higher than in normal mice up to day 14 after exposure to allergens, with the dominant cytokines TH2. It was also demonstrated *in vitro* that administration of IL-4 on T-lymphocyte cell cultures decreased the Fas expression. This suggests that the regulation of lymphocyte apoptosis was not only through the intrinsic (mitochondrial) pathway, but also the extrinsic (death receptor) pathway in which the deficiency of Fas/Fas -L is likely to be more involved in the inhibition of apoptosis.

Besides Bax, Bak also had a big role in the inhibition of mitochondrial apoptosis path as evidenced in research by Lindsten et al. (2000). Mice with a deficiency of either Bax or Bak only experienced mild abnormalities in development, whereas in mice with deficiency of both Bax and Bak, 90% experienced perinatal mortality, while 10% had multiple abnormalities. This suggests that these two proteins two proteins have complementary roles in tissue homeostasis. However, further research is still needed because there is no research that examines the role of Bak in apoptotic lymphocytes in asthma.

There is a clear balance between the pro- and anti-apoptotic proteins in the process of inflammation linkage (Hallett et al., 2008). Pro-and anti-apoptosis which influenced apoptosis of lymphocytes in this study were described by using the ratio of Bcl-2/Bax. In linear regression analysis, it was a significant negative relationship between the ratio of Bcl-2/Bax expression and lymphocyte apoptosis ratio, where the ratio of lymphocyte apoptosis is influenced by the ratio of Bcl-2 expression of lymphocytes by 31% ($r = -0.56$, $r^2 = 0.31$; $p = 0.017$) (Table 3). Apoptosis of lymphocytes can be predicted by the formula: Lymphocyte apoptosis ratio = 0.66 to 0.21 (the ratio of Bcl-2/Bax expression.) This indicates that an increase in Bcl-2/Bax ratio will decrease the apoptosis of lymphocytes. Because the increased Bcl-2/Bax ratio of 31% only had a role in reducing apoptosis of lymphocytes, mean decrease in apoptosis of lymphocytes in this study was also caused by pro-and anti-apoptosis factor proteins.

Studies conducted by Abdulamir et al. (2008) found that the Bcl-2/Bax ratio was higher in severe asthma compared with mild asthma and healthy controls. The number of leukocytes in peripheral blood cells also increased with increasing ratio of Bcl-2/Bax. Bcl-2/Bax ratio is associated with asthma severity and confirmed that the protein Bcl-2 can inhibit the expression and effects of pro-apoptotic protein Bax. Progressive decrease in apoptosis resulted in increased severity, chronicity, and persistence of inflammation in asthma.

This study showed that there was inhibition of apoptosis of lymphocytes in asthma, which is influenced by a variety of complex factors interaction. The existence of various factors that influence the apoptosis of lymphocytes in this study, due to the design of *in vivo* studies that illustrate the pathogenesis and patho-physiology of asthma, are more similar to the mechanism of the real disease. Partially, this research was also able to describe the pathogenesis of asthma from the point of failure of apoptosis. This research was able to demonstrate the role of intrinsic point (especially Bcl-2 lymphocytes) in the inhibition of apoptosis of lymphocytes in order to consider the use of asthma therapy via this route.

In this experiment, female Balb/c mice was chosen because they have better response to allergens (Epstein, 2004) and act as representative to provide a snapshot of asthma in humans. This animal model showed a Th2-mediated allergic inflammation, airway mucosal eosinophilia, and airway hyper-responsiveness (Nials and Uddin, 2008), and also showed a goblet cell hyperplasia, epithelial hypertrophy, and sub-epithelial fibrosis and peribronchial. These phenomena were settled after cessation of exposure to allergens, and airway remodeling picture were obtained (Kumar et al., 2004; McMillan and Lloyd, 2004). In this study, the asthma group can be analoged to the description of asthma in humans in the form of airway sensitization in mice and is characterized by an increase in IgE ovalbumin (Table 2).

There are limitations in our study: (1) parameter examination were conducted only once after chronic exposure to ovalbumin; (2) determination of lymphocyte was based solely on morphology (not using a marker, so the lymphocyte subsets of lymphocytes observed were not known); (3) there are limitations of imunohistochemistry in the determination of cells expressing Bcl-2 and Bax; and (4) other factors that influence lymphocyte apoptosis was not examined. Therefore, further research is necessary to: (1) perform serial examination starting from initial exposure until the exposure end (chronic exposure), (2) examine marker(s) lymphocytes to determine the subset of lymphocytes (mainly CD4 + T cells), (3) examine technique with flow cytometry as it can be a measured expression of Bcl-2 and Bax along with cells that are expressed, and (4) examine other factors that may affect lymphocyte apoptosis, among others: IFN-γ, Fas/Fas-L, Bak, pro-and anti-apoptosis proteins.

Conclusion

This study showed an increased expression of Bcl-2 lymphocytes, decreased Bax expression and decreased lymphocyte ratio of lymphocyte apoptosis in mice with asthma. Increased expression of Bcl-2 decreases the ratio of apoptotic lymphocytes by 43%. However, the

decreased expression of Bax was not significantly associated with decreased lymphocyte apoptosis. Increased expression of Bcl-2/Bax ratio decreases the ratio of apoptotic lymphocytes by 35%. This shows that increasing the ratio of Bcl-2/Bax inhibit lymphocyte apoptosis, where Bcl-2 play more roles than Bax.

REFFERENCES

Abdulamir AS, Hafidh RR, Abubakar R, Abbas KA (2008). Changing survival, memory cell compartment, and T-helper balance of lymphocytes between severe and mild asthma. BMC Immunol. 9:73-82.

Abdulamir AS, Kadhim HS, Hafidh RR, Ali MA, Faik I, Abubakar F, Abbas KA (2009). Severity of Asthma: The Role of CD25+, CD30+, NF-κB, and Apoptotic Markers. J. Investig. Allergol. Clin. Immunol. 19:218-224.

Adams JM (1998). The Bcl-2 Protein Family: Arbiters of Cell Survival. Science 281:1322-1326.

Akbar AN, Borthwick NJ, Wickremasinghe RG, Panayiotidis P, Pilling D, Bofill M, Krajewski S, Reed JC, Salmon M (1996). Interleukin-2 receptor common y -chain signaling cytokines regulate activated T cell apoptosis in response to growth factor withdrawal: selective induction of anti-apoptotic (bcl-2, bcl-x,) but not pro-apoptotic (bax, bcl-x,) gene expression. Eur. J. Immunol. 26:294-299.

Busse WW, Lemanske RF (2001). Asthma. N. Engl. J. Med. 344:350-362.

Darveau ME, Jacques E, Rouabhia M, Hamid Q, Chakir J (2008). Increased T-cell survival by structural bronchial cells derived from asthmatic subjects cultured in an engineered human mucosa. J. Allergy Clin. Immunol. 121:692-699.

Epstein MM (2004). Do mouse models of allergic asthma mimic clinical disease? Int. Arch. Allergy. Immunol. 133:84-100.

GINA Executive Committee (2009). Global Initiative for Asthma: global strategy for asthma management and prevention. Ontario, Canada.

Guicciardi ME, Gores GJ (2005). Apoptosis: a mechanism of acute and chronic liver injury. Gut 54:1024-1033.

Hallett JM, Leitch AE, Riley NA, Duffin R, Haslett C, Rossi AG (2008). Novel pharmacological strategies for driving inflammatory cell apoptosis and enhancing the resolution of inflammation. Trends Pharmacol. Sci. 29(5):250-257.

Hamzaoui A, Hamzaoui K, Salah H, Chabbou A (1999). Lymphocytes apoptosis in patients with acute exacerbation of asthma. Mediators Inflamm. 8:237-243.

Hanafiah, Ali K (1991). Rancangan percobaan. Rajawali Pers, Jakarta.

Hildeman DA, Zhu Y, Mitchell TC, Kappler J, Marrack P (2002). Molecular mechanisms of activated T cell death in vivo. Curr. Opin. Immunol. 14:354-359.

Kim H, Rafiuddin-Shah M, Tu H-C, Jeffers JR, Zambetti GP, Hsieh JJ-D, Cheng EH-Y (2006). Hierarchical regulation of mitochondrion-dependent apoptosis by BCL-2 subfamilies. Nat. Cell. Biol. 8:1348-1358.

Krammer PH (2000). CD95's deadly mission in the immune system. Nature 407:700-706.

Kumar RK, Herbert C, Kasper M (2004). Reversibility of airway inflammation and remodelling following cessation of antigenic challenge in a model of chronic asthma. Clin. Exp. Allergy 34:1796-1802.

Lamb JP, James A, Carroll N, Siena L, Elliot J, Vignola AM (2005). Reduced apoptosis of memory T-cells in the inner airway wall of mild and severe asthma. Eur. Respir. J. 26:265-270.

Lindsten T, Ross AJ, King A, Zong WX, Rathmell JC, Shiels HA, Ulrich E, Waymire KG, Mahar P, Frauwirth K, Chen Y, Wei M, Eng VM, Adelman DM, Simon MC, Ma A, Golden JA, Evan G, Korsmeyer SJ, MacGregor GR, Thompson CB (2000). The combined functions of proapoptotik Bcl-2 family members Bak and Bax are essential for normal development of multiple tissues. Mol. Cell 6:1389-1399.

McMillan SJ, Lloyd CM (2004). Prolonged allergen challenge in mice leads to persistent airway remodelling. Clin. Exp. Allergy 34:497-507

Muller M, Grunewald J, Hoglund CO, Dahlen B, Eklund A, Stridh H (2006). Altered apoptosis in bronchoalveolar lavage lymphocytes after allergen exposure of atopic asthmatic subjects. Eur. Respir. J. 28:513–522.

Nials AT, Uddin S (2008). Mouse models of allergic asthma: acute and chronic allergen challenge. Dis. Model. Mech. 1:213-220.

Pierce JD, Pierce J, Stremming S, Fakhari M, Clancy RL (2007). The Role of Apoptosis in Respiratory Diseases. Clin. Nur. Specialist 21(1):22-28.

Salmon M, Scheel-Toellner D, Huissoon AP, Pilling D, Shamsadeen N, Hyde H, D'Angeac AD, Bacon PA, Emery P, Akbar AN (1997). Inhibition of T cell apoptosis in the rheumatoid synovium. J. Clin. Investig. 99:439-446.

Simon HU, Yousefi S, Schranz C, Schapowal A, Bachert C, Blaser K (1997). Direct demonstration of delayed eosinophil apoptosis as a mechanism causing tissue eosinophilia. J. Immunol. 158:3902-3908.

Spinozzi F, de Benedictis D, de Benedictis FM (2008). Apoptosis, airway inflammation and anti-asthma therapy: from immunobiology to clinical application. Pediatr. Allergy Immunol. 19:287–295.

Tong J, Bandulwala HS, Clay BS, Pilling D, Shamsadeen N, Hyde H, D'Angeac AD, Bacon PA, Emery P, Akbar AN (2006). Fas-positive T cells regulate the resolution of airway inflammation in a murine model of asthma. J. Exp. Med. 203:1173-1184.

Tumes DJ, Wong ACH, Sewell WA, McColl SR, Connolly A, Dent LA (2008). Differential rates of apoptosis and recruitment limit eosinophil accumulation in the lungs of asthma-resistant CBA/Ca mice. Mol. Immunol. 45:3609–3617.

UKK Pulmonologi (2004). Pedoman nasional asma anak. Jakarta: PP Ikatan Dokter Anak Indonesia.

Vignola AM, Chanez P, Chiappara G, Siena L, Merendino A, Reina C, Gagliardo R, Profita M, Bousquet J, Bonsignore G (1999). Evaluation of apoptosis of eosinophils, macrophages, and T lymphocytes in mucosal biopsy specimens of patients with asthma and chronic bronchitis. J. Allergy Clin. Immunol. 103:563-573.

White SR, Dorscheid DR (2002). Corticosteroid-Induced Apoptosis of Airway Epithelium. Chest 122:278S-284S.

Zosky GR, Larcombe AN, White OJ, Burchell JT, Janosi TZ, Hantos Z, Holt PG, Sly PD, Turner DJ (2007). Ovalbumin-sensitized mice are good models for airway hyperresponsiveness but not acute physiological responses to allergen inhalation. Clin. Exp. Allergy 38:829–838.

academicJournals

Vol. 5(4), pp. 198-205, April 2013
DOI: 10.5897/IJMMS12.0888
ISSN 2006-9723 ©2013 Academic Journals
http://www.academicjournals.org/IJMMS

International Journal of Medicine and Medical Sciences

Full Length Research Paper

Psychogenic activation phenomenon of specific anti-tumor immunity in cancer patients

Oleg V. Bukhtoyarov* and Denis M. Samarin

Laboratory of Psychoimmunology, Immanuel Kant Baltic Federal University, Kaliningrad, Russian Federation.

Accepted 26 February, 2013

The present study aimed to identify the influence of the mind on specific anti-tumor immunity of cancer patients. The study involved 90 cancer patients (78 women and 12 men) with 18 various types of cancer. The presence of psychogenic medical history before diagnosis of cancer was the basic criterion of cancer patients selection for this study. The psychometric parameters, quality of life, delayed-type hypersensitivity skin reaction on the tumor-associated antigens of human melanoma cell line BRO, cytokines (30 Plex) in native plasma and 24 h blood supernatants were investigated before and after psycho-correction. The delayed-type hypersensitivity skin reaction on the tumor-associated antigens in all cancer patients significantly increased (p= 0.0001) after elimination of psycho-emotional disorders by psychotropic drugs. In this case, there were no detected reliable changes in the concentrations of cytokines. The described new pathophysiological phenomenon consists in a spontaneous (non-immunogenic) increase of specific anti-tumor activity of the immune system of cancer patients who coped effectively with psycho-emotional disorders.

Key words: Cancer patients, specific anti-tumor immunity, delayed-type hypersensitivity reaction, psychogenic medical history, psycho-emotional disorders.

INTRODUCTION

The cancer process is still uncontrollable (Boyle and Ferlay, 2005; Herbst et al., 2006), despite significant progress in understanding of cancer biology (Coleman and Tsongalis, 2006). Largely, it is caused by unpredictability of the state of anti-tumor immunity of cancer patients at all stages of the cancer disease course and the difficulty of its valid and reliable evaluation (Armstrong and Hawkins, 2001). It is understood that the appearance of cancer and the lack of its recurrence depends on the supervisory functions of the immune system, and its specific anti-tumor activity (Kim et al., 2007). Earlier, attention was drawn to the possibility of forming psychogenic induced immunosuppression in cancer patients (Kiecolt-Glaser et al., 2002). In addition, there were shown relationships between the activity of NK-cells, psychological factors and the activity of cerebral cortex and the limbic system. In particular, in observing patients with various malignant tumors, a reduction of cerebral metabolism was noted in the limbic brain structures (Tashiro et al., 2001), ensuring the formation of motivations, emotions, and behavioral reactions, appropriate adaptation of the body to the external environment and the preservation of homeostasis.

There is some reason to believe that the huge potential of the brain is capable to supervise and modulate the processes connected with genesis and progression of cancer (Mravec et al., 2008). We have repeatedly observed in our clinical practice that effective treatment of psycho-emotional disorders of cancer patients with psychogenic medical history was followed by disease-free

*Corresponding author. E-mail: bukhtoyarov@mail.ru. Tel: +74012 563398. Fax: +74012 563397.

survival. This disease-free survival would not be possible without active participation of anti-tumor immunity. It is known that cancer patients with high levels of functional activity of cytotoxic T lymphocytes and natural killer cells exhibit longer terms of survival (Blake-Mortimer et al., 2004). However, the relationship of a specific anti-tumor activity of the immune system and higher nervous activity in cancer patients was not clear yet. The purpose of this study was to investigate the possibility of the mental state influence to a specific anti-tumor activity of the immune system of cancer patients.

METHODOLOGY

Ethical approval for this study was obtained at the Ethical Committee of the Institute of Clinical Immunology, Siberian Branch of the Russian Academy of Medical Sciences (Novosibirsk, Russian Federation), protocol No. 48, April 8, 2010. All patients gave written informed consent.

Characteristics of cancer patients

The presence of psychogenic medical history before diagnosis of cancer was the basic criterion of selection of cancer patients. Psychogenic medical history was studied for each patient in anamnesis morbi (history of present illness) and included the presence of massive traumatic events (death of a close person, divorce, appearance of the disabled person in family, frequent family conflicts, change of residence and work, etc.) with the formation of helplessness, hopelessness, despair. The study involved 90 cancer patients (78 women and 12 men) with 18 cancers: breast cancer [34 (37.8%)], ovarian cancer [10 (11.1%)], melanoma [6 (6.7%)], uterine cancer [6 (6.7%)], kidney cancer [6 (6.7%)], rectal cancer [5 (5.6%)], colon cancer [4 (4.4%)], other sites (cervical, vulva, stomach, lung, bladder, thyroid, laryngis, pleural mesothelioma, lymphoma, lymphosarcoma, acute myeloblastic leukemia) [19 (21%)]. Cancer stage: I [15 (16%)], II [33 (35%)], III [26 (29%)], IV [16 (19%)]. Age characteristics: 30 to 40 years [6 (6.7%)], 41 to 50 years [27 (30%)], 51 to 60 years [41 (45.5%)], over 61 to 70 years [11 (12.2%)], 71 to 80 years [5 (5.6%)] patients. Smokers [17 (18.9%)] and non-smokers [73 (81.1%)]. All patients were administered the standard combined treatment for malignant tumors: only surgery [39 (43.3%)], surgery and chemotherapy [26 (28.9%)], surgery, chemotherapy and radiotherapy [14 (15.7%)], surgery and radiotherapy [4 (4.4%)], only chemotherapy [4 (4.4%)], only chemo- and radiotherapy [1 (1.1%)] patients. No treatment was given to 2 (2.2%) of patients. The time after surgery varied from 1 month to 12 years and among those from 1 month to 1 year [42 (50.6%)], 1 to 3 years [25 (30.1%)], 3 to 5 years [10 (12%)]; and over 5 years [6 (7.3%)].

Forty-five patients of the 90 cancer patients after elimination of psycho-emotional disorders by psychotropic drugs agreed to participate in further studies. This group (n = 45) included: 39 women and 6 men with 12 cancer types: breast cancer [17 (37.8%)], ovarian cancer [6 (13.3%)], uterine cancer [5 (11.1%)], melanoma [4 (8.9%)], kidney cancer [4 (8.9%)], colon cancer [3 (6.7%)], and one patient each with rectal cancer, stomach cancer, cervical cancer, bladder cancer, lung cancer and cancer laryngis [6 (13.3%)]. Cancer stage include: I [10 (22.2%)], II [15 (33.4%)], III [14 (31.1%)], and IV [6 (13.3%)]. Age characteristics: 30 to 40 years [4 (8.9%)], 41 to 50 years [18 (40%)], 51 to 60 years [19 (42.2%)], 61 to 70 years [3 (6.7%)], 71 to 80 years [1 (2.2%)] patients. Smokers [9 (18.9%)] and non-smokers [36 (81.1%)]. All patients were randomly assigned after the completion of the standard combined treatment of malignant tumors: only surgery [21 (46.7%)], surgery and chemotherapy [14 (31.1%)], surgery, chemotherapy and radiotherapy [7 (15.6%)], surgery and radiotherapy [1 (2.2%)], and only chemotherapy [2 (4.4%)] patients. The time after surgery varied from 1 month to 12 years and among them from 1 month to 1 year were [26 (58.1%)], 1 to 3 years [11 (23.3%)], 3 to 5 year [7 (16.3%)]; and over 5 years [1 (2.3%)].

Psychometric testing and quality of life investigation in cancer patients

The diagnosis of psycho-emotional disorders along with evaluation of psycho-correction was carried out by clinical method with additional usage of the following psychometric tests and rates: Hospital anxiety and depression scale (HADS) for anxiety and depression; State-trait anxiety inventory scale (STAI) for stait anxiety and trait anxiety; Symptom Checklist 90 (SCL-90) for somatization, obsessive-compulsive, interpersonal sensitivity, depression, anxiety, hostility, phobic anxiety, paranoid ideation, psychoticism, and global severity index. The quality of life was measured with the 36-Item short form health survey (SF-36) for physical functioning, role-physical functioning, bodily pain, general health, vitality, social functioning, role-emotional, and mental health.

The psychotropic medicines in psycho-correction of cancer patients

The main purpose of adjustment of psycho-emotional disorders by psychotropic drugs was the complete elimination of psycho-emotional disorders in cancer patients. The following medications were used: coaxil (tianeptine), valdoxan (agomelatine), velaxin (venlafaxine), fluoxetine, afobazol, diazepam (microdoses). Mental disorders treatment was carried out strictly individually. In case of the absence of the clinical effect or the occurrence of side effects, the drug and treatment scheme were replaced.

Evaluation of specific anti-tumor activity of the immune system in cancer patients

Anti-tumor activity of the immune system of cancer patients was assessed by skin test of the delayed type hypersensitivity (DTH) reaction on the tumor-associated antigens (TAA), which were used as a lysed human melanoma cell line BRO in the amount of 25 thousand cells in a test. Human melanoma cell line BRO (Lockshin et al., 1985) was obtained at the Institute of Cytology of Russian Academy Sciences (St. Petersburg, Russia). We investigated the DTH skin reaction after the intradermal administration on the forearm at 9, 12 and 24 h.

Preparation of tumor associated antigens for diagnostic test-DTH skin reaction

The human melanoma cell line BRO were maintained in Roswell Park Memorial Institute (RPMI) 1640 supplemented with 10% heat-inactivated fetal calf serum, L-glutamine (2 mmol/ml), 25 mmol hydroxyethyl piperazineethanesulfonic acid (HEPES) buffer, and 25 µg/ml gentamicin at 37°C in 5% CO_2 humidified air. Cells were detached from the dish by treating with trypsin-Ethylenediaminetetraacetic acid (EDTA) followed by washing three times with Dulbecco's phosphate-buffered saline (DPBS), precipitated by centrifuging, counted and diluted with 0.9% saline solution with 0.1% EDTA. Cells were lysed by repeated (8 times)

freezing and stored at -20°C until use.

Multiplex cytokine analysis (Human 30-Plex)

Multiplex testing service (Human Cytokine Panel 30-Plex, 96 Assay-Points): IL-1β, IL-1RA, IL-2, IL-2R, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12 (p40/p70), IL-13, IL-15, IL-17, TNF-α, IFN-α, IFN-γ, GM-CSF, MIP-1α, MIP-1β, IP-10, MIG, Eotaxin, RANTES, MCP-1, VEGF, G-CSF, EGF, FGF-basic, HGF, was carried out in Institute of Medical Microbiology and Hygiene, Regensburg, Germany. The materials for investigation were native plasma and 24 h blood supernatants in cancer patients before and after adjustment of psycho-emotional disorders by psychotropic drugs. In order to obtain this, the blood supernatant was used in RPMI 1640 medium in a 1:4 ratio followed by cultivating at 37°C in atmosphere with 5% CO_2. The native plasma and 24 h blood supernatant samples were stored at -80°C until analysis.

Statistical analysis

The statistical data processing was done using ALGLIB numerical analysis and data processing library which is available to the public. The minimal p-value in this library is 0.0001. The level of statistical significance (so-called alpha level for a p-value) was accepted as 0.05. Because majority (> 50%) of parameters under investigation were not normally distributed (by Kolmogorov-Smirnov test), a non-parametric test procedure was used. For all tests, two-tailed modifications were considered. Wilcoxon signed-rank test was used to find differences in mean values of psychometric and immunologic parameters before and after psycho-correction. To study relationship between psychometric and immunologic parameters, correlation analysis was done by Spearman's rank correlation test.

RESULTS

Psycho-emotional disorders in cancer patients with psychogenic medical history

Clinical and psychometric studies of 90 cancer patients with psychogenic medical history showed that 95.6% of patients have had various psycho-emotional disorders. According to ICD-10, the disorders distribution was as follows: generalized anxiety disorder (F41.1) [31 (36.0%)], mixed anxiety and depressive disorder (F41.2) [10 (11.6%)], prolonged depressive reaction (F43.21) [10 (11.6%)], mixed anxiety and depressive reaction (F43.22) [28 (32.6%)] and organic anxiety disorder (F06.4) [7 (8.2%)] which in our opinion was a complication chemotherapy. It should be noted that 4 out of 90 cancer patients had no psycho-emotional disorders, and simultaneously registered higher indicators of DTH skin reaction (hyperemia diameter 15 to 16 mm). There were three cancer patients with breast cancer and one with colon cancer, in which time after surgery was an average of 4.8 years. There were 100% of mental disorders in the group of 45 cancer patients who agreed to participate in further studies. According to ICD-10, structure of disorders was as follows: generalized anxiety disorder (F41.1) [17 (37.8%)], mixed anxiety and depressive disorder (F41.2) [2 (4.4%)], prolonged depressive reaction (F43.21) [2 (4.4%)], mixed anxiety and depressive reaction (F43.22) [20 (44.5%)] and organic anxiety disorder (F06.4) [4 (8.9%)], which in our opinion was a complication chemotherapy which is also called 'chemo brain' phenomenon (Staat and Segatore, 2005).

Results of adjustment of psycho-emotional disorders in cancer patients with psychogenic medical history

The main purpose of therapy was the complete elimination of psycho-emotional disorders in cancer patients using the psychotropic drugs. This psycho-correction was followed by achievement of significant clinical effects, which are confirmed by data of psychometric measurement. Results are shown in Table 1. In addition, the improvement of cancer patients' quality of life was achieved on the background of psycho-correction. Results are shown in Table 2. It should be noted that the process of drug correction of psycho-emotional disorders in cancer patients has been fraught with considerable difficulties. It was often necessary to change an antidepressant for several times or to combine it with anxiolytic. The greatest clinical benefit was obtained by the combined administration of the following drugs: coaxil (tianeptine), 1 tablet 3 times a day and diazepam, 1/6 tablet (microdoses) 3 times a day. The duration of this treatment regimen is 2 to 3 months. In general, the duration of the treatment course with other drugs to achieve the desired clinical effect varied widely, from 1 through 6 months.

Psychogenic activation phenomenon of specific anti-tumor immunity in cancer patients with psychogenic medical history

It was observed that there was no or minimal (hyperemia diameter before 2 mm) DTH skin reaction to the TAA of human melanoma cell line BRO before the beginning of psycho-correction in almost all cancer patients regardless of the cancer type, cancer stage and time after surgery. The peak response to BRO cell line lysate in most cases (the diameter of redness in mm) was observed after 12 h. Results are shown in Table 3. The DTH skin reaction in all cancer patients significantly increased from 2.62 ± 0.54 to 7.80 ± 0.96 mm after effective psycho-correction (Figure 1). The DTH skin reactions to administration of TAA according to the ages of cancer patients are shown in Table 4. The degree of immune responses of 30 to 50 years old and 51 to 60 years old cancer patients were not differerent. The degree of immune response against TAA

Table 1. Psychometric indicators in cancer patients before and after treatment of psycho-emotional disorders.

Indicator	Before (n=45) Mean±SEM*	After (n=45) Mean±SEM*	P
Symptom checklist 90 (SCL-90)			
Somatization	1.31±0.10	0.90±0.10	0.0001
Obsessive-Compulsive	1.16±0.10	0.74±0.08	0.0001
Interpersonal Sensitivity	0.95±0.16	0.60±0.09	0.0001
Depression	1.36±0.11	0.82±0.08	0.0001
Anxiety	0.91±0.09	0.66±0.13	0.0001
Hostility	0.77±0.09	0.52±0.08	0.0001
Phobic Anxiety	0.47±0.07	0.26±0.07	0.0014
Paranoid Ideation	0.67±0.08	0.56±0.08	0.0002
Psychoticism	0.74±0.09	0.50±0.07	0.0001
Global Severity Index	1.00±0.07	0.60±0.05	0.0001
Hospital anxiety and depression scale (HADS)			
Anxiety	9.37±0.63	4.44±0.46	0.0001
Depression	7.62±0.64	3.28±0.54	0.0001
State-trait anxiety inventory scale (STAI)			
Stait Anxiety	39.16±1.65	28.58±1.95	0.0001
Trait Anxiety	40.82±1.63	29.95±1.87	0.0001

*Means and standard errors means .

Table 2. Quality of life (SF-36) in cancer patients before and after treatment of psycho-emotional disorders.

Indicator		Before (n=45) Mean±SEM*	After (n=45) Mean±SEM*	P
Physical health	Physical functioning	68.29±3.45	79.29±2.49	0.0006
	Role-physical functioning	29.27±6.47	58.57±5.07	0.0002
	Bodily pain	52.73±3.92	73.74±2.93	0.0003
	General health	41.66±3.42	56.80±2.81	0.0012
Mental health	Vitality	45.98±3.69	67.00±2.31	0.0001
	Social functioning	57.85±4.49	82.06±2.15	0.0001
	Role-emotional	35.78±6.23	64.77±5.11	0.0004
	Mental health	44.59±3.47	69.31±1.78	0.0001

*Means and standard errors means.

Table 3. Dynamics of DTH skin reaction on TAA of human melanoma cell line BRO (hyperemia diameter, mm) before and after treatment of psycho-emotional disorders.

Time after administration (h)	Before (n=45) Mean±SEM*	After (n=45) Mean±SEM*	P
9	3.13±0.74	7.43±0.87	0.0001
12 (peak response)	2.62±0.54	7.80±0.96	0.0001
24	1.67±0.44	5.74±0.72	0.0001

*Means and standard errors means.

of 61 to 80 years old cancer patients is difficult to assess because of the small number of patients (n = 4).

In the correlation analysis were identified inverse connection between psychometric parameters “stait anxiety” (r = -0.22, p = 0.047), “psychoticism” (r = -0.22, p = 0.041) and indicated the activity of specific anti-tumor immunity of cancer patients (DTH skin reaction on the TAA). At the same time, was found a positive correlation

Table 4. DTH skin reaction on TAA of human melanoma cell line BRO (hyperemia diameter, mm) before and after psycho-correction according to the ages.

Age (years)	Before (Mean±SEM*)	After (Mean±SEM*)	P
30-50 (n=22)	1.73±0.41	7.86±1.07	0.0001
51-60 (n=19)	2.72±0.68	7.11±1.05	0.0001
61-80 (n=4)	5.00±3.79	6.75±1.93	2.7670^{NS}

*Means and standard errors means; $^{NS}p>0.05$ not significant at 5%.

Table 5. Cytokines in native plasma of cancer patients before and after treatment of psycho-emotional disorders by psychotropic drugs.

Cytokines in native plasma (pg/ml)	Before (n=45) Mean±SEM*	After (n=45) Mean ±SEM*	P
VEGF	8.380±0.94	6.830±0.80	0.679^{NS}
EGF	34.99±7.16	28.20±7.81	0.778^{NS}
HGF	199.2±41.0	204.1±75.3	1.000^{NS}
FGF-basic	17.62±4.01	10.84±1.19	0.383^{NS}
IL-2R	232.5±16.3	226.3±30.5	0.552^{NS}
IL-6	13.03±2.63	15.44±5.55	0.585^{NS}
IL-8	152.1±47.7	128.9±42.3	0.769^{NS}
IL-12p40/p70	59.09±3.76	54.78±4.19	0.835^{NS}
RANTES	1732.5±133.9	1872.5±190.8	0.766^{NS}
Eotaxin	47.98±2.94	48.44±5.95	0.795^{NS}
MIP-1alpha	48.21±8.46	46.63±9.75	0.771^{NS}
MIP-1beta	70.50±11.7	68.34±14.9	0.989^{NS}
MCP-1	635.6±31.5	605.9±36.8	0.742^{NS}
TNF-alpha	11.36±4.19	17.86±8.52	0.188^{NS}
IP-10	49.90±5.08	38.07±3.94	0.809^{NS}
MIG	57.60±7.97	51.15±7.56	0.320^{NS}

*Means and standard errors means; $^{NS}p > 0.05$ not significant at 5%.

correlation between “mental health” (the SF-36) and the DTH skin reaction ($r = -0.46$, $p = 0.003$). The specific anti-tumor activity of the immune system is associated with mental health as well as anxiety which is an obligate symptom in cancer patients (Miller and Massie, 2006).

The native plasma of cancer patients from 30 examined cytokines were very low, far below the lower limits of detection concentrations following 14 cytokines: IL-1b, IFNα, IL-1RA, IL-2, IL-4, IL-5, IL-7, IL-10, IL-13, IL-15, IL-17, G-CSF, GM-CSF, IFN-γ. A 24 h blood supernatants of 30 cytokines could not determine the concentration of the following 13 cytokines: IFNα, IL-2, IL-4, IL-5, IL-7, IL-10, IL-13, IL-15, IL-17, G -CSF, GM-CSF, IFN-γ, MIG. Comparative analysis showed no significant change in the concentrations of studied native plasma cytokines and 24 h blood supernatants before and after treatment ($P>0.05$). Results are shown in Table 5 and Table 6. In addition, levels of cytokine concentrations did not correlate with DTH skin reaction before and after psycho-correction of cancer patients.

DISCUSSION

In our opinion, cancer patients with psychogenic medical history are affected by both massive psycho-trauma: stressful life events such as before cancer diagnosis and the fact of a cancer diagnosis itself. These circumstances explain the high level (95.6%) of psychopathology in cancer patients with psychogenic medical history. We assumed that these cancer patients, regardless of the type of cancer, have both psychologically caused psycho-emotional disorders, and psychologically caused de-pression of anti-tumor immunity. This provision became the basis for psycho-immunological studies of cancer patients in one group, without dividing them into separate

Table 6. Cytokines in 24 h blood supernatants of cancer patients before and after treatment of psycho-emotional disorders by psychotropic drugs.

Cytokines in 24h blood supernatants (pg/ml)	Before (n=45) Mean±SEM*	After (n=45) Mean±SEM*	P
VEGF	10.08 ± 1.18	12.49 ± 2.41	0.483^{NS}
EGF	19.71 ± 1.51	23.06 ± 2.22	0.094^{NS}
HGF	75.95 ± 4.43	71.88 ± 6.32	0.654^{NS}
FGF-basic	15.91 ± 0.56	14.92 ± 0.84	0.234^{NS}
IL-2R	84.9 ± 12.3	123.3 ± 29.0	0.154^{NS}
IL-6	143.4 ± 63.8	346.2 ± 150.3	0.137^{NS}
IL-8	600.1 ± 230.6	2057.4 ± 738.4	0.084^{NS}
IL-12p40/p70	84.67 ± 11.7	105.1 ± 18.4	0.194^{NS}
RANTES	1143.0 ± 97.3	1571.7 ± 230.2	0.496^{NS}
Eotaxin	31.44 ± 1.69	30.29 ± 1.96	0.826^{NS}
MIP-1alpha	222.7 ± 97.8	801.2 ± 418.9	0.269^{NS}
MIP-1beta	515.7 ± 215.3	1595.1 ± 600.7	0.300^{NS}
MCP-1	401.6 ± 118.9	753.3 ± 236.3	0.928^{NS}
TNF-alpha	48.24 ± 20.1	73.83 ± 27.3	0.191^{NS}
IL-1RA	282.5 ± 67.9	501.8 ± 144.2	0.313^{NS}
IP-10	28.11 ± 2.91	20.46 ± 1.98	0.898^{NS}
IL-1β	25.76 ± 7.20	61.53 ± 24.8	0.578^{NS}

*Means and standard errors means; $^{NS}p > 0.05$ not significant at 5%.

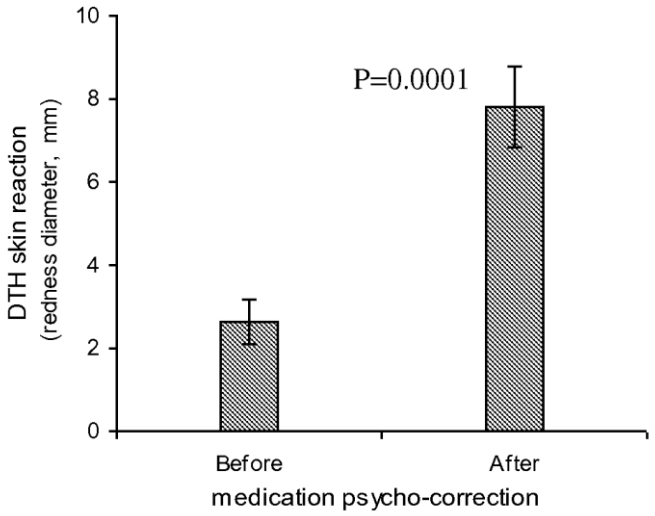


Figure 1. Increase of the delayed type hypersentivity reaction on tumor-associated antigens in cancer patients background medication psycho-correction. Initially suppressed specific anti-tumor immunity (low DTH skin reaction on tumor-associated antigens) in cancer patients with psychogenic anamnesis was activated (increase in performance DTH skin reaction) as a result of effective medication of the psycho-emotional disorders.

nosologies. The time spent on adjustment of psycho-emotional disorders of cancer patients with psychogenic medical history by psychotropic drugs did not depend on the form of the cancer stage, time after surgery treatment and kind of psycho-emotional disorders.

Another feature was a bad compliance in some cancer patients after the effective cupping of their psycho-emotional disorders. Patients feeling very good and their continued participation in the rehabilitation program did not consider it necessary (perhaps this behavior of cancer patients is associated with psychological and cultural characteristics of the patients).

It is known that there is maximal spectrum of the tumor-associated antigens typical for different histological types of malignant solid tumors expressed on melanoma cells (Wang, 1997). In our preliminary studies, particularly BRO cell line lysate allowed us to obtain maximum DTH skin reaction with the minimum number of cells (in the amount of 25 thousand cells in a test). Selecting a minimum number of TAA to the skin test DTH (according to our preliminary studies) is due to a desire to obtain specific anti-tumor immunological response in cancer patients without their prior vaccination, and without the possibility of vaccine effect in most of the diagnostic test.

In comparison with our colleagues who used the DTH skin reaction on TAA of human melanoma cell lines in the study of clinical efficacy of anti-tumor polyvalent vaccine 'CancerVax' (developed from three allogeneic human melanoma cell lines) (Habal et al., 2001), a diagnostic dose nearly 100 times smaller (2.5×10^4 cells versus 2.4×10^6 cells) was used. In addition, our patients did not receive any immunotropic therapy during the study. The selected dose does not cause allergic and other pathological

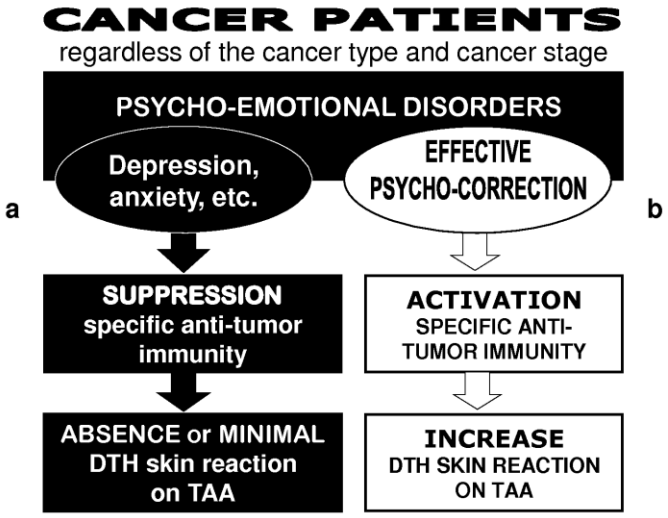


Figure 2. Influence of mind on specific antitumor immunity in cancer patients. (a) Psycho-emotional disorders in cancer patients with psychogenic anamnesis regardless of the cancer type and stage are by suppression of specific anti-tumor activity of the immune system. DTH skin reaction melanoma cell line BRO is the absence or minimal (hyperemia diameter before 2 mm): black part of figure. (b) Effective psycho-correction leads to activation of anti-tumor immunity in cancer patients with a significant increase of DTH skin reaction on TAA of human melanoma cell line BRO: white part of figure.

reactions. It is known that the delayed type hypersensitivity reaction is a specific immune response and begins to manifest later (8 to 12 h) after ingestion antigen, and in most cases the reaction reaches a peak after 48 to 72 h (Carroll, 2011). In our case, (early 12 h) the peak responses which is due to the absence of prior immunization of cancer patients and is the largest contribution of cellular reactions in the DTH skin test was achieved (Jacysyn et al., 2001).

It should be emphasized that the increase of specific anti-tumor activity of the immune system in cancer patients with psychogenic medical history as response to elimination of psycho-emotional disorders is the spontaneous reaction of the immune system. So it is not related to preliminary immunostimulation. We believe that such spontaneous reaction of the immune system is the result of elimination of psychogenic immunosuppression. Elimination of psycho-emotional disorders was not accompanied by changes in concentrations of cytokines determined either in native plasma, neither in the 24 h blood supernatants. This is a confirmed data that most cytokines act only over a short distance and their investigation in native plasma and 24 h blood supernatants in cancer patients does not reflect changes in the activity of specific anti-tumor immunity in the presented phenomenon. The present research did not reveal cytokine mechanism of the observed pathophysiological phenomenon.

In 4 out of 90 cancer patients with psychogenic medical history, we have not found any psychopathology. Anamnestic investigation of these cases showed that in the standard of cancer treatment in these patients, there were spontaneous cupping of psycho-emotional disorders caused by positive changes in their personal life and an active social support. The absence of psychopathology, and high levels of specific anti-tumor activity of the immune system (indicators of DTH skin reaction, 15 to 16 mm) in these cancer patients are likely to have caused favorable course without recurrence of cancer disease in an average of 4.8 years since the surgery. Therefore, the described phenomenon indicated that at cancer patients with psychogenic medical history, the mind plays a key role in suppression of specific anti-tumor immunity (Figure 2). It should be noted that the index of DTH skin reaction used by us as a biological marker is of effectiveness in cupping psycho-emotional disorders in cancer patients.

Conclusion

There are some cancer patients who have psychogenic medical history. Such cancer patients demonstrate extremely high levels of psycho-emotional disorders. However, due to administration of psychotropic drugs, their psycho-emotional disorders disappear and this causes spontaneous (non-immunogenic) increase of specific anti-tumor activity of the immune system determined by specific DTH skin reaction against TAA. These immunological reactions were observed in cancer patients with psychogenic medical history regardless of the cancer type, cancer stage and ages. Discovered new pathophysiological phenomenon requires further, more in-depth study on a larger number of cancer patients including patients without psychogenic medical history. The results of future studies will help to make a certain contribution to the understanding of the hidden mechanisms of cancer disease and cancer management.

ACKNOWLEDGEMENTS

We would like to thank Alexey Palchevsky (Kaliningrad, Russia) and Nikolay Tamodin (Moscow, Russia) for understanding the problem and providing the financial support for this scientific work. The authors gratefully acknowledge the support of Dr. Andrei Kovalevskii (Windsor, Canada) for translation of this article in English.

Abbreviations: TAA, Tumor-associated antigens; **DTH,** delayed type hypersensitivity; **IL,** interleukin.

REFERENCES

Armstrong AC, Hawkins RE (2001). Vaccines in oncology: background and clinical potential. Br. J. Radiol. 74(887):991-1002.

Blake-Mortimer JS, Sephton SE, Carlson RW, Stites D, Spiegel D (2004). Cytotoxic T lymphocyte count and survival time in women with metastatic breast cancer. Breast J. 10(3):195-199.

Boyle P, Ferlay J (2005). Cancer incidence and mortality in Europe, 2004. Ann. Oncol. 16(3):481-488.

Carroll EW (2011). Cell and tissue functions. In: Porth CM (Ed), Essentials of Pathophysiology: Concepts of Altered Health States, Philadelphia. pp. 56-58.

Coleman WB, Tsongalis GJ (2006). Molecular mechanisms of human carcinogenesis. Experientia Suppl. 96:321-349.

Habal N, Gupta RK, Bilchik AJ, Yee R, Leopoldo Z, Ye W, Elashoff RM, Morton DL (2001). CancerVax, an allogeneic tumor cell vaccine, induces specific humoral and cellular immune responses in advanced colon cancer. Ann. Surg. Oncol. 8(5):389-401.

Herbst RS, Bajorin DF, Bleiberg H, Blum D, Hao D, Johnson BE, Ozols RF, Demetri GD, Ganz PA, Kris MG, Levin B, Markman M, Raghavan D, Reaman GH, Sawaya R, Schuchter LM, Sweetenham JW, Vahdat LT, Vokes EE, Winn RJ, Mayer RJ (2006). Clinical cancer advances 2005: major research advances in cancer treatment, prevention, and screening – a report from the American Society of Clinical Oncology. J. Clin. Oncol. 24(1):190-205.

Jacysyn JF, Abrahamsohn IA, Macedo MS (2001). Modulation of delayed-type hypersensitivity during the time course of immune response to a protein antigen. Immunology 102(3):373-379.

Kiecolt-Glaser JK, Robles TF, Heffner KL, Loving TJ, Glaser R (2002). Psycho-oncology and cancer: psychoneuroimmunology and cancer. Ann. Oncol. 13(4):165-169.

Kim R, Emi M, Tanabe K (2007). Cancer immunoediting from immune surveillance to immune escape. J. Immunol. 121(1):1-14.

Lockshin A, Giovanella BC, De Ipolyi PD, Williams LJ Jr, Mendoza JT, Yim SO, Stehlin JS Jr (1985). Exceptional lethality for nude mice of cells derived from a primary human melanoma. Cancer Res. 45(1):345-350.

Miller K, Massie MJ (2006). Depression and anxiety. Cancer J. 12(5):388-397.

Mravec B, Gidron Y, Hulin I (2008). Neurobiology of cancer: interactions between nervous, endocrine and immune systems as a base for monitoring and modulating the tumorogenesis by the brain. Semin. Cancer Biol. 18(3): 150-163.

Staat K, Segatore M (2005). The phenomenon of chemo brain. Clin. J. Oncol. Nurs. 9(6):713-721.

Tashiro M, Itoh M, Kubota K, Kumano H, Masud MM, Moser E, Arai H, Sasaki H (2001). Relationship between trait anxiety, brain activity and natural killer cell activity in cancer patients: a preliminary PET study. Psychooncology 10(6):541-546.

Wang RF (1997). Tumor antigens discovery: perspectives for cancer therapy. Mol. Med. 3(11):716-731.

academicJournals

Vol. 5(4), pp. 206-209, April 2013
DOI: 10.5897/IJMMS2013.0914
ISSN 2006-9723 ©2013 Academic Journals
http://www.academicjournals.org/IJMMS

International Journal of Medicine and Medical Sciences

Full Length Research Paper

Prevalence of human herpesvirus 8 infection in Iranian patients with hematological malignancies

Seyed Hamidreza Monavari[1]*, Hossein Keyvani[1], Hamidreza mollaie[2], Mehdi fazlalipour[2], Farzin sadeghi[2], Mostafa Salehi –Vaziri[2] and Farah Bokharaei-Salim[1]

[1]Department of Virology and Antimicrobial Resistance Research Center, Tehran University of Medical Sciences, Tehran, Iran.
[2]Medical Virology, Tehran University of Medical Sciences, Tehran, Iran.

Accepted 17 October, 2012

Human herpesvirus 8 (HHV-8) is a gamma-herpesvirus linked causally to Kaposi's sarcoma and many malignancies such as multicentric Castleman's disease, primary effusion lymphoma, some lymphoproliferative diseases and post transplantation bone marrow failure. The aim of this study was to determine the prevalence of HHV-8 infection in patients with malignant hematological diseases. From September, 2009 to April, 2010, 62 patients with hematological diseases were recruited for the study. Five milliliter of ethylenediaminetetraacetic acid (EDTA) anti-coagulated peripheral blood was collected from each subject. The presence of HHV-8 DNA was tested using a real time technology for the sequences from HHV-8 ORF65. The mean age of patients was 33.9 ± 18.0 years. Four of the patients were found to be HHV-8 polymerase chain reaction (PCR) positive using Real time-PCR and viral prevalence was 6.5%. HHV-8 was found in 1 (25%) patient with acute myelogenous leukemia (AML), 3 (75%) patients with chronic myelogenous leukemia (CML), and none was detected in patients with acute lymphoblastic leukemia (ALL) and lymphoma. The results of this study show that patients with malignant hematological diseases may have HHV-8 infection, therefore, it seems that considering HHV-8 infection in patients with hematological malignancies might be beneficial.

Key words: Human herpesvirus 8 (HHV-8), malignant hematological diseases, prevalence, Iran.

INTRODUCTION

Human herpesvirus 8 (HHV-8), also known as Kaposi's sarcoma-associated herpesvirus (KSHV), was initially identified in AIDS-associated Kaposi's sarcoma (KS) in 1994 (Chang et al., 1994). Human herpesvirus 8 has also been implicated in a number of hematological malignancies such as primary effusion lymphoma (Cesarman et al., 1995), multicentric Castleman's disease (Soulier et al., 1995), B cell lymphoma (Deloose et al., 2005), germinotropic B cell plasmablast lymphoproliferative disorder (Du et al., 2002) and post transplantation bone marrow failure (Luppi et al., 2000) or lymphoproliferative disorders (Cesarman et al., 1999). Unlike most other human herpes viruses, KSHV infections presumably begin with a primary infection of susceptible hosts; following this, latency is established, from which intermittent reactivation of replication is possible (Knipe et al., 2007). The global prevalence of HHV-8 infection can be categorized into three major patterns of prevalence: high (>50%) in sub-Saharan Africa and parts of Amazon basin, intermediate (between 5 and 20%) in Mediterranean, Middle East Countries, Caribbean , and low (up to 5%) in Northern and Western Europe and in the United States (Knipe et al., 2007).

Kaposi's sarcoma-associated herpesvirus may be transmitted by sexual intercourse, saliva, solid organ transplantation(Regameyetal.,1998)andblood transfusion

*Corresponding author. E-mail: hrmonavari@yahoo.com.

(Parisi et al., 2007). Patients with hematological malignancies are usually associated with multiple transfusions and immunocompromised state. Whether they are at a higher risk of HHV8, infection has not been determined. In Iran HHV-8 have been studied in hemodialysis, renal transplant recipients and HIV patients diagnosed clinically with Kaposi's sarcoma (Somayeh et al., 2011), but have not been studied in patients with malignant hematological diseases. We conducted this study to determine the prevalence of HHV8 infection among 62 patients with hematological malignancies.

MATERIALS AND METHODS

Study population

In this cross-sectional study, 62 consecutive patients with established malignant hematological diseases who were referred from September, 2009 to April, 2010 to oncology Unit of Hazrate Rasul Hospital, Tehran, Iran, were used for this study. All of the patients received chemotherapy for their malignancy

Collection and preparation of samples

About 5 ml of peripheral blood of each subject were collected into ethylenediaminetetraacetic acid (EDTA)-containing vacutainer tubes. Blood buffy coat were separated from EDTA-treated blood by centrifugation and stored at -70°C until analysis. Informed consents were obtained from all of the subjects, which conform to the guidelines of the 1975 Declaration of Helsinki. EDTA anti-coagulated peripheral blood specimens from 20 consecutive blood donors as well as genomic DNA from HHV8 harboring cells were used as negative and positive controls, respectively.

HHV-8 DNA detection in blood buffy coat samples by real time-polymerase chain reaction (PCR)

Detection of HHV-8 DNA in blood buffy coat specimens was performed by Real Time-polymerase chain reaction (Real Time - PCR) method. Briefly, DNA was extracted from 200 µl blood buffy coat sample using the High Pure Extraction Kit (Roche Diagnostics GmbH, Mannheim, Germany) in accordance with the instructions provided by the manufacturer and subjected to PCR with a Light Cycler system with the Light Cycler Fast Start DNA Master Hybridization probes kit (Roche Applied Science) and Taqman probes for HHV-8. The primer set for the HHV-8 ORF65 was HHV8-F:CCTCTGGTCCCCATTCATTG and HHV8-R:CGTTTCCGTCGTGGATGAG and the probe for HHV-8 ORF65 was HHV8-P: FAM-CCGGCGTCAGACATTCTCACAACC-TAMRA (Sugita et al., 2008). The thermal cycler profile is optimized and validated with Heat activation (15 min at 95°C) of hot-start; *Taq* polymerase was followed by 40 cycles of denaturation (30 s at 95°C), annealing (30 s at 50°C), and extension (30 s at 72°C).

Statistical analysis

All data were analyzed using SPSS software version 11.0 (SPSS Incorporated, Chicago, IL, USA). The analyses were carried out using descriptive statistical indexes including standard deviation, mean, confidence interval at 95%, and *t* test. $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Sixty-two (62) patients with established malignant hematological diseases were recruited in this study. The mean age of patients was 33.9 ± 18.0 years. Of the 62 patients, 45 (72.6%) were male. According to the type of hematological malignancy, 27 (43.5%) with acute myelogenous leukemia (AML), 22 (35.5%) with chronic myelogenous leukemia (CML), 3 (4.8%) with lymphoma, and 10 (16.1%) with acute lymphoblastic leukemia (ALL) consist our study population. The Molecular method of Real time-PCR that amplifies sequences from the ORF65 provided a viral prevalence of 6.5%.

Human herpesvirus type 8 is an unusual herpesvirus because it encodes a huge number oncoproteins or cell signaling proteins. There is a large body of evidence linking HHV-8 to at least three malignancies, Kaposi's sarcoma, multicentric Castleman's disease, and primary effusion lymphomas (Mikala et al., 1999). HHV-8 infections presumably begin with a primary infection of susceptible hosts; following this, latency is established (mostly in B cells), from which intermittent reactivation of replication is possible. The information and understanding of HHV-8 prevalence in different population and patients groups is crucial because it may be useful in establishing prophylactic measures to decrease rates of viral transmission from infected individuals.

Most HHV-8 epidemiological studies have been based on antiviral antibodies detection but detection of HHV-8 genome in the specimen of patients is more reliable than that used in the present study and demonstrate that the prevalence of HHV-8 infection in patients with malignant hematological diseases in Iran is 6.5% (Table 1).

In Iran, HHV-8 has not been studied in patients with malignant hematological diseases and most studies found HHV-8 seroprevalence. In Iranian population a noticeable higher seroprevalence of HHV8 has been reported in hemodialysis (16.9%), renal transplant recipients (25%) and HIV (45.7%) patients compared to blood donors (2%) (Jalilvand et al., 2011a). Einollahi et al found Kaposi's sarcoma is the most common malignancy after renal transplantation in Iran (Eyn et al., 2001). Jalilvand et al. (2011b) found that the HHV-8 variants among classic Iranian Kaposi's sarcoma are largely related to Eurasian genotypes previously identified in Kaposi's sarcoma from Mediterranean, Middle East, and East Asian regions.

Lin et al. (2008) study showed that the prevalence of plasma HHV-8 DNA was 10.6% for HIV infection through sexual contact and 7.1% for HIV infection through intravenous injection. There are several reports in world which most of all determined viral prevalence in patients with lymphocytic (B cell) disorders and a small number of studies demonstrated information about lymphocytic and myelogenous malignancies. Chen et al. (2005) reported the prevalence of HHV-8 DNA in peripheral blood

Table1. Demographic characteristic and prevalence of HHV-8 infection among Iranian patients with Hematological Malignancies.

Hematological malignancy	Mean age	Male/female	Patient No	Age (years)	HHV-8 PCR positive (%)
Acute Myelogenous Leukemia (AML)	29.2 ± 12.0	19/8	15	<30	0
			11	30 - 60	0
			1	61 - 90	1
Chronic Myelogenous Leukemia (CML)	50.5 ± 14.4	17/5	1	<30	0
			16	30 - 60	0
			5	61 - 90	3
Lymphoma	27.3 ± 2.5	2/1	3	<30	0
			-	30 - 60	0
			-	61 - 90	0
Acute Lymphoblastic Leukemia (ALL)	12.3 ± 6.2	7/3	10	<30	0
			-	30 - 60	0
			-	61 - 90	0
Total	33.9 ± 18.0	45/17	62	-	4 (6.5)

Table 2. Demographic characteristics of patients positive to HHV-8.

Case	PCR result	Syndrome	Duration of blood transfusion in month	Age/Gender
1	+	AML	2	68/M
2	+	CML	2	83/F
3	+	CML	2	76/M
4	+	CML	1	65/M

mononuclear cells (PBMCs) in Taiwanese leukemia populations 10.29%. Tattevin et al. (2002) reported a HHV-8 infected patient with concomitant chronic lymphocytic lymphoma and multiple myeloma. Vey et al. (2001) in France report a HHV- 8-infected patient with chronic myelogenous leukemia. Wang et al. (2001) in a study for the determination of prevalence of HHV-8 DNA in acute myelogenous leukemia (AML) found HHV-8 DNA sequences in one case.

In the present study, difference was seen between patients with and without HHV-8 infection but this difference was not statistically significant ($p < 0.04$). Therefore, HHV-8 infection may be as a result of transfusion with contaminated blood or blood products. The present study suggests that serious consideration must be given to prevent this infection via transfusion in hematological malignant patients, and assessing HHV-8 serology assay of the patients, before any transfusion, is a useful tool to get information about patient HHV-8 status.

Hudnall et al. (2003) study showed that there is a significant association between seropositivity of HHV-8 and older age. Interestingly in the present study, a significant difference was seen between HHV-8 DNA positive results and older age ($p < 0.01$), although increasing HHV-8 positive results (seroprevalence or/and DNA) with age is consistent lifelong persistence of antibody against this virus as seen with other herpesvirus infections and with life-long susceptibility to this infection (Table 2).

In conclusion, the results of this study suggest that patients with malignant hematological diseases may have HHV-8 infection. Therefore, the possibility of HHV-8 infection should be considered in patients who suffer from hematological malignancies.

ACKNOWLEDGEMENTS

This investigation has been funded by Tehran University of Medical Sciences, and had not other financial support. The code of this project was MT 392.

Abbreviation: HHV-8, Human herpesvirus 8; **EDTA,** ethylenediaminetetraacetic acid; **AML,** acute myelogenous leukemia; **CML,** chronic myelogenous leukemia; **ALL,** acute lymphoblastic leukemia.

REFERENCES

Cesarman E, Chang Y, Moore PS, Said JW, Knowles DM (1995). Kaposi's sarcoma–associated herpesvirus-like DNA sequences in AIDS-related body-cavity–based lymphomas. N. Engl. J. Med. 332(18):1186-91.

Cesarman E, Knowles DM (1999). The role of Kaposi's sarcoma-associated herpesvirus (KSHV/HHV-8) in lymphoproliferative diseases. Semin. Cancer Biol. 9(3):165-74.

Chen CH, Chang CP, Wu FY, Liu CL, Peng CT, Lin CW (2005). Prevalence of human herpesvirus 8 DNA in peripheral blood mononuclear cells of acute and chronic leukemia patients in Taiwan. FEMS Immunol. Med. Microbiol. 2011 61(3):356-8.

Chang Y, Cesarman E, Pessin MS, Lee F, Culpepper J, Knowles DM (1994). Identification of herpesvirus-like DNA sequences in AIDS-associated Kaposi's sarcoma. Science 266(5192):1865.

Deloose STP, Smit LA, Pals FT, Kersten MJ, Van Noesel CJM, Pals ST (2005). High incidence of Kaposi sarcoma-associated herpesvirus infection in HIV-related solid immunoblastic/plasmablastic diffuse large B-cell lymphoma. Leukemia 19(5):851-5.

Du MQ, Diss TC, Liu H, Ye H, Hamoudi RA, Cabeçadas J, Harris NL, Chan JK, Rees JW, Dogan A, Isaacson PG (2002). KSHV-and EBV-associated germinotropic lymphoproliferative disorder. Blood 100(9):3415.

Eyn EB, Khatami MR, Simforoush N, Firouzan A, Nafar Mohsen NMM, Lesan Pezeshki M (2001). Incidence of postrenal transplantation malignancies: a report of two centers in Tehran, Iran. Transplant Proc. 33(5):281-2.

Jalilvand S, Shoja Z, Mokhtari-Azad T, Nategh R, Gharehbaghian A (2011a). Seroprevalence of Human herpesvirus 8 (HHV-8) and incidence of Kaposi's sarcoma in Iran. Infect. Agents Cancer 6(1):5.

Jalilvand S, Tornesello ML, Buonaguro FM, Buonaguro L, Naraghi ZS, Shoja Z, Ziaee AA, Hamkar R, Shahmahmoodi S, Nategh R, Mokhtari-Azad T (2011b). Molecular epidemiology of human herpesvirus 8 variants in Kaposi's sarcoma from Iranian patients. Virus Res. 163(2):644-9.

Hudnall SD, Chen T, Rady P, Tyring S, Allison P (2003). Human herpesvirus 8 seroprevalence and viral load in healthy adult blood donors. Transfusion 43(1):85-90.

Knipe DM, Howley PM, Griffin DE, Lamb RA, Martin MA, Roizman B, Straus SE (2007). Fields Virology, 5th Edition. Lippincott, Williams & Wilkins, Philadelphia, PA.

Lin CW, Chang CP, Wu FY, Liu CL (2008). Comparative prevalence of plasma human herpesvirus 8 DNA in sexual contact and intravenous injection routes of HIV transmission. FEMS Immunol. Med. Microbiol. 52:428–30.

Luppi M, Barozzi P, Schulz TF, Setti G, Staskus K, Trovato R, Narni F, Donelli A, Maiorana A, Marasca R, Sandrini S, Torelli G (2000). Bone marrow failure associated with human herpesvirus 8 infection after transplantation. N. Engl. J. Med. 343(19):1378-85.

Mikala G, Xie J, Berencsi G, Kiss C, Márton I, Domján G, Vályi-Nagy I (1999). Human herpesvirus 8 in hematologic diseases. Pathol. Oncol. Res. 5(1):73-9.

Parisi SG, Cruciani M, Palù G (2007). Transmission of human herpesvirus 8 by blood transfusion. N. Engl. J. Med. 356(1):87-9.

Regamey N, Tamm M, Wernli M, Witschi A, Thiel G, Cathomas G, Erb P (1998). Transmission of human herpesvirus 8 infection from renal-transplant donors to recipients. N. Engl. J. Med. 339(19):1358-63.

Soulier J, Grollet L, Oksenhendler E, Cacoub P, Cazals-Hatem D, Babinet P, d'Agay MF, Clauvel JP, Raphael M, Degos L (1995). Kaposi's sarcoma-associated herpesvirus-like DNA sequences in multicentric Castleman's disease [see comments]. Blood 86(4):1276.

Somayeh J, Zabihollah S, Talat MA, Rakhshandeh N, Ahmad G (2011). Seroprevalence of Human herpesvirus 8 (HHV-8) and incidence of Kaposi's sarcoma in Iran. Infect. Agents Cancer 6-5

Sugita S, Shimizu N, Watanabe K, Mizukami M, Morio T, Sugamoto Y, Mochizuki M (2008). Use of multiplex PCR and real-time PCR to detect human herpes virus genome in ocular fluids of patients with uveitis. Br. J. Ophthalmol. 92(7):928.

Tattevin P, Davi F, Merle Béral H, Calvez V, Hermine O, Papo T (2002). Chronic lymphocytic lymphoma and multiple myeloma in a patient infected with human herpesvirus 8 (HHV 8). Am. J. Hematol. 71(2):138-9.

Vey N, Camerlo J, Xerri L, Petit N, Dermeche S, Maraninchi D (2001). Simultaneous Occurrence of Kaposi's Sarcoma and Chronic Myelogenous Leukemia. Leuk. lymphoma 41(3-4):425-8.

Wang M, Song Y, Ma X, Han M, Bi Y, Mu G, Lin Y, Li G, Wu K (2001). Detection of human herpesvirus 8 DNA in acute leukemia patients. Chin. Med. J. 114(8):873.

UPCOMING CONFERENCES

24th International Congress for the History of Science, Technology and Medicine, Manchester, UK, 22 Jul 2013

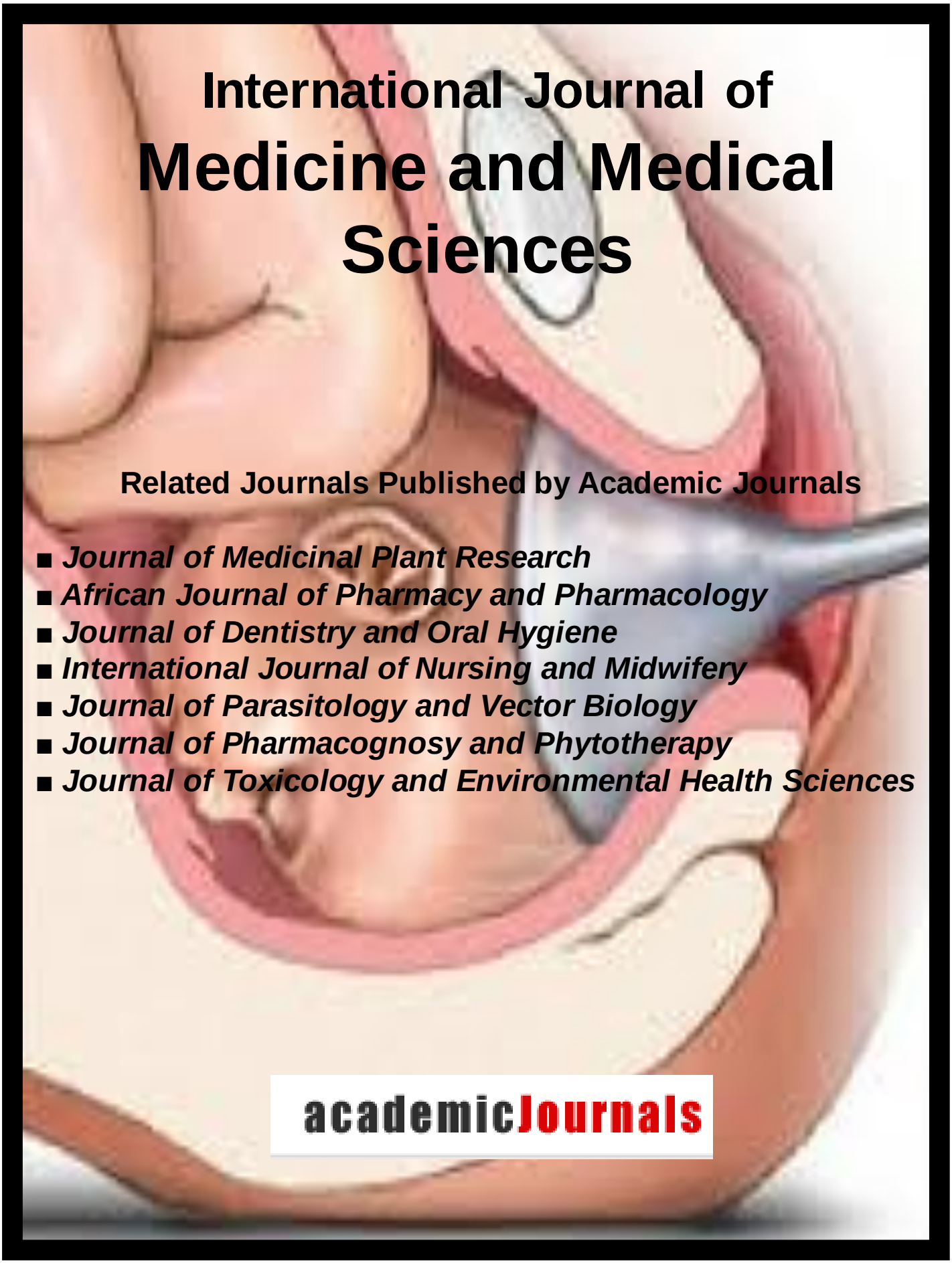
13th Congress of the Asia-Pacific Federation for Clinical Biochemistry and Laboratory Medicine (APCCB 2013), Bali, Indonesia, 27 Oct 2013

Conferences and Advert

August 2013
Association of Institutions for Tropical Veterinary Medicine (AITVM) 14th International Conference, Pretoria, South Africa, 25 Aug 2013

September 2013
13th Asian Federation of Sports Medicine Conference 2013, Kuala Lumpur, Malaysia, 25 Sep 2013

December 2013
20th World congress on Parkinson's Disease and Related Disorders, Geneva, Switzerland, 8 Dec 2013



International Journal of Medicine and Medical Sciences

Related Journals Published by Academic Journals

- *Journal of Medicinal Plant Research*
- *African Journal of Pharmacy and Pharmacology*
- *Journal of Dentistry and Oral Hygiene*
- *International Journal of Nursing and Midwifery*
- *Journal of Parasitology and Vector Biology*
- *Journal of Pharmacognosy and Phytotherapy*
- *Journal of Toxicology and Environmental Health Sciences*

academicJournals