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, Neuropsychology 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.
Editors

Dr. J. Ibekwe
Acting Editor-in-chief,
International Journal of Medicine and Medical Sciences
Academic Journals
E-mail: ijmms.journals@gmail.com
http://www.academicjournals.org/ijmms

Nargis Albert Labib
Editor, Medicine and Surgery
Training Consultant for CDC
Surveillance Unit
Ministry of Health, Cairo,
Egypt

Anil Aggrawal
Editor, Forensic Medicine
Department of Forensic Medicine,
Maulana Azad Medical College,
New Delhi-110002,
India

Afrozul Haq
Editor, Laboratory Medicine
Department of Laboratory Medicine
Sheikh Khalifa Medical City
P.O. Box S1900, ABU DHABI
United Arab Emirates

Basavaraj K. Nanjwade
Editor, Pharmacutics
Department of Pharmaceutics
KLE University
Belgaum –590010, India.

Chang-Gu Hyun
Editor, Pharmacutics
Research Institute (JBRI) & Jeju Hi-Tech Industry
Development Institute (HiDI),
Korea

Osmond Ifeanyi Onyeka
Editor, Alternative Medicine
IUCM/Global Foundation for Integrative Medicine,
U.S.A.

Vahideh Moin-Vaziri
Editor, Parasitology
Department of Parasitology and Mycology,
School of Medicine, Shahid Beheshti
University of Medical Sciences and health services,
Tehran, Iran

Donovan Anthony McGrowder
Editor, Chemical Pathology
University Hospital of The West Indies,
Kingston,
Jamaica

Panagiotis Christopoulos
Editor, Obstetrics and Gynaecology
1 Hariton Street,
Kifisia 14564, Athens,
Greece

Shuiyuan Xiao
Editor, Psychiatry
Professor of social medicine and psychiatry
29 mailbox Xiangya Medical School
110 Xiangya Road,
Changsha, Hunan 410078,
China

Ajai Kumar Srivasta
Editor, Basic Medical Sciences
D.D.U. Gorakhpur University,
India

Tonukari N. J.
Editor, Basic Medical Sciences
Department of Biochemistry
Delta State University, Abraka,
Delta State,
Nigeria

Oluwafemi O. Oguntibeju
Editor, Basic Medical Sciences
Department of Biomedical Sciences,
Faculty of Health & Wellness Sciences,
Cape Peninsula University of Technology,
Bellville 7535,
South Africa

Maysaa El Sayed Zaki
Editor, Clinical Pathology
Faculty of Medicine
Department of Clinical Pathology
Mansoura University
Mansoura,
Egypt
Editorial Board

Chandrashekhar T. Sreeramareddy
Department of Community Medicine,
P O Box No 155, Deep Heights
Manipal College of Medical Sciences,
Pokhara,
Nepal

Sisira Hemananda Siribaddana
259, Temple Road, Thalapathpitiya,
uNugegoda, 10250
Sri Lanka

Dr. Santi M. Mandal
Internal Medicine
UTMB, Galveston, TX,
USA

Konstantinos Tziomalos
Department of Clinical Biochemistry
(Vascular Prevention Clinic),
Royal Free Hospital Campus,
University College Medical School, University College
London, London,
United Kingdom

Cyril Chukwudi Dim
Department of Obstetrics & Gynaecology
University of Nigeria Teaching Hospital (UNTH)
P.M.B. 01129, Enugu. 400001,
Nigeria

Mojtaba Salouti
School of Medical and Basic Sciences,
Islamic Azad University- Zanjan,
Iran

Imtiaz Ahmed Wani
Srinagar Kashmir, 190009,
India

Professor Viroj Wiwanitkit
Wiwanitkit House, Bangkae,
Bangkok
Thailand 10160

Dr. Srinivas Koduru
Dept of Clinical Sciences
Collage of Health Sciences
University of Kentucky
Lexington USA

Weiping Zhang
Department of Oral Biology
Indiana University School of Dentistry
1121 West Michigan Street, DS 271
Indianapolis, IN 46202
USA

Lisheng Xu
Ho Sin Hang Engineering Building
Department of Electronic Engineering
The Chinese University of Hong Kong
Shatin, N.T. Hong Kong,
China

Dr. Mustafa Sahin
Department of Endocrinology and Metabolism
Baskent University,
Ankara,
Turkey

Dr. Harshdeep Joshi
Maharishi Markandeshwar
Institute of Medical Sciences and Research
Ambala, (Haryana).
India.
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:


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.
ARTICLES

Research Articles

Serum albumin as a rough guide to the assessment of nutritional status of hospitalized patients: A study from Zaria, Northern Nigeria

Akuyam S. A, Anaja, P. O, Aliyu, I. S, Mai A and Dahiru I. L

Lercanidipine effect on polymorphonuclear leukocyte-related inflammation and insulin resistance in essential hypertension patients

Raymond Farah and Revital Shurtz-Swirski

Intestinal parasitism in school children periodically treated with albendazole in 2 sampling periods

Quihui-Cota Luis and Morales-Figueroa Gloria Guadalupe

Giant aortic arch thrombus, methylenetetrahydrofolate reductase (MTHFR) A1298C heterozygous gene mutation, smoking and hormonal replacement therapy

Abdallah K. Alameddine, Jonathan Freeman, John Rousou, Yvonne Alameddine, Joseph E. Flack and Victor K. Alimov

Importance of diet on disease prevention

Francesco Sofi, Rosanna Abbate, Gian Franco Gensini and Alessandro Casini
## ARTICLES

### Research Articles

**Retrospective incidence of wound infections and antibiotic sensitivity pattern: A study conducted at the Aminu Kano Teaching Hospital, Kano, Nigeria**  
Mohammed, A., Adeshina, G. O and Ibrahim, Y. K. E  

**Application of area to point Kriging to breast cancer incidence in Ashanti Region of Ghana**  
Ebenezer Bonyah, L. Munyakazi, N.N.N. Nsowah-Nuamah, D. Asong and I.I. Saeed  

**Validation of pharmacokinetic model of propofol in Indian population**  
Avinash Puri and Sanju dhawan  

**Sex differences in the cranial and orbital indices for a black Kenyan population**  
Munguti Jeremiah, Mandela Pamela and Butt Fawzia  

**A retrospective study on the outcomes of tuberculosis treatment in Felege Hiwot Referral Hospital, Northwest Ethiopia**  
Fantahun Biadglegne, Berhanu Anagaw, Tewodros Debebe, Belay Anagaw, Woghata Tesfaye, Belay Tessema, Arne C. Rodloff and Ulrich Sack
Full Length Research Paper

Serum albumin as a rough guide to the assessment of nutritional status of hospitalized patients: A study from Zaria, Northern Nigeria

Akuyam S. A.1*, Anaja, P. O.1, Aliyu, I. S.1, Mai A.2 and Dahiru I. L.3

1Department of Chemical Pathology, Ahmadu Bello University Teaching Hospital, Zaria, Nigeria.
2Department of Surgery, Ahmadu Bello University Teaching Hospital, Zaria, Nigeria.
3Department of Traumatic and Orthopedic Surgery, Ahmadu Bello University Teaching Hospital, Zaria, Nigeria.

Accepted 4 February, 2013

Several studies have revealed that malnutrition is a common finding in hospitalized patients. The situation in our hospitals is not known. The objective of the present study was to compare serum albumin (ALB) among hospitalized, out-patients and apparently healthy subjects, with a view to assessing its status during hospitalization. Serum ALB concentrations were measured each in 50 of hospitalized and out-patients, as well as apparently healthy individuals. The data obtained were analyzed using Statistical Package for Social Sciences (SPSS 11.0) for Windows (SPSS, Chicago, IL). Student’s t-test and one way analysis of variance (ANOVA) were employed for the comparison of the results obtained from different groups of the subjects. A p-value of equal to or less than 0.05 (p ≤ 0.05) was considered as statistically significant. Serum ALB concentrations in hospitalized patients, out-patients and apparently healthy subjects were 35.88 ± 1.56, 39.70 ± 1.77 and 42.32 ± 1.34 g/L, respectively. The differences in these values between hospitalized patients and apparently healthy subjects were statistically significant (p < 0.01), while there were no significant differences between out-patients and apparently healthy subjects. It is concluded from the findings of this study that serum ALB concentrations were significantly lower in hospitalized patients than in apparently healthy subjects, while it is similar in out-patients and apparently healthy subjects. It is recommended that serum ALB concentrations be routinely measured as part of the assessment of nutritional status in hospitalized patients.

Key words: Serum albumin, assessment of nutritional status, hospitalized patients, out-patients, apparently healthy individuals.

INTRODUCTION

Albumin (ALB) is the most abundant protein in human plasma, representing 55 to 65% of the total protein (Peters, 1975; Javed and Waqar, 2001). It is a low molecular weight protein (about 65,000) synthesized in the hepatocytes of the liver (Schultze and Heremans, 1970). Serum ALB estimation is one of the commonly requested tests in the Clinical Chemistry Laboratory for evaluation of protein disorders and in several disease conditions, including nephrotic syndrome, liver diseases, malnutrition and water and electrolytes imbalance (Grant et al., 1987; Margaron and Soni, 1998; Banh, 2006; Sundell, 2007).

Malnutrition is highly prevalent in hospitalized patients, most especially in the elderly ones and is associated with increased morbidity and mortality (Lowenstein, 1982; Mowe and Bohmer, 1991; Gariballa, 2001; Visvanathan, 2003; Singh et al., 2006). Malnutrition has been reported...
in up to 15% of community-dwelling and home-bound elderly individuals. It is also reported in up to 62% of hospitalized elderly patients and up to 85% of residents of nursing homes (Compan et al., 1999; Morley and Thomas, 1999; Visvanathan, 2003).

Nutritional assessment is an important part of management in every patient, including hospitalized ones. The objective of this assessment is to identify those patients who are already malnourished or who are at increased risk of developing malnutrition (Baron, 1986). Dozens of assessment techniques are currently available and in common use. Blackburn et al. (1977) have recommended an extensive panel of clinical and laboratory assessments which include anthropometric measurements, and laboratory analysis including serum albumin measurement. The use of serum ALB and total protein concentrations and other biochemical tests as indices of nutritional status have been fully documented (World Health Organization (WHO), 1966). The levels of serum proteins, including ALB have been reported previously in several Nigerian and African subjects (Edozien, 1957; Isichei, 1975; Onwuameze, 1989; Anaja et al., 1997).

The aspect of nutritional assessment is neglected in the management of patients in most of the Nigerian hospitals, including ABUTH, Zaria. Moreover, there is paucity of data on the nutritional assessment in hospitalized patients in Nigeria, including Zaria. The reported studies on this aspect were carried out elsewhere in the world (Baron, 1986; Chima et al., 1997; Kagansky et al, 2005; Beckman Coulter, 2007). In view of its clinical importance, there is therefore the need to carry out a study on the nutritional assessment in hospitalized patients in Ahmadu Bello University Teaching Hospital (ABUTH), Zaria. This could help for the diagnostic, therapeutic and prognostic purposes, including provision of nutritional support for the malnourished hospitalized patients and therefore reduces the morbidity and mortality among this group of patients in our hospitals. The objective of the present study was therefore to compare serum ALB among hospitalized, out-patients and apparently healthy subjects with a view to assessing its status during hospitalization.

### MATERIALS AND METHODS

The study was conducted in the Department of Chemical Pathology of ABUTH, Zaria, Northern Nigeria. It is a cross-sectional study which was approved by the Ethical Committee of the ABUTH, Zaria in accordance with Helsinki declaration. A total of 150 subjects (88 males and 62 females) were recruited for this study. This consisted of 50 [31 males and 19 females; mean age 41 ± 26 (range 15 to 70 years)] hospitalized patients (in-patients), 50 [25 males and 25 females; mean age 46 ± 31 (range 15 to 70 years)] out-patients with different disease conditions and 50 (32 males and 18 females; mean age 36 ± 21 (range 15 to 60 years)] apparently healthy individuals (control). The target populations were adult patients who were on admission in various wards or who were attending various out-patients clinics of ABUTH, Zaria, respectively. The apparently healthy individuals were recruited from the population of staff and students of ABUTH, Zaria.

Patients aged between 15 and 70 years of age presenting to any one of the above mentioned facilities of ABUTH, Zaria with various disease conditions, such as diabetes mellitus (DM), hypertensive heart disease (HHD), peptic ulcer disease (PUD) and others were included in the study. Apparently healthy individuals who have not been diagnosed to have any one of the above mentioned disease and who were within the same age range with patients were included in the study as controls. Patients with any one of the disease conditions known to affect serum ALB, such as liver and kidney diseases, malnutrition, cancer and others were excluded from the study. Similarly, all subjects who were below 15 and above 70 years were excluded from this study. All subjects who declined to give consent for inclusion were also excluded from the study.

At the respective locations, arrangements were made with the clinicians whereby consecutive subjects who satisfied the study inclusion criteria were selected. Informed consent for inclusion into the study was obtained from the subjects. The nature of the study was explained to the subjects using an appropriate language. A full history was obtained from the selected subjects. This was followed by clinical examination and collection of blood specimens. The main findings were documented. Blood specimen (about 5 ml) was collected from a peripheral vein (antecubital venepuncture). It was transferred into a plain bottle and allowed to clot for about 30 min. This was then centrifuged for 5 min at 1,200 g. The serum was separated from the cells and stored frozen at - 20°C until the time for analysis. Serum ALB concentrations were measured using method of Doumas et al. (1971) by the use of reagent diagnostic kit which was procured from RANDOX Laboratories Ltd. (United Kingdom).

The data obtained were analysed using Statistical Package for Social Sciences (SPSS 11.0 for Window) (SPSS Inc., Chicago, IL). One way analysis of variance (ANOVA) statistical method was employed for comparison of the results of serum ALB obtained from 3 different groups of the subjects and Student’s t-test was used for post-hoc analysis, for the comparison of results between 2 different groups of studied subjects. A p-value of equal to or less than 0.05 (p ≤ 0.05) were considered as statistically significant.

### RESULTS

The results of serum ALB in hospitalized and out-patients, as well as apparently healthy individuals are presented in Table 1. These results show significant differences between the 3 groups of the studied subjects (p < 0.05, ANOVA). The results of serum ALB in hospitalized patients and controls are shown in Table 2.

---

**Table 1. Serum albumin (mean ± SEM) in hospitalized patients, out-patients and apparently healthy subjects.**

<table>
<thead>
<tr>
<th>Subject</th>
<th>n</th>
<th>Serum albumin (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hospitalized patients</td>
<td>50</td>
<td>35.88±1.56</td>
</tr>
<tr>
<td>Out-patients</td>
<td>50</td>
<td>39.70±1.77</td>
</tr>
<tr>
<td>Apparently healthy subjects</td>
<td>50</td>
<td>42.32±1.34</td>
</tr>
</tbody>
</table>

*p-value*<0.05

\[ n = \text{sample size and SEM = standard error of the mean, *this was based on one way analysis of variance (ANOVA).} \]
The findings of the present study show that the results of serum ALB obtained from hospitalized patients were significantly lower than those obtained from apparently healthy individuals, while there was no statistically significant difference in serum ALB between out-patients and apparently healthy individuals. Similarly, the concentrations of serum ALB obtained from hospitalized patients and out-patients were not statistically different. These results therefore demonstrate that serum ALB was decreased during hospitalization. These findings agree well with the previous reports by Margarson (1998), Banh (2006) and Gariballa (2001) who reported that serum ALB concentrations deteriorate steadily during hospitalization period which results in hypoaalbuminaemia and immediately following discharge. It has been reported that the low serum levels of ALB found in hospitalized patients positively correlated with poor nutritional status in these patients (Gariballa, 2001).

The use of serum ALB and total protein concentrations and other biochemical tests as indices of nutritional status have been fully documented (World Health Organization, 1966). Serum albumin is the most widely measured biochemical analyte for the assessment of nutritional status in both patients and for the nutrition surveys. This is because of ease of its measurement as regard to cost, short turn-around time and ability to detect malnutrition. The fact that serum ALB concentrations were significantly low in hospitalized patients supports the report that malnutrition is common in hospitalized patients and may be associated with increased morbidity and mortality.

Several studies across the world revealed that malnutrition is highly prevalent in hospitalized patients most especially in the elderly ones, and this is associated with increased morbidity and mortality (Lowenstein, 1982; Mowe and Bohmer, 1991; Gariballa, 2001; Visvanathan, 2003; Kagansky et al., 2005; Singh et al., 2006). Malnutrition has been reported in up to 15% of community-dwelling and home-bound elderly individuals and in up to 62% of hospitalized elderly patients and 85% of residents of nursing homes (Compan et al., 1999; Morley and Thomas, 1999; Visvanathan, 2003). Many studies have shown that poor nutrition leads to complications during hospitalization and increases mortality (Potter et al., 1988; Constans et al., 1992; Sullivan and Walls, 1995). The poor nutritional status in hospitalized patients is associated with various factors, including chronic diseases, anorexia, medications, isolation, psycho-social problems and decline in cognitive and functional status (Kagansky et al., 2005). It has been suggested that the causes of malnutrition in hospitalized patients can be divided into 3 major categories: (1) decreased oral intake, (2) increased nutritional losses and (3) decreased nutrient requirements, which may be due to psycho-social problems (Baron, 1986).

### Table 2. Serum albumin (mean ± SEM) in hospitalized patients and apparently healthy individuals.

<table>
<thead>
<tr>
<th>Subject</th>
<th>n</th>
<th>Serum albumin (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hospitalized patients</td>
<td>50</td>
<td>35.88±1.56</td>
</tr>
<tr>
<td>Apparently healthy subjects</td>
<td>50</td>
<td>42.32±1.34</td>
</tr>
</tbody>
</table>

*p-value* <0.01

n= sample size and SEM= Standard error of the mean, *this was based on Student's t-test.

### Table 3. Serum albumin (mean ± SEM) in out-patients and apparently healthy individuals.

<table>
<thead>
<tr>
<th>Subject</th>
<th>n</th>
<th>Serum albumin (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Out-patients</td>
<td>50</td>
<td>39.70±1.77</td>
</tr>
<tr>
<td>Apparently healthy subjects</td>
<td>50</td>
<td>42.32±1.34</td>
</tr>
</tbody>
</table>

*p-value* >0.05

n = sample size and SEM = standard error of the mean, *this was based on Student's t-test.

### Table 4. Serum albumin (mean ± SEM) in hospitalized patients.

<table>
<thead>
<tr>
<th>Subject</th>
<th>n</th>
<th>Serum albumin (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hospitalized patients</td>
<td>50</td>
<td>35.88±1.56</td>
</tr>
<tr>
<td>Out-patients</td>
<td>50</td>
<td>39.70±1.77</td>
</tr>
</tbody>
</table>

*p-value* >0.05

n = sample size and SEM = standard error of the mean, *this was based on Student's t-test.

The results in this table show that serum ALB in hospitalized patients were significantly lower than those obtained in controls (p < 0.01, Student’s t-test).

Serum ALB obtained from out-patients and controls are shown in Table 3. These results show that serum ALB concentrations in out-patients were not significantly different from that observed in controls (p > 0.05, Student’s t-test). The results of serum ALB concentrations obtained from hospitalized and out-patients are presented in Table 4. The results show that ALB values in hospitalized were not significantly different from that observed in out-patients counterparts (p > 0.05, Student’s t-test).

**DISCUSSION**

ALB concentrations have been measured in hospitalized patients, out-patients and apparently healthy individuals to compare the results between these groups of subjects, with a view to assessing its status during hospitalization to serve as a baseline study. The findings of the present study show that the results of serum ALB obtained from hospitalized patients were significantly lower than those obtained from apparently healthy individuals, while there was no statistically significant difference in serum ALB between out-patients and apparently healthy individuals. Similarly, the concentrations of serum ALB obtained from hospitalized patients and out-patients were not statistically different. These results therefore demonstrate that serum ALB was decreased during hospitalization. These findings agree well with the previous reports by Margarson (1998), Banh (2006) and Gariballa (2001) who reported that serum ALB concentrations deteriorate steadily during hospitalization period which results in hypoaalbuminaemia and immediately following discharge. It has been reported that the low serum levels of ALB found in hospitalized patients positively correlated with poor nutritional status in these patients (Gariballa, 2001).

The use of serum ALB and total protein concentrations and other biochemical tests as indices of nutritional status have been fully documented (World Health Organization, 1966). Serum albumin is the most widely measured biochemical analyte for the assessment of nutritional status in both patients and for the nutrition surveys. This is because of ease of its measurement as regard to cost, short turn-around time and ability to detect malnutrition. The fact that serum ALB concentrations were significantly low in hospitalized patients supports the report that malnutrition is common in hospitalized patients and may be associated with increased morbidity and mortality.

Several studies across the world revealed that malnutrition is highly prevalent in hospitalized patients most especially in the elderly ones, and this is associated with increased morbidity and mortality (Lowenstein, 1982; Mowe and Bohmer, 1991; Gariballa, 2001; Visvanathan, 2003; Kagansky et al., 2005; Singh et al., 2006). Malnutrition has been reported in up to 15% of community-dwelling and home-bound elderly individuals and in up to 62% of hospitalized elderly patients and 85% of residents of nursing homes (Compan et al., 1999; Morley and Thomas, 1999; Visvanathan, 2003). Many studies have shown that poor nutrition leads to complications during hospitalization and increases mortality (Potter et al., 1988; Constans et al., 1992; Sullivan and Walls, 1995). The poor nutritional status in hospitalized patients is associated with various factors, including chronic diseases, anorexia, medications, isolation, psycho-social problems and decline in cognitive and functional status (Kagansky et al., 2005). It has been suggested that the causes of malnutrition in hospitalized patients can be divided into 3 major categories: (1) decreased oral intake, (2) increased nutritional losses and (3) decreased nutrient requirements, which may be due to psycho-social problems (Baron, 1986).
The striking reduction of serum ALB in the hospitalized patients of the present study and the previous reports could be due partly to the poor appetite and hence decrease calorie intake which may be as a result of psychologic trauma caused by hospitalization. The significant reduction of serum ALB in hospitalized patients could be attributed to immobilization which has been suggested to be one of the causes of hypoalbuminaemia (Czajka-Narins, 1987; Veldee, 1999). Prolonged immobilization, as in the case of hospitalization, is associated with haemodilution which is known to cause significant reduction of serum ALB (Peralta and Rubery, 2006). The haemodilution in this group of patients could be due to ascites and oedema which are secondary to increased vascular permeability, which permits the loss of ALB into these spaces (Johnson et al., 1999). The limitations of the present study were inability to measure other markers for the nutritional assessment such as transferrin, which are more sensitive than the ALB and inability to measure the serum ALB before and after admission for better assessment than measuring it only once during admission.

CONCLUSIONS AND RECOMMENDATIONS

It is concluded from the findings of the present study that serum ALB concentrations were significantly lower in hospitalized patients than in apparently healthy individuals, while serum ALB concentrations in out-patients and apparently healthy individuals were similar. It is recommended from the findings of the present study that serum ALB concentrations be routinely measured as part of the assessment of nutritional status in hospitalized patients, and that assessment of nutritional status be part of the management of every patient, most particularly hospitalized ones. This is to complement the findings from history, physical examination and anthropometric measurements. This could aid in identifying those patients who are already malnourished or who are at increased risk of developing malnutrition and its complications. Hence it could help for the diagnostic, therapeutic and prognostic purposes, including protein and amino acid requirements, increased risk of developing malnutrition and its complications. Hence it could help for the diagnostic, therapeutic and prognostic purposes, including protein and amino acid requirements.

ACKNOWLEDGEMENT

We gladly acknowledge the assistance of Mr. S. B. Danborno and Mr. F. O. Ayegbusi in the area of statistical analysis.

REFERENCES


Full Length Research Paper

Lercanidipine effect on polymorphonuclear leukocyte-related inflammation and insulin resistance in essential hypertension patients

Raymond Farah1* and Revital Shurtz-Swirski2

1Department of Internal Medicine B, Ziv Medical Center, Safed, Israel.
2Faculty of Medicine in the Galilee, Bar-Ilan University, Safed, Israel.

Accepted 19 November, 2012

Inflammation, insulin resistance and oxidative stress (OS), are among the mechanisms that have been implicated in pathogenesis of essential hypertension (EH). Peripheral polymorphonuclear leukocytes (PMNLs) are primed in EH patients, releasing uncontrolled superoxide anion contributing to OS in these patients. PMNL priming correlates with insulin resistance and with PMNL intracellular calcium ([Ca2+]i). Recent studies have attributed to the anti-hypertensive drug lercanidipine, a third generation calcium-channel blocker, and additional anti-ischemic and anti-oxidative characteristics. To evaluate the possible non-traditional effect of two months of lercanidipine treatment on insulin resistance and on PMNL-related inflammation in EH patients. Non-smoking EH patients with untreated mild to moderate high blood pressure (BP) were included. Low-graded inflammation was reflected by WBC and PMNL counts and by PMNL apoptosis. Systemic inflammation was measured by plasma fibrinogen, C-reactive protein (CRP) and albumin levels. Fasting serum insulin levels served as a marker of insulin resistance. Two months of lercanidipine treatment showed significant decrease in BP, WBC and PMNL counts, and also in PMNL apoptosis, CRP and serum insulin levels and significant increase in serum albumin levels. Rates of superoxide release from PMNLs, WBC and PMNL counts and insulin levels positively correlated with mean arterial blood pressure values. We imply that use of lercanidipine can be favored in EH patients due to its combined anti-PMNL priming and anti-inflammatory effects, in addition to its anti-hypertensive characteristics.

Key words: Essential hypertension, lercanidipine, low-graded inflammation, primed polymorphonuclear leukocytes, oxidative stress.

INTRODUCTION

Essential hypertension (EH) is a substantial public health problem affecting 25% of the adult population in industrialized societies (Burt et al., 1995). This multifactorial and multi-genetic disorder is a major risk factor for many common causes of mortality and morbidity, including stroke, myocardial infarction, congestive heart failure, and end stage renal disease (Mosterd et al., 1999). Insulin resistance is seen in more than half of patient with EH (Swislocki et al., 1989). Despite the important role of EH as a cause of disease, its pathogenesis remains largely unknown.

Abnormalities in endothelial function and morphology appear to play a central role in the pathogenesis of hypertension-related atherosclerosis (Zanchetti et al., 1993). Among the mechanisms causing endothelial dysfunction that have been recently implicated in EH, are OS that may impair endothelium-dependent vasodilatation,
tion, inflammation and insulin resistance (Alexander, 1995). Primed PMNLs are one of the main types of inflammatory cells; once activated, primed PMNLs release reactive oxygen species (ROS), contributing to OS, low-grade inflammation, endothelial damage and atherosclerosis in the long run (Smedly et al., 1986; Weiss, 1989).

Recently, we have previously reported that primed PMNLs contribute to the OS and inflammation in correlation with insulin resistance and PMNL intracellular calcium ([Ca$^{2+}$]) in EH (Kristal et al., 1998; Sela et al., 2002a). In addition, we have recently implicated the PMNL priming as a key mediator of low-grade inflammation and OS associated with renal failure (Sela et al., 2005), thus constituting a common denominator in chronic infection, inflammation, receiving medication, vitamins or antioxidants, smoking and secondary causes of hypertension (Apolipoprotein (Apo) A1, HDL cholesterol level, and triglyceride level). However, a recent study demonstrated the various effects of this drug on systemic and PMNL-related inflammation and on insulin resistance during two months of treatment. Thus, the objective of the present study was to determine the effect of lercanidipine on these parameters in EH.

### MATERIALS AND METHODS

#### Patients

Fifteen untreated EH patients (12 males/3 females), with mild to moderate hypertension (age range 20 to 65 years) and 15 age and gender-matched healthy controls (NC) were enrolled in this prospective study. Inclusion criteria of the EH group were: sitting diastolic blood pressure (DBP) > 90 mmHg (average of three outpatient visits), sitting systolic blood pressure (SBP) > 140 mmHg (average as above), body mass index < 30 kg/m², no evidence of target organ damage and systemic diseases supported by microalbumin/creatinine ratio, fudus examination, echocardiogram test and kidney function tests. Subjects with evidence of acute or chronic infection, inflammation, receiving medication, vitamins or antioxidants, smoking and secondary causes of hypertension were excluded. The selection of all participants was based upon a clinical examination and laboratory confirmation. All the subjects had normal fasting (>14 h), serum cholesterol (<200 mg/dl), triglycerides (<150 mg/dl) and glucose levels, with normal kidney and liver function (Table 1). The study was approved by signing an informed consent for blood sampling approved by the institutional committee.

#### Table 1. The changes in measurements of EH patients.

<table>
<thead>
<tr>
<th>Ocular Fundus</th>
<th><strong>HC</strong></th>
<th><strong>Untreated EH</strong></th>
<th><strong>1 month treatment</strong></th>
<th><strong>2 months treatment</strong></th>
<th><strong>P</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>negative</td>
<td>negative</td>
<td>negative</td>
<td>negative</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>120±3.2$^a$</td>
<td>162±4</td>
<td>146±3$^a$</td>
<td>143±3$^a$</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>69.1±2$^a$</td>
<td>100±1</td>
<td>89±3$^a$</td>
<td>87±2$^a$</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>86.8±2$^a$</td>
<td>120±2</td>
<td>108±2</td>
<td>107±2$^a$</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>205±1.7</td>
<td>230±9.3</td>
<td>225±13.2</td>
<td>226.3±15.6</td>
<td>ns</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>120±3.2</td>
<td>158±26</td>
<td>148.6±19.2</td>
<td>130±23.7</td>
<td>ns</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>58±0.7</td>
<td>42.8±1.4</td>
<td>40.4±2</td>
<td>39.2±3.3</td>
<td>ns</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>114±1.3</td>
<td>155±6.6</td>
<td>155.1±10.8</td>
<td>161±11.8</td>
<td>ns</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>94±1.9</td>
<td>89.1±4.5</td>
<td>98.4±3.4</td>
<td>100.8±5.0</td>
<td>ns</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.93±0.02</td>
<td>0.96±0.03</td>
<td>0.98±0.04</td>
<td>0.95±0.04</td>
<td>ns</td>
</tr>
<tr>
<td>ALT (U/l)</td>
<td>20±0.5</td>
<td>38±6.2</td>
<td>30.7±6.3</td>
<td>38.4±5</td>
<td>ns</td>
</tr>
<tr>
<td>AST (U/l)</td>
<td>19.6±0.3</td>
<td>26.6±3.3</td>
<td>25.4±5.1</td>
<td>24±3.6</td>
<td>ns</td>
</tr>
<tr>
<td>ALP (U/l)</td>
<td>75±6.1</td>
<td>87.8±5.5</td>
<td>83.7±5.4</td>
<td>81.9±5.0</td>
<td>ns</td>
</tr>
<tr>
<td>LDH (U/l)</td>
<td>283±1.9</td>
<td>294±11.9</td>
<td>311±17</td>
<td>313±13</td>
<td>ns</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>14.3±0.1</td>
<td>14.7±0.25</td>
<td>14.6±0.3</td>
<td>14.7±0.2</td>
<td>ns</td>
</tr>
<tr>
<td>Insulin (U/ml)</td>
<td>8.4±0.9$^a$</td>
<td>15.1±1.1</td>
<td>16.4±4.1</td>
<td>10.1±1.1$^a$</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Values are means ± SEM, $^a$versus untreated EH patients; ns = non significant.
in accordance with the Helsinki declaration.

Blood was drawn in the morning after an overnight fast from all EH patients and NC subjects for the determination of biochemical and hematological parameters and for PMNL isolation. Blood was drawn from EH patients before and following treatment with 10 mg/day lercanidipine (Vasodip®TM, Dexon, Israel) for 1 and 2 months. PMNL isolation was carried out from a 20 mL heparinized blood sample as previously described (Klebanoff and Clark, 1977; Sela et al., 2005). The separated PMNLs (>98% pure, approximately 10⁷ cells per isolation) were resuspended in phosphate buffered saline (PBS) containing 0.1% glucose. Sera and plasma were frozen at -20°C for determining the clinical and biochemical characteristics of the participants and for systemic inflammation parameters.

PMNL priming

Rate of superoxide release

The measurements of the rate of superoxide release are based on superoxide dismutase (SOD) inhibitable reduction of 80 µM cytochrome C (Sigma, St. Louis, MO., USA) to its ferrous form (Babior et al., 1973). The rate of superoxide release was monitored from 10⁶ separated PMNLs, after stimulation with 0.32 x 10⁻⁷ M phorbol myristate 13-acetate (PMA; Sigma, St. Louis, MO., USA), at 22°C for 50 min. This parameter was used as a measure for PMNL priming.

PMNL-derived inflammation

WBC and PMNL counts

Counts of WBC and PMNLs from blood drawn in Ethylenediamine-tetraacetic acid (EDTA) were performed by an automated cell counter (Coulter STKS, Miami, Fla., USA) and used as a measure of low-graded inflammation.

Analysis of apoptotic PMNLs

Apoptosis was analyzed in whole blood from EH patients and NC subjects of each group by flow cytometry according to Kuypers et al. (1996). Blood samples were assayed for apoptosis after lysis of red blood cells by Q prep (Beckman Coulter) and incubated with fluorescein isothiocyanate (FITC)-labeled monoclonal antibodies using the Annexin V kit (Bender MedSystems, Vienna, Austria). PMNLs were defined by forward scatter/side scatter and by R-phycoerythrin (PE)-labeled monoclonal anti-CD16.

Systemic inflammation

Measurement of plasma fibrinogen

Fibrinogen was measured in a Cobas Mira plus instrument by Roche (Mannheim, Germany), in all plasma samples using the kit of K-Assay® (Kamiya Biomedical Company, Germany).

Measurement of C-reactive protein (CRP), transferrin and albumin

C-reactive protein (CRP), transferrin and albumin were routinely assayed in the routine biochemistry lab by Hitachi 917 Automatic analyzer (Roche Diagnostics, Mannheim, Germany) in separated sera obtained from all the above EH patients and NC subjects after an overnight fast.

Insulin as a marker of insulin resistance

Fasting serum insulin levels served as a measure for insulin resistance using the electrochemiluminescence immunoassay kit (Roche Diagnostics, Mannheim, Germany). Insulin resistance was also verified by homeostasis model assessment–insulin resistance (HOMA-IR) test.

Statistical analysis

Data values are means ± standard deviation (SD). The two groups were compared by student t-test, using Prism version 3.0 statistical software (GraphPad software, San Diego, California, USA). Correlations between different study parameters were performed using Pearson correlation coefficients. P < 0.05 was considered significant.

RESULTS

Study population

Table 1 summarizes the clinical and biochemical characteristics of the participants. All studied groups of patients showed similar serum cholesterol, serum creatinine, serum triglycerides, liver enzymes and serum glucose levels, without showing target organ damage. Most traditional risk factors were similar during lercanidipine treatment period. Blood pressure values, namely diastolic blood pressure (DBP), systolic blood pressure (SBP) and mean arterial pressure (MAP) decreased significantly following 1 and 2 months of lercanidipine treatment (Table 1).

PMNL priming

Rate of superoxide release

Significantly faster rates of superoxide release from PMA-stimulated PMNLs were found in EH patients before and following 2 months of lercanidipine treatment (Table 2), as compared to NC (18.2 ± 1.2 nmol/10⁶ cells/10 min), reflecting a higher priming state in these groups (EH). Two months of treatment reflected a slight though significant decrease in the rate of superoxide release from PMA-stimulated PMNLs (Table 2).

PMNL-derived Inflammation

WBC and PMNL counts

EH patients had significantly higher numbers of WBC and PMNLs (Table 2), as compared to NC subjects (6.7 ± 0.2 and 3.9 ± 0.2 x 10⁷, respectively), although all values fell within the upper quartile of the normal range. Two months of lercanidipine treatment reduced significantly WBC and PMNL numbers (Table 2).
Table 2. PMNL-related inflammation and priming and systemic inflammation parameters.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>HC</th>
<th>Untreated EH</th>
<th>1 month treatment</th>
<th>2 month treatment</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC × 10^9</td>
<td>7.2±0.1</td>
<td>7.8±0.5</td>
<td>7.4±0.4</td>
<td>7.1±0.2^a</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>PMNL × 10^9</td>
<td>3.9±0.2^a</td>
<td>4.8±0.4</td>
<td>4.4±0.4</td>
<td>4.2±0.2^a</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>PMNL apoptosis (%)</td>
<td>2.8±0.7^a</td>
<td>15.4±1.8</td>
<td>11.5±2</td>
<td>7.2±1.0^a</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Rate of superoxide release (nmoles/10^6 cells/10 min)</td>
<td>18.2±1.2</td>
<td>29±1.6</td>
<td>31.7±1.3</td>
<td>27.5±1.3^b</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Fibrinogen (mg/dl)</td>
<td>289±12^a</td>
<td>393±48</td>
<td>387±34</td>
<td>367±30</td>
<td>ns</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>4.6±0.05^a</td>
<td>4.5±0.06</td>
<td>4.6±0.07</td>
<td>4.64±0.05^a</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Transferrin (g/dl)</td>
<td>273±5^a</td>
<td>288±8</td>
<td>276±6</td>
<td>274±7</td>
<td>ns</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>1.5±0.08^a</td>
<td>3.91±0.9</td>
<td>3.04±0.9</td>
<td>1.67±0.6^a</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SEM. ^a versus untreated EH patients; ^b versus 1 m lercanidipine treated EH patients.

**Percentage of apoptotic PMNLs**

The percentage of apoptotic PMNLs, assayed immediately after blood withdrawal in whole blood, was significantly higher in EH group of patients (Figure 1), as compared to NC (7.7 ± 0.4%). One month of lercanidipine treatment reduced significantly the percentage of apoptotic PMNLs, a reduction that further amplifies after 2 months of treatment, down to NC levels (Figure 1).

**Systemic inflammation**

**Measurement of plasma fibrinogen**

Plasma fibrinogen levels fell within the upper quartile of the normal range and were higher than NC levels (289 ± 12.3 mg/dl). A slight non-significant reduction in plasma fibrinogen levels was found after 2 months of lercanidipine treatment (Table 2).

**Measurement of C-reactive protein (CRP), albumin and transferrin**

A significantly decreased serum CRP levels could be shown after 2 months of lercanidipine treatment to NC levels (1.46 ± 0.08 mg/l) (Figure 2). Increased serum albumin levels were found in HC and treated EH patients compared with untreated EH patients, although all fell within normal range. No significant change was found in serum transferrin levels (Table 2).

**Insulin as a marker of insulin resistance**

Fasting serum insulin levels serve as a measure for insulin resistance (Sela et al., 2002). Figure 3 shows a significant decrease of serum insulin levels after 2 months of lercanidipine treatment, although this was still higher than NC levels (8.41 ± 0.95 µU/ml) after one month of treatment. It has to be emphasized that in these mild to moderate untreated EH patients, most serum insulin levels were within the normal range, although in the upper quartile.
PMNL priming and inflammation in relation to MAP

PMNL priming expressed by the rate of superoxide release in NC and EH patients (treated and untreated with lercanidipine) was positively correlated with MAP: \( r = 0.45, P < 0.001 \) (n = 106, Figure 4A), the higher the blood pressure parameter, the higher the superoxide release. The WBC counts from NC and EH patients (treated and untreated with lercanidipine) were positively correlated with MAP: \( r = 0.25, P = 0.009 \) (n = 90, Figure 4B). The peripheral PMNL counts from NC and EH patients (treated and untreated with lercanidipine) were also positively correlated with MAP: \( r = 0.2, P = 0.04 \) (n = 109, Figure 4C).

Systemic inflammation parameters in relation to MAP

Fibrinogen and CRP, the accepted positive systemic inflammation markers, determined in NC and in EH patients (treated and untreated with lercanidipine), were correlated with MAP. Plasma fibrinogen levels positively correlated with MAP: \( r = 0.27, P = 0.007 \) (n = 101, Figure 5A). However, no correlation could be found between serum CRP levels and MAP: \( r = 0.05, P = 0.6 \) (n = 109,
DISCUSSION

The present study evaluates the role of lercanidipine, a dihydropyridine CCB, in mild and moderate hypertensive patients and his non-traditional effects on PMNL priming, PMNL-related inflammation, systemic inflammation markers and on insulin resistance. Our previous studies showed that EH is accompanied by a primed state of PMNLs, inducing OS and inflammation (Kristal et al., 1998; Sela et al., 2005). We have defined PMNL priming as a common denominator in other clinical states such as hypertension, diabetes and cigarette smoking known to be associated with endothelial dysfunction, accelerated atherosclerosis and increased prevalence of cardiovascular morbidity and mortality (Kristal et al., 1998; Shurtz-Swirski et al., 2001; Tulenko et al., 2001; Sela et al., 2005). In addition, we have recently shown that PMNL priming constitutes a key mediator of low-grade inflammation and OS associated with renal failure (Sela et al., 2005).

A novel interesting observation was the significantly higher percentage of the apoptotic PMNL in EH patients as compared to normal control and the significant decrease in percentage of apoptotic PMNLs already one month after treatment with lercanidipine, a reduction further amplifies after two months of treatment to NC level. PMNL apoptosis has already been shown by us as part of PMNL-related low-grade inflammation parameters, along with WBC and PMNL counts (Sela et al., 2005), which constitute a mortality predictor in HD patients (Pifer et al., 2002; Reddan et al., 2003) and as a predictor for developing CKD (Erlinger et al., 2003).

In the present study WBC and PMNL counts were higher in EH patients as well, and declined significantly after treatment with lercanidipine, exhibiting a reduction in the PMNL-related low-grade inflammation.

In parallel, other systemic inflammation markers as CRP, fibrinogen, transferrin and albumin were also assessed. Serum albumin is a negative acute-phase protein whose low level is attributed to inflammation (Tsirpanlis et al., 2005); although in the normal range, we could show a significantly increase following Lercanidipine treatment. The reduction in fibrinogen was slight and non-significant, and transferrin levels did not change, possibly due to the relative small number of the patients. An interesting observation from the present study is the significant decrease in CRP level during this treatment to low levels as observed in NC, which are predictive of reduced cardiovascular risk. Numerous studies have demonstrated that elevated CRP levels and upper

Figure 5. (A) Correlation between plasma fibrinogen levels and MAP; (B) Correlation between serum CRP levels and MAP. Data refers to values from all EH patients (treated and untreated) and NC subjects (n = 106).

Figure 6. Correlation between serum insulin levels and MAP. Data refers to values from all EH patients (treated and untreated) and NC subjects (n = 106).

Serum insulin levels in relation to MAP

Fasting serum insulin levels, serving as a measure for insulin resistance, were positively correlated with MAP: \( r = 0.36, P = 0.001 \) (n = 110, Figure 6).
quintile of normal levels are highly predictive of an increased incidence of cardiovascular events in healthy males and females (Kuller et al., 1996; Koenig et al., 1999; Ridker et al., 2001). The low-graded inflammation derived from PMNL priming does not correlate with CRP. These findings imply that different processes are involved in inflammation, which need to be further clarified.

In the present study, lercanidipine treatment significantly lowered fasting serum insulin levels. EH patients have higher plasma insulin levels in response to glucose load, whether obese or of normal body weight (DeFronzo and Ferrannini 1991). This hyperinsulinemia is a consequence of resistance to the effects of insulin on peripheral glucose utilization and to decreased hepatic uptake of insulin (Reaven and Laws, 1994). Elevated [Ca\(^{2+}\)]\(_i\) has been described in various cells in insulin resistant states such as uremia, diabetes and EH (Ware et al., 1989; Draznin, 1993; Ohno et al., 1996). We have previously described in EH PMNLs a link between PMNL [Ca\(^{2+}\)]\(_i\), plasma insulin and EH, adding PMNLs to previously described cells exhibiting elevated [Ca\(^{2+}\)]\(_i\), contributing to OS and inflammation (Sela et al., 2002). Furthermore, the reported correlation of individual blood pressure with both PMNL [Ca\(^{2+}\)]\(_i\), and plasma insulin levels, together with the fact that elevated PMNL [Ca\(^{2+}\)]\(_i\); mediates PMNL priming, suggest that elevated PMNL [Ca\(^{2+}\)]\(_i\); and insulin are involved in the pathogenesis of hypertension-induced vascular injury in EH. The cause of slight increases in glucose value of normal upper limit after 2 months treatment, not exactly known, this could be related to high activity of the enzyme hormone sensitive lipase (HSL) due to low concentration of insulin (Claus et al., 2005), but follow-up after several months did not develop diabetes or pre-diabetes in any of the participant patients. We showed in this study a correlation between MAP and PMNL-related priming and inflammation parameters. A significant link between blood pressure and ROS formation by PMNLs has been observed by Yasunary et al. (2005).

In addition, they reported an inhibition of ROS formation by PMNLs by treatment with benidipine, a long acting CCB, which can be attributed in part to the decreased blood pressure. However, they did not completely rule out the possibility that the drug itself served as an antioxidative agent (Yasunari et al., 2005). In our study, lercanidipine was chosen for treating hypertension, as a long acting CCB, because it has high efficacy on mild and moderate hypertension, low incidence of adverse effects and good tolerability by most patients. Several studies demonstrated that lercanidipine shows anti-ischemic and anti-oxidative effects (Bellosta and Bernini, 2000; Bang et al., 2003; Tomlinson and Benzie, 2003; Farah and Shurtz-Swirski, 2008; Martinez et al., 2008) due to its ability to inhibit the growth of smooth muscle cell and their migration to the blood vessel wall, indicating a possible anti-atherosclerotic effects of the drug (Bellosta and Bernini, 2000; Wu et al., 2009), thus may be useful in the treatment of insulin resistant hypertensive patients.

In summary, lercanidipine, in addition to its effect as an antihypertensive drug, carries anti-inflammatory features improving most inflammation markers, systemic and PMNL-related, and can improve insulin sensitivity. The amelioration in the inflammatory parameters can be attributed in part to the decrease in blood pressure. In vitro future experiments are needed to find a direct effect of lercanidipine on PMNL-contributed low-grade inflammation.

**Limitation of the study**

The small sample and not randomized and UN blinded, because we had to select the appropriate patients with no smoking and no other chronic illness without any hypertensive treatment that may cause a change in inflammatory markers. It was not so simple to find patients who meet the criteria. Other limitation was the control group were those patients themselves at baseline. The examined drug has not been tested in vitro, and only small sample with positive results not yet published were present.

**ACKNOWLEDGEMENT**

This work was partially supported by Dexion Ltd.

**REFERENCES**


Full Length Research Paper

Intestinal parasitism in school children periodically treated with albendazole in 2 sampling periods

Quihui-Cota Luis* and Morales-Figueroa Gloria Guadalupe

Department of Public Health and Nutrition, Research Center for Food and Development, Carretera a La Victoria, Km 0.6 Hermosillo, Sonora C.P. 83304, Mexico.

Accepted 23 January, 2013

This transversal study estimated the prevalence of intestinal parasitic infections in school children twice yearly treated by the national campaign of albendazole during two consecutive years in Northwestern Mexico. 450 and 389 children showed prevalences of 46 and 35% for intestinal parasites, 42 and 30% for protozoa, and 11 and 12% for helminths in 2005 and 2006, respectively. Giardia duodenalis and Entamoeba histolytica/dispar/moshkovskii showed high and low prevalences, respectively. The prevalence of infection increased with age. 50 (September 2005) and 42 children (September 2006) excreted medians of 520 and 630 of eggs per gram (epg) of Hymenolepis nana, respectively. Albendazole alone is not sufficient approach to overcome intestinal parasitic infections in school children. Educational strategies should be integrated to the national deworming campaign in Northwest Mexico to obtain more effective results.

Key words: Intestinal parasitism, albendazole, de-worming campaign, school children, Northwest Mexico.

INTRODUCTION

Intestinal parasitism has been recognized as a public health problem worldwide for several years (Albonico et al., 1999; Crompton, 1999), because they are associated with malabsorption and growth disturbances (Brown et al., 1980; Solomons, 1993). Therefore, intervention programs were introduced for the control of parasitic helminth infections in different regions. They have significantly reduced the prevalence, intensity and morbidity of chronic infections in Seychelles, Zanzibar, and Sri Lanka, using mebendazole and albendazole (WHO, 1996). In Mexico, intestinal infections remain a serious public health problem, associated with high morbidity in the general population (SINAIS, 2005). In 1987, Mexican school children were considered the most vulnerable group to these infections, and 35.2 and 83.2 million Mexicans were affected by helminths and protozoa, respectively (Martuscelli, 1987). This motivated the launch of a Mexican deworming campaign in 1993 influenced by the effectiveness of global programs for control of helminths, the recommendation by the World Health Organization, the political will of the Mexican government and the infrastructure provided by the Health National Week (Velasco et al., 1993). The Ministry of Health determined that albendazole was provided to 95% of children (ages 6 to 14 years old) to reduce not only the prevalence and excretion of helminth eggs, but also re-infection rates and morbidity (Velasco et al., 1993). Evaluations between 1993 and 1998 in more than 90,000 Mexican children demonstrated the effectiveness of the program, reducing the national prevalence of Ascaris lumbricoides and Trichuris trichiura from 20 to 8% and 15 to 11%, respectively (Velasco et al., 1993). In 1995, the prevalence of Giardia duodenalis was estimated at 32%...
in Mexico (Tay et al., 1995) and remained the most important protozoan infection in Northwestern Mexico with prevalences ranging from 14 to 49% (Gomez et al., 1996; SS, 2006). *Entamoeba histolytica* is another pathogen protozoan capable of presenting a prevalence up to 50% in Southern Mexico (Morales-Espinoza et al., 2003) but appears to be less predominant than giardiasis in Northwestern Mexico (Gomez et al., 1996; SS, 2006). Currently, the Ministry of Health continues to administer a single dose of albendazole twice a year to school children in Mexico, but the intestinal parasites are possibly contributing to the high gastrointestinal infections rates in the childhood population of Northwestern Mexico (SS, 2006). Therefore, the aim of this study was to investigate the current prevalence of intestinal parasites in children who are receiving periodical albendazole in Northwestern Mexico.

**MATERIALS AND METHODS**

**Study area and population**

This cross-sectional study was conducted in two consecutive years (September 2005 and September 2006) in the State of Sonora, Northwest Mexico. Sonora is bordering to the east with the state of Chihuahua, south to the state of Sinaloa, west to the Gulf of California, and north to the US state of Arizona. Ninety-six percent of the region of Sonora is dry and semidry. The summer average temperature is 38°C (June to August) and 5 to 30°C from September to January. In 2005, the total population of Sonora was estimated in 662,000 and 60% of this population were under 15 years of age (INEGI, 2011). Ten public primary schools of 3 municipalities of Sonora [Guaymas, Hermosillo, and Navojoa] were selected based on high rates of gastrointestinal infections in the population (SS, 2006); low socioeconomic status in areas around the schools (Alvarez et al., 2009) and the administration of twice a year of a single dose (400 mg) of albendazole by the national de-worming campaign (Velasco et al., 1993). To date, no epidemiological surveillance to investigate the prevalence of intestinal parasitic infections has been conducted in the study sites. A total of 2152 children were enrolled in the primary schools selected between September 2005 to September 2006 (SEC, 2005). The purpose of this study was described to the personnel of health services, municipalities, schools, parents and students. All children were invited to participate while plastic containers were distributed for stool sample collection (three per subject). A total of 839 out of the 2152 children, participated in September 2005 and 2006, and they represented 39% of the enrolled population. Academic personnel confirmed the administration of albendazole during official visits of the de-worming campaign.

**Ethical consideration**

A written consent was obtained from parents or guardians of all participating children. From the remaining 1313 of the total 2152 children, and who did not take part in this study, 1159 children were unwilling to participate and 154 who did not meet the study criteria (disabled, supplemented or medicated). Approval to conduct this study was granted by the Ethical Review Committee of the Research Center for Food and Development. Children infected with intestinal parasites were referred to the Ministry of Public Health for the appropriate treatment.

**Collection of feces and parasite analysis**

Stool samples were collected and transported to the parasitology laboratory of the Research Center for Food and Development in Hermosillo. Samples were stored at 5 and 7°C for 24 to 72 h prior to analysis by the techniques of Faust and Kato-Katz (Markell et al., 1976). The technique of Faust was used for identification of protozoan cysts of *G. duodenalis*, *E. histolytica* / *dispar* and *moshkovskii* (Cheng et al., 2004), *Entamoeba coli*, *Endolimax nana* and *Iodamoeba butschlii* and helminth eggs of *A. lumbricoides*, *T. trichiura* and *Hymenolepis nana*. The intensity of infection was estimated indirectly by Kato-Katz, counting the number of eggs per gram of feces (epg) of helminth infections using the 40x objective, the final value was the average of epg divided per the sample number provided (3, 2 or 1) per child. The epg was calculated by multiplying twenty times the number of eggs counted in 50 mg of feces. Infection was defined as the state with one or more species of parasites, poliparasitism with two or more species of parasites, helminth infection only with species of helminth parasites, protozoa infections only species of protozoan parasites.

**Statistical analysis**

The prevalence of intestinal parasitism was expressed as the percentage of children with parasitic species in any of the fecal samples provided. The Fisher exact test was used to test the differences between proportions (prevalence of intestinal parasites). The intensity of infection was defined as epg of helminth species expressed as a median with confidence interval and Kruskal Wallis to test the difference between the age-intensity (epg) data. Data was analyzed using the Number Crunching Statistical System 2001, Version 1.6.0. (329 North 1000 East Kaysville, Utah 84037.com. USA).

**RESULTS**

A total of 450 and 389 school children participated voluntarily in September 2005 and 2006, respectively. The mean ages of the children were 7.7 (± 1.2) and 8.1 (± 1.3) in both sampling periods, respectively. 220 (49%) and 217 (56%) were girls in 2005 and 2006, respectively. No differences were found between the proportions of boys and girls ($\chi^2 = 2.342, df = 1, P = 0.7003$ in 2005; $\chi^2 = 3.476, df = 1, P = 0.6231$ in 2006). The overall prevalence of intestinal parasites in boys and girls was 48 versus 44% ($P = 0.708$) in 2005; and 29 versus 35% ($P = 0.357$) in 2006. In addition, no difference was found between the prevalence of parasites species by gender (data not shown). A total of 1231 and 861 fecal samples were collected in September 2005 and 2006, respectively. 62 and 26% of the children provided 3 and 2 stool samples in September 2005, and 47 and 26% of the children provided 3 and 2 samples in September 2006. High prevalence for intestinal parasitic infections and protozoan infections were found in 2005 and 2006, respectively (Table 1). *H. nana* and *G. duodenalis* also...
a low prevalence in both periods. Non-pathogenic parasites such as $E$. nana, $E$. coli and I. butschlii were also detected.

**Prevalence of intestinal parasitism with age**

The prevalence of intestinal parasites showed an increased trend with age (groups 6 - 7.9 vs. 8 - 9.9, $P = 0.6060$; groups 6 - 7.9 vs. 10 - 11.9, $P = 0.5103$ in 2005; groups 6 - 7.9 vs. 8 to 9.9, $P = 1.000$; groups 6 - 7.9 vs. 10 - 11.9, $P = 0.6262$ in 2006) (Fisher exact test) (Table 2).

**Intensity of $H$. nana by age**

During the study, 50 of 450 (September 2005) and 42 of 389 children (September 2006) excreted a median of 520 and 630 epg of $H$. nana, respectively. From these children, 16 (32%) and 16 (32%) children in 2005, and 20 (48%) and 8 (19%) children in 2006 showed intensities ≤ 100 and ≥ 1000 epg, respectively. The intensity of infection with $H$. nana showed an increased tendency with age in both sampling periods (Table 3).

**DISCUSSION**

Almost half of our study children were suffering from intestinal parasites. Earlier epidemiological records by the Ministry of Health and published information by research studies recognized $G$. duodenalis as the predominant pathogenic protozoan (SS, 2006) and $H$. nana as the persistent helminth species causing infections in the school children of Northwest Mexico. Our study confirms that $G$. duodenalis is the predominant protozoan species and revealed that $H$. nana is the only helminth detected in the study sites. Our results have shown that prevalence...
of intestinal parasites remains unchanged in albendazole treated school children of the study sites. Before the national campaign was established in 1993, prevalences of 58.9% in 1957 and 19.5% in 1968 for ascariasis (Tay et al., 1976) and prevalences of 39.7% in 1957 and 19.5% in 1978 for trichuriasis were observed in some Mexican regions (Bayona et al., 1968; del Villar Ponce et al., 1978). Similarly, the prevalence of giardiasis and amebiasis showed prevalences ranging from 14 to 16% and from 12 to 21%, respectively from 1982 to 1984 in children under 15 years of age (Alonso Guerrero, 1983; Duarte-Zapata et al., 1984; Salazar Schettino et al., 1981). After 1993 (Guevara et al., 2003; Gutierrez-Rodriguez et al., 2007; Martínez et al., 1998; Rodríguez et al., 1997), the prevalences of T. trichiura, A. lumbricoides, H. nana, G. duodenalis and E. histolytica were peaking around 16, 8, 15, 24 and 60%, respectively in the general population. A substantial reduction of trichuriasis and ascariasis, but persistent giardiasis, hemoenoleopiasis and amebiasis were found in Southern Mexico. Probably this pattern is associated with the deworming campaign and this may explain the absence of ascariasis and trichuriasis in our study. Furthermore, no differences were found in the prevalence of intestinal infections between our girls and boys. Studies in Mexico and other Latin American countries have also found similar findings. Probably the children in our study are developing the same transmission risk activities related to poor hygiene (Sánchez de la Barquera et al., 2010). On the other hand, the prevalence of intestinal parasites showed an increase with age in our study. Khosrow et al. (2011) published a similar finding in 405 Iranian school children with ages of 6 to 10 years without identifying the causative associated factors. This probably reflects the major attention from parents to young children neglecting the fact that the older children are infected more easily. It is also probable that, the higher the prevalence of H. nana, the greater the intensity of H. nana in this study. In spite of the deworming campaign, the persistence of intestinal parasites may be a reflection of poor hygiene practices of our participating children’s families and the limited basic services in the study areas where they are living. This study was not designed to evaluate the effectiveness of the deworming campaign, due to inappropriate sample size and lack of a methodological strategy of sampling. We recognized that the Mexican campaign is aimed primarily at the soil-transmitted helminthiasis, and albendazole is the drug of choice. However, it is evident that our study site’s population is at high risk of acquiring giardiasis and hemoenoleopiasis. The school children in this study had received 400 mg of albendazole in April 2005 and 2006 and our findings have suggested that health education strategies should be integrated into the deworming campaign, since albendazole alone will not improve health conditions of our study children. In addition, this is the first study conducted to investigate the prevalence of intestinal parasitism in the study sites where a deworming campaign is implemented. Our results encouraged the design of a study to assess the effectiveness of the campaign in the study sites and to identify the causative factors responsible of the persistent prevalence of parasitic infections in our study children.

ACKNOWLEDGEMENTS

Authors thank the support provided by the Q.B. Carmen Maria Lugo Flores in preparing the manuscript. This work was based on the knowledge and contribution of my Professors, DWT Crompton and Stephen Phillips. We also thank the primary schools, academic staff and the participating school children. The authors thank the National Council of Science and Technology (Funds SON-2004-C01-005 MIXTOS), CONACYT, the Ministry of Health of the State of Sonora, and the Research Center for Food and Development for providing financial support for this study.

REFERENCES

Parasitol. 10(1):112-117.
macronutrients from a rice-vegetable diet before and after treatment 
Crompton DW (1999). How much human helminthiasis is there in 
characterization of peroxiredoxin from Entamoeba moshkovskii and a 
Incidence of intestinal parasitosis in children treated at the Clinical 
Hospital No. 68 of IMSS, Tultepec, State of Mexico. Salud Publica 
Duarte-Zapata L, Escalante-Triay F, Lopez-Novelo de Ceballos M 
(1984). Prevalence of intestinal parasitosis in the middle-class 
Gomez RN, Mada VJ, Durazo GN, Matty OM, Vazquez PE, Robles MG 
(1996). Helminthiasis en los Niños, Informe de 543 Casos. Hospital 
Infantil del Estado de Sonora. 13:30-34.
Guevara Y, De-Haro I, Cabrera M, Garcia G, Salazar-Schettino PM 
(2003). Enteroparasitoses in Indigenous and Mestizo individuals 
from the Nayarit Mountain Range, Mexico. Parasitol Latinoam. 58:30-34.
Gutierrez-Rodriguez C, Trujillo-Hernandez B, Martinez-Contreras A, 
Pineda-Lucatero A, Millan-Guerrero RO (2007). Frequency of 
intestinal helminthiasis and its association with iron deficiency and 
143(4):297-300.
Estadistica y Geografia.
intestinal parasitic infections among primary school attending 
students in Barandooz-Chay rural region of Urmia, West Azerbaijan 
Philadelphia.
Enterobiosis en niños de la delegación Iztapalapa, Distrito Federal, 
Martuscelli QA (1987). Frecuencia de helmintiosis en niños de la 
Moraes-Espinoza EM, Sanchez-Perez HJ, Garcia-Gil Mdel M, Vargas- 
parasites in children, in highly deprived areas in the border region of 
Rodriguez GR, Sánchez-Maldonado MI (1997). Frecuencia de 
parasitosis en niños de Minaltiñán, Veracruz. Rev. Fac. Med. UNAM. 
40:170-171.
Salazar Schettino PM, Garcia Yanez Y, Ruiz Hernandez AL, Alonso 
Guerrero T, Quintero Garcia ME, de Auajare Cinta SV, Rodriguez 
Ramos MG (1981). Incidence of intestinal parasitoses in populations 
23(2):179-182.
Sánchez de la Barquera MI, Miramontes-Zapata M (2010). Parasitosis 
intestinales en 14 comunidades rurales del altiplano de México. Rev. 
SEC (2005). Información de niños escolares inscritos durante el ciclo 
05-06 en las escuelas públicas primarias en el Estado de Sonora. 
Sonora, Mexico: Secretaria de Educación y Cultura.
http://www.sinais.salud.gob.mx/basesdedatos/index.html 
Solomons NW (1993). Pathways to the impairment of human nutritional 
enteroparasitoses en el Estado de Sonora. Departamento de Estadística 
y Evaluación, Dirección de Planeación y Desarrollo. Gobierno del 
Estado de Sonora. México.
Tay J, Ruiz A, Sanchez Vega JT, Romero-Cabello R, Robert L, Becerril 
Parasitol. 50(1-2):10-16.
Tay J, Salazar-Schettino PM, de Haro Arteaga I, Bucio Torres MI 
helmintiasis intestinales en México (No. 24). Secretaria de Salud. 
Mexico, D. F.
WHO (1996). Informal consultation on the use of chemotherapy for the 
control of morbidity due to soil-transmitted nematodes in humans. 
Full Length Research Paper

Giant aortic arch thrombus, methylenetetrahydrofolate reductase (MTHFR) A1298C heterozygous gene mutation, smoking and hormonal replacement therapy

Abdallah K. Alameddine1*, Jonathan Freeman2, John Rousou1, Yvonne Alameddine1, Joseph E. Flack1 and Victor K. Alimov1

Divisions of Cardiac Surgery, Baystate Medical Center, Springfield, MA and Tufts School of Medicine, Boston MA. Divisions of Pathology, Baystate Medical Center, Springfield, MA and Tufts School of Medicine, Boston MA.

Accepted 19 December, 2012

We report the case of a mobile aortic arch thrombus possibly induced by the combination of postmenopausal hormonal replacement therapy (HRT) and cigarette smoking in a woman with methylenetetrahydrofolate reductase (MTHFR) A1298C mutation. No other cause for her illness could be identified despite an extensive laboratory work-up for thrombophilic state. Surgical exploration showed the floating aortic arch thrombus attached on a histologically normal aortic wall. At an 8-year follow-up, she remained free of recurrence after discontinuation of HRT and counseling to quit smoking. The probable synergistic impact of tobacco smoking as an additional risk factor for thrombophilic events in women with MTHFR variant and using HRT has yet to be determined. Previous studies and case reports focusing on MTHFR variation and the incidence of thrombotic events have provided conflicting evidence of an association. With the understanding that this case does not yet ascribe cause-and-effect relationship between MTHFR variant and clot formation, important public health concerns are raised. The prevalence of MTHFR A1298C genotype is population-specific, implying that permissive gene-environment interactions other than genetic mutation alone may also be relevant in establishing a clinically overt disease. Causality remains to be proven in prospective evaluation across diverse geographic areas taking into account interactions with dietary and other life-style risk factors. Furthermore, in such genetically predisposed patients, future genome-wide association studies to identify loci variants that determine the overall susceptibility to thrombosis may prove helpful to derive preventive interventions.

Key words: Aortic arch thrombus, hormone replacement therapy, methylenetetrahydrofolate reductase (MTHFR) A1298C gene mutation, smoking.

INTRODUCTION

Thrombus formation in aortic arch is a devastating condition. Herein, we present such a case that was associated with methylenetetrahydrofolate reductase (MTHFR) A1298C genetic variant and normal homocysteine level. Does heterozygous MTHFR A1298C allelic gene mutation without hyperhomocystenemia increase arterial thrombophilia? The answer to this question remains a subject of debate. The etiology of increased tendency to clotting is thought to be a multigene disorder (Seligsohn and Zivelin, 1997; Ehrenforth et al., 2004; Sacher, 1999), making this genotype variant as a cause of thrombus formation difficult to ascertain. Furthermore, such genetic variation is ethnic and population specific. For example, the overall prevalence of this allelic variant in population-based
The most common missense mutation identified in the MTHFR gene is the C to T substitution (C677T). Deficiency in this gene, an autosomal recessive disorder, leads to a reduced enzymatic function with a mild hyperhomocysteinemia and coronary artery diseases. Hyperhomocysteinemia has an important role in inducing hypercoagulability state on the venous system in the general population (Den Heijer et al., 1996). However, arterial clot formation in the less common heterozygous allelic mutation MTHFR A1298C has thus far not been reported. In this report, the role of the gene-environmental interaction for vascular damage in case of MTHFR mutation is also highlighted.

**METHOD**

**Case presentation**

A 51-year-old female presented with multiple recent bilateral cerebellar infarcts and found to have heterozygous methylenetetrahydrofolate reductase MTHFR A1298C. For three months prior to admission, she had been on oral daily postmenopausal HRT for symptoms control. Each tablet contains norethindrone acetate, ethinyl estradiol (1 mg/ 5 mcg). There was no personal or family history of venous or arterial thrombotic disease or coronary artery disease, hypertension, diabetes mellitus or hyperlipidemia, and she has never been on oral contraceptives.

The patient had no history of weight loss, trauma, and no clinical manifestations of inflammatory bowel disease, malignancy, cutaneous ulcers or nodules, tuberculosis, syphilis or vasculitis. She had 13 pack-years history of cigarette smoking and did not drink alcohol or use illicit drugs.

Transsthoracic and transesophageal echocardiography demonstrated a large soft mobile echogenic mass with an irregular shape visualized in the aortic arch with absence of intramural hematoma (Figure 1). The heart rhythm, ventricular function and the cardiac valves were normal and no intracadiac source of emboli was identified. With the risk of embolization, and absence of angina symptoms, coronary catheterization was thought to be unwise and therefore was not performed. Computed tomography scans of the head, abdomen and pelvis did not reveal evidence of malignancy. The carotid ultrasound was unrevealing. Results of laboratory evaluation showed normal platelets count and no abnormalities of coagulation factor II, or V, and normal levels of protein C, protein S, and antithrombin III; tests for complement, anticiardiolipin antibodies and lupus anticoagulant also yielded negative results. The erythrocyte sedimentation rate and the thyrothropin level were normal. Subsequent screening for thrombophilic state showed heterozygous mutation in gene encoding for 5, 10-methylenetetra-
hydrofolate reductase (MTHFR) A1298C without hyperhomocysteinemia.

Thrombolysis of the clot could not be undertaken because of risk of partial lysis or dislodgment of the thrombus, therefore urgent surgical intervention was indicated to prevent fatal thromboembolic events. Exploration via a mid sternotomy revealed a friable thrombotic mass measuring 3.5 × 4.0 cm, localized in the aortic arch and prolapsing outward into the distal aorta beyond the innominate artery (Figure 2).

Excision of a small button of the aortic wall surrounding the thrombus and local patch graft repair were performed. Histopathologic examination revealed a thrombus attached to a normal aortic wall with the absence of protruding atherosclerotic plaque ulcerations or malignancy at the site of insertion of the thrombus (Figure 3).

RESULTS

The patient was discharged home 6 days postoperatively after an uneventful recovery on a regimen of aspirin and folate after discontinuation of HRT and advised smoking cessation. The role of long-term anticoagulant therapy in the treatment of idiopathic arterial thrombosis is controversial, but antiplatelet agents have been shown to be effective in the prevention and treatment of arterial thrombosis (Guidelines for the Primary Prevention of Stroke, 2011; Baigent et al., 2002). The patient continues to do well at 8 years of follow-up and she reported no further episodes of cerebral infarcts. After that last visit to her internist, the patient moved out of state and was lost to follow-up.

DISCUSSION

The mechanism underlying aortic thrombus formation is complex and likely multifactorial. Aortic atheroma (plaque thickness ≥ 4 mm, ulcerated or with mobile component, is an important non-cardiac source of peripheral or cerebral emboli (Aldons, 2000). There is a lack of data showing direct association between MTHFR A1298C and arterial thrombus formation. Although heterozygous mutation in the gene encoding MTHFR have been identified in this patient, it remains uncertain whether this genetic polymorphism without hyperhomocysteinemia can cause thromboembolic events in the arterial system (Spiroski et al., 2008; Spiroski et al., 2008; Trabetti, 2008; Schwahn and Rozen, 2001; Contractor et al., 2011; Domagala et al., 2002; Kim and Becker, 2003).
On microscopical examination (hematoxylin and eosin) the aortic wall has no underlying atheromatous plaque. The aortic luminal thrombus (A) was focally adherent to the aortic wall (B). Magnification of the aortic wall (inset C) showed minimal mucinous degeneration without atherosclerotic plaque or other abnormality.

Since this patient had no other cause for her clotting disorder that could be ascertained, a synergistic effect of smoking, estrogen intake, along with her genetic profile may have contributed to her arterial thrombotic event through a loss of endothelial protection, enhanced activity of thromboxane A₂ and initial platelet activation (Leone, 2007; Pretorius et al., 2010; Khullar and Maa, 2012; Herrington and Howard, 2003; Petitti, 2012).

Role of HRT and smoking in arterial thrombosis

There are indirect estimates of postmenopausal women smokers on HRT in the US general population. According to data drawn from national information sources (Third National Health and Nutrition Examination Survey, conducted in the US between 1988 and 1994), an estimated 37% of postmenopausal women took HRT pills for 1 to 5 years (Women-Health Facts, 2012).

Interestingly, the prevalence of smoking in the US has decreased; however, an estimated 17.4% women continued to smoke in 2007 (Women-Health Facts, 2012). With such a large number of women smokers using HRT, a minute increase in prothrombogenic states brought about by other frequent risk factors for thrombophilia such as overweight or obesity, inflammation, malnutrition, malignancy and factor V Leiden, will affect many (Nelson et al., 2012; Cushman et al., 2004; Miller et al., 2002). Multiple studies have shown a moderate increased risk for arterial thrombosis (stroke/myocardial infarction) due to HRT intake (Slooter et al., 2005; Lidegaard et al., 2012; Hannaford, 2000). Together, the data suggest that HRT increases the risk of thrombophilia. This conclusion is congruent with the recommendation by the Agency for Healthcare Research and Quality (US) which does not recommend long-term use of HRT for the same reason (Nelson et al., 2012; Miller et al., 2002; Rossouw et al., 2002).

The MTHFR mutation affects genomic methylation through an interaction with folate (Friso et al., 2002). Consequently, it interacts with multiple other factors. These factors include the genetic make-up of individual patients, geographic regions, ethnicity, associated prothrombotic or inflammatory states, dietary habits, and multiple lifestyle factors and nutritional supplementation (Zheng et al., 2000; Zhao et al., 2011; Gürsoy et al., 2011). Lidegaard et al. (2012) have reported an increased risk by a factor of 1.5 to 2 among users of oral estrogen-progestin. This risk in arterial thrombotic events could be minimized by abstinence from smoking (Hannaford, 2000).

Is MTHR polymorphism without hypercystenemia associated with idiopathic thrombosis?

In the setting of a population-specific but prevalent MTHFR A1298C polymorphism, the effect of combining smoking and HRT raises important public health concerns in the generation of venous as well as arterial thrombi. As mentioned earlier, this conclusion is difficult to prove, mainly because of unforeseen confounding factors that influence final phenotype or so called “phenotype modifiers” (Girirajan et al., 2012; Girirajan and Eichler, 2010; Dipple and McCabe, 2000). In our case, homocysteine level was normal. However, low dietary intakes of folate and riboflavin, vitamins B₁₂/B₆, all have been implicated to playing a role in plasma level of
homocysteine (Domagala et al., 2002; Kanth, 2011; Dawson and Waters, 1994).

Impact and prevalence of MTHFR genotype on thrombotic diathesis

Our patient was white of European heritage, and after discontinuation of both smoking and HRT intake, there were no additional reported neurological vascular events up to 8 years after discharge.

Considered in isolation, the risk of increased clotting in MTHFR mutation is still equivocal (Schwahn and Rozen, 2001; Gürsoy et al., 2011; Dölek et al., 2007). Noteworthy, based on data derived from case/control reports, genetic susceptibility to thrombosis and the prevalence of MTHFR may vary in different ethnic populations worldwide (Hannaford, 2000; Dipple and McCabe, 2000; Dawson and Waters, 1994; Gürsoy et al., 2011). A Macedonian case-control study suggested that the prevalence of C677T and A1298C genotypes are connected with increased homocysteinemia level among patients with deep venous thrombosis (DVT) (Domagala et al., 2002). In that study, Domagala et al. (2002) observed a 15% incidence of MTHFR variant in healthy Polish cohort.

According to recent literature, A1298C mutation was equally distributed in the Turkish patient group with DVT compared with the control group (Dölek et al., 2007). Similarly, the report by Solomon et al. (2001), as well as the report by Zetterberg et al. (2002) found no increase in DVT or vascular disease. A 2003 metaanalysis review and a recent study comparing patients with venous thromboembolism and healthy subjects also failed to demonstrate such a link (Domagala et al., 2002; Kim and Becker, 2003). Therefore on the basis of this case and other data, MTHFR A1298C polymorphism alone may not be sufficient to confer clinically overt thrombophilia. There must exist other permissive environmental factors leading to increase clotting propensity. Exposure to smoking and hormone replacement therapy each elicits integrated risk of increased thromboembolic events in parallel to genetically-controlled hypercoagulable response by such factor as the enzyme MTHFR. Moreover, other lifestyle factors and nutrients in the diet have been shown to interact with that enzyme. For example the findings by Huang et al. (2011) in a cross-sectional study further support that notion. In that study, of Puerto-Rican men and women residing in the Boston metropolitan area, subjects with MTHFR A→C displayed significant interactions with alcohol intake, smoking and physical activity in determining plasma homocysteine level. There have also been reports confirming interactions between genetic MTHFR variant and the risk for esophageal cancer in former moderate and heavy drinkers or smokers in the Chinese population (Zhao et al., 2011).

Similarly, recent Medline search to identify association between MTHFR mutation and arterial circulatory events has suggested that the individual propensity for those events is due to other systemic mechanisms (Kim and Becker, 2003).

Finally and consistent with this view, association between MTHFR mutation and cerebral infarction and DVT has also been reported in the Chinese population by Zheng et al. (2000). In these cohorts of patients, the prevalence of the 677 C→T allele in normal control subjects was 30.7%, similar to that in Caucasians and Japanese.

Taken together, these data show that the prevalence of MTHFR varies among different populations and that in addition to the traditional risk factors (such as tobacco use, hypertension, dyslipidemia, HRT intake, diet and sedentary life-style) complex strong gene-gene and gene-environmental interactions form significant effects on the incidence of overt thrombotic events (Schwahn and Rozen, 2001).

What does this case inform us?

The patient’s findings imply the need in maintaining a wide-ranging view of carefully assessing the genetic causes of arterial thrombotic events especially when the reason is not identified in women who are smokers and on HRT. This case also suggests that gene-environment interactions in genetic disorder confer rather very different clinical manifestations among populations that may be relevant in this and other genetic disorders.

Conclusions

The data supporting the relationship between MTHFR mutation and inclination to thrombosis are conflicting. Available clinical and epidemiologic evidence show that there is broad ethnic and regional variability in the clinical response to MTHFR polymorphism. If a link exists between smoking and HRT that predisposes female patients to arterial thrombosis in the setting of this allelic gene mutation that will certainly raise important matter in clinical practice and in public health. Specifically, is it necessary to endorse genetic testing for such mutation in thromboembolic events for risk stratification and therapeutic decisions? Perhaps, the answer to this question remains a matter of individual judgment. There may be specific subgroups of women with certain predisposition to traditional thrombotic risks who are more likely to benefit from a genetic testing for MTHFR before prescribing HRT, even if the use of HRT is intended for the short-term. Future studies of genome-wide sequencing and the interactions with environmental elements-in different geographic areas-may clarify the susceptibility to thrombophilia and may yield results that foster causal relationship. Special attention should be
given to higher thrombotic risk groups to intervene and modify risk factors.

REFERENCES


Women-Health Facts: both sources were accessed August 11, 2012 @ cdc.gov/nchs/nhanes and statehealthfacts.org/women-health.


Importance of diet on disease prevention

Francesco Sofi\textsuperscript{1,2,3}, Rosanna Abbate\textsuperscript{1}, Gian Franco Gensini\textsuperscript{1,3} and Alessandro Casini\textsuperscript{1,2}

\textsuperscript{1}Department of Clinical and Experimental Medicine, University of Florence, Italy
\textsuperscript{2}Agency of Nutrition, Azienda Ospedaliero-Universitaria Careggi, Florence, Italy
\textsuperscript{3}Don Carlo Gnocchi Foundation Italy, Onlus IRCCS, Florence, Italy.

Accepted 21 November, 2012

Over the last decades, a considerable body of evidence supported the hypothesis that diet and dietary factors play a relevant role in the occurrence of diseases. To date, all the major scientific associations as well as the World Health Organization, scientific and non-scientific organizations place an ever-increasing emphasis on the role of diet in the strategies able to prevent non-communicable diseases. Many studies have evaluated the associations between food groups, foods, or nutrients and chronic diseases, and a consensus about the role of nutritional factors in the etiology of non-communicable diseases such as cardiovascular and neoplastic diseases has gradually emerged. Indeed, data from analytical and experimental studies indicated a relation between increased consumption of some food categories such as fruits and vegetables, fiber and whole grains, fish and moderate consumption of alcohol and reduced risk of major chronic degenerative diseases, whereas increased total caloric intake, body weight, meat and fats are associated with greater risk. However, the appropriate dietary strategy to prevent chronic degenerative diseases remains a challenging and a highly relevant issue. Recently, Mediterranean diet has been extensively reported to be associated with a favorable health outcome and a better quality of life.

Key words: Diet, nutrition, diseases, health.

INTRODUCTION

During the past decades, a rapid expansion in the number of relevant scientific fields, and in particular, the amount of population-based epidemiological evidence has clearly demonstrated the role of diet in preventing and controlling morbidity and premature mortality resulting from non-communicable diseases (NCDs) (World Health Organization Study Group, 2003). The burden of NCDs is rapidly increasing worldwide. It has been calculated that, in 2001, chronic diseases contributed approximately 60\% of the 56.5 million total reported deaths in the world and approximately 46\% of the global burden of disease. Moreover, the proportion of the burden of NCDs is expected to increase to 57\% by 2020 (World Health Organization, 2005). Almost half of the total chronic disease deaths are attributable to cardiovascular diseases; obesity and diabetes are showing worrying trends, whereas neoplastic diseases are still one of the commonest causes of mortality and morbidity in Western countries, as well as neurodegenerative diseases which showed in the last years an increasing trend of incidence. Moreover, the chronic disease problem is far from being limited to the developed regions of the world.

Contrary to widely held beliefs, developing countries are increasingly suffering from high levels of public health problems related to chronic diseases (World Health Organization, 2005). The World Health Organization (WHO) in its recent documents places a great emphasis on the prevention of NCDs (World Health Organization Study Group, 2003; World Health Organization, 2005, 2006). The most important risk factors for NCDs include
high blood pressure, high concentrations of cholesterol in the blood, inadequate intake of fruit and vegetables, overweight or obesity, stress, physical inactivity and tobacco use. Five of these risk factors are closely related to diet. Indeed, unhealthy diet is among the leading causes of NCDs, including cardiovascular diseases, type 2 diabetes and certain types of cancer, and contribute substantially to the global burden of disease, death and disability.

Currently, the relationship between diet and diseases has been studied intensively for nearly a century. The first evidence of a possible relationship between dietary habits and occurrence of diseases dates back to the years following the World War II, when significant variations in the incidence of major NCDs such as cardiovascular diseases and certain cancers were observed in studies conducted in migrants that moved from countries with a favourable dietary profile to a country with an unfavourable and industrialized profile. Subsequently, many studies investigated the influence of diet and nutrition on the pathogenesis of the disease states through using analytical, ecologic and epidemiological approaches (Sofi et al., 2008).

To date, diet and nutrition are important factors in the promotion and maintenance of good health throughout the entire life course, and their role as determinants of chronic degenerative diseases is well established, thus occupying a prominent position in prevention activities.

**DIET AND CARDIOVASCULAR DISEASES**

Cardiovascular diseases are the first cause of mortality and morbidity in Western countries (World Health Organization, 2006). During the last decades, clinical investigation on the prevention of cardiovascular diseases has defined in an unquestionable manner, the role of diet as a modifiable risk factor. Currently, it has been largely demonstrated from epidemiologic studies that increased consumption of fruits, vegetables, non-refined cereals, and fish can reduce cardiac events and related mortality in the whole population (World Health Organization, 2005). The recent result from the “InterHeart” study, a large case-control study that investigated risk factors for myocardial infarction within 52 countries including non-developed, developing and industrialized countries, demonstrated that diet is one of the most important risk factors for the occurrence of myocardial infarction, independently from all the other parameters. In fact, consumption of fruit and vegetables has been reported to be responsible for a significant and relevant protection against the occurrence of myocardial infarction in all the countries (Yusuf et al., 2004). Furthermore, the significant interrelationships between some of the most important risk factors such as diabetes, hypertension, and dyslipidemia and dietary habits gave further evidence towards the role of nutrition in preventing cardiovascular diseases.

The preliminary scientific evidence about the role of nutrition in the pathogenesis of cardiovascular diseases has been supplied by the “Seven Countries’ Study”, an epidemiologic study designed by Ancel Keys, the pioneer of nutritional studies, at the beginning of the 1950s (Keys et al., 1986). This study enrolled nearly 13,000 male subjects of age ranging from 40 to 59 years, living in 7 different countries (Italy, Greece, the Netherlands, United States, Finland, Japan, former Yugoslavia), with the aim of evaluating the possible association between diet and lifestyle habits and mortality and incidence of cardiovascular and neoplastic diseases. Since the first results of the study, it became evident that there was a significant difference in terms of incidence of diseases, as well as of mortality among the cohorts of the study. At the end of the 25 years follow-up, about one half of these death cases were due to a coronary disease with mortality rates remarkably differing in the various study countries (Menotti et al., 1993).

In particular, a lower mortality rate for coronary heart disease was recorded in Greece and in the South of Italy, with 25 death cases every 1,000 inhabitants in a 25-year period, whereas the highest mortality rate was recorded in Finland with 268 death cases every 1,000 inhabitants in a 25-year period. The low rate of cardiovascular diseases in the Mediterranean regions of Europe stimulated an increasing interest for the potential role of their traditional diet in the protection from these diseases.

From that time onward, several studies have been conducted in different study populations with the aim of identifying the real relationship between nutrients, foods, food groups and diseases, by showing that a dietary profile typical of the Mediterranean regions is associated with a reduced incidence of NCDs, as well as with a reduced rate of mortality and morbidity (Sofi et al., 2008). In the Mediterranean diet, olive oil rich in monounsaturated fatty acids is the prevalent visible fat, the intake of saturated fat is relatively low, while fish guarantees a substantial provision of polyunsaturated fats (n-3 polyunsaturated fatty acids). The Mediterranean diet is, in fact, characterized by a high amount of vegetables, fruits and whole grain products, which represent a good source of fiber, complex carbohydrates, proteins, potassium, antioxidant substances, and vitamins. Finally, the moderate consumption of red wine associated with the food is prevalent with respect to other types of alcoholic beverages.

The association between these nutrients and foods and the occurrence of cardiovascular diseases has been largely demonstrated in the last decades (World Health Organization, 2005; Sofi et al., 2008). However, the failure of several recent clinical trials supplementing single
nutrients, suggested that the global Mediterranean nutrition pattern, rather than specific nutrients, might have protective effects on cardiovascular diseases. This is in agreement with some intervention studies, main ones being the Lyon Diet Heart Study and the Dietary Approaches to Stop Hypertension trial, which indicated that interventions to change dietary patterns into a Mediterranean-like pattern could be highly effective in reducing cardiovascular risk (de Lorgeril et al., 1999; Sacks et al., 2001).

The Lyon Heart Study conducted among those with existing heart disease, found a Mediterranean-type diet high in omega-3 fatty acids reduced recurrent infarction by 70%, compared with an American Heart Association diet (de Lorgeril et al., 1999). More recently, an intervention study led by Shai et al. (2008) and published in the New England Journal of Medicine, reported a benefit for Mediterranean diet on reducing cardiovascular risk profile of a population of obese. The authors considered a comparison of three diet regimens with regard to the body weight of more than 200 obese subjects: a typical low-calorie diet low in fat, a Mediterranean-type diet, and a low-calorie and low-carbohydrate diet without caloric restriction. After approximately two years of follow-up, the low-carbohydrate diets were more effective in obtaining weight loss in the short-term, but the long-term benefits obtained in addition to the weight loss, which included improvement of the metabolic parameters were obtained in the subgroup of people following the Mediterranean diet (Shai et al., 2008).

However, the intervention diets in those trials were very different from common dietary patterns in Western populations. People choose foods and combinations of foods rather than isolated nutrients, and practical dietary advice to the public in terms of foods is preferred. Dietary changes may be more readily achieved if recommended foods are compatible with existing patterns of food consumption. Until recently, research efforts to identify dietary means of reducing disease risk have focused on single-nutrient interventions to affect responses in single medical conditions. Determining appropriate dietary recommendations for improved health is further complicated by the paucity of information of the clinical value and feasibility of the interactive effects of multiple nutrients consumed in combination. Recognizing that nutrients are not ingested in isolation, but rather as interactive constituents of a complete diet, much of the focus in nutrition and cardiovascular research in recent years has shifted from assessment of single-nutrient effects on medical conditions associated with increased risk to that of the effects of the total diet or dietary pattern. Therefore, research efforts in this field switched progressively to the evaluation of a score for the adherence to the Mediterranean dietary pattern, rather than to the identification of single nutrients in association with the disease.

The most important attempt to define the degree of adherence to the Mediterranean diet has been released by Trichopoulou et al. (2003) on the frame of the European Prospective Investigation into Cancer and Nutrition (EPIC) study. The authors established a score of adherence that takes into account the main dietary variables, divided into food groups, typical of the Mediterranean diet. This adherence score, based on food groups typically present in the Mediterranean diet (bread, pasta, fruit, vegetables, fish, legumes, moderate red wine consumption, and olive oil), gives a positive score to people who consume more than the median of the overall population for foods typical of the Mediterranean diet, and a negative score to those who consume a higher amount of foods which are not typical of the Mediterranean diet. Hence, a score of 0 represents the lowest adherence to the Mediterranean diet, while a score of 9 represents the highest adherence to the Mediterranean diet.

In recent meta-analyses, we have demonstrated that a greater adherence to the Mediterranean diet, estimated through a computational score, was associated with a reduced incidence of overall mortality (-8%), as well as of cardiovascular mortality and/or incidence (-10%) (Sofi et al., 2010, 2008).

DIET AND NEOPLASTIC DISEASES

Cancer is a major cause of mortality throughout the world, and in the developed world, it is generally exceeded only by cardiovascular diseases (World Health Organization Study Group, 2003; World Health Organization, 2005; World Health Organization, 2006). An estimated 10 million new cases and over 6 million deaths from cancer occurred in 2000. As developing countries become urbanized, patterns of cancer, including those most strongly associated with diet, tend to shift towards those of economically developed countries. Between 2000 and 2020, the total number of cases of cancer in the developing world is predicted to increase by 73%, and in the developed world, to increase by 29%, largely as a result of an increase in the number of old people.

Dietary factors are estimated to account for approximately 30% of cancers in industrialized countries, making diet second only to tobacco as a theoretically preventable cause of cancer (Key et al., 2004). This proportion is thought to be about 20% in developing countries, but may grow with dietary change. Many of the prominent hypotheses for effects of diet on cancer risk are derived from examination of the associations between dietary patterns and cancer rates in different populations around the world. It was noted in the 1970s that developed Western countries have diets high in
animal products, fat and sugar, and high rates of cancers of the colorectum, breast and prostate developing countries typically have diets based on one or two starchy staple foods, low intakes of animal products, fat and sugar, low rates of these 'Western' cancers, and sometimes high rates of other types of cancer such as cancers of the esophagus, stomach and liver. Other studies have shown that cancer rates often change in populations that migrate from one country to another, and change over time within countries.

During the last 30 years, hundreds of studies that examined the association between diets of individuals and their risk for developing cancer have been published. Some studies have investigated the possible role of Mediterranean diet and the occurrence of neoplastic diseases showing a beneficial effect of such dietary pattern in the general population. The results of recent meta-analyses published by our group clearly showed that a strict adherence to the rules of the classical Mediterranean diet determines a 6% reduced risk of incidence and/or mortality from neoplastic diseases (Sofi et al., 2008, 2010).

**DIET AND NEURODEGENERATIVE DISEASES**

An interest association between diet and disease states is the one related to the reduced risk of incidence of neurodegenerative diseases such as Alzheimer’s and Parkinson’s disease that has been observed in some recent studies (Sofi et al., 2008). Indeed, several observations hypothesised a potentially important role for diet in the prevention and occurrence of Alzheimer’s disease.

The links proposed between dietary factors and neurocognitive diseases are different. Neurodegenerative diseases are characterized in their prevalent forms, by an increased oxidative stress and inflammation (Rinaldi et al., 2003). To date, oxidative stress and inflammation can be modulated and influenced by many dietary compounds, hence supporting the hypothesis that nutritional habits may play a role on the pathogenesis of Alzheimer’s disease. Moreover, another possible link between diet and such diseases are that related to the presence of high levels of homocysteine, an intermediate compound of the metabolic cycle of methionine in patients affected by cognitive impairment (Seshadri and Wolf, 2003). Finally, additional interesting links between diet and neurocognitive disorders are those related to dietary fats, alcohol and inflammatory parameters (Mukamal et al., 2003; Wärnberg et al., 2009). High intake of cholesterol has been shown to increase the deposition of beta-amyloid in animal brains and high intake of fats may also determine oxidative stress. In addition, some findings in animal models demonstrated that alcohol is a neurotoxin, so acting as a modulator of the oxidative brain damage.

In the last few years, researches on diet and nutrition in relation to the occurrence of neurodegenerative diseases have been reported with interesting findings on Alzheimer’s and Parkinson’s diseases (Sofi et al., 2008). In fact, a greater adherence to a Mediterranean-type diet has been shown to decrease the risk of occurrence of both Parkinson’s and Alzheimer’s disease. The results of our meta-analyses showed that an increase of 2 points in the adherence score to Mediterranean diet is associated with a reduction of over than 10% of the risk of occurrence of such pathologies, by demonstrating the beneficial role of diet and dietary habits in the prevention of neurocognitive disorders (Sofi et al., 2008, 2010).

**CONCLUSION**

There is a vast amount of literature, to date, that reports a healthy dietary habit to be one of the strongest preventive measure for the general population, as well as for the population of patients with a manifested disease. Diet is able to decrease the risk of mortality and reduce the incidence of some of the most important disease states.


Retrospective incidence of wound infections and antibiotic sensitivity pattern: A study conducted at the Aminu Kano Teaching Hospital, Kano, Nigeria

Mohammed, A., Adeshina, G. O.* and Ibrahim, Y. K. E.

Department of Pharmaceutics and Pharmaceutical Microbiology, Ahmadu Bello University, Zaria, Nigeria.

Accepted 19 November, 2012

Infection continues to be a major complication of wounds with significant increase in costs, morbidity and potential mortality. Retrospective study of incidences of wound infection and antibiotic sensitivity pattern in patients that visited Aminu Kano Teaching Hospital, Kano, Nigeria, which involved the analysis of the medical records of 651 patients diagnosed from April, 2009 to September, 2010, was carried out. The medical records of the patients with wound infections showed that 77.9% of the wound sites were contaminated with various bacteria isolates, notably Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Klebsiella spp. in decreasing order of frequency. The most common infection site was surgical sites with amoxicillin, gentamicin and ceftriaxone, being the most commonly prescribed antibiotics for the treatment of resulting infections based on the culture and sensitivity results. The study shows that there is a high rate of wound infection in Kano, Nigeria.

Key words: Wound infections, retrospective studies, antibiotics.

INTRODUCTION

Human skin acts as an excellent barrier to infection, provided it is not breached. A wound is a type of injury in which the skin is torn, cut or punctured (open wound) or where blunt force trauma causes a contusion (closed wound). Wounds can further be classified as accidental, pathological or post-operative according to its nature (Collier, 2003). Certain parasites (for example, Hook-worm larvae) and bacteria (Treponema pallidum) can penetrate intact skin, but certain primary skin infections like impetigo is caused by Streptococcus pyogenes or S. aureus, or both gain access through abrasions, as minor trauma to skin is a part of everyday life (Bhatt and Lahkey, 2007).

Infection of a wound is the successful invasion and proliferation by one or more species of microorganisms anywhere within the body’s sterile tissues, sometimes resulting in pus formation (Calvin, 1998). Development of wound infection depends on the interplay of many factors. The breaking of the host protective layer, the skin, and thus disturbing the protective functions of the layer, will induce many cell types into the wound to initiate host response (Collier, 2003). Wound infections may occur following accidental trauma and injections, but post-operative wound infections in hospital are most common. Some infections are endogenous in which infection occurs from patient’s own bacterial flora such as S. aureus from skin and anterior nares or coliforms. Many infections are exogenous; skin and anterior nares are important sources of Staphylococci, spread of organisms from hospital staff and visitors occur by direct and indirect airborne routes.

At present, more than 60% of hospital-acquired infections are due to gram-negative enteric bacilli and only in
30% cases are gram-positive cocci responsible (Bhatt and Lahkey, 2007). Organisms commonly found in infected wounds include Gram positive cocci such as S. aureus, Streptococcus spp. Gram negative bacilli mostly Acinetobacter, Enterobacter, E. coli, Proteus spp, Ps. aeruginosa and anaerobic bacteria such as Propionibacterium spp. and Klebsiella spp. (Taiwo et al., 2002).

The current spread of multi-drug resistant bacteria pathogens has added a new dimension to the problem of wound infections (Sule and Olusanya, 2000). This is particularly worse in resource poor countries where sale of antibiotics is under poor control (Onile, 1997). A regular bacteriological review of infected wounds is therefore a necessity if affected patients must receive qualitative health care, particularly when blind treatment is a necessity, as in underdeveloped and developing nations (Fadeyi et al., 2008).

This study aims at investigating the incidence of wound infection and the antibiotic sensitivity pattern at the Aminu Kano Teaching Hospital, Kano, Nigeria.

### MATERIALS AND METHODS

#### Study area

This study was carried out at Aminu Kano Teaching Hospital, Kano in North West Nigeria. It is the largest Tertiary Health Institution in Kano State. It has a bed capacity of four hundred and twenty two.

#### Ethical considerations

Written Ethical approval for this study was obtained from the Medical Advisory Committee of the Teaching Hospital.

#### Data collection and sample size

Method of data collection was by Review of Records. A 17 months retrospective study of patients diagnosed with wound infections was carried out. A total of 651 patients were recorded over this period at the medical records department. Relevant data such as age, sex, aetiology of wound were obtained. Results of culture and sensitivity carried out by the microbiology department using standard biochemical tests were also obtained from the patient’s medical records.

#### Statistical analysis

Medical records data was used for analysis. Data was organized in Microsoft excel and the general descriptive analysis and correlation coefficient was used to analyze occurrence and extent of factors and the statistical relationship using Microsoft excel and statistical package for social science (SPSS) windows 16.0 (Standard Version SPSS Inc., Chicago, IL, USA).

### RESULTS

Out of the 651 wound samples received at the General Culture bench of the Microbiology Department of Aminu Kano Teaching Hospital from April, 2009 to September, 2010, 484 representing 74.35% yielded single organism, 23 (3.53%) yielded two organisms while 144 (22.12%) yielded no growth. The gender distribution amongst the 507 samples that showed growth, 308 (79.6%) were males, while 199 females also had almost equal proportion (75.4%) with the males. The age distribution of patients with wound infections in Aminu Kano Teaching Hospital with 392 (82.5%) being adults (from 13 years and above) and 115 (65.3%) being children (5 to 12 years) is shown in Table 1.

The percentage distribution of isolates from the different wound sites showed that the Surgical site wounds which amounted to 199 (39.9 %) of the isolates was found to be the most commonly infected. This was closely followed by wound sepsis 130 (26.1%). All acute soft tissue infections such as road traffic accidents, lacerations, domestic violence and gunshot injuries were classified under wound sepsis and then burn sites. Infections at diabetic and non-diabetic ulcer sites were least frequent (Figure 1).

### Table 1. Gender and age distributions of patients with wound infections in Aminu Kano Teaching Hospital.

<table>
<thead>
<tr>
<th>Gender</th>
<th>No and percentage of samples with bacteria isolates (%)</th>
<th>Age</th>
<th>No and percentage of samples with bacteria isolates (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male (n=387)</td>
<td>308 (79.6)</td>
<td>Adult (n=475)</td>
<td>392 (82.5)</td>
</tr>
<tr>
<td>Female (n=264)</td>
<td>199 (75.4)</td>
<td>Child (n=176)</td>
<td>115 (65.3)</td>
</tr>
<tr>
<td>Total (n=651)</td>
<td>507 (77.9)</td>
<td>Total (n=651)</td>
<td>507 (77.9)</td>
</tr>
</tbody>
</table>
The most frequently isolated organisms from diabetic ulcer sites were *S. aureus* (36.8%), *Pr. mirabilis* (26.3%), *E. coli* (19.3%) and *Ps. aeruginosa* (12.3%). Less frequently isolated from this site were *Klebsiella* spp and *Streptococcus* spp. (Figure 5). *S. aureus* continued to be the predominant organism from the surgical wound sites, constituting about 40% of the isolates. The Enterobacteriaceae such as *E. coli* and *Pr. mirabilis* were the next most frequent, followed distantly by *Ps. aeruginosa*. Only one isolate of *Streptococcus* spp. was obtained from this site (Figure 6).

Data presented in Table 2 showed that the most commonly used antibiotics in the treatment of wound infections based on the culture and sensitivity results in the hospital were β-lactam antibiotics (Penicillins and Cephalosporins) and the aminoglycosides, followed closely by the quinolones. Tetracyclines and anti-metabolites such as sulphonamides were less prescribed; only used in infections caused by *S. aureus*.

**DISCUSSION**

Generally, inadequate antimicrobial treatment defined as ineffective treatment of infection is an important factor in emergence of antibiotic resistant bacteria. Factors that contribute to inadequate antimicrobial treatment of hospitalized patients include: the prior use of antibiotic, broad spectrum antibiotics, prolonged hospital stay and the presence of invasive medical devices. The relatively high percentage of wound samples with infection in the retrospective studies indicated that there is high prevalence of wound infection within the study environment. Although, the number of samples from male patients with wound infections were much higher than those from female patients (308 males compared to 199 females), the differences in the proportions with infection in each gender class were much less (79.59% males and 75.38% females). There was negligible correlation ($r = 0.12$) between gender and contracting wound infection. A similar result was also reported in India, the slight difference in the number of males to females with wound.
Figure 2. Percentage distribution of bacteria isolates from wound sepsis sites of patients attending Aminu Kano Teaching Hospital from April, 2009 to September, 2010.

Figure 3. Percentage distribution of bacteria isolates from burn sites of patients attending Aminu Kano Teaching Hospital from April, 2009 to September, 2010.
Figure 4. Percentage Distribution of Bacteria Isolates from Ulcer Sites of Patients Attending Aminu Kano Teaching Hospital from April, 2009 to September, 2010

Figure 5. Percentage distribution of bacteria isolates from diabetic ulcer sites of patients attending Aminu Kano Teaching Hospital from April, 2009 to September, 2010.
**Table 2.** Percentage distribution of antibiotic prescription pattern on wound infections in Aminu Kano Teaching Hospital.

<table>
<thead>
<tr>
<th>Bacteria isolates</th>
<th>Pen</th>
<th>Ceph</th>
<th>Aminog</th>
<th>Quinol</th>
<th>Mac</th>
<th>Tet</th>
<th>Sulph</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em> (n = 92)</td>
<td>36.9</td>
<td>25.4</td>
<td>21.9</td>
<td>13.7</td>
<td>2.1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Ps. aeruginosa</em> (n = 49)</td>
<td>16.2</td>
<td>57.3</td>
<td>16.2</td>
<td>10.3</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>S. aureus</em> (n = 267)</td>
<td>38.2</td>
<td>21.2</td>
<td>20.0</td>
<td>17.2</td>
<td>2.8</td>
<td>0.2</td>
<td>0.4</td>
</tr>
<tr>
<td><em>Pr. mirabilis</em> (n = 7)</td>
<td>30.4</td>
<td>21.5</td>
<td>20.7</td>
<td>24.5</td>
<td>2.9</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Pr. vulgaris</em> (n = 1)</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>E. faecalis</em> (n = 1)</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Streptococcus</em> spp (n = 6)</td>
<td>36.4</td>
<td>27.1</td>
<td>27.4</td>
<td>9.1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Klebsiella</em> spp (n = 37)</td>
<td>29.1</td>
<td>28.9</td>
<td>23.7</td>
<td>18.3</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Pen = penicillins, Ceph = cephalosporins, Aminog = aminoglycosides, Quinol = quinolones, Mac = macrolides, Tet = tetracyclines, Sulph = sulphonamides.

infection is due to the social behavior where males are given superiority to the females, and if contacted disease are brought immediately to hospitals in comparison to female for treatment (Aizza et al., 2007). The proportion of adults with wound infection was much higher than children, and there was a moderate correlation ($r = 0.43$) between age and contracting wound infection. This could be due to the fact that more adults are likely to undergo a...
surgical operation which is the site that is mostly infected. It is not surprising that the three microorganisms most frequently isolated from the wound samples in the retrospective study were *S. aureus*, *E. coli* and *Ps. aeruginosa*. The frequent occurrence of *S. aureus* and the Gram negative organisms has also been reported (Olayinka et al., 2004; Sani et al., 2012). The preponderance of *S. aureus* (58%) in the retrospective data is in conformity with findings from other studies (Taiwo et al., 2002).

It was observed that Surgical site infection ranked highest among wound infections. This report is in agreement with the result of Taiwo et al. (2002). This is attributable to the fact that patients are likely to undergo surgical operations and more likely to have breaks in their local defence system. Wound sepsis which includes acute soft tissue infections follows surgical site infection in prevalence. Similar findings have also been reported (Aizza et al., 2007). In wound sepsis, *S. aureus* was also the most prevalent infectious organism caused by incision or fluid collection under the skin surface. This finding is similar to that obtained by Akinjogunla et al. (2009). The susceptibility of burn wound to opportunistic colonization by bacteria and fungi results from several factors, including the presence of coagulated proteins, the absence of blood-borne immune factors, and the avascularity of the burn wound (Jefferson and João, 2005).

Further analysis of the retrospective studies also showed that *S. aureus*, *Ps. aeruginosa*, *E. coli* and *Pr. mirabilis* are associated with surgical site infections. This finding is similar to that reported by (Nwachukwu et al., 2009) who found that 41.2, 21.3, 19 and 10.9% were *S. aureus*, *E. coli*, *Pr. mirabilis* and *Ps. aeruginosa* respectively. The relatively high number of Enterobacteriaceae isolated in this study points to the fact that the presence of enteric organisms in the wounds at operation probably resulted to subsequent sepsis. This finding, therefore, infers that enteric organisms are important determinants of healing in surgical wounds. The incidence of the enteric bacteria also confirms the observation that most wound infections arising from abdominal procedures are presently acquired from the patient's own faecal flora (Jonathan et al., 2008).

Although, several antibiotics were in use, based largely on the organisms isolated from the wound sites; it has been suggested that treatment should be based on the patient as a whole and not the infection alone, and that management strategies must be based on data derived from a holistic assessment of the needs of the individual (Collier, 2003).

**Conclusion**

Bacteria isolates associated with wound infections in the retrospective study, which were mostly *S. aureus*, enteric bacteria and *Ps. aeruginosa* are consistent with reports of similar studies conducted globally and in various parts of the country. The most commonly prescribed antibiotics in the facility were the penicillins, cephalosporins, aminoglycosides and quinolones. The correct choice of antibiotics should be made only after antibiotic sensitivity testing.

**REFERENCES**


Sule AM, Olu wynaha O (2000). In-vitro antimicrobial activities of fluoroquinolones compared with common antimicrobial agents against clinical bacterial isolates from parts of South Western Nigeria. Nig. Quarterly J. Hospital Med.10 (1):18-21.

Full Length Research Paper

Application of area to point Kriging to breast cancer incidence in Ashanti Region of Ghana

Ebenezer Bonyah¹*, L. Munyakazi², N.N.N. Nsowah-Nuamah³, D. Asong⁴ and Bashiru I.I. Saeed¹

¹Lecturers at Department of Mathematics and Statistics, Kumasi Polytechnic Institute, Kumasi, Ghana
²Associate Professor and Head Department of Mathematics and Statistics, Kumasi Polytechnic Institute
³Professor in Statistics and Rector of Kumasi Polytechnic Institute, Kumasi, Ghana.
⁴Komfo Anoyke Teaching Hospital and Senior Lecturer at Kwame Nkrumah Universtiy of Sciences and Technology, Kumasi, Ghana

Accepted 11 December, 2012

This paper provides a spatial analysis of breast cancer incidence in the Ashanti region area during the period of 2010 to 2011. Breast cancer disease has prevalence in Ghana particularly in the major cities including Ashanti region. Geographical units vary in shape and size and incidence count is non homogeneous in nature. For this reason, assigned area to point kriging approach is adopted as methodology. There is a large range of spatial autocorrelation in ages above 40 years than that of below 40 years in the various administrative units. The surrounding administrative units in the regional capital are less endemic for women whose ages are above 40 years. However, for those whose ages are below 40 years in all the surrounding administrative units are endemic but the capital is not. Most of the endemic districts share boundaries with Kumasi metropolis, the regional capital, where the only Teaching hospital is located. Most of the districts do not have good health facilities where women report for early treatment.

Key words: Area to point kriging, breast cancer, area to area, kriging.

INTRODUCTION

The leading malignancy in Ghana is breast cancer (Archampong, 1977). This is responsible for 15.4% of all malignancies and seems to be on the increase (Archampong, 1977). In 2011 there has been rise in admission for malignant neoplasms at the Komfo Anoyke Teaching hospital of which most of the cases were breast cancers. Ghana has seen tremendous public education about breast cancer within the last few years and some of the non-governmental organizations such as Mammocare Ghana, Cancer Society of Ghana and others have been playing pivotal role in dissemination of information about this disease. More than fifty percent of Ghanaian women have reported the issue of breast cancer at the hospitals when the disease may be at its advanced stage (Badoe and Baako 2000). In terms of average most of these women report eight months or more after observing a change in their breast (Biritwum et al., 2000). These patients are referred to the Komfo Anoyke Teaching hospital where they go for treatment at the surgical outpatient’s clinic.

Breast cancer disease incidence rate recorded at districts level are areal data which is good for areal data mapping in geostatistics. This has been implemented by several authors including Goovaerts and Jacquez (2005) and Kyriakidis (2004) to predict areal values. This approach is referred to as “area-to-point” (ATP) or “area to area” (ATA) kriging as following Kyriakidis (2004). The unique feature about ATP kriging is that it allows the mapping of variability within geographical unit (polygon) and at the same time ensuring the coherence of the prediction. For instance, disaggregated estimates of count data are non-negative and the sum is equal to the

*Corresponding author. E-mail: ebbonya@yahoo.com.
original aggregated count.

Kerry et al. (2010a) applied ATP and ATA for analyzing the geography of offenses and for identifying significant clusters of crimes on car-related thefts in the Baltic states. Shao el. al. (2009) applied ATP to introduce sex for the cancer rates, and observed the difference between age-adjusted rates and age-sex-adjusted rates.

Goovaerts (2006a) used this technique for cancer data analysis. This approach applied areal supports to predict point values by taking into account the spatial support of data as well as the varying population size. ATP and ATA are capable of analyzing cancer count and mortality maps making it possible to incorporate the shape and size of administrative units into the smoothing of choropleth maps and the creation of isopleths risk maps, respectively.

This paper presents a geostatistical analysis of breast cancer incidence data that consists of three steps: (1) filtering of noise in the data using Poisson kriging where the shape and size of administrative units is incorporated into the filtering, (2) the mapping of the corresponding risk at a fine scale and (3) geographical clustering of the disease at the administrative units.

METHODOLOGY
Study area

The Ashanti Region is centrally located in the middle belt of Ghana. It lies between longitudes 0.15° W and 2.25° W, and latitudes 5.50° N and 7.46° N. The region shares boundaries with four of the ten political regions, Brong-Ahafo in the north, Eastern Region in the east, Central Region in the south and Western Region in the south west. The region occupies a total land area of 24,389 km² representing 10.2% of the total land area of the country.

It is the third largest region after Northern (70,384 km²) and Brong Ahafo (39,557 km²) regions. The region has a population density of 194 persons per square kilometer, the third after Greater Accra and Central Regions.

The total population of the region is 4,725,046 made up of 2,288,325 males and 2,436,721 females (Ghana Statistical Service, 2010). The average daily temperature is about 27°C. Much of the region is situated between 150 and 300 m above sea level. The region has one Teaching hospital situated at the regional capital Kumasi and serves the entire region and beyond for tertiary cases. The rest of the 26 administrative units mostly do not have district hospitals and where they exist there are not enough qualified personnel to manage these facilities.

Data sources

The Ashanti region has a Disease Control Units (DCU) to which all District Health Directorates (DHD) report confirmed cases of various diseases at the end of year. In addition to this, the Teaching Hospital within the region has a research unit where various database of diseases are kept. Some of the confirmed cases of cancer were obtained from this centre and compared to that of the various District Disease Control Units.

Data for the analysis were classified based on ages below and above 40 years within each administrative unit. This was to find out the incidence rates deference between the two groups of women.

Population data obtained from Ghana Statistical Service was used in computing the raw rates of cancer. Raw rates were calculated as the number of cancer cases in each district divided by the estimated Population in 2010. In order to put the risk better, the raw rates were rescaled by multiplying it by a factor of 100,000. This expresses the raw rates as per 100,000 people.

Spatial and non-spatial data input

The basic data inputs were topographic map data, geographic location of the study area where breast cancer cases with patients ages been reported. Topographic map of Ashanti region at a scale of 1:25000 was obtained from Accra Survey and Mapping unit (Figure 1). This was georeferenced and digitized in ArcGIS version 10.0 where coordinates per polygon were extracted from the map.

Reported cases of breast cancer and ages of patients with confirmed breast cancer cases obtained from Komfo Anokye Teaching Hospital (KATH) and Disease Control Units (DCU) were entered as attributes of the polygon features (that is, the District) in the software. Application software are ArcGIS version 10.0 developed by ESRIL and SpaceStat 3.6.1 developed by BioMedware USA.

Geostatistical analysis

Area to Area (ATA) Poisson kriging

Given number N of geographical units \( V_i \) (administrative units), represent the observed mortality rates Cancer areal data as 
\[
v_{a} = d(v_{a}) / n(v_{a}) ,
\]
where \( d(v_{a}) \) is the number of Cancer counts and \( n(v_{a}) \) is the size of the population at risk. The Cancer incidence is explained as realization of a random variable \( D(v_{a}) \) that obeys a Poisson distribution with one parameter (expected number of count) simply the product of the population size \( n(v_{a}) \) by the local risk \( R(v_{a}) \).

The risk is computed as a linear combination of rate \( z(v_{a}) \) and the rates observed in (K-1) neighbouring entities \( V_i \):
\[
\hat{r}(v_{a}) = \sum_{i=0}^{K} \lambda_i z(v_i)
\]  \hspace{1cm} (1)

We compute weights \( \lambda_i \) assigned to the K rates by solving the number of system of linear equations known as “Poisson kriging” system:
\[
\sum_{j=1}^{K} \lambda_j \left[ \overline{C}_R(v_i,v_j) + \delta_{ij} \frac{m^*}{n(v_j)} \right] + \mu(v_{a}) = \overline{C}_R(v_i,v_{a})
\]  \hspace{1cm} (2)
\[
\sum_{j=1}^{K} \lambda_j = 1
\]

Where \( \delta_{ij} \) if i=j and 0 otherwise and m* is the population-weighted mean of the N rates. The “error variance”, \( m^*/n(v_i) \) help to locate
Figure 1. Map of Ashanti region of Ghana indicating District names.

small weightes for less reliable data. The spatial correlation among geographical units through the area-to-area covariance terms \( \overline{C}_R(v_i,v_j) = \text{Cov}(Z(v_i), Z(v_j)) \) and \( \overline{C}_R(v_i,v_\alpha) \).

Covariance is then between any two locations discretizing the area \( v_i \) and \( v_j \):

\[
\overline{C}_R(v_i,v_j) = \frac{1}{P_i \times P_j} \sum_{s=1}^{P_i} \sum_{s'=1}^{P_j} \text{wss}' C(u_s,u_{s'})
\]

(3)

Where \( P_i \) and \( P_j \) are the number of points used to discretize the two areas \( v_i \) and \( v_j \) respectively. We compute the weights \( \text{wss}' \) as the product of two population sizes assigned to each discretizing point \( u_s \) and \( u_{s'} \):

\[ \text{wss}' = n(u_s) \times n(u_{s'}) \quad \text{with} \quad \sum_{s=1}^{P_i} n(u_s) = n(v_i) \quad \text{and} \quad \sum_{s=1}^{P_j} n(u_{s'}) = n(v_j) \]

(4)

The uncertainty about the cancer mortality risk prevailing within the geographical unit \( v_\alpha \) can be modeled using the conditional cumulative distribution function (ccdf) of the risk variable \( R \), \( \text{Prob}(R(v_\alpha) \leq r | K) \). Based on assumption of normality of the prediction errors, ccdf is modeled as a Gaussian distribution with the mean and variance corresponding to the Poisson kriging estimate and variance are computed as:

\[
\sigma^2(v_\alpha) = \overline{C}_R(v_\alpha,v_\alpha) - \sum_{i=1}^{K} \lambda_i \overline{C}_R(v_\alpha,v_\alpha) \mu(v_\alpha)
\]

(5)

Where \( \overline{C}_R(v_\alpha,v_\alpha) \) is the within-area covariance that is calculated
according to Equation (3) with \( v_i = v_j = v_{\alpha} \).

**Area- to- Point (ATP) Point kriging**

The prediction support being small as point \( u_s \) resulting area-to-point Poisson kriging estimator and kriging variance:

\[
\hat{r}_{PK}(u_s) = \sum_{i=1}^{K} \hat{\lambda}_i(u_s) z_i
\]

(6)

\[
\sigma_{PK}^2(u_s) = C_R(0) - \sum_{i=1}^{K} (u_s) \overline{C}_R(v_i, u_s) - \mu(u_s)
\]

(7)

We compute the kriging weights and the Langrange parameter \( \mu(u_s) \) by solving system similar to the ATA kriging system (2), apart from the right-hand side term where the area-to-area covariance \( \overline{C}_R(v_i, u_s) \) are replaced by area-to-point point covariance \( \overline{C}_R(v_i, u_s) \), that are simplified as:

\[
\overline{C}_R(v_i, u_s) = \frac{1}{P_j} \sum_{j=1}^{P_j} \text{wss}^j C(u_s, u_s)
\]

(8)

Where \( P_j \) and wss' are defined as in expression (3). ATP reduces visual bias and has coherence property. The population-weighted average of the risk values estimated at the \( P_{\alpha} \) points \( \mu_s \) discretizing a given entity \( v_{\alpha} \) produces the ATA risk estimate for this entity:

\[
\hat{r}_{PK}(v_{\alpha}) = \frac{1}{n(v_{\alpha})} \sum_{s=1}^{P_{\alpha}} n(u_s) \hat{r}_{PK}(u_s)
\]

(9)

Constraint (8) is fulfilled if the same K areal data used for the ATA \( \hat{r}_{PK}(v_{\alpha}) \) are also used for the ATP kriging of the \( P_{\alpha} \) risk values.

**Deconvolution of the semivariogram of the risk**

In ATA and ATP kriging we need knowledge of point support covariance of the risk \( C(h) \) or similarly the semivariogram \( \gamma(h) \).

We cannot obtain this straightforwardly since only the areal data is available. The regularized semivariogram of the risk can be estimated as:

\[
\hat{r}_s(h) = \frac{1}{2 \sum_{j=1}^{n(v_{w})} n(v_{w})} \sum_{j=1}^{n(v_{w})} \sum_{j=1}^{n(v_{u})} \frac{n(v_{w}) n(v_{u})}{n(v_{w}) + n(v_{u})} |z(v_{w}) - z(v_{u})|^2 - m^*
\]

(10)

where \( N(h) \) is the number of pairs of administrative units \( (v_{\alpha}, v_{\beta}) \) whose population-weighted centroids are separated by the vector \( h \). The varying spatial increment \( [z(v_{w}) - z(v_{u})]^2 \) are weighted by a function of their respective population size \( n(v_{w}) n(v_{u}) / [n(v_{w}) + n(v_{u})] \), a term which is inversely proportional to their standard deviations (Monetiez et al., 2006).

Determination of a point-support semivariogram from the semivariogram \( \gamma R(h) \) fitted to areal data is known as "deconvolution", a common operation in geostatistics and it typically involves regular areas or blocks (Journel and Huijbregts, 1978). In this paper, we adopted the iterative procedure introduced for rate data measured over irregular geographical units in which one seeks the point-support model that, once regularized, is the closest to the model fitted to areal data; more details and simulation studies are found in Goovaerts (2006b).

**Cluster analysis**

A common task in disease analysis is to examine administrative units in adjacent geographical locations that are significantly similar or different. Similarity between the breast cancer incidence rate observed within area \( v_{\beta} \) and those recorded in the \( j(v_{\alpha}) \) neighboring areas \( v_{\alpha} \) can be computed by the local Moran statistic (Anselin et al., 2000) as:

\[
I(v_{\alpha}) = \left[ \frac{z(v_{\alpha}) - m}{s} \right] \times \left[ \sum_{j=1}^{j(v_{\alpha})} \frac{1}{f(v_{\alpha})} \left[ \frac{z(v_{j}) - m}{s} \right] \right]
\]

(11)

where \( m \) and \( s \) are the mean and standard deviation of the set of \( N \) area incident rates respectively. This Local Indicator of Spatial Association (LISA) is simply the product of the kernel rate and the average of the neighboring rates.

The distribution of the local Moran statistic under the null hypothesis of complete spatial randomness is usually obtained through a random of shuffling all the count(s) except at \( v_{\alpha} \) each time calculating (10) to get the distribution of simulated LISA values.

The empirical values of (10) are compared with this distribution to compute the \( P \) value for the rest. This randomization ignores the population size associated with each areal unit (Goovaerts and Jacquez, 2005).

**RESULTS AND DISCUSSIONS**

The Figures 2 and 3 show the omnidirectional variogram of breast cancer below and above 40 years risk computed from district-level rates using estimator (10). The experimental variogram was fitted using a Cubic model with a range of 13.4 km for breast cancer below 40 year and 32.52 km for above 40 years (Table 1).

However, breast cancer incidence above 40 years has better range of spatial autocorrelation than incidence below 40 years for each administrative unit. Each model was deconvoluted using the iterative procedure. In two
situations, the procedure ended once a small (that is, <2%) decrease in D statistic occurred four times, after 24 iterations for breast cancer 40 year and 13 for above 40 years.

The deconvoluted variogram model was used to estimate aggregated risk values at the district level in both region (ATA and ATP kriging) (Figure 4). In all cases, the estimation was based on K=32 closest observations which were selected according to the population-weighted district for ATA kriging. All maps are smoother than the map of raw rates since the noise due to small population sizes is filtered.

The breast cancer incidence rate below 40 years (Figure 4 A) indicates that the disease is more endemic around the regional capital Kumasi. The regional capital has the only teaching hospital in the northern sector of the country. There is similarity between the breast cancer incidence rate and ATA (Figure 4 A and B).

This provides the isopleths map which does not reflect the viability at various administrative units. Almost all the administrative units surrounding the regional capital are more endemic and other areas (Figure 4 A and C) for ages below 40 years. The ATP provides the variability within each administrative unit also shows that all the administrative units surrounding Kumasi metropolis have high risk of cancer disease except Atwima Nwabiagya and Kwabre. These two places have seen good infrastructure development in terms of health facilities. There are other places such as Ofinso North and Asante Akim South which is also endemic and these areas are poorly developed in terms of infrastructure and human resource for them to manage the health facilities even if they exist. In Obuasi municipality there is variability of risk of breast cancer and there is a need to do further research to

Table 1. Semivariogram parameters for both below and above 40 years breast cancer disease.

<table>
<thead>
<tr>
<th>Name</th>
<th>Model type</th>
<th>Sill</th>
<th>Nugget</th>
<th>Range (m)</th>
<th>MSS Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast Cancer Below 40 years</td>
<td>Cubic</td>
<td>0.1669</td>
<td>0</td>
<td>13435.76</td>
<td>2.667</td>
</tr>
<tr>
<td>Average Age Above 40 years</td>
<td>Cubic</td>
<td>0.237</td>
<td>0</td>
<td>32522.30</td>
<td>0.018</td>
</tr>
</tbody>
</table>
identify these specific places so that enough education could be conducted for early attendance to health facilities. There are some remote administrative units such as Amansie West, Sekyere Afram Plains and others have very low risk of breast cancer disease within the region.

The breast cancer incidence rate above 40 years for rate per 10,000 persons and ATA risk are similar (Figure 5 A and B). There are risk in most of the areas including Sekyere Afram Plain, Offinso North, Ahafo Ano North, Kwabre, Adansi North and Adansi South. Some of these administrative units do not have even health post for primary medical care. Therefore these women whose ages are above 40 do not report the disease to the health facilities for early treatment.

These women who are above 40 years within the various administrative units are mostly unemployed to get money for treatment. In ATP risk (Figure 5F) which explains the variability within each administrative unit indicates the regional capital Kumasi which is endemic but the surrounding administrative units are less endemic for women above 40 years. Notwithstanding, there are some that are far from the regional capital and endemic including Sekyere Afram Plains, Offinso North, Asante Akim South and others. These places share boundaries with other regions such Brong Ahafo and Eastern region. The proximity to health facility may account for the low reporting of the disease and some resort to herbal and spiritual healing. Kumasi has a renowned breast cancer NGO known as Breast Care International. They have been organizing free breast cancer screen for women within and around Kumasi metropolis. In most cases special attention is given to women above 40 years who are known to be prone to the disease.

The Local Moran statistics (Figure 6) shows that only Ofinso North and the regional capital Kumasi are significant for women with ages below 40 years. However, it is only significant in Amansie West for women with ages above 40 years in Ashanti region (Figure 6H). This could be one of the reasons why there is a low awareness in most of the administrative units especially those that share boundaries with other region. The regional capital Kumasi which has the teaching hospital and well trained personnel for screening for breast cancer and frequent outreach programme for various suburbs of the cities has improved the awareness of this disease.

However, the majority of the administrative units are not significant (p-value > 0.05). This does not imply that

---

**Figure 4.** Maps of breast cancer incidence rate estimated by breast cancer rate per 10,000 person, ATA Poisson kriging and ATP kriging on ages below 40 years at various administrative units (A, B and C) respectively.
these places are breast cancer free. The clustering of the disease in the central part for women with ages below 40 year (Figure 6G) is where we have the regional capital and is a densely populated area.

**Conclusions**

This study has demonstrated how the breast cancer incidence data can be analysed by considering average ages. ATP kriging is used to create a continuous risk surface that reduces the visual bias associated with large administrative units. This approach of ATP kriging may also give insight into more localized potential “hot spots” that are not evident when areal count on rates are employed. There is large spatial dependency which exist in breast cancer data (Figures 1 and 2). The risk associated with breast cancer (Figures 3 and 4) is centered in the regional capital and administrative units that share boundary with Kumasi the regional capital. In both situations ages below and above 40 years the disease is endemic in administrative units that are far away from
Kumasi. The risk of people developing breast cancer in Ashanti is heterogeneous during the period 2010 to 2011.

REFERENCES


Full Length Research paper

Validation of pharmacokinetic model of propofol in Indian population

Avinash Puri¹ and Sanju Dhawan²

¹Department of Anesthesia, Post Graduate Institute of Medical Education and Research, Chandigarh, India.
²University Institute of Pharmaceutical Sciences, Punjab University, Chandigarh, India.

Accepted 17 December, 2012

The pharmacokinetics of propofol has been evaluated extensively in a variety of patients groups after either bolus doses or continuous infusions. Presently there are multiple models available based on western data including China. So far, the pharmacokinetics of propofol has not been studied in the Indian population. With this background we planned to evaluate pharmacokinetics of propofol in Indian patients which will help in better management of these patients undergoing surgery using propofol infusion in total intravenous anesthesia. Venous blood samples (3 ml) for estimation of propofol concentrations were taken at different time intervals. Plasma propofol concentration was estimated by using High Performance Liquid Chromatography (HPLC) method. Maximum performance error occurred at 2 min with a median of -3.85 and it varied from -1.7 to -9.5 showing a consistent over prediction of the concentration at two minutes after the loading dose and start of infusion. Subsequently the error decreased to median of -0.9 (-0.9 to 4.6) at 10 minutes and median of -0.3 (range-0.3 to 2.8) at 30 and in 60 min -1.55(-0.28 to 1). When we compare the performance of our pharmacokinetic model of propofol in this study with other western studies, we observed less error with our pharmacokinetic model.

Key words: Propofol, pharmacokinetics, median performance error (MDPE), median absolute performance error (MDAPE).

INTRODUCTION

Propofol is an intravenous hypnotic agent which is widely used for induction and maintenance of general anesthesia. Its tremendous body uptake as well as the rapid elimination caused by huge volume of distribution and a high clearance makes propofol the best controllable intravenous anesthetic for maintenance of anesthesia at present. The pharmacokinetics of propofol has been evaluated extensively in a variety of disease states and different patients groups after either bolus doses or continuous infusions (Kay et al., 1986; Gepts et al., 1987; White and Kenny, 1990; Kirkpatrick et al., 1988; Cockshott et al., 1987). Presently there are multiple models available based on western data (Marsh et al., 1991). So far, the pharmacokinetics of propofol has not been studied in the Indian population. Previously we have studied pharmacokinetics of propofol following single bolus dose of 2 mg/kg in healthy Indian adult patients followed by serial plasma propofol concentration estimation and found Pharmacokinetic model of Propofol (Puri et al., 2012). In this present study, we planned to validate the pharmacokinetic data by targeting specific plasma propofol concentration and maintaining target plasma concentration based on our model.

MATERIALS AND METHODS

After approval from the Institutional Ethics Committee and written informed consent, 10 ASA grade 1 20 to 40 years old Indian patients were included. All patients underwent surgeries requiring general anesthesia for less than two hours and expected blood loss less than 10% of total blood volume. Patients with previous adverse exposure to propofol and who received propofol bolus or infusion within 15 days were excluded from the study. Patients with hepatitis, HIV infection, hepatic, renal, hematological and cardiovascular diseases were excluded from the study. No pregnant
patient and no patient with history of smoking or alcohol intake were included in the study.

Patients were premedicated with Tab Diazepam 5 mg night before as well as 2 h before induction. Before induction of anesthesia two large bore intravenous lines were secured. One in the antecubital vein and other in dorsum of the contralateral hand. The antecubital vein was used for blood sampling. Morphine 0.12 mg/kg was injected 5 min before starting propofol injection. Injection Lignocaine 2% 1 ml was injected in the iv line before injecting Propofol.

Propofol was administered as bolus dose followed by decreasing infusion rate calculated based on pharmacokinetics data of present pharmacokinetic model of propofol. The propofol infusion rate was delivered by syringe infusion pump (Pilot C Fresenius cabi) by using computer controlled. The propofol concentration was set at 3 µg/ml in 3 patients. 3.25 µg/ml in 3 patients and 3.5 µg/ml in 4 patients.

Blood sampling

Venous blood samples (3 ml) for estimation of propofol concentrations were taken at the following intervals after propofol injection and at 0 min (just before injection) and then at 2, 10, 30, and 60 min after administration of propofol bolus and infusion. Plasma propofol concentration was estimated by using HPLC method (Pavan and Buglione, 1992).

Infusion rate calculation

In the present Propofol model, we found significant correlation in between volume of central compartment and weight of the patients and based on the equation,

\[ Y = 147.18x + 4181.9 \]

After the body weight and target concentration had been entered into the computer, loading dose was calculated based on target concentration and volume of central compartment using formula given below

\[ LD = \text{Target concentration} \times \text{Volume of central compartment} \]

Immediately following bolus dose, in each patient, fixed plasma concentration of propofol (3, 3.25 and 3.5 µg/ml) were maintained till the end of the surgery using decreasing infusion rate.

\[ R_I = LD \times (K_{12} e^{-K_{21}t} + K_{13} e^{-K_{31}t} + K_{10}) \]

R is the continuously decreasing infusion rate to match the distribution into the second and third compartment; LD is the loading dose;

\[ K_{12} = \text{rate constant from central to tissue compartment} \]
\[ K_{21} = \text{rate constant from tissue to central compartment} \]
\[ K_{13} = \text{rate constant from central to deep tissue compartment} \]
\[ K_{31} = \text{rate constant from deep tissue compartment to central compartment} \]
\[ K_{10} = \text{Elimination constant} \]

And t is the time in seconds following bolus.

Mean values of rate constant obtained from present pk model

\[
K_{12} = 0.13 \quad K_{21} = 0.10 \quad K_{13} = 0.05 \quad K_{31} = 0.01 \quad K_{10} = 0.08
\]

Decreasing infusion rate was calculated using equation ‘A’ every 10 s and rate of infusion changed every 10 s by computer.

Validation of Model

Validation of model was assessed by measuring the plasma concentration at specific time intervals (2, 10, 30 and 60 min) calculating median performance error (MDPE), median absolute performance error (MDAPE), wobble, divergence (time related trends) using methods described by Varvel et al. (1991).

The predicted and measured values of propofol concentration were compared and various variables were derived as below.

\[ \text{Offset} = \text{Measured concentration} - \text{Predicted concentration} \]

The performance error was calculated by the formula

\[ \text{Performance error (\%)} = \frac{(Cp \text{ (measured)} - Cp \text{ (predicted)})}{Cp \text{ (predicted)}} \times 100 \]

Median Performance error (MDPE)

The percentage median performance error (MDPE) which reflects the bias in the ith subject is a signed value and represents the direction (over or underprediction) of the performance error.

\[ MDPE_i = \text{median} \left( |PE|_{ij} \right) \quad i = 1, \ldots, Ni \]

It is used to measure the systematic tendency of the system to underestimate or overestimate the measured concentration of blood propofol, that is, if bias has a positive value; it indicates that measured value is on an average greater than the system prediction and vice versa.

Median absolute performance error (MDAPE)

The percentage median absolute performance error (MDAPE) indicates the measure of inaccuracy in the ith subject.

\[ MDAPE_i = \text{median} \left( |PE|_{ij} \right) \quad i = 1, \ldots, Ni \]

Where Ni is the number of |PE| values obtained for the ith subject.

Wobble

Wobble is another index of the time related changes in performance and measures the intrasubject variability in performance errors. In the ith subject the percentage wobble is calculated as follows:

\[ \text{Wobble} = \text{median} \left( |PE|_j - \text{MDAPE}_{ij} \right) \quad j = 1, \ldots, Ni \]

RESULTS

The mean age of patients in this study was 28.4 ± 6.8 years and mean weight was 55.1 ± 9.2 Kg and the mean height was 154 ± 5.2 cm (Table 1).

Propofol concentration measured in plasma at different time points in all the patients followed the target concentration fairly well (Table 2).

Maximum performance error occurred at 2 min with a median of -3.85 and it varied from -1.7 to -9.5 showing a consistent over prediction of the concentration at two
Table 1. Demographic data of the patients

<table>
<thead>
<tr>
<th>Parameter</th>
<th>mean ± SD</th>
<th>Min.</th>
<th>Max.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Years)</td>
<td>28.4± 6.8</td>
<td>20</td>
<td>40</td>
</tr>
<tr>
<td>Weight (Kg)</td>
<td>55.1 ± 9.2</td>
<td>40</td>
<td>67</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>154 ± 5.2</td>
<td>148</td>
<td>165</td>
</tr>
</tbody>
</table>

(Data expressed as mean ± SD)

Table 2. Showing predicted and measured concentration at different time intervals in different patients.

<table>
<thead>
<tr>
<th>S/ no</th>
<th>T.C ug/ml</th>
<th>Rate(ml/h) at 2 min</th>
<th>Rate(ml/hr) at 10 min</th>
<th>MC at 2 ug/ml at 10 min</th>
<th>Rate(ml/h) at 30min</th>
<th>MC at 30 min ug/ml</th>
<th>Rate(ml/h) at 60 min</th>
<th>MC at 60 min ug/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3</td>
<td>42.6</td>
<td>2.89</td>
<td>31.3</td>
<td>2.97</td>
<td>22.3</td>
<td>3.09</td>
<td>19.5</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>50.1</td>
<td>2.88</td>
<td>36.8</td>
<td>2.85</td>
<td>26.3</td>
<td>3.04</td>
<td>22.9</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>56.3</td>
<td>2.89</td>
<td>41.9</td>
<td>3.14</td>
<td>29.5</td>
<td>3.01</td>
<td>25.8</td>
</tr>
<tr>
<td>4</td>
<td>3.25</td>
<td>48.2</td>
<td>3.02</td>
<td>35.4</td>
<td>3.22</td>
<td>25.3</td>
<td>3.18</td>
<td>22.0</td>
</tr>
<tr>
<td>5</td>
<td>3.25</td>
<td>58.3</td>
<td>3.09</td>
<td>42.9</td>
<td>3.17</td>
<td>30.6</td>
<td>3.22</td>
<td>27.2</td>
</tr>
<tr>
<td>6</td>
<td>3.25</td>
<td>60.4</td>
<td>2.94</td>
<td>44.4</td>
<td>3.11</td>
<td>31.6</td>
<td>3.22</td>
<td>28.2</td>
</tr>
<tr>
<td>7</td>
<td>3.5</td>
<td>69.4</td>
<td>3.37</td>
<td>51</td>
<td>3.35</td>
<td>36.4</td>
<td>3.46</td>
<td>31.7</td>
</tr>
<tr>
<td>8</td>
<td>3.5</td>
<td>67.9</td>
<td>3.3</td>
<td>49.9</td>
<td>3.52</td>
<td>35.6</td>
<td>3.4</td>
<td>31.1</td>
</tr>
<tr>
<td>9</td>
<td>3.5</td>
<td>54.1</td>
<td>3.41</td>
<td>39.8</td>
<td>3.54</td>
<td>28.4</td>
<td>3.6</td>
<td>24.7</td>
</tr>
<tr>
<td>10</td>
<td>3.5</td>
<td>60.7</td>
<td>3.29</td>
<td>44.6</td>
<td>3.44</td>
<td>31.8</td>
<td>3.51</td>
<td>27.7</td>
</tr>
</tbody>
</table>

TC--target concn, MC--Measured concn, Concentration in µg/ml.

Table 3. The performance error showing at various time points during the study in each patient.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Name</th>
<th>Sex</th>
<th>Age (Yrs)</th>
<th>Weight (KG)</th>
<th>Height (cm)</th>
<th>Surgery</th>
<th>TC (ug/ml)</th>
<th>LD (ml)</th>
<th>Rate (ml/h) at 2 min</th>
<th>MC at 2 (ug/ml) at 10 min</th>
<th>Rate (ml/h) at 30min</th>
<th>MC at 30 min (ug/ml)</th>
<th>Rate (ml/h) at 60 min</th>
<th>MC at 60 min (ug/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PK</td>
<td>F</td>
<td>22</td>
<td>40</td>
<td>150</td>
<td>Tympanoplasty</td>
<td>3</td>
<td>3.5</td>
<td>42.6</td>
<td>31.3</td>
<td>2.97</td>
<td>22.3</td>
<td>3.09</td>
<td>19.5</td>
</tr>
<tr>
<td>2</td>
<td>Suj</td>
<td>M</td>
<td>26</td>
<td>52</td>
<td>160</td>
<td>Cleft rhinoplasty</td>
<td>3</td>
<td>3.5</td>
<td>50.1</td>
<td>36.8</td>
<td>2.85</td>
<td>26.3</td>
<td>3.04</td>
<td>22.9</td>
</tr>
<tr>
<td>3</td>
<td>SR</td>
<td>F</td>
<td>39</td>
<td>62</td>
<td>148</td>
<td>Hysterolap</td>
<td>3</td>
<td>3.5</td>
<td>56.3</td>
<td>41.9</td>
<td>3.14</td>
<td>29.5</td>
<td>3.01</td>
<td>25.8</td>
</tr>
<tr>
<td>4</td>
<td>Su</td>
<td>F</td>
<td>32</td>
<td>43</td>
<td>155</td>
<td>Hysterolap</td>
<td>3.25</td>
<td>3.4</td>
<td>48.2</td>
<td>30.2</td>
<td>3.05</td>
<td>25.3</td>
<td>3.18</td>
<td>22.0</td>
</tr>
<tr>
<td>5</td>
<td>San</td>
<td>M</td>
<td>24</td>
<td>58</td>
<td>165</td>
<td>MF amputation</td>
<td>3.25</td>
<td>4.1</td>
<td>58.3</td>
<td>42.9</td>
<td>3.17</td>
<td>30.6</td>
<td>3.22</td>
<td>27.2</td>
</tr>
<tr>
<td>6</td>
<td>Raj</td>
<td>F</td>
<td>28</td>
<td>61</td>
<td>155</td>
<td>Cystectomy</td>
<td>3.25</td>
<td>4.2</td>
<td>60.4</td>
<td>44.4</td>
<td>3.11</td>
<td>31.6</td>
<td>3.22</td>
<td>28.2</td>
</tr>
<tr>
<td>7</td>
<td>Bim</td>
<td>F</td>
<td>30</td>
<td>67</td>
<td>154</td>
<td>Hysterolap</td>
<td>3.5</td>
<td>4.9</td>
<td>69.4</td>
<td>51</td>
<td>3.35</td>
<td>36.4</td>
<td>3.46</td>
<td>31.7</td>
</tr>
<tr>
<td>8</td>
<td>Sat</td>
<td>M</td>
<td>20</td>
<td>65</td>
<td>155</td>
<td>NHU R Leg</td>
<td>3.5</td>
<td>4.8</td>
<td>67.9</td>
<td>49.9</td>
<td>3.52</td>
<td>35.6</td>
<td>3.4</td>
<td>31.1</td>
</tr>
<tr>
<td>9</td>
<td>San</td>
<td>F</td>
<td>23</td>
<td>48</td>
<td>148</td>
<td>PBC neck</td>
<td>3.5</td>
<td>3.8</td>
<td>54.1</td>
<td>39.8</td>
<td>3.54</td>
<td>28.4</td>
<td>3.6</td>
<td>24.7</td>
</tr>
<tr>
<td>10</td>
<td>Sha</td>
<td>F</td>
<td>40</td>
<td>55</td>
<td>155</td>
<td>Salphingophrectomy</td>
<td>3.5</td>
<td>4.2</td>
<td>60.7</td>
<td>44.6</td>
<td>3.44</td>
<td>31.8</td>
<td>3.51</td>
<td>27.7</td>
</tr>
</tbody>
</table>
minutes after the loading dose and start of infusion (Table 3 and Figure 1). Subsequently the error decreased to median of -0.9 (-0.9 to 4.6) at 10 min and median of -0.3 (range-0.3 to 2.8) at 30 and in 60 min -1.55(-0.28 to 1).

Table 4 shows the analysis of MDPE, MDAPE, WOBBLE for 10 patients. The Median prediction error (MDPE%) was found to be -2.1. The Median absolute performance error (MDAPE%) was 2.2. The wobble calculated was 1.67.

Table 5 shows the comparison of performance errors of present study with Western data. In comparison to earlier studies of Marsh et al. (1991), Dyck et al. (1991), Tackley et al. (1989) and Hung et al. (2003) evaluating various pk models of propofol (Table 5) validation of present model

<table>
<thead>
<tr>
<th>S.no</th>
<th>Patients</th>
<th>MEAN PE</th>
<th>MEAN OFFSET</th>
<th>MDPE*</th>
<th>MDAPE**</th>
<th>WOBBLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PK</td>
<td>-0.16</td>
<td>-0.005</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>Suj</td>
<td>-2.3</td>
<td>-0.07</td>
<td>-2.8</td>
<td>2.8</td>
<td>1.6</td>
</tr>
<tr>
<td>3</td>
<td>SR</td>
<td>-2.9</td>
<td>-0.095</td>
<td>-1.8</td>
<td>1.8</td>
<td>0.61</td>
</tr>
<tr>
<td>4</td>
<td>Su</td>
<td>-2.8</td>
<td>-0.092</td>
<td>-2.7</td>
<td>2.7</td>
<td>1.87</td>
</tr>
<tr>
<td>5</td>
<td>San</td>
<td>-5.4</td>
<td>-0.177</td>
<td>-5.6</td>
<td>5.6</td>
<td>2.6</td>
</tr>
<tr>
<td>6</td>
<td>Raj</td>
<td>-2.1</td>
<td>-0.075</td>
<td>-2.4</td>
<td>2.4</td>
<td>1.5</td>
</tr>
<tr>
<td>7</td>
<td>Bim</td>
<td>-2.5</td>
<td>-0.09</td>
<td>-2.5</td>
<td>2.5</td>
<td>1.74</td>
</tr>
<tr>
<td>8</td>
<td>Sat</td>
<td>0.28</td>
<td>0.01</td>
<td>0.42</td>
<td>1.8</td>
<td>1.57</td>
</tr>
<tr>
<td>9</td>
<td>San</td>
<td>-1.6</td>
<td>-0.05</td>
<td>-0.7</td>
<td>1.28</td>
<td>1.28</td>
</tr>
<tr>
<td>10</td>
<td>Sha</td>
<td>0.25</td>
<td>0.0075</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

MEAN -1.923±1.7 -0.063±0.05 -1.8±1.8 2.4±1.18 1.67±0.5
MEDIAN -0.0725 (-0.005~ -0.17) -2.1(0~5.6) 2.2(1.8~5.6) 1.67(0.61~2.6)

(MDPE)* Median Performance error. (MDAPE)** Median absolute performance error. *Data are expressed as medians (range)

<table>
<thead>
<tr>
<th>VARIABLE</th>
<th>GROUP</th>
<th>MEDIAN</th>
<th>10%</th>
<th>90%</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDPE %</td>
<td>PRESENT STUDY</td>
<td>-2.1</td>
<td>-3.08</td>
<td>0.042</td>
</tr>
<tr>
<td></td>
<td>Marsh et al. (1991)</td>
<td>-7</td>
<td>-42.6</td>
<td>42.7</td>
</tr>
<tr>
<td></td>
<td>Dyck et al. (1991)</td>
<td>36.4</td>
<td>14.3</td>
<td>76.5</td>
</tr>
<tr>
<td></td>
<td>Tackley et al. (1989)</td>
<td>-4.6</td>
<td>-35.6</td>
<td>24.6</td>
</tr>
<tr>
<td></td>
<td>Hung et al. (2003)</td>
<td>14.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDAPE %</td>
<td>PRESENT STUDY</td>
<td>2.488</td>
<td>1.748</td>
<td>3.08</td>
</tr>
<tr>
<td></td>
<td>Marsh et al. (1991)</td>
<td>18.2</td>
<td>8.3</td>
<td>43.1</td>
</tr>
<tr>
<td></td>
<td>Dyck et al. (1991)</td>
<td>39.3</td>
<td>15.4</td>
<td>76.5</td>
</tr>
<tr>
<td></td>
<td>Tackley et al. (1989)</td>
<td>20.6</td>
<td>8.3</td>
<td>43.1</td>
</tr>
<tr>
<td></td>
<td>Hung et al. (2003)</td>
<td>23.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DIVERGENCE %</td>
<td>PRESENT STUDY</td>
<td>0.2876</td>
<td>0.019</td>
<td>0.701</td>
</tr>
<tr>
<td></td>
<td>Marsh et al. (1991)</td>
<td>6.5</td>
<td>-15.1</td>
<td>21.9</td>
</tr>
<tr>
<td></td>
<td>Dyck et al. (1991)</td>
<td>14.6</td>
<td>-61.1</td>
<td>42.2</td>
</tr>
<tr>
<td></td>
<td>Tackley et al. (1989)</td>
<td>6.9</td>
<td>-8.4</td>
<td>28.9</td>
</tr>
<tr>
<td></td>
<td>Hung et al. (2003)</td>
<td>-1.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WOBBLE %</td>
<td>PRESENT STUDY</td>
<td>1.67</td>
<td>1.2</td>
<td>2.06</td>
</tr>
<tr>
<td></td>
<td>Marsh et al. (1991)</td>
<td>10</td>
<td>4.5</td>
<td>29.6</td>
</tr>
<tr>
<td></td>
<td>Dyck et al. (1991)</td>
<td>12</td>
<td>7.7</td>
<td>21.9</td>
</tr>
<tr>
<td></td>
<td>Tackley et al. (1989)</td>
<td>14</td>
<td>7.5</td>
<td>21.6</td>
</tr>
<tr>
<td></td>
<td>Hung et al. (2003)</td>
<td>18.9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
DISCUSSION

When we compare the performance of our pharmacokinetic model of propofol in this study with other western studies, we observed less error with our pharmacokinetic model. Though we did not evaluate other western model in our population but we compared the performance of our model obtained in present study with the performance of other models evaluated by Coetzee et al. (1995) in their respective studies. As obvious from the Table 4 we observed less error with our pharmacokinetic model. It has been suggested that the performance of a TCI system is clinically acceptable if the bias (MDPE) is no greater than 10 to 20% (Glass et al., 1990). Performance bias may be minimized by using pharmacokinetic model derived from local population and including co-variates such as, weight etc. to improve the performance of such model, that is, adjusting the pharmacokinetic model to individual patient optimize the precision of TCI. The precision (MDAPE) in Marsh model was 18.3% while in our study was 2.4%. Another reason for better performance in present study may be that we kept stable propofol concentration in each patient. Variation of plasma concentration with in patients during the study may have produce different performance results. Thus in our pharmacokinetic model derived from the pharmacokinetic data from Indian population is more acceptable as the performance error calculated were less compared to the western models.

REFERENCES

UPCOMING CONFERENCES

The Fifth International Conference on eHealth, Telemedicine, and Social Medicine
eTELEMED 2013
February 24 - March 1, 2013 - Nice, France

The 7th International Conference on Microtechnologies in Medicine and Biology
MMB 2013
April 10-12, 2013
Conferences and Advert

July 2012
International Congress on Naturopathic Medicine, Paris, France, 7 Jul 2013

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

September 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