Afro-Egyptian Journal of Infectious and Endemic Diseases
المجلة الافريقية المصرية للامراض المعدية والمتوطنة

ISSN (Online): 2090-7184
ISSN (Print): 2090-7613

An Official Publication of Endemic and Tropical Medicine Department ,Faculty of Medicine ,Zagazig University ,Zagazig 44519 ,Egypt

Published by: Communication and Information Technology Center(CITC),Zagazig University , Zagazig ,Egypt
Atef Eraky
E mail:atef_eraky@yahoo.com
Wafaa Metwally
E mail:wafaa@zu.edu.eg

Scope of the Journal

The Afro-Egyptian Journal of Infectious and Endemic Diseases (AJIED) is a peer-reviewed journal that publishes clinical, parasitological, microbiological, physiological, biochemical, immunological and pathological studies in the field of infectious, endemic and tropical diseases. The scope of the journal includes also articles of endemic gastroenterology and hepatology. The journal is published quarterly by Endemic and Tropical Medicine Department, Faculty of Medicine, Zagazig University, Zagazig, 44519, Egypt.

Submission Process

The Journal accepts online submissions only. Manuscripts can be submitted at

http://mis.zu.edu.eg/ajied/home.aspx. Once the manuscript has been uploaded, our system automatically generates an electronic pdf, which is then used for reviewing. All correspondence, including notification of the Editor's decision and requests for revisions, will be managed through this system. Authors can follow the progress of their paper using this system to final decision. For any problems please contact the Editorial Office at ajied@zu.edu.eg.
Due to editorial policy to accept high quality articles, the journal accept only 50% of received articles.

Authorship

All authors should have made substantial contributions to all of the following:

(1) the conception and design of the study, or acquisition of data, or analysis and interpretation of data
(2) drafting the article or revising it critically for important intellectual content
(3) final approval of the version to be submitted.

Article types

The following types of manuscripts are routinely accepted:

1- **Original Articles:** This should include an abstract, keywords, introduction, patients/material and methods, results, discussion and references. They should be no longer than 5000 words (word count excludes tables, figures and legends).
2- **Reviews:** An abstract and keywords are required. The text should be divided into sections by suitable headings. Tables and figures may be used as appropriate for the text. They should be no longer than 6000 words.
3- **Opinions, Commentaries and Letters to the editor:** These take the same form as a review.
4- **Short Communications:** These should be no more than 2,500 words, with up to 15 references and a maximum of 3 figures or tables.
5- **Case Reports:** Case reports should present only cases of exceptional interest including presentation, diagnosis and management of disease. They should contain short summaries, an introduction, the case report, discussion, a reference list, tables and figure legends.
6- **Images in Infectious and Endemic Diseases:** These consist of interesting cases with high quality images with a short text and no more than 10 references.
7- **Video case**:By invitation.

Preparation of the manuscript

Please ensure that the following are including in your submission: -One author designated as corresponding author: Their E-mail address ,full postal address Telephone and fax numbers -Keywords -Cover letter addressed to the Editor, introducing the manuscript and confirming that it is not being submitted concurrently elsewhere -All figure captions -All tables (including title, description, footnotes) -All necessary files have been uploaded -Manuscript has been spell checked -All text pages have been numbered -References are in the correct format for this journal -All references mentioned in the Reference list are cited in the text and vice versa -Permission has been obtained for use of copyrighted material from other sources (including the Web) -Color figures are clearly marked as being intended for color reproduction or to be reproduced in black-and-white.-Manuscripts :Please type all pages with double spacing and wide margins on one side of the paper. Title page, abstract, tables, legends to figures and reference list should each be provided on separate pages of the manuscript. Use font such as Times New Roman or Arial. The text should be in single-column format. Number the pages. Keep the layout of the text as simple as possible. Most formatting codes will be removed and replaced on processing the article. In particular, do not use the options to justify text or to hyphenate words. However, do use bold face, italics, subscripts, superscripts etc. Do not embed 'graphically designed' equations or tables, but prepare these using the facility in Word or as a separate file in Excel. When preparing tables, if you are using a table grid, use only one grid for each individual table and not a grid for each row. Do not prepare tables in PowerPoint. To avoid unnecessary errors you are strongly advised to use the spellchecker. The title page should include: the title, the name(s) and affiliation(s) of the author(s), an address for correspondence, and telephone/fax numbers for editorial queries. All articles should include an Abstract of no more than 300 words and 3-6 key words for abstracting and indexing purposes. Please write your text in good English. Use decimal points (not commas); use a space for thousands (10 000 and above).
Provide the following data in your submission (in the order given).

1- **Title page (separate page):** Title should be concise and informative. Avoid abbreviations and formulae where possible. Author names and affiliations. Where the family name may be ambiguous (e.g., a double name), please indicate this clearly. Present the authors' affiliation addresses (where the actual work was done) below the names. Indicate all affiliations with an Arabic number immediately after the author's name and in front of the appropriate address. Corresponding author: This should be indicated after authors affiliations. Clearly indicate who is willing to handle correspondence at all stages of refereeing and publication, also post-publication. . Ensure that telephone and fax numbers (with country and area code) are provided in addition to the e-mail address and the complete postal address.

2- **Abstract**: (separate paper). A concise and informative abstract is required (maximum length 300 words). The abstract should state briefly the purpose of the research, the principal results and major conclusions. Do not cite references in the abstract. Non-standard or uncommon abbreviations should be avoided in the abstract, but if essential they must be defined at their first mention in the abstract itself. The abstract should be divided into: Background and study aims, patients/material and methods, results and conclusion. Keywords Immediately after the abstract, provide a maximum of 6 keywords.

3- **Abbreviations:** Define abbreviations that are not standard in this field at their first occurrence in the article (even if mentioned in the abstract). Ensure consistency of abbreviations throughout the article

4- **Introduction:** State the objectives of the work and provide an adequate background, avoiding a detailed literature survey or a summary of the results.

5- **Patients/Materials and methods**: Provide sufficient detail to allow the work to be reproduced. Methods already published should be indicated by a reference. Only relevant modifications should be described. Include in figure legends and table texts, technical details of methods used, while describing the methods themselves in the main text.

6- **Results:** This should explore the significance of the results of the work, not repeat them. A combined Results and Discussion section is often appropriate in a Short Communication but not in an Original Article. Ensure that the chapter results stands by itself and explain all results of your work. Note that all tables and figures should be presented in separate papers.

7- **Discussion:** Discuss your results and avoid extensive citations and discussion of published literature.

8- **Acknowledgement:** Collate acknowledgements in a separate section at the end of the article and do not, therefore, include them on the title page, as a footnote to the title or otherwise. When the work included in a paper has been supported by a grant from any source, this must be indicated. A connection of any author with companies producing any substances or apparatus used in the work should be declared in this section. All contributors who do not meet the criteria for authorship as defined above should be listed in an acknowledgements section. Examples of those who might be acknowledged include a person who provided purely technical help, writing assistance, or a department chair who provided only general support. Authors should disclose whether they had any writing assistance and identify the entity that paid for this assistance.

9- **References:** References should be numbered consecutively (with parentheses) as they appear in the text e.g. [5]. Type the reference list with double spacing on a separate sheet. This includes family name and first name initial, up to 6 authors are required and more authors are marked with et al. Examples: 1- Abdel-Wahab M, Esmat G, El-Boraey Y, Ramzy I , Medhat E, Strickland G. The epidemiology of schistosomiasis in Egypt: methods , training, and quality control of clinical and ultrasound examinations . *Am J Trop Med Hyg* 2000 ; 62 (suppl) :17-20. 2- Wright W. Geographical distribution of schistosomes and their intermediate hosts. Ansari N, ed. Epidemiology and control of schistosomiasis (bilharziasis) .*Baltimor* ;*University Park Press* 1973 ;42-48.. Do not include references to personal communications, unpublished data or manuscripts either 'in preparation' or 'submitted for publication'. If essential, such material may be incorporated into the appropriate place in the text. Recheck references in the text against reference list after your manuscript has been revised. All references listed in the text should be included in the reference list and all references in the reference list should be included in the text.

10- **Illustrations:** Photographs should be presented as high quality jpg. Illustrations will not be redrawn by the Publisher: line figures should be suitable for direct reproduction. They should be prepared with black on white background, or be black-and-white images; ; they should be completely and consistently lettered, the size of the lettering being appropriate to that of the illustration, taking into account the necessary reduction in size. Colour figures will be included

11- **Tables:** Number tables consecutively in accordance with their appearance in the text. Place footnotes to tables below the table body and indicate them with superscript lowercase letters. Avoid vertical rules. Be sparing in the use of tables and ensure that the data presented in tables do not duplicate results described elsewhere in the article.

Editorial Review

All manuscripts are subject to peer review. If changes are requested, revisions received later than 2 months after this request will be treated as new submissions. When changes are made, the corresponding author should go into resubmission under title of submission of revised manuscript, and a word document should be uploaded that indicates changes and modifications done.

Publication charges

No publication charges are needed .

Off prints

The corresponding author, at no cost, will be provided with a PDF file of the article via e-mail. Authors can download the PDF from the journal web page and in the same way the journal cover image can be downloaded.

Policy and Ethics Declarations

Upon submission you will be required to complete this form to declare funding, conflict of interest and to indicate whether ethical approval was sought. This information must also be inserted into your manuscript under the acknowledgements section. If you have no declaration to make please insert the following statements into your manuscript: Funding: None, Competing interests: None declared ,Ethical approval: Not required . Work on human beings that is submitted to AJIED should comply with the principles laid down in the Declaration of Helsinki; Recommendations guiding physicians in biomedical research involving human subjects. Adopted by the 18th World Medical Assembly, Helsinki, Finland, June 1964, amended by the 29th World Medical Assembly, Tokyo, Japan, October 1975, the 35th World Medical Assembly, Venice, Italy, October 1983, and the 41st World Medical Assembly, Hong Kong, September 1989. The manuscript should contain a statement that the work has been approved by the appropriate ethical committees related to the institution(s) in which it was performed and that subjects gave informed consent to the work. Studies involving experiments with animals must state that their care was in accordance with institution guidelines.

Competing interests

All authors must disclose any financial and personal relationships with other people or organizations that could inappropriately influence (bias) their work. Examples of potential conflicts of interest include employment, consultancies, stock ownership, honoraria, paid expert testimony, patent applications/registrations, and grants or other funding. Role of the funding source all sources of funding should be declared. Authors should declare the role of study sponsors, if any, in the study design, in the collection, analysis and interpretation of data; in the writing of the manuscript; and in the decision to submit the manuscript for publication. If the study sponsors had no such involvement, the authors should so state.

Indexing

The Journal is indexed currently in ENSTINET, Google Scholar and Index Copernicus (IC 2012:4.47).

Contents

Eosinophilic Esophagitis in Refractory GERD

Mostafa Elshami
Tropical Medicine Department, Faculty of Medicine, Zagazig University, Egypt
See article pages:59-68

Background

Eosinophilic esophagitis (EE) is a disease in which upper intestinal symptoms is associated with dens eosinophilic infiltration of squamous epithelium or deeper eosophageal tissue. Neither symptoms nor eosinophilia respond to treatment with proton pump inhibitors (PPI), but it responds well to topical steroids. The pathophysiological mechanisms are likely related to allergic inflammation, not to underlying motility disorder as in gastroesophageal reflux disease (GERD) [1].

Diagnosis of EE is made by esophageal biopsy during endoscopic examination with the presence of eosinophiles (>15 per HPF) in the tissue with or without peripheral eosinophilia. There is an overlap between EE and refractory GERD as regard tissue eosinophils as refractory GERD patients may have eosinophilic infiltration in esophageal biopsy but with counts less than 15 per HPF [2]. The etiology of EE is poorly understood, allergic response has been implicated. The offending agent of this allergic reaction may be ingested or inhaled allergen as proved by skin hypersensitivity test. In EE, Th2- type immune response is present with increased level of eosinophilic active Th2 cytokines, as IL-4, IL-5, IL-13 [3].

EE is an uncommon disease and there are insufficient studies to know its exact prevalence and incidence, it is common in males than females, in children more than adults, it must be considered in patients with refractory GERD [4].

Summary of the paper

The paper entitled "Eosinophilic Esophagitis in Patients with Refractory Gastro-esophageal Reflux Disease (GERD) ". The authors enrolled 100 patients with GERD ; 50 patients of them have refractory GERD aiming to evaluate the frequency of EE in patients with refractory GERD and, the clinical and pathological differences between both groups. They performed basic investigations together with endoscopy with esophageal biopsy, esophageal manometry, 24-hour PH metry, pathological and immunohistochemical examination of the biopsy specimen. They found that the frequency of EE is 4% in patients with refractory GERD and tissue eosinophilia is significantly increased in refractory GERD patients while peripheral eosinophilia was the same. They also stated that pathological examination with ordinary stains gave the same results as immunohistochemical staining.

Comment on the study

The frequency of EE is not exactly known but the frequency in special population groups is suggested in clinical trials, but the number of patients in this study was too small to achieve statistical significance, but the results support the role of EE in the differential diagnosis of refractory GERD. The paper clarify the difference between GERD and refractory GERD without stress on EE patients as regard clinical findings this is because of little number of patients. The authors describe that rings and furrows are the most common endoscopic findings in EE patients while these findings were present in only 2 patients.

Recommendations:

Large population sample is required to better determination of EE frequency, and proper understanding the clinical, laboratory and endoscopic data of this disease. Better understanding of clinical outcomes, complications and response to treatment.

References

1- Fox VL, Nurko S , Furuta GT. Eosinophilc eosophagitis, it is not just kid's stuff. *Gastro-enterointest Endoscopy* 2002; 56: 260-270.

2- Mishra A, Hogan SP, Lee JJ. Fundamental signals that regulate eosinophil homing to the gastrointestinal tract. *J Clin Invest* 1999; 103:1719-1727.

3- Blanchard C, Wang N, Stringer. Eotaxin-3 and uniquely conserved gene-expression profile in eosinophiliv eosophagitis. *J Clin Invest* 2006; 116: 536-547.

4- Potter JW, Saiean K , Staff D. Eosinophilic eosophagitis in adults: an emerging problem with unique eosophageal features. *Gastrointest Endoscopy* 2004; 59 : 355-361.

Eosinophilic Esophagitis in Patients with Refractory Gastro-esophageal Reflux Disease (GERD).

Hamed M. Soliman[1], Rashed M. Hassan[1], Mahmoud W. Emara[2], Mohammed E. Ali[1], Tarik I. Zaher[1],Sherif M. Galal[1]

[1] *Endemic and Tropical Medicine Department, Faculty of Medicine, Zagazig University, Egypt*

[2] *Pathology Department, Faculty of Medicine, Zagazig University, Egypt*

Corresponding Author
Tarik I Zaher

Email:tareqzaher@zu.edu.eg

Key words:Eosinophilic; esophagitis; refractory;GERD

Background and study aim : Eosinophilic esophagitis (EE) is defined as the presence of an abundant eosinophil infiltrate of the esophageal mucosa observed at biopsy. For some time, the existence of a patho-physiological relationship between GERD and EE has been speculated. The aim of this work is to estimate the frequency of eosinophilic esophagitis among patients with refractory reflux esophagitis.

Patients and Methods: We selected fifty patients diagnosed previously as refractory GERD(group I) . Another fifty patients corresponding in age and sex with group I with esophageal and extra-esophageal clinical symptoms suggestive of GERD were enrolled in this study as control (group II). All patients were subjected to upper GIT endoscopy (multiple biopsies were taken), 24 hrs esophageal PH monitoring, esophageal motility study, histopathology and immune -histochemistry of esophageal biopsies.

Results: Two patients with EE were found among patients with refractory GERD. Dysphagia, heart burn, and food impaction were the common presenting symptoms of patients with EE. Rings and furrow were the most common and significant endoscopic pictures in cases of EE. Eosinophils, microabces, basal zone hyperplasia and increased lamina propria papillae were the significant specific finding for EE in esophageal biopsies. Esophageal dysmotility and occurrence of reflux were common and significant in patients with EE. Immuno-histochemistry had high sensitivity in detection of eosinophils and its degradation product in esophageal biopsies.

Conclusion: eosinophilic esophagitis is one of the causative factors of refractory GERD and its frequency is about 4%.It should be put in differential diagnosis of cases of refractory GERD. Endoscopic picture is suggestive but biopsy for EE is confirmatory.

INTRODUCTION

Eosinophilic esophagitis (EE) is defined as the presence of an abundant eosinophil infiltrate of the esophageal mucosa observed at biopsy [1]. While it is true that there is still controversy about the number of eosinophils that must be observed per high power field (HPF) for the diagnostic standard of EE, most authors have used a number equal or greater than 15 cells [2].

This disease was described in children and in adults [3]. An increased prevalence has been observed which may be partially explained by the more diligent search for the disease or etiology-related changing mechanisms [4].

The prevalence of EE is highly variable and appears to depend on the characteristics of the study population. In a prospective studies indicated a low prevalence (0.05–0.4%) in general population [5]. However, it may be up to 15% in patients with dysphagia [6] or as high as 48% in patients with food bolus impaction [7].

The clinical features of EE are not completely known due to the fact that few prospective studies have been conducted. However, EE is frequently associated with dysphagia, esophageal food impaction, allergic processes (aeroallergens, food, asthma) and some endoscopic abnormalities (concentric rings, longitudinal furrows, mucosal white plaques, strictures and narrowing of the esophageal lumen [8]. EE may also be associated with clinical symptoms suggestive of gastro-esophageal reflux disease (GERD) such as heartburn and regurgitation in about 30% of cases [9]. Also cases with non-specific symptoms and normal endoscopy have been reported from 8% to 28% of cases [10].

For some time, the existence of a pathophysio-logical relationship between GERD and EE has been speculated, as eosinophilic infiltrates have been observed in the esophageal mucosa of patients with GERD. It has also been suggested that EE may cause secondary GERD [11]. Some authors have reported more than 20 eosinophils × HPF in patients with GERD, which disappeared after treatment with gastric acid inhibitors [12]. In other results it was observed that patients with EE are hypersensitive to acid perfused in the oesophagus, thus acid may play a role in the symptoms [11].

Although there may be overlapping of the two diseases, it is currently accepted that the EE is a pathological entity different from GERD, whose etiology seems to be related to allergic and genetic factors [13]. Consequently, EE symptoms respond well to topical steroid administration, while the response to gastric acid inhibitors is very limited.

We hypothesized that some patients with GERD symptoms who do not respond to conventional treatment may have EE. Currently this disease is not routinely considered in the differential diagnosis of refractory GERD. For this reason, we proposed as main objective to prospectively evaluate the frequency of EE in a consecutive population of patients with GERD who were refractory to conventional therapy, and as secondary objective to describe the clinical features and predictors of EE in this subset of patients.

The aim of this work is to estimate the frequency of eosinophilic esophagitis among patients with refractory reflux esophagitis, to study its effect on the course of the disease and to find in what way it can be differentiated from non refractory GERD.

PATIENTS AND METHODS

Patients and study design :

This study was done in Tropical Medicine and Pathology departments Faculty of Medicine Zagazig University, from July 2008 to December 2012.We prospectively studied patients who were treated at the outpatient clinic of the Gastroenterology Unit. We selected fifty patients diagnosed previously as refractory GERD as they had persistent esophageal or extra esophageal symptoms suggestive of GERD and are refractory to full dose PPI (40 mg) treatment for 8 weeks (group I).Failure of response to 8 weeks treatment is taken as a base of refractory GERD according to Locke et al. [14]. Another fifty patients corresponding in age and sex with group I with esophageal and extra-esophageal clinical symptoms suggestive of non refractory GERD according to the GERD Symptoms Checklist were enrolled in this study as control (group II).

Exclusion Criteria :

We excluded patients diagnosed with :

1- Crohn's disease.
2- Scleroderma.
3- Fungal infections.
4- Collagen disease.
5- Esophageal carcinoma.
6- Drug induced esophagitis.

This study was performed on these patients to study the frequency and the role of eosinophilic infiltration on the course of GERD. All patients underwent a complete history taking included demographic data, history of environmental, food, and drug allergies, clinical symptoms suggestive of GERD, length of symptoms, type and duration of GERD treatment, adherence to treatment, and associated co-morbid states and diagnostic testing for GERD.

All patients are exposed to the following investigations :

1- CBC especially eosinophilic count.
2- Chemical laboratory tests including LFTs, KFTs and fasting blood sugar to exclude hepatic, renal and diabetes which affect esophageal motility.
3- Esophagogastroduodenoscopy: Using Pentax (EG-2940) endoscope to assess the presence of esophagitis, multiple biopsies were taken from the upper, middle and lower third of the esophagus. Esophageal biopsy specimens were obtained by direct endoscopic vision using fenestrated, ellipsoid, spiked 7mm open

span biopsy forceps. Four quadrant biopsies were taken from each subject essentially including areas suffer from sings of esophagitis.

Grading were done according to Los Angeles classification of esophagitis [15] :

Grade A	One (or more) mucosal break no longer than 5 mm that does not extend between the tops of two mucosal folds
Grade B	One (or more) mucosal break more than 5 mm long that does not extend between the tops of two mucosal folds
Grade C	One (or more) mucosal break that is continuous between the tops of two or more mucosal folds but which involve less than 75% of the circumference
Grade D	One (or more) mucosal break which involves at least 75% of the esophageal circumference

4- Oesophageal Manometry Study : We studied our cases using Sandhill Smart Lab. Computer Polygraph Manometry system.

Interpretation of the results :

1- Resting lower esophageal sphincter (LOS) Pressure : Two popular ways to measure the LOS pressure are from gastric baseline to the either mid-expiration or end-expiration pressure at the highest point. We used mid-expiration pressure because it provides a resting LOS pressure measurement that most reliably distinguishes patients with normal of gastro-oesophageal reflux from those with abnormal amounts. Thus the pressure component contributed by the diaphragm during respiration may be an important component of the antireflux mechanism of the LOS and should be included in the assessment of overall resting pressure. We considered normal value of mid-expiration LOS pressure 24.4±10.1 mmHg according to Ott et al. [16].

2- Oesophageal body motility : Measures are made for the peristaltic parameters: amplitude, and duration. Usually 10 wet swallows are assessed and parameters are based on the mean.

The values given below are considered to be standard for wet swallow [16]:

Normal oesophageal body pressure data :

Wet swallows	Duration (sec)
Amplitude (mmHg)	
62 ± 29	2.8 ± 0.8
70 ± 32	3.5 ± 0.7
99 ± 40	3.9 ± 0.9

5- Ambulatory Oesophageal Ph Monitoring Interpretation : Acid reflux was defined where the PH in the oesophagus dropped to 4.0 or less. In the analysis of oesophageal PH recording, different parameters were estimated including :

The variables advocated by Johnson and Demeester [17] :

a- Parameter : Percent of time pH <4	**Normal value**
% time reflux upright	< 6.3
% time reflux supine	< 1.2
% time reflux total	< 4.2
b- Number of episodes:	
Total episodes	< 50
Episodes longer 5 min	< 3
Longest episode (min)	< 9.2
c- Composite score	< 22

6- Histological Examination : Diagnosis of EE is based on criteria described in diagnosis and treatment guidelines published in 2007. According to these guidelines, diagnosis is confirmed in patients with :

- Symptoms suggesting esophageal dysfunction,
- ≥15 eosinophils per HPF (×400) in at least one esophageal biopsy samples, and
- Exclusion of other causes of esophageal eosinophilia [18].

Biopsy specimens were fixed in 4% formalin, embedded in paraffin, serially, sectioned and then stained with hematoxylin and eosin. Biopsy. Preparation and step serial sections of biopsy specimens were performed to enhance detection of eosinophils [19].

7- Immunohistochemistry : To detect eosinophilic count in oesophageal biopsies in both groups which refers to the process of detecting antigens of eosinophiles in tissue sections by exploiting the principle of antibodies binding specifically to antigens in biological tissues [20].

RESULTS

The mean , SD (X ± SD) and range of patients ages with refractory GERD (group I) were 37 ± 14 and 20-70 years respectively. In addition 23(46%) were males and 27(54%) were females.While the demographic data in patients with GERD (group II) show the mean , SD and range of ages were 32.9 ± 13 and 18-68 respectively in addition 24 (48%) were males and 26 were females (52%).There is no statistical significant difference in demographic data between the two groups.

The clinical picture of the studied patients showed that in group I heart burn, epigastric pain, vomiting, dysphagia, haematemsis and cough were 60%, 40%, 32%, 30%, 8%, 18% respectively while in group II they were 82%, 50%, 24%, 14%, 22% and 20% respectively.There is statistical highly significant difference between the two groups only as regard heart burn.

The associated allergic diseases in the studied patients such as bronchial asthma, allergic rhinitis, allergic conjunctivitis and autoimmune diseases in group I were 10%, 10%, 4% and 8% while in group II were 12%, 4%,4% and 2% respectively without statistical significant differences between both groups.

The endoscopic finding in the studied groups showed pathological changes as esophageal rings and furrows in 2 patients of group I (eosinophilic esophagitis patients).Los Angles classification showed that grade A,B,C and D were 50%, 26%, 16% and 8% in group I while in group II were 82%, 2%, 2% and 2% respectively. There was highly statistically significant difference between the two groups as regard endoscopic grading (table1).

The motility study in group I showed hypo peristalsis in 94%, hyper peristalsis in 6% and hypotensive lower oesophageal sphincter(LES) in 92% while in group II were 70% hypo peristalsis, 30% hyper peristalsis and 80% hypotensive LES. There was highly significant difference between the two groups as regard the peristalsis but no significant statistical difference in the lower esophageal sphincter tone. 24- pH monitoring of group I showed that reflux state was present in 18% and absent in 82% of patients but in group II present in 86% and absent in 14%. Reflux frequency were few in 10% and many in 90% in group I patients while was few in 68% and many in 32% in group II. There is highly statistical difference between both groups as regard the state of reflux (frequency and number of attacks).

There was no statistical significant difference between both groups as regard the count of blood eosinophils and esophageal biopsies eosinophils by histopathology . The eosinophilic count in the esophageal biopsies by immunohistochemistry revealed mean ± SD of eosinophilic count in esophageal biopsies of group I was 2.72 ± 4.6 (range 0-27) while in group II were 0.76 ± 1.5 (range 0-5).There was highly significant difference between both groups (table 2). There was strong correlation between the eosinophilic count in esophageal biopsies by histopathology and immunohistochemistry ($r \geq .97$, $p < 0.001$).

The histopathological features in patients of EE patients (2 patients) showed the characters of EE; eosinophils ≥15/HPF, increased lamina propria papillae in 100%, basal zone hyperplasia in 50% and microabcess in 50% while in other refractory GERD patients(48 patients) showed oedema in 58%, neutrophils in 16%, fibrosis in 4.8% and eosinophils ≤6 cells /HPF in 6.3% with highly significant statistical difference between the two groups in microabcess, lamina propra papillae, oedema, basal zone hyperplasia, fibrosis and eosinophilic count and significant difference in neutrophils count (table 3).

Table (4) shows the histopatholigical findings of studied groups that reveal eosinophilic count (mean ± SD) in group I: 2.6 ± 4.5 (rang 0-27), lymphocyte not detected in 78% and detected in 22%, microabcess in 4%, fibrosis of lamina propria detected in 2% while leucocytes detected in 2% but group II showed eosinophilic count: 8 ± 1.5 (range 0-5), lymphocyte, fibrosis, microabcess and leucocyte were not detected.There was highly significant difference between both groups as

regard eosinphilic count and fibrocytes detected in the esophageal biopsies.

Table (1) : Upper GIT Endoscopy of studied patients (Los Angeles classification)

	Group I		Group II		X^2	P
	No	%	No	%		
Pathological finding						
Rings	2	4	0	0	2.04	0.15 (NS)
Furrows	2	4	0	0	2.04	0.15 (NS)
Stricture	2	4	0	0	2.04	0.15 (NS)
Hiatus hernia	10	20	41	82	2.99	0.08 (NS)
Grade of GERD						
A	25	50.0	41	82.0	9.2	0.001* (HS)
B	13	26.0	1	2.0	8.9	0.004* (HS)
C	8	16.0	1	2.0	7.8	0.002* (HS)
C	4	8.0	1	2.0	12.9	0.004* (HS)

Table (2) : Immunohistochemistry of esophageal biopsies of the studied patients

	Group I	Group II	X^2	P
Eosinophils				
X ± SD	2.72 ± 4.6	0.76 ± 1.5	10.09	0.0014** (HS)
Range	0 - 27	0 - 5		

Table (3) : Histopathological features in patients with EE and gastro-esophageal reflux

	EE patients (N = 2) Mean (range)		Refractory GERD (N = 48) Mean (range)			
Eosinophil/HPF	20 (12-25)		3 (0-6)			
	No	%	No	%	X^2	P
Micoabcess	1	50	0	0	9.4	0.001 (HS)
Increasea lamina propra papillae	2	100	3	6.3	12.1	0.003(HS)
Basal zone hyperplasia	1	50	4	8.3	8.1	0.004 (HS)
Oedema	0	0	28	58.3	7.6	0.004 (HS)
Neutophils	0	0	8	16.6	5.4	0.03 (SD)
Eosinophils	2	100	3	6.3	10.4	0.001(HS)
Fibrosis	1	50	2	4.8	6.7	0.003(HS)

Table (4) : Histopathology of studied groups

	Group I		Group II		X^2	P
	No	%	No	%		
Eosinophil count						
X ± SD	2.6 ± 4.5		0.8 ± 1.5		8.6	0.003* (HS)
Range	0 - 27		0 - 5			
Lymphocytes						
Not detected	39	78.0	50	100.0	12.36	0.001* (HS)
Detected	11	22.0	0	0.0		
Leucocytes						
Not detected	48	96.0	50	100.0	2.04	0.15
Detected	2	4.0	0	0.0		(NS)
Microabscess						
Not detected	48	96.0	50	100.0	2.04	0.15
Detected	2	4.0	0	0.0		(NS)
Fibrosis lamina pro.						
Not detected	48	96.0	50	100.0	2.04	0.15
Detected	2	4.0	0	0.0		(NS)

DISCUSSION

Although we had known that eosinophilic esophagitis (EE) prevalence is low among the population, this study was conducted to through the light towards this disease.

One hundred patients were enrolled in this study, 50 patients with refractory GERD (group I) and the other with non refractory GERD (group II). All patients were studied clinically, endoscopically, manometerically and histopathologically to evaluate the presence of eosinophilic infiltration and its role in refractory esophageal pathology.

The demographic data of this study showed no statistical significant difference among the patients sex of two groups, with GERD either with or without refractory entity. This finding is in agreement with Vindigni et al. [21] but in contrary with Forountan et al. [22] who mentioned that the disease show female predominance. This difference can be explained by age and parity difference. The same findings of non-statistical significant difference were founded as regarding the age. This is consistent with Dellon et al. [23], but in contrary with Liacouras et al. [24] who mentioned that refractory GERD was more predominant in old ages. This can be explained by the effect of aging on the LES tone [25].

The clinical picture showed no statistical significant difference between both groups as regard dysphagia, vomiting, haematemsis, itching, cough, pallor, wheezes and urticaria, but there is a statistical significant difference as regard heart burn. These results were in agreement with Molina-Infante et al. [26], and not in agreement with Aceves et al. [27] who mentioned that the dysphagia is the most common presenting symptoms in patients with refractory GERD. This difference can be explained by variability in the duration of disease. Where Richter [28] reported that the more advanced pathological findings were encountered in patients with long history of disease, as they selected their patients with long history of GERD.

The associated disease as allergic rhinitis, allergic conjunctivitis, bronchial asthma and auto immune diseases showed no statistical significant difference between the studied groups and these results were in agreement with Martinez et al. [29], but in contrary with Liacouras et al. [24] who stated that 28% of their patients suffer from associated allergic disease and this can be explained by relative low rate of allergic diseases in both groups of patients in this study.

The endoscopic finding in the studied groups showed pathological changes as esophageal rings and furrow in eosinophilic esophagitis patients (2 patients) and this was in agreement with Venge et al. [30] but in contrary with Remedios et al. [9] who stated that, plaques were the most common endoscopic findings in their patients but none was diagnosed with a ringed esophagus which is one of the most typical findings in the previous studies, together with linear furrows. This difference can be explained by seasonal variation and multiple variety in the endoscopic picture of EE [31]. There was a statistically significant difference between the two groups as regard endoscopic grading of esophagitis. These result

was in agreement with Dellon et al. [23], but in contrary with Shah et al. [32] who stated that no endscopic difference among their studied groups. This variation can be explained by difference in operator experience, prevalence of EE and treatment used before endscopy. The more pathological changes may be attributed to the events which reported by Hirano [33] as prolonged exposure of esophageal mucosa to acid, partial response of EE to PPI therapy and toxic effect of eosinophilic degranulation into the tissue.

The motility study of both groups showed highly statistical significant difference between the two groups as regard the peristalsis but no statistical difference in the lower esophageal sphincter tone. These result were in agreement with Pandolfino et al. [34] but in contrary with Tian et al. [35] and this difference can be explained by finding of Mueller et al. [36] as variations in factors controlling esophageal peristalsis and lower esophageal sphincter such as diet, hormonal, nervous, drugs and eosinophilic granule constituents which are toxic to a variety of tissues, including esophageal epithelium. Study of 24-ph monitoring of patients group shows a significant statistical difference between both groups as regard the state of reflux(frequency and number of attacks).These result in agreement with Weusten et al. [37], but differ with Mattioli et al. [38] who stated that 25% of GERD with esophagitis have 24 h pH monitoring within normal range. This variation can be explained by difference in defensive factories such as LES and luminal clearance of acids [39] or variation of injurious factors such as prolonged transient esophageal sphincter relaxation, hiatus hernia and blood supply [40].

As regard the eosinophilic count in peripheral blood which showed no statistical significant difference between patients of both groups and this finding is in agreement with Gonsalves et al. [41]. It is known that EE is a chronic immuno-allergic disorder characterized by clinical symptoms related to esophageal dysfunction and eosinophilic infiltration in the esophagus regardless the peripheral blood eosinophilic count [42]. The relation between peripheral blood eosinophils and esophageal biopsies eosinophilis showed no statistical significant difference. This result was in agreement with Attwood et al. [25], however, the result of this study was differing with Brown et al. [43]. This variation can be explained by more advances in diagnostic tools and nowadays EE is considered a local allergy of esophagus to foods (asthma of esophagus) [44]. Regarding to the eosinophilic count in the esophageal biopsies by immuno-histochemistry revealed a statistical significant difference between both groups. These result was in agreement with Leader et al. [45]. Study of esophageal eosinophilia by H&E and immune histochemistry showed highly statistical significant difference between eosinophilic count by H&E stain and immuno-histochemistry stain. This result was in agreement with Mueller et al. [36] who stated that the immunohistochemistry detected up to two times more eosinophils than routine haematoxylin and eosin staining and can perhaps be used to discover minimal change EE. We did not find any study differ with this result as eosinophils morphologically are easily to see in conventional histology. Moreover, it is almost impossible to identify degranulated eosinophils with haematoxylin and eosin staining [46].

Correlation between eosinophilic count in esophageal biopsies by histopathology and immune histochemistry showed highly statistical significant correlation. This results were in agreement with Onbasi et al, [47] who reported that immune histochemistry using monoclonal anti body is the most sensitive method for eosinohils detection.

The histopatholigical finding in group I that showed a highly statistical significant difference between the 2 EE patients and other 47 refractory GERD patients as regard eosinphilic count, fibrocytes, oedema, basal cell hyperplasia and fibrosis detected in the esophageal biopsies these finding were in agreement with Spergel et al. [48] who founded that eosinoplic infiltration of esophageal mucosa ≥15 cells/hpf, oedema, basal cell hyperplasia and fibrosis are charachterstic of EE.

The histopathological finding in both groups showed highly statistical significant differences in tissue eosinophilic count and lymphocyte but no statistical differences in leucocyte, microabcess and fibrosis lamina propria. These were in agreement with Straumann et al. [49]. The cause of eosinophilic infiltration is poorly understood, but allergy has been implicated. The majority of patients have evidence of food and aeroallergen hypersensitivity, as defined by skin prick test responses, however, only a minority have a history of food anaphylaxis [50], this indicating distinct mechanisms compared with classical IgE-mediated mast cell/basophil activation. Substantial evidence is accumulating that EE is associated with TH2-type immune responses. In

particular, increased levels of eosinophil-active TH2 cytokines (eg, IL-4, IL-5, and IL-13), as well as mast cells, are present in the esophagi of patients with EE [51].

CONCLUSION

Finally it can be concluded that the eosinophilic esophagitis is one of the causative factors of refractory GERD and its frequency is about 4%.It should be put in differential diagnosis of cases of refractory GERD. Endoscopic picture is suggestive but biopsy for EE is confirmatory. H&E stain appearance is cost-effective approach for diagnosis.

Funding: Non.

Conflicts of interest: The authors declare no conflict of interest.

Ethical approval: The study was approved by the Ethical Committee of Zagazig Faculty of Medicine and a written informed consent was taken from each participant that follows principles in the Declaration of Helsinki.

REFERENCES

1. Yan BM, Shafer EA. Eosinophilic esophagitis. A newly established cause of disphagia. *World J Gastroneterol* 2006; 12: 2328–2334.
2. Sgouros SN, Bergele C, Mantides A. Eosinophilic esophagitis in adults: a systematic review. *Eur J Gastroenterol Hepatol* 2008; 18: 211–217.
3. Moawad FJ, Veerappan GR, Wong RK. Eosinophilic esophagitis. *Dig Dis Sci* 2009; 54: 1818–1828.
4. Prasad GA, Alexander JA, Schleck CD. Epidemiology of eosinophilic esophagitis over three decades in Olmsted Country, Minnesota. *Clin Gastroenterol Hepatol* 2009; 7: 1055–1061.
5. Ronkainen J, Talley NJ, Aro P. Prevalence of eosinophilia and eosinophilic esophagitis in adults in the community: a random population based study. *Gastroenterology* 2007; 130, A575.
6. Mackenzie SH, Go M, Chadwick B. Eosinophilic oesophagitis in patients presenting with dysphagia - a prospective analysis. *Aliment Pharmacol Ther* 2008; 28: 1140–1146.
7. Kerlin P, Jones D, Remedios M, Campbell C. Prevalence of eosinophilic esophagitis in adults with food bolus obstruction of the esophagus. *J Clin Gastroenterol*. 2007 Apr; 41(4):356-61.
8. Müller S, Pühl S, Vieth M. Analysis of symptoms and endoscopic findings in 117 patients with histological diagnoses of eosinophilic esophagitis. *Endoscopy* 2007; 39: 339–344.
9. Remedios M, Campbell C, Jones DM. Eosinophilic esophagitis in adults: clinical, endoscopic, histologic findings, and response to treatment with fluticasone propionate. *Gastrointest Endosc* 2006; 63: 3–12.
10. Sgouros SN, Bergele C, Mantides A. Eosinophilic esophagitis in adults: a systematic review. *Eur J Gastroenterol Hepatol* 2006; 18: 211–217.
11. Krarup AL, Villadsen GE, Mejlgaard E. Acid hypersensitivity in patients with eosinophilic oesophagitis. *Scand J Gastroenterol* 2010; 45:273–281.
12. Ngo P, Furuta GT, Antonioli DA. Eosinophils in the esophagus: peptic or allergic eosinophilic esophagitis? Case series of three patients with esophageal eosinophilia. *Am J Gastroenterol* 2006; 101: 1666–1670.
13. Penfield JD, Lang DM, Goldblum JR. The role of allergy evaluation in adults with eosinophilic esophagitis. *J Clin Gastroenterol* 2010; 44: 22–27.
14. Locke GR, Talley NJ, Weaver AL. A new questionnaire for gastroesophageal reflux disease. *Mayo Clin Proc* 1994; 69: 539–547.
15. Lundell LR, Dent J, Bennett JR. Endoscopic assessment of oesophagitis: clinical and functional correlates and further validation of the Los Angeles classification. *Gut* 1999; 45: 172–180.
16. Ott DJ, Joel ER, Yu MS. Esophageal radiology and manometry correlation in 187 patients with dysphagia. *A.J.R* 1987; 149:307-3011.
17. Johnsom L, De.,Meester. Tewenty four hour ph monitoring of the distal oesphagus.*Am.J Gastro-entrology* 1974;62:322-325.
18. Furuta GT, Liacouras CA, Collins MH. Eosinono-philic esophagitis in children and adults: a systematic review and consensus recommendations for diagnosis and treatment. *Gastroenterology* 2007; 133: 1342–1363.
19. Haggitt R.C., Rubin. Adenocarcinoma in Barrett´s oesophagus:a new epidemic?. *Human pathology* 1992; 23:475-276.
20. Ramos-Vara. "Technical Aspects of Immunohisto-chemistry". *Vet Pathol* 2005;42 (4): 405–426.
21. Vindigni C, Villanacci V, Marini M. Eosinophilic esophagitis: an Italian experience. *Rev Esp Enferm Dig* 2010; 102 (1): 15–19.
22. Foroutan M, Norouzi A, Molaei M. Eosinophilic esophagitis in patients with refractory gastro-esophageal reflux disease. *Dig Dis Sci* 2010; 55 (1): 28–31.
23. Dellon ES, Aderoju A, Woosley JT. Variability in diagnostic criteria for eosinophilic esophagitis: a systematic review. *Am J Gastroenterology* 2007; 184: 14-18.

24. Liacouras CA, Spergel JM, Ruchelli E. Eosinophilic esophagitis: a 10-year experience in 381 children. *Clin Gastroenterol Hepatol* 2005; 3: 1198–1206.

25. Attwood SE, Smyrk TC, Demeester TR. Esophageal eosinophilia with dysphagia. A distinct clinico-pathologic syndrome. *Dig Dis Sci* 1993; 38:109–116.

26. Molina-Infante J, Ferrando-Lamana L, Ripoll C. Esophageal eosinophilic infiltration responds to proton pump inhibition in most adults. *Clin Gastroenterol* 2011; 9: 110–117.

27. Aceves SS, Newbury RO, Chen D. Resolution of remodeling in eosinophilic esophagitis correlates with epithelial response to topical corticosteroids. *Allergy* 2010; 65 (1): 109–116.

28. Richter J.E. Peptic stricture of the esophagus Gastroenterology. *Clinics of North America* 1999; 31: 875–891.

29. Martinez SD, Malagon IB, Garewal HS, Cui H, Fass R. Non-erosive reflux disease (NERD)-acid reflux and symptom patterns. *Aliment Pharmacol Ther* 2003; 15; 17(4):537-45.

30. Venge P, Byström J, Carlson M, Hâkansson L, Karawacjzyk M, Peterson C et al. Eosinophil cationic protein (ECP): molecular and biological properties and the use of ECP as a marker of eosinophil activation in disease. *Clin Exp Allergy* 1999; 29(9):1172-86.

31. Rodrigo S, Abboud G, Oh D, DeMeester SR, Hagen J, Lipham J, DeMeester TR, Chandrasoma P. High intraepithelial eosinophil counts in esophageal squamous epithelium are not specific for eosinophilic esophagitis in adults. *Am J Gastroenterol* 2008; 103(2):435-42.

32. Shah A, Kagalwalla AF, Gonsalves N, Melin-Aldana H, Li BU, Hirano I. Histopathologic variability in children with eosinophilic esophagitis. *Am J Gastroenterol* 2009; 104(3):716-21

33. Hirano I. Eosinophilic esophagitis and gastro-esophageal reflux disease: there and back again. *Clin Gastroenterol Hepatol* 2011; 9(2):99-101.

34. Pandolfino JE, Bianchi LK, Lee TJ, Hirano I, Kahrilas PJ. Esophagogastric junction morphology predicts susceptibility to exercise-induced reflux. *Am J Gastroenterol* 2004; 99(8):1430-6.

35. Tian ZQ, Liu JF, Wang GY, Li BQ, Wang FS, Wang QZ, Cao FM, Zhang YF. Responses of human clasp and sling fibers to neuromimetics. *J Gastroenterol Hepatol* 2004; 19(4):440-7.

36. Mueller S, Aigner T, Neureiter D, Stolte M. Eosinophil infiltration and degranulation in oesophageal mucosa from adult patients with eosinophilic oesophagitis: a retrospective and comparative study on pathological biopsy. *J Clin Pathol* 2006; 59(11):1175-80.

37. Weusten BL, Roelofs JM, Akkermans LM, Van Berge-Henegouwen GP, Smout AJ. The symptom-association probability: an improved method for symptom analysis of 24-hour esophageal pH data. *Gastroenterologl* 1994; 107(6):1741-5.

38. Mattioli S, Pilotti V, Spangaro M, Grigioni WF, Zannoli R, Felice V, Conci A, Gozzetti G. Reliability of 24-hour home esophageal pH monitoring in diagnosis of gastroesophageal reflux. *Dig Dis Sci* 1989; 34(1):71-8.

39. Orlando RC. Reflux esophagitis. In: YamadaT, Alpers DH, OwyangC, PowellDW, SilversteinFE, eds. Textbook of Gastroenterology. Philadelphia: JB Lippincott 1999; 1123.

40. Tobey NA, Hosseini SS, Caymaz-Bor C, Wyatt HR, Orlando GS, Orlando RC. The role of pepsin in acid injury to esophageal epithelium. *Am J Gastroenterol* 2001; 96(11):3062-70.

41. Gonsalves N, Policarpio-Nicolas M, Zhang Q, Rao MS, Hirano I. Histopathologic variability and endoscopic correlates in adults with eosinophilic esophagitis. *Gastrointest Endosc*. 2006; 64(3):313-9.

42. Franciosi JP, Hommel KA, Bendo CB, King EC, Collins MH, Eby MD et al. PedsQL eosinophilic esophagitis module: feasibility, reliability, and validity. *J Pediatr Gastroenterol Nutr* 2013; 57(1): 57-66.

43. Brown LF, Goldman H, Antonioli DA. Intraepithelial eosinophils in endoscopic biopsies of adults with reflux esophagitis. *Am J Surg Pathol* 1984; 8(12): 899-905.

44. Rothenberg ME, Mishra A, Brandt EB, Hogan SP. Gastrointestinal eosinophils in health and disease. *Adv Immunol* 2001;78:291-328.

45. Leader M, Patel J, Makin C, Henry K. An analysis of the sensitivity and specificity of the cytokeratin marker CAM 5.2 for epithelial tumours. Results of a study of 203 sarcomas, 50 carcinomas and 28 malignant melanomas. *Histopathology* 1986 ;10(12):1315-24.

46. Levy AM, Yamazaki K, Van Keulen VP, Burgart LJ, Sandborn WJ, Phillips SF et al. Increased eosinophil infiltration and degranulation in colonic tissue from patients with collagenous colitis. *Am J Gastroenterol* 2001 ;96(5):1522-8.

47. Onbasi K, Sin AZ, Doganavsargil B, Onder GF, Bor S, Sebik F. Eosinophil infiltration of the oesophageal mucosa in patients with pollen allergy during the season. *Clin Exp Allergy* 2005; 35(11): 1423-31.

48. Spergel JM, Beausoleil JL, Mascarenhas M, Liacouras CA. The use of skin prick tests and patch tests to identify causative foods in eosinophilic esophagitis. *J Allergy Clin Immunol* 2002; 109(2): 363-8.

49. Straumann A, Bauer M, Fischer B, Blaser K, Simon HU. Idiopathic eosinophilic esophagitis is associated with a T(H)2-type allergic inflammatory response. *J Allergy Clin Immunol* 2001; 108(6): 954-61.

50. Fox VL, Nurko S, Furuta GT. Eosinophilic esophagitis: it's not just kid's stuff. *Gastrointest Endosc* 2002; 56(2):260-70.

51. Blanchard C, Wang N, Stringer KF, Mishra A, Fulkerson PC, Abonia JP et al. Eotaxin-3 and a uniquely conserved gene-expression profile in eosinophilic esophagitis. *J Clin Invest* 2006; 116(2):536-4.

Peer reviewer:Mostafa Elshami ;Professor of Tropical Medicine and Hepatogastroenterology,Faculty of Medicine , Zagazig University, Egypt.**Editor:Mohamad Emara**; Lecturer of Tropical Medicine and Hepatogastroenterology, Faculty of Medicine , Zagazig University, Egypt.

Epidemiological Studies on *Strongyloides stercoralis* at Dilla District, Ethiopia

Feleke Eriso
Parasitologist, Department of Biology, Dilla University, Dilla, Ethiopia

Corresponding Author
Feleke Eriso

Mobile:
+251916514682

E mail:
feleke.eriso@yahoo.com

Key words:Strongyloides stercoralis; Diagnosis; Morbidity; Mortality; Treatment; Immunocompetent

Background and study aim: Some authors have accepted parthenogenesis or asexual reproduction and hermaphroditism (protandrogony) to be the only mode of reproduction of parasitic female *Strongyloides stercoralis* in human hosts as parasitic males of it did not exist in human hosts. Therefore, the first objective was to work out the infection rate of *Strongyloides stercoralis* in the population of elementary schools children at Dilla district; secondly, to produce a visible evidence for the prese nce of many parasitic males of *Strongyloides stercoralis* as there are parasitic femal -es in fresh stools samples of human hosts; and thirdly, to replace the unfit term by a correct one.

Patients and methods: Stools samples were collected from student children of elementary schools, and observed under microscope in the laboratory of parasitology after employing Baermann apparatus technique.

Results: In the study a total of 710 student children were examined for *Strongyloides stercoralis* infection out of whom 142 (in 1st study) were positive, confirming the infection rate to be 20% or 198 positive (in 2nd study) the infection rate being 28% by the parasite. Then, the average infection rate was 24%. All developmental stages and sexes of the parasite were obtained in the study executed.

Conclusion: The presence of many parasitic males with everted spicules observed in fresh stools samples during this study had been a very strong evidence for the fact that male and female copulation & fertilization were naturally taking place among parasitic worms of *Strongyloides stercoralis* in human hosts. Parasitic and free living males of *S. stercoralis* have the same morphology including the curved or coiled posterior body part.

INTRODUCTION

Strongyloides stercoralis is known to have two life cycles: an internal sexual cycle, involving parasitic worms that constitute the parasitic generation, and the external sexual cycle, interacting among free-living worms that represent the free-living generation in soil [1-3]. It had been stated by authors in modern textbooks, journals, and on the internet that the type of reproduction in the parasitic generation of *S. stercoralis* in human hosts was only by parthenogenesis of parasitic females in the absence of parasitic males [4-7]. Due to this concept of parthenogenesis the parasitic male had been omitted in the figures that demonstrated the life cycle of the parasitic generation of *S. stercoralis* in all modern and relevant textbooks, journals, and on the internet. Not only that, there was an article on the internet which stated that the parasitic generation of *S. stercoralis* was known not to have parasitic males and the parasitic females used to reproduce only by the asexual method of reproduction [8]. In the parasitic generation, when the filariform larvae are in contact with skin, they penetrate the small cutaneous blood vessels and are carried through the right heart to the lungs [9]. Then, sexually mature parasitic females settle in the tissues of epithelial mucosa to lay eggs that hatch soon and are discharged in the stools each day [10,11]. When all or some larvae metamorphose into infective filari-form larvae autoinfection may be onset by invading the mucosa of the ileum or colon, travel to lungs a nd then return to the intestine to mature in the mucosa [12-15].

Disseminated strongyloidiasis had been reported in both of two recipients of kidney allografts from a single cadaver donor [16]. It was also reported that in a 53-year-old man who had lung cancer, fulminantly fatal strongyloidiasis had developed following postchemotherapy of immunosuppression, resulting in the death of the patient within 48 hrs [16]. The development of a florid strongyloidiasis was observed in a 45-year-old man, following anticancer chemotherapy when eggs of *S. stercoralis* were seen in the stools [17]. One scientific study has reported that almost all deaths due to helminths in the United States result from *S. stercoralis* hyperinfection mortality rates because the occurrence of hyperinfection can be as high as 87% [18].

Aim of the study

The aim of the study has three objectives to work out:

- First, to determine the infection rate of *S. stercoralis* in the population of elementary schools children at Dilla district;
- Second, to produce a visible evidence for the presence of many parasitic males of *S. stercoralis* as there are parasitic females in fresh stools samples of human hosts; and
- Thirdly, to replace the unfit morphologic term by a correct one. Is parthenogenesis or asexual reproduction true in the parasitic generation of *S. stercoralis* in human hosts?

Concerning some morphological features of this parasite, the part of the worm's body that is known as the tail is the posterior part of body beginning from cloaca in the parasitic males or beginning from anus in the parasitic females. Cloaca is the opening through which spicules are everted at times of copulation & fertilization and it is also the outlet of the digestive tract. The 3 stages of human strongyloidiasis are Intestinal Strongyloidiasis, Gastropulmonary Strongyloidiasis and Disseminated Strongyloidiasis [19-21]. Some of the clinical presentations of strongyloidiasis can be highlighted as:

- Cutaneous with larva currens (racing larvae), pruritic linear or serpiginous, creeping urticarial eruption, dermatologic lesions, and petechiae;
- Pulmonary with persistent wheezing, cough, and deteriorating respiratory status; and
- Intestinal with vomiting, abdominal pain, watery diarrhea and constipation.

PATIENTS AND METHODS

The suitable type of study selected to answer the question of this research work was the Cross Sectional Study. The statistical methods pre-planned to be employed in analyzing and interpreting the results were the expression by percentage and standard deviation.

Eight different elementary schools found at Dilla district were selected to be the sites of fresh stools sample collection from student children. It is important, from the ecological and geographical points of view, to notify that Dilla district is located in Gedio Zone that is found in:

- Southern Ethiopia,
- The continent of Africa, and
- The northern hemisphere between the tropic of cancer and the equator.

Collection of fresh stools samples and documenting related information from the schools had been carried out from 6/12/2006 to 10/06/2007 and again repeated in depth from 10/9/2008 to 25/6/2009. However, the deliberate follow up to be certain about the prevalence and persistence of the parasite at Dilla district, was performed by taking fresh stools samples of ten students from each of the eight different elementary schools every year from 2000 up to the beginning of 2014.

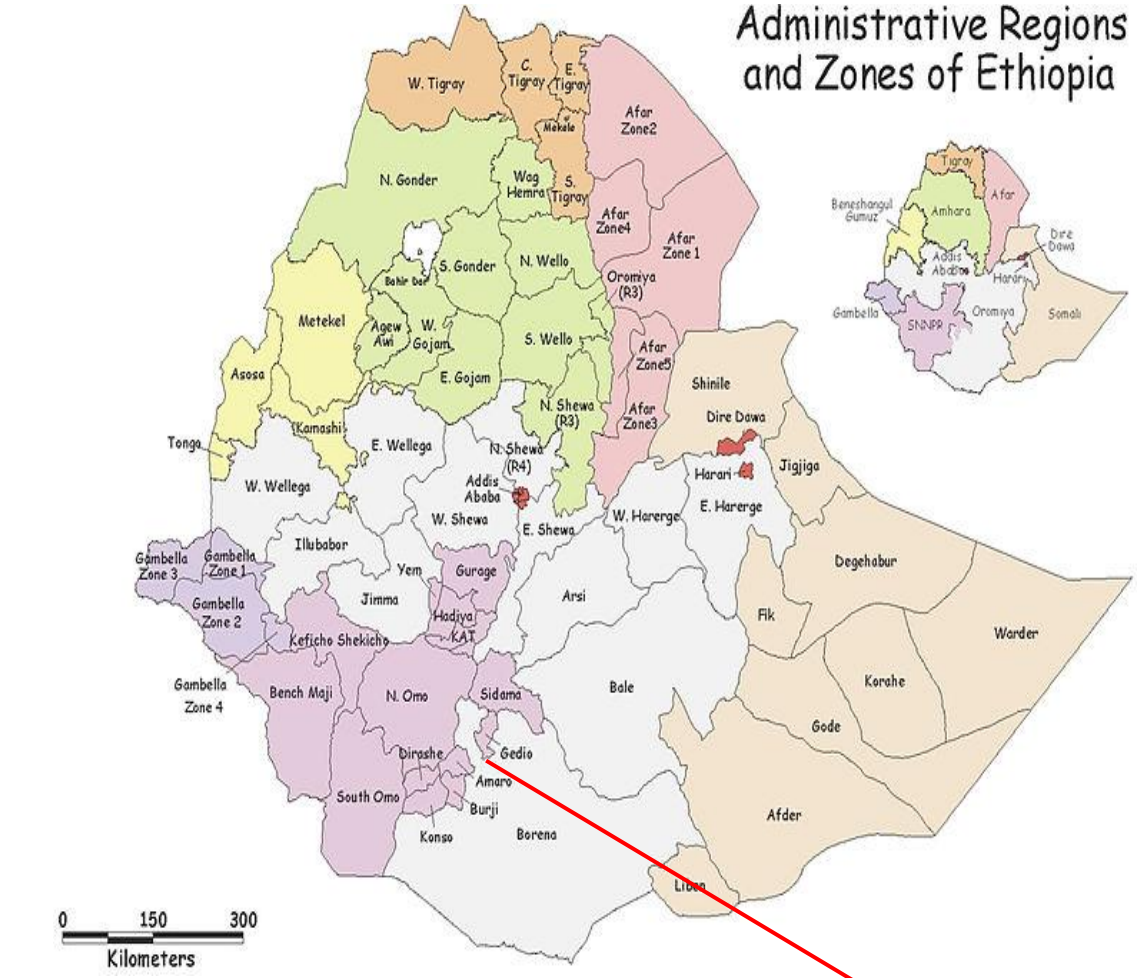


Figure (1): Map of administrative regions and zones of Ethiopia.

Specific sample size:

The sample size taken from the participant student children was 710. Each day, Monday through Friday (i.e. every week), fresh stools samples, of ten student children were taken to parasitology laboratory of Dilla University. When the sample was taken from the student child, he/she gave fresh stools sample in a bottle on which his/her I.D. No. was written. In addition to this, on that very day and moment a table that had columns with the headings of Date, Name of Child, I.D. No. of child, Class (grade), Age in Year, Sex, *S. stercoralis*, Education of parents, and Job of parents were filled by including the participation of the child for the necessary information, with the exception of the column under *S. stercoralis*, because it had to be filled either "–" or "+" for *S. stercoralis*, by the researcher after examining the fresh stools sample. Writing the name of the student child in the steps of raw data collection was important to identify the child for giving treatment if he/she had been found to be positive for the parasite, because many children could not remember their I.D. No. Of course, it was decided not to write the name of the student child in the report of the article.

Diagnostic Examination:

The diagnostic examination of fresh stools sample of each student child involved the following nine steps.

- Baermann funnel apparatus was constructed and the lower opening of the rubber tubing fitted to the stem of the funnel was closed.
- Water warmed to 40^0 C was poured into the funnel of the Baermann apparatus and the cheese cloth, that contained the fresh stools sample of the student child and tied with its peripheral edges to the rim of the funnel, was partially immersed in the water warmed to 40^0 C. This was done because if adults as well as juveniles of *S. stercoralis* were present in the stools, they would be attracted by the warm temperature of water (about 37.5^o C as there was dissipation of heat from the initial 40^o C of the added water to the surrounding materials and equipment) and escape into the warm water through the pores of the cheese cloth.
- After staying 1 hour and 30 minutes, the closed lower end of the rubber tubing was opened, releasing the water found in the funnel of Baermann apparatus into a 500 ml beaker. The stools left behind in the cheese cloth was thrown into the tube of toilet after being treated with a disinfectant (iodine solution) and washed away by a current of water.
- The water released and collected in the 500 ml beaker was centrifuged at a speed of 1000 rpm (revolutions of the rotor per minute) for 2 minutes using a manual centrifuge loaded with 4 centrifuge tubes and anchored to the edge of a table.
- From each centrifuge tube the supernatant was poured off into a waste collecting bucket to be thrown into the tube of toilet drainage line by treating with the disinfectant.
- Using a dropper, about 2 ml of the supernatant was added to the sediment of one of the 4 sediment containing centrifuge tubes and shaked well by closing its mouth with its own fittingly tight lid. The action of shaking was to change the sediment into a transferable suspension. The same suspension was
- Transferred to each of the remaining 3 centrifuge tubes one by one where in each case the centrifuge tube was shaked well and the sediment was changed into suspension.
- Next, the sediment collected in the form of suspension from 4 centrifuge tubes was poured into a test tube labeled with the I.D. No. of the student child from whom the fresh stools sample was taken to be examined. These seven steps were repeated for the fresh stools sample of each of the remaining 9 student children.
- Using a dropper, a drop of suspension was taken from the surface of bottom sediment of the labeled test tube suspension and placed on a clean glass slide and then covered with a cover slip. The preparation was examined under the low power objective of a research microscope to confirm the presence or absence of *S. stercoralis* in the fresh stools sample of the student child. The sample of each student child was examined in this way. The column under the heading of *S. stercoralis* for each student child was marked "–" indicating the absence or "+" confirming the presence of *S. stercoralis* in the fresh stools sample taken.
- The suspensions positive for *S. stercoralis* were fixed and preserved by adding 10% formaldehyde. Each container bottle of preservation in 10% formaldehyde was labeled *S. stercoralis* larvae/other stages including the date of collection and kept in a safe place in the laboratory of parasitology. Water emergence semi-concentration technique for detecting strongyloides larvae in feces was also used when there were needs to supplement the Baermann method **[22]**.

Water emergence semi-concentration technique for detecting S. stercoralis larvae in feces:

- A fresh (not more than 2 hours old) formed or semi-formed fecal specimen is required. The method is as follows:
- Using a piece of stick, make a central depression in the specimen contained in a vial or bottle. Fill the depression with warm water (about 37.50ºC).
- Incubate the specimen in a 35-37.50ºC incubator for 1.5 to 3 hours during which time the larvae will migrate out of the feces into the warm water.
- Using a plastic bulb pipette or Pasteur pipette, transfer some of the water to a slide and cover with a cover glass. Alternatively, transfer all the water to a conical tube, centrifuge, and transfer the sediment to a slide.
- Examine the preparation, under the low or middle power objective lens of a compound light microscope, for motile larvae of *S. stercoralis*.

Treatment :
The drug, that was available to treat the student children infected with *S. stercoralis* and ordered by the medical doctor assigned to assist the researcher of this study, was albendazole (Avion: Nabros, England) in the study project of 6/12/2006 to 10/06/2007. On the other hand, the drug of choice ordered by the medical doctor in the study of 10/09/2008 to 25/06/2009 was ivermectin (Ochoa: Ravenbhel, India).

The dose of albendazole:
Each infected child whose age was 9 years and above was advised to take two albendazole tablets at one time after dinner immediately before going to bed for night sleep daily for two consecutive days whereas those whose ages were 8 years and below were given 1 bottle (20 ml) albendazole oral suspension to take after dinner immediately before going to bed for night sleep daily for three consecutive days. It was notified that each tablet contained 200 mg albendazole USP whereas each bottle (20 ml) contained 400 mg albendazole USP.

The dose of ivermectin:
The prescription was stated as follows in proportion to individual student child's body weight. (Note: in this particular ivermectin 1 tablet is 6 mg in weight).

Body-weight	6 mg tablet of ivermectin
15-24 kg	0.5 tablet, single dose on empty stomach.
25-35 kg	1 tablet, single dose on empty stomach
36-50 kg	1.5 tablets, single dose on empty stomach
51-65 kg	2 tablets, single dose on empty stomach
66-79	2.5 tablets, single dose on empty stomach

Each student child was advised to take the tablet/s with a glass of water in the morning after waking up from bed and begin taking meal at noon.

Growth of free-living generation of *S. stercoralis* in the autoclaved topsoil in petridish incubated at 28^0C.

Topsoil that contained organic substance was taken and put into three different petridishes. Each of the petridishes was closed with its own lid and labeled 1, 2, and 3.

- Next, the petridishes with their contents of topsoil were autoclaved.
- The topsoil autoclaved in each of the Petri-dishes was inoculated with *S. stercoralis* from fresh stools sample obtained from a student child, infected with *S. stercoralis*, before he had been given treatment.
- Excess water was added to the topsoil of all the three petridishes and were incubated at 28^0 C on the same day.
- The topsoil of petridish No. 1, 2, and 3 were examined, using Baermann funnel apparatus technique to check the growth of free-living generation of *S. stercoralis*, after 11, 30, and 48 days of initial incubation respectively.
- The worms of the free-living generation of *S. stercoralis* collected from the three petridishes of topsoil using Baermann techniquewere fixed and preserved in 10% formaldehyde to be used for the preparation of permanent slides **[5]**.

Method of Safranin stain preparation :
I.1. Safranin O stock solution: Dissolve 2.5g safranin O Certistain in 100 ml of 96% ethanol. This is a stock solution.
2. For use: 10 ml of stock solution should be di luted with 90 ml of distilled water [23,24].
OR
II. 1. Safranin powder……0.1 g
2. Distilled water…….100 ml
The safranin powder is dissolved in the distilled water measured above.

Preparation of permanent slides and microphotographs :
In short, the preparation of *S. stercoralis* permanent slides was effectively done by applying the following Yetwin mounting medium **[25]**.

Yetwin Mounting Medium:
i 1. 10% bacto-gelatin, granular, aqueous 150.0 ml
2. Glycerin 50.0 ml
3. 1% chromium potassium sulfate aqueous (Chromium) 100.0 ml
4. Phenol (carbolic acid), melted 1.0 ml

ii. Gelatin was dissolved in boiling water (i.e., a 400 ml beaker, into which 10 g of gelatin & 90 ml of pure water were added, was immersed in a volume of boiling water in a larger heat-resistant dish) and glycerin was added to it. After mixing glycerin and 10% gelatin solution, 1% chromium potassium sulfate solution and phenol were added to the mixture of glycerin and 10% gelatin solution. The medium was liquefied in 15 minutes at 65ºC.

iii. Thereafter, the *S. stercoralis* worms were transferred from 10% formaldehyde directly into a drop of mounting medium, placed on a clean slide. The mounting medium with the worms was covered with a cover slip.

iv. Then, within overnight the gelatin hardened to form a permanent slide of *S. stercoralis* worms.

From the permanent slides prepared microphotographs of the larvae and other stages of S. stercoralis were taken using a digital camera from the fields of vision under suitable objective lenses of the compound light microscope.

RESULTS

The infection rate of *S. stercoralis* in the population of student children of elementary schools at Dilla district was 20% in the first study project (conducted during 6/12/2006 to 10/6/2007), but in the second one (done during 10/9/2008 to 25/6/2009), it went up to 28%. Why was that so? That was so, because a larger amount of sample size was taken & included, in the second study project than in the first one, from student children who were living in a remote village with poor environmental sanitation and covered with diversity of perennial plants, shrubs of densely planted coffee together with other giant trees where the soil was moist and warm, and the majority of student children were bare-footed as they used to come from poor parent families. As the result of those environmental conditions the worm-load of *S. stercoralis* in the population of student children was far higher in this particular remote village than in any other site school selected for sample taking. Due to those environmental and economic factors, the infection rate of *S. stercoralis* grew up to 28% in the second study project.With those practical results in mind, the infection rate of *S. stercoralis* at Dilla district was adjusted to 24%, taking the average infection rate of those two study projects

$$(\text{i.e.,}\ \frac{20\% + 28\%}{2} = \underline{\underline{24\%}}).$$

Several risk factors have been associated with human strongyloidiasis, including coinfection with HIV (Human Immunodeficiency Virus); HTLT-1 (Human T-cell Lymphotropic Virus type1) infection; diabetes mellitus; chronic alcoholism; asthma; tuberculosis; malnutrition; chronic pulmonary disease; leprosy; chronic renal failure; impaired bowel motility; immunosuppressive therapy for diseases such as rheumatic disease, malignancy or cancer, and organ transplants; and promiscuous defecation.

The difference in the infection rate of *S. stercoralis* in children due to the difference in the status of environmental sanitation & economic income in the families' residence areas of the children was analyzed by the statistic of standard deviation. In this case, the larger the standard deviation meant the greater the infection rate than the mean rate, manifesting at the epidemic level.

This was the statistical evidence for the fact that the poor status of environmental sanitation and poor economic income in the parent families' residence areas of elementary schools children had been one of the obvious causes for the increase of infection rate in the student children with *S. stercoralis*. This sanitation in the residence areas of the children was poor so that the pathogenic worm-load in the soil would be high and infect the bare-footed student children whose parents were poor and could not buy shoes for them.

Both parasitic male and female adults of *Strongyloides stercoralis* including the developmental stages had been isolated from fresh stools samples of the participant student children.

Growth of free-living generation of *S. stercoralis* in the autoclaved topsoil in petridishes incubated at 28ºC, showed the following result. In each of the three petridishes that were observed after 11, 30, and 48 days from the date of initial incubation, adults (males & females) and a large number of larvae were present. The purpose of growing free-living generation to compare the morphology of free-living males with that of parasitic males.

Safranin stain is not known at all to stain protozoa or any other parasite here before. When it was tried to stain the worms of *S. stercoralis*, for the first time, it gave a very good dyeing effect. It stained the worms red.

Tables 1 & 2, and Fig. 2 are given on following 3 consecutive pages.

Table (1): The infection rate with *Strougyloides stercoralis* and the cure rate of the drug albendazole against human stronglyoidiasis, 6/12/2206 to 10/06/2007

No. of students examined	No. of students positive for S. stercoralis	The drug used for treatment	No. of students cured by the treatment
710	142 (20%) [#]	Albendazole	138 (97%)[†]

[#]The percentile quantity in parenthesis adjacent to the value that meant "No. of Students positive for *S. stercoralis*," represented the infection rate of *S. stercoralis* in the population of student children whereas the one adjacent to the value that meant "No. of Students cured by the treatment," i.e.,

[†]represented the cure rate of the drug albendazole against human strongyloidiasis found at Dilla district.

Table (2): The increase of infection rate with *Strougyloides stercoralis* due to the poor status of sanitation and economic income

Infection rate of *S. stercoralis* in children from families of better (sanitation and economic) status-residence areas	Infection rate of *S. stercoralis* in children from families of poor (sanitation and economic) status-residence areas
38%	12%
11%	50%
10%	40%
15%	20%

$\overline{X}_1$=12%; S_1 =2.2% $\overline{X}_2$=37%; S_2 =12.5%

$\overline{X}_1$ or $\overline{X}_2$ stands for a sample mean and S_1 or S_2 represents the standard deviation of a sample. $\overline{X}_1$ and S_1 are variables for the children from families of better status in sanitation and in economic income whereas $\overline{X}_2$ and S_2 are for those from families of poor status in sanitation & in economic income.

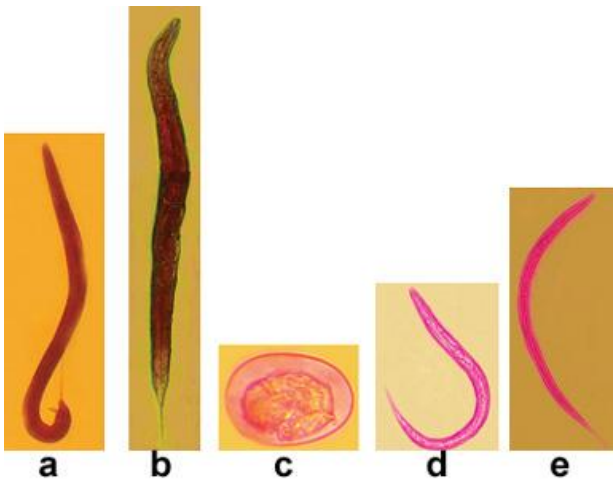


Figure (2): Microphotographs of different developmental stages and sexes of *Strongyloides stercoralis* isolated from fresh stools samples.

(a) parasitic adult male (stained with Safranin), magn[‡]. X64 ;
(b) parasitic adult female, magn. X64;
(c) egg, magn. X640; (d) rhabditiform larva, magn. X640; and
(e) filariform larva, magn. X320. Pictures (b), (c), (d) and (e) were colored by a Computer Adobe Phot oshop▪ CS. Each of these five pictures was transformed from its original magnified size to the resolution of 1200 pixels/inch with the quality of 12 (maximum) and large file compatible with A4 page format .

[‡]magn. stands for the term magnification that gives the value of how many times the actual size of the specimen was magnified.

DISCUSSION

The results obtained in this study project can be defined as a set of achievement scored by way of cross-sectional type of study. Standard deviation of infection rate, in student children from families of better environmental sanitation and economic status, was far less (S_1 =2.2%) than in those from poor environmental sanitation and economic status (S_2 =12.5%). Larger S_2 indicated that the observed infection rate went up beyond the mean infection rate in the population of student children. Student children from families of better economic status did live a relatively more hygienic mode of life as they used to get water supply lines to wash their hands, clothes and bodies at their homes. Families might be in a better economic position by having enough capital to carry out their own private business work in the central part of the city with better sanitation that would be comfortable to be hygienic and buy shoes for their student children that could not be afforded and done by poor families. Parents who had educational skill and government job were economically self-sufficient so that they were able to buy shoes for their student children, resulting in reduction in the infection rate with *S. stercoralis*. The fact that poor environmental sanitation and poor economic income did form one of the obvious causes for the increase of infection rates in the student children was evidenced by the statistic of standard deviation and other angles.

When the safranin stain was tried to stain the worms of *S. stercoralis*, for the first time, it gave a very good dyeing effect. It stained the worms red. Actually, safranin is well known as the secondary stain (counter stain) applied to the fixed preparations of bacteria. If the bacteria are decolorized with alcohol, they will take up the safranin and appear red (gram-negative). If the bacterial cells are not decolorized , the safranin will have no effect on the already stained preparation, and the bacteria will remain blue or purple (gram-positive) [23, 24].

The student children who were positive for *S. stercoralis* infection were not revealing or not manifesting affectedness with the disease, being active in their daily lives like other student children who were without strongyloidiasis. On the other hand, the larvae of *S. stercoralis*, recovered from fresh stools samples of those infected student children, were practically observed moving actively in the fields of vision under the objectives of compound light microscopes. With this truth in mind, the student children who were positive for *S. stercoralis* infection and did not manifest affectedness with strongyloidiasis should be immunocompetent. In these infected children, adults and larvae of the parasite were confined to the digestive tract in which case the children were symptomless and the *S. stercoralis* infection they had was asymptomatic intestinal strongyloidiasis. In other words, it is neither at the "gastropulmonary strongyloidiasis" nor "disseminated strongyloidiasis" stage in these infected participant student children.

Here it could be understood that the parasite was silently hiding in the intestine of each of the infected student children to develop to the lethal conditions of strongyloidiasis whenever the immunity of the student child was broken down (weakened) by some risk factors. Such a hidden pathogenic parasite was found out from where it was hiding by carefully employing standard diagnostic procedures such as Baermann technique and displayed with all its developmental stages and sexes. Hence, the parasitic males of *S. stercoralis* are present together with their parasitic females in the bodies of human hosts and this verified evidence is a spectacularly targetful answer to the major question and objective of this study.

Some authors had generalized that parthenogenesis and protandrogony (i.e., hermaphroditism) were the methods of reproduction for *S. stercoralis* in human hosts as the parasitic males did not exist in human body [6]. *S. stercoralis* in humans are parthenogenetic and can produce offspring without being fertilized by the male. But the fact that parasitic males do exist can be demonstrated in experimentally infected dogs [5]. In other words, this group of thought stated that the adult female *S. stercoralis* is parthenogenetic & hermaphroditic in the mechanism of reproduction. No adult male *S. stercoralis* is known to exist, the adult female is considered as being parthenogenetic [26]. Another division of thought had concluded that asexual reproduction was the method for

the parasitic females of *S. stercoralis* to reproduce in human hosts for the very reason that parasitic males did not exist in the body of humans [8]. However, let us take that both parthenogenesis and asexual reproduction have the same meaning for the method of reproduction. Is there any evidence to generalize that adult female *S. stercoralis* is parthenogenetic and hermaphroditic? Each of these groups of thought did not have any trace of substantiated and persuasive scientific proof to be accepted in science. This was so because many parasitic males of *S. stercoralis* with spicules everted out of their spicule pouches were practically observed in fresh stools samples of participant student children. The presence of many parasitic males of *S. stercoralis* with everted spicules in fresh stools samples together with parasitic females was a very strong evidence for the fact that there was copulation & fertilization. Everted spicules of males are seen only at times of mating.

CONCLUSION

- The result of this study had identified the concepts of both parthenogenesis/asexual reproduction and protandrogony, in the parasitic generation of *S. stercoralis*, to be unscientific conclusions. The reports without adequate assessment and evidences, on the reproduction of *S. stercoralis* in the parasitic generation in human hosts that had been reacted to by this paper could not be denied because they were reported straight forward by authors in modern textbooks, journals, and on the internet. Due to those reports, in all modern human parasitology textbooks, journal, and on the internet, the males of *S. stercoralis* had been excluded (omitted) from the life cycle of its parasitic generation in human hosts. It was possible for copulation to take place between the parasitic males and females to result in fertilization in the lumen of the human host's gut and then the fertilized parasitic female could burrow into the intestinal mucosa to lay eggs that would hatch soon. It is just like a domestic cock and a hen where it is the hen which goes to a nest after mating to lay and incubate eggs and not the cock.
- Applying efficient preventive measures and devising effective treatment under clinical supervision against a pathogenic parasite depend on deep and detailed understanding about the biology and life cycle of the parasite.
- The term curved tail was used by authors for the posterior body part of males that belong to free living generation found in soil **[4]**. The term was not inclusive and unfit to define the actual taxonomic morphology of both free-living and parasitic males of *S. stercoralis*. The degree (extent) of being curved in the posterior body part of male *S. stercoralis* is greatly variable among the male worms of both free-living and parasitic ones in a similar way in extent. The morphology of both parasitic and free living males is the same. This was verified by growing free-living males in autoclaved topsoil that was inoculated with fresh stools sample obtained from an infected child before giving him treatment and incubated at 28ºC. When the morphology, including the variation in the degree of curvature or coiling of the posterior body part, of these free-living males was compared with that of parasitic males, it was found to be similar in both free living and parasitic ones. Therefore, the term practically ascertained to be correct to differentiate both the parasitic and free-living males from their respective females of *S. stercoralis* was a ventrally "curved or coiled posterior body part" in the males of this very parasite whereas that of the females was straight.
- In the life cycle of parasitic generation of *S. stercoralis* both parasitic male & female must be included just like the free-living male & female in their life cycle.
- This article is a realistic response to a chronic global problem that has remained unsolved for generations of man until now and needs world-wide attention of human parasitologists.

Funding:

The financial support, covering the cost of medicine used to treat the infected children and technical assistance by assigning a medical doctor for prescription and clinical

supervision were given by the Ethiopian Catholic Church (Dilla Don Bosco).

Conflict of interest:

I confirm that I don't have any competitive conflict of interest with any body.

Ethical approval:

Ethical permission/clearance to perform the research work for the well-being of human subjects was obtained from:- Dilla University, the Office of Gedio-Zone Administration, and the Directors of the schools involved in the study. The demand for the continuity of this study project and participation by the participant student children and their parents was unusually high.

ACKNOWLEDGEMENTS

Don Bosco, i.e., Dilla Ethiopian Catholic Church was very kind and quick to accept the request forwarded by the researcher of this study for technical and medical assistance. Contribution to and participation in this study project by Don Bosco was immense. It was this very organization that rescued the student children infected with *Strongyloides stercoralis* when it understood the potential risk the infection could entail. Then it reacted promptly by covering the financial cost of the drug of choice for treatment and assigned a medical doctor (Dr. Corazon B. Jaca FMA) for prescription and clinical supervision in the process of treatment. I am extremely thankful to Don Bosco for its extensive financial contribution and participation in favour of the infected student children.

I am very grateful to the office of the President of Dilla University and the main Administrator Office of Gedio Zone for their writing letters to the Directors of elementary schools, requesting them to be cooperative for my research activities with their students.

I am very strongly thankful to Mr. Tilahun Kebede, Mr. Mingizem Birhan, and Mr. Tsegaye Hylu who are computer science teachers & technicians in Dilla University, for their providing me with and installing technical computer tools such as Equation Editor and Adobe Photoshop▪CS that were essential in processing the data.

REFERENCES

1. Mitreva M, McCarter JP, Martin J, Dante M, Wylie T, Chiapelli B, et al. Comparative genomics of gene expression in the parasitic and free-living nematodes *Strongyloides stercoralis* and *Caenorhabditis elegans*. Genome Res [serial on the Internet]. 2004 [cited 2012 Oct.24]14: [about 6p.]. Available from:http://genome.cship.org/content/14/2/209. full
2. Concha R, Harrington WJR, Rogers AI. Intestinal strongyloidiasis: recognition, management, and determinants of outcome. *J Clin Gastroenterol*. 2005; 39: 203-11.
3. Hunter CJ, Petrosyan M, Asch M. Dissemination of *Strongyloides stercoralis* in a patient with systemic lupus erythematous after initiation of albendazole: a case report. J Med Case Reports [serial on the Internet]. 2008 May 14 [cited 2012 Oct. 24];2: [about 5p.]. Available from: http: //www.jmedicalcasereports.com/content/2//156
4. Gilles HM. Soil-transmitted helminths (geohelminths). In: Cook GC. editor. Manson's tropical diseases. 20th ed., London: WB Saunders; 1996; 1394-8, 1627.
5. Paniker CKJ. Textbook of medical parasitology. 6th ed., New Delhi; Jaypee Brothers Medical Publishers 2007; 169-74.
6. Bogitsh BJ, Carter CE, Oeltmann TN. Human parasitology. 3rd ed., USA; Academic press 2005; 334-9.
7. Dillard KJ, Saari SAM, Anttila M. *Strongyloides stercorali* infection in a Finnish kennel. Acta Vet Scand [serial on the Internet]. 2007 Dec 12 [cited 2012 Oct 24]; 49: [about 5p.].Available from: http://www.actavetscand.com/content/49/1/37
8. Keiser PB, Nutman TB. *Strongyloides stercoralis* in the immunocompromised population. Clin Microbiol Rev [serial on the Internet]. 2004 Jan [cited 2012 Oct 24];17(1):[about 3p.]. Available from:http://www.ncbi.nlm.nih.gov>…>Clin Microbiol Rev>v.17(1); Jan 2004
9. Nolan TJ, Bhopale VM, Schad GA. Hyperinfective strongyloidiasis: *S. stercoralis* undergoes autoinfective burst in neonatal gerbils. *J Parasitol* 1999; 85: 286-9.
10. Dionisio D, Manneschi LL, Lollo SD, Orsi A, Tani A, Esperti F. *Strongyloides stercoralis*: ultrostructural study of newly hatched larvae within human duodenal mucosa. *J Clin Pathol* 2000; 53: 110-6.
11. Massey HCJ, Nishi M, Chaudhary K, Pakpout N, Lok JB. Structure and developmental expression of *Strongyloides stercoralis*. *Int J Parasitol* 2003; 33: 1537-44.

12. Garg PK, Perry S, Dorn M, Hardcastle L, Parsonnet J. Risk of intestinal helminth and protozoan infection in a refugee population. *Am J Trop Med Hyg* [serial on the Internet]. 2005 Mar 20 [cited 2012 Oct 24]; 83(3): [about 4 p.]. Available from: http: //www.ajtm.org/content/ 73/ 2/386.
13. Siddiqui AA, Berk SL. Diagnosis of *Strongyloides stercoralis*. In: Ericsson CD, Steffen R, editors. *Clin Infect Dis* [serial on the Internet]. 2012 Oct 15 [cited 2012 Oct 24]; 55(8): [about 8 p.]. Available from: http: //cid.oxfordjournals. org/content/33/7/ 1040.full
14. Siegel MO, Simon GL. Is human immuno-deficiency virus infection a risk factor for *Strongyloides stercoralis* hyperinfection and dissemination? *PLoS Negl Trop Dis* [serial on the Internet]. 2012 Jul 31 [cited 2012 Oct 24]; 6(7): [about 4 p.].Available from: http:// www. ncbi. nlm.nih.gov>…>PLoS Negl Trop Dis> v.6(7); Jul 2012
15. Ramanathan R, Nutman TB. *Strongyloides stercoralis* Infection in the Immuno-compromised Host. *Japan J Parasitol* [serial on the Internet]. 2008 May [cited 2012 Oct. 24]; 10(2):[about 4 p.]. Available from: http://www. ncbi.nlm.gov/pubmed/18462583
16. Poonam P, Jayshree RS, Acharya RS, Hema S, Govind B, Suresh TM. Fulminant fatal *Strongyloides stercoralis* infection in a postchemo-therapy immuno-suppressed cancer patient. *Med Pediatric Oncol* 1999; 33: 504-5.
17. Jayshree RS, Hema S, Govind B, Suresh TM. *Strongyloides stercoralis* eggs in the stools during anticancer therapy. *Indian J Gastroenterol* 2001; 20: 160- 1.
18. Kim J, Joo HS, Ko HM, Na MS, Hw ASH, Im JC. A case of fatal hyperinfective strongyloidiasis with discovery of autoinfective filariform larvae in sputum. *Korean J Parasitol* 2005; 43: 51-5.
19. Fardet L, Genereau T, Poirot JL, Guidet B, Kettaneh A, Cabane L. Severe strongyloidiasis in corticosteroid-treated patients: case series and literature review. J Infect 2007; 54:18-27.
20. Kerepesi LA, Hess JA, Leon O, Nolan TJ, Schad GA, Abraham D. Toll-like receptor 4(TLR4) is required for protective immunity to larval *Strongyloides stercoralis* in mice. *Microb Infect* 2007; 9: 28-34.
21. Rahif RH, Emara GY, Al-saqur IM. Epidemio-logical study of intestinal nematodes in dogs in Basrah(Iraq): prevalence of helminthic ova in the fecal deposits. *Iraqi J Vet Sci* 2002; 16: 71-9.
22. Cheesbrough M. District laboratory practice in tropical countries. Part 1. India; Cambridge University Press 1998; 214-5.
23. Merck KGA. Microscopy: Safranin 0 (C.I. 50240) Certistain. [serial on the Internet]. 2011; [about 1 p]. Available from: www.merck-chemicals.com/ microscopy
24. Boyd PF. Basic medical microbiology. 5th ed. USA: Little, Brown and Co. (Inc). 1995; 557.
25. Beadle GW, Emerson R, Whitaker DM, Lawson CA, Lewis RW, Biirmester MA et al. Full text of "Animal tissue techniques". [serial on the Internet]. USA: W.H. Freeman and Co. 1962; [about 1]. Available from: http://archive. org/stream/ animaltissuech00hurr
26. Zeibig EA. Clinical parasitology: a practical approach. London; W.B Saunders Co. 1997; 148.

Peer reviewer: Samia Etewa: Professor of Parasitology, Faculty of Medicine, Zagazig University, Egypt.

Editor: Tarik Zaher: Professor of Tropical Medicine, Faculty of Medicine, Zagazig University, Egypt.

ERCP in Management of Common Bile Duct Stones in Children: Safety and Efficacy

Khalid Safwat[1], Khalid Abd EL-Aziz[1], Hany Fawezy[2], Ahmed Yousef[1]
[1]*General Surgery Department, Faculty of Medicine, Zagazig University, Egypt*
[2]*Pediatrics Department, Faculty of Medicine, Zagazig University, Egypt*

Corresponding Author
Ahmed Yousef

E mail:
ahmadattia85@yahoo.com

Key words:
Endoscopic retrograde cholangiopancreatography (ERCP); choledocholithiasis; Common bile duct; Children.

Background and study aim: Choledocholithiasis due to common bile duct (CBD) stones is a critical condition in children. The aim of this study was to evaluate the efficacy and safety of endoscopic retrograde cholangiopancreatography (ERCP) in management of CBD stones in children.
Patients and methods: Twenty five children suffering from obstructive jaundice were diagnosed and selected after completion of full laboratory and radiological investigations in the period from June 2010 till December 2013 and were admitted to the Surgery Department Zagazig University Hospitals, Egypt. Patients were classified into 2 age groups below and above 6 years to decide the diameter of ERCP scope and the stents used in the procedure. Group A; 0-6 years were 12 patients and Group B; 6-15 years were 13 patients.
Results: ERCP was adequate in helping to predict biliary ductal stones and removal of them in all patients with no reported diagnostic or therapeutic failure. The stent placement was indicated in 21 of the 25 (84%) children, the other 4 children (16%) just sludge was cleared using basket forceps without the need for stent insertion. Two to three weeks later laparoscopic cholecystectomy was successfully performed to all indicated children with removal of the stent after 2 to 3 weeks. There rate of ERCP complications was 12% (3 cases), one case reported mild pancreatitis and 2 cases reported mild cholagnitis.
Conclusion: The encouraging results of this study with very minimal insignificant complications of ERCP (safety) and good results of ERCP in treatment of CBD stones in children (efficacy) recommend that children with choledocholithiasis due to CBD stone or sludge should be treated by endoscopic CBD stone or sludge removal.

INTRODUCTION

Although still rare pediatric calcular obstructive jaundice appears to have been on the rise since the early 1970s [1]. The causes of this increase are multifactorial and include an improved ability to detect gallstones as well as an actual increased incidence [1,2]. Endoscopic retrograde cholangiopancreatoraphy (ERCP) use in children, has been limited by technical difficulties, low incidence of pancreaticobiliary diseases and lack of knowledge of ERCP by pediatric surgeons and pediatricians [3]. ERCP provides a good definition of the extrahepatic and intrahepatic bile ducts, its most important role is to define usual extrahepatic biliary obstruction and in the diagnosis of sclerosing cholangitis. It also has a role in detecting stones in the CBD. It is also useful in the diagnosis of bile duct patency and thus excluding atresia in cholestatic infants [4]. Therapeutic ERCP reduces the need for surgery and preoperative ERCP provides surgeons with a "Road map" when surgery is required. Success in neonates has also been reported [5]. The aim of this study was to evaluate the efficacy and safety of ERCP in management of CBD stones in children.

PATIENTS AND METHODS

This study was conducted between June 2010 and December 2013 at the endoscopy unit, Surgery Department Zagazig University Hospitals, Egypt, and included 25 children with cholestasis due to CBD stones. Patients were referred by the out patient clinic of Pediatric Gastroenterology unit and General Surgery Department Zagazig University Hospitals, they were 11 females and 14 males. Patients were classified into 2 groups according to the age below and above 6 years to decide the diameter of ERCP scope and the stents used. Group (A) 0-6 years; were 12 patients, Group (B) above 6 to 15years; were 13 patients (Table 1). All of them had been diagnosed to have cholestasis due to CBD stones and diagnosis was based on clinical, biochemical and ultrasonic evidence. Their medical records were reviewed and the following information were obtained: age, sex, clinical presentation, investigations and abdominal ultrasound results. All the 25 children were subjected to ERCP (using Pentax 402 ED3485T or Olympus JIF T20 side viewing duodenoscope) under general anesthesia in the operative room using the C-arm (TCA4PLUS) for biliary visulitzation. Of them 23 children were proposed for elective laparoscopic cholecystectomy (LC) within two to three weeks after ERCP. Two to three weeks after LC stent removal under deep sedation (midazolam, ketamine and fentanyl) in the operative room was performed. A detailed informed written consent was obtained from the parents or guardian of all children. This study was approved by our Institutional Review Board.

Statistical Analysis:

Analysis of the data was carried out using the SPSS (version 12, Chicago, Illinois, USA) software. Data were expressed as number, percentage and mean ± standard deviation. Comparison between the mean values was performed using the Student's t-test. P values lower than 0.05 was considered statistically significant.

RESULTS

The study included 25 pediatric patients with age ranged from 2.3 to 13 years (mean age of 6.76 ± 3.9 years). There was no statistical significance between both groups regarding the age and sex (Table 1). All patients presented with manifestations of cholestasis including jaundice, dark urine and pale stools associated with abdominal colic and fever up to 39°C (Table 2).

Table (1): Age groups in relation to sex distribution of the studied cases

Sex	Age groups					
	Group A (n = 12)		Group B (n = 13)		Total	
	No	%	No	%	No	%
Male	7	28	7	28	14	56
Female	5	20	6	24	11	44
Total	12	48	13	52	25	100

P value = 0.22 (NS)

Table (2): Presenting symptoms among the studied cases

	No	%
Abdominal colic	25	100
Vomiting and fever	25	100
Color of urine (dark)	25	100
Color of stool (pale)	25	100
Jaundice	25	100

Regarding the ultrasound findings (Table 3), 18 patients (72%) with calcular obstructive jaundice [11 in group B and 7 in group A]. In six patients (24%) ultrasound diagnosed as obstructive jaundice due to thick bile (Inspissated bile syndrome, Mucovicidosis) [2 in group B and 4 in group A]. Dilated intrahepatic biliary radicles (IHBR) reported in 7 patients (28%), [3 patients in group A, 4 patients in group B]. One patient in group A (4%) had in addition parasitic worm (*Fasciola*) obstructing the CBD.

Table (3): Interpretation of the main ultrasound findings in the studied cases

Item	Age group		Total (n = 25)
	Group A	Group B	
CBD stones (Choledocholithiasis)	7	11	18
Obstructive jaundice by thick bile	4	2	6
Dilated IHBR	3	4	7
Obstructive jaundice by worm	1	-	
P value	**0.01 (S)**		

The final diagnosis in relation to the sex showed that there was statistical significance between males and females regarding the CBD stones and the inspissated bile syndrome, one male child in group A was diagnosed as obstructive jaundice by parasitic worm (Table 4).

Table (4): Final diagnosis of different age groups in relation to sex distribution of studied cases

Sex	Final diagnosis	Age groups		Total
		Group A (n = 12)	Group B (n = 13)	
Female (n = 11)	**Calcular obstructive jaundice**	4	5	9
	Inspissated bile syndrome	1	1	2
	Obstructive jaundice with worm	0	0	0
P value		**0.002 (S)**		
Male (n = 14)	**Calcular obstructive jaundice**	4	6	10
	Inspissated bile syndrome	2	1	3
	Obstructive jaundice with worm	1	0	1
P value		**0.045 (S)**		

The CBD cannulation was successful in all patients (100%). Diagnostic ERCP for cholestasis of obscure etiology was performed in a total of 8 patients (32%), 4 patients in group A (33.3%) and 4 patients in group B (33.3%), while therapeutic ERCP was done in 17 patients (68%) [8 patients in group A (32%), 9 patients in group B (36%)] (Table 5).

Table (5): Type of ERCP performed in different age groups of the cases

Item	Age group				Total	
	Group A		Group B			
	No	%	No	%	No	%
Diagnostic for Cholestasis of obscure etiology	4		4		8	32
Therapeutic in Cholestasis of known etiology	8		9		17	68
Total	12		13		25	100

Findings during ERCP in the studied patients are shown in Table 6. CBD stones were found in 19 child, while biliary parasite (*Fasciola*) was extracted in one male child in group A. Six cases were diagnosed with mucovicidosis.

Table (6): ERCP diagnosis in the studied patients

Item	Age group		Total	
	Group A	Group B		
	No	No	No	%
Calcular obstructive jaundice	7	11	18	72
Mucoviscidosis	4	2	6	24
Obstructive jaundice with worm	1	0	1	4
Failed	0	0	0	0
Dilated IHBR	2	7		
P value	**< 0.001 (HS)**			

As regard the different ERCP procedures done in the two age groups of the studied patients. Clearance of bile ducts from stones (Figure1 and 2) or worm or thick bile using basket forceps and/or the extraction balloon done in all patients(100%).

Figure (1): Cholangiography showing multiple filling defects (stones)

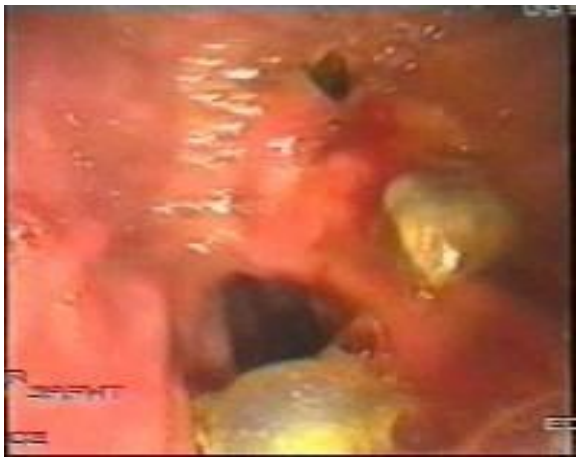


Figure (2): Stone clearance using the extraction balloon

Endoscopic sphincterotomy (ES) was performed in all patients (Figure 3). Placement of internal biliary prosthesis (stent) was done (Figure 4) in 21 patients (84%) [11 patients in group B and 10 patients in group A] (Table 7).

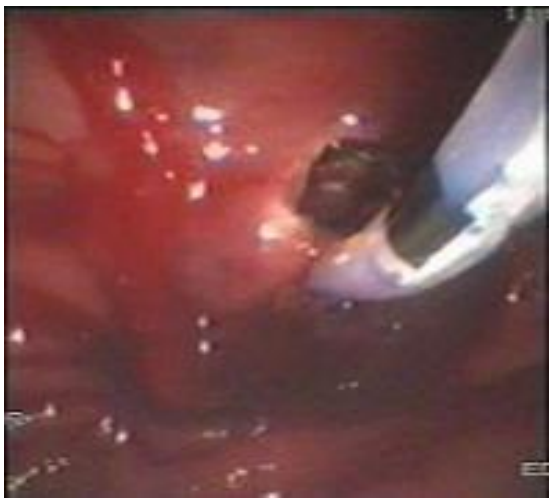


Figure (3): Sphincterotomy of the papilla

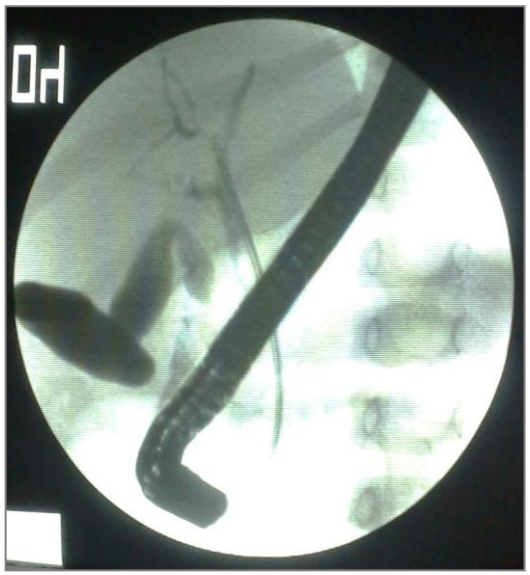


Figure (4): Plastic stent in the common bile duct

Table (7): Therapeutic ERCP procedures done in the studied patients

ERCP procedures	Age group		Total
	Group A	Group B	
Endoscopic sphincterectomy (ES)	12	13	25
Clearance of bile duct using basket forceps and/or balloon	3	2	5
Stent	10	11	21

A total of 17 cases diagnosed with choledocholithiasis were referred to surgical management by laparoscopic cholecystectomy. The one case diagnosed as parasitic worm (*Fasciola*) improved following ERCP and was treated medically. The cases diagnosed with Mucovicidosis improved following ERCP clearance of the duct and was treated medically (Table 8).

Table (8): Outcome in the age group of studied patients

Outcome	Group A	Group B	Total
Laparoscopic cholecystectomy then stent removal later on	8	9	17
Stent removal followed by medical treatment	2	2	4
Improved with medical treatment as no stent	2	2	4

The rate of ERCP complications encountered in both groups was 12% (3/25). One case had cholangitis following ERCP in group A and one case developed pancreatitis in each group. All cases were treated medically and there was no mortality (Table 9).

Table (9): Complications of ERCP in the studied children

Item	Age group				Total	
	Group A (n = 12)		Group B (n = 13)			
	No	%	No	%	No	%
Biliary pancreatitis	0	0	1	4	1	
Cholangitis	1	4	1	4	2	
Death	0	0	0	0	0	0
Total	**1**		**2**		**3**	

DISCUSSION

Endoscopic retrograde cholangiopancreatography is not as widely used in children as in adults and is performed in few specialized centers. ERCP is an effectual and safe therapeutic procedure in children and adolescents of different ages in a variety of pancreatobiliary disorders [6]. A major advantage of ERCP is that it offers the opportunity to perform endoscopic examination. The cause of the patient's symptoms may be evident on visualization of the papilla of Vater itself. It is reported that ERCP is an established modality for the diagnosis and treatment of pancreatobiliary diseases in adults. Repeated experience with diagnostic and therapeutic ERCP in pediatric patients is limited due to the relatively low incidence of pancreatobiliary diseases, limitations in the size of duodenoscopes, the need for general anesthesia and the lack of highly trained and experienced endoscopists familiar with these special procedures in pediatric patients.

With the refinement of technique and improvement of endoscope design, excellent results have been reported in the pediatric population especially the management of biliary diseases (biliary sphincterotomy to facilitate drainage or stone extraction, stent placement or stricture dilatation) [7]. The incidence of gallstones in children increased in the last years due to both the regular use of the noninvasive detection technique (ultrasonography) and actual increased incidence. ERCP has become a validated diagnostic and therapeutic modality in children with choledocholithiasis, these patients were treated successfully by endoscopic sphincterotomy and stone removal [8-11].

Our study included 25 patients with clinical, laboratory and ultrasound evidence of cholestasis, with calcular biliary obstruction and all had strong suspicion of stone common bile duct. There was no statistical significance when comparing the age groups with the sex of the studied patients. Coinciding with the findings of others in their studies the most common presenting symptoms in all patients was abdominal colic, vomiting, fever and triad of jaundice, dark urine and pale stools (100%) [6]. Also On clinical examination we noticed that jaundice was the commonest finding in the studied group (100%).

Enlarged liver and/or spleen were found in all age groups. These findings were supported by ultrasonographic examination of the abdomen [7].

The different indications for ERCP examination in our study were, confirming the diagnosis of other imaging procedures and temporary relief of obstruction until definitive management was amenable while the most common indications for ERCP in Western children are choledocholithiasis and pancreatitis [8,12]. Indications differ in Asian countries [11,13,14]. For example, in Saudi Arabia the most common indication was choledocholithiasis in patients with sickle-cell anemia [11], whereas indication in Japan and India was mostly cholodecal cyst [13-16]. As others demonstrated in recent studies that ERCP was an important diagnostic modality in infants and children of all ages with cholestasis offering valuable detailed information on the biliary and pancreatic ductal system and that it has the advantage over MRCP and CT in offering diagnostic as well as therapeutic capabilities with very minimal insignificant complications [6,13, 14,17-19].

The overall success rate in cannulation of the common bile duct and pancreatic duct was 100% in our study. The higher success rate reported in our study was slightly different than rates reported by many authors [6,12,18,20,21], and this can be explained by the fact that the youngest child in our study was older than 2 years and also the small number of cases in our study is another factor. The stent placement was indicated in 21 of 25 (84%) child; the other 4 children (16%) just sludges were cleared using basket forceps without the need for stent insertion. Two to three weeks later laparoscopic cholecystectomy was successfully performed to all indicated children with removal of the stent after 2 to three weeks. As reported in other studies, Endoscopic sphincterotomy (ES) and basket extraction of living *Fasciola* worm from the CBD was performed successfully and without complications in one patient and the children then treated medically [20,21].

In conclusion, the encouraging results of this study with very minimal insignificant complications of ERCP (safety) and good results of ERCP in treatment of CBD stones in children (efficacy) recommend that children with choledocholithiasis due to CBD stone or sludges as (thick bile or worms) should be treated by endoscopic CBD stone or sludges removal to prevent the potential complications of choledocholithiasis which lead to major risks, discomfort, and repeated hospitalization.

Funding: Non.

Conflict of interest:Non.

Ethical approval: Approved by Ethical Committee of Faculty of Medicine, Zagazig University.

REFERENCES

1. Bailey PV, Connors RH, Tracy TF Jr, Sotelo-Avila C, Lewis JE, Weber TR. Changing spectrum of cholelithiasis and cholecystitis in infants and children. *Am J Surg*. 1989; 158 : 585-588.
2. Waldhausen JHT, Benjamin DR. Cholecystectomy is becoming an increasingly common operation in children. *Am J Surg*. 1999; 177: 364-367.
3. Hsu RK, Draganov P, Leung JW, Tarnasky PR, Yu AS, Hawes RH, et al. Therapeutic ERCP in the management of pancreatitis in children. *Gastrointest Endosc*. 2000; 51: 396-400
4. Wilkinson MI, Mieli-Vergani O, Ball C, Portmann B, Mowat AP. Endoscopic retrograde cholangio-pancreatography in infantile cholestasis. *Arch. Dis. Child* 1991; 66 : 121-123.
5. Otto AK, Neal MD, Slivka AN, Kane TD. An appraisal of endoscopic retrograde cholangio-pancreatography (ERCP) for pancreaticobiliary disease in children: our institutional experience in 231 cases. *SurgEndosc*. 2011; 23:143-151.
6. Guelrud M. Endoscopic retrograde cholagio-pancreatography. *Gastrointest Endosc Clin N Am.* 2001; 11(4): 585 – 601.
7. Cheng CL, Fogel EL, Sherman S, McHenry L, Watkins JL, Croffie JM, et al. Diagnostic and therapeutic endoscopic retrograde cholangio-pancreatography in children: a large series report. *J Pediatr Gastroenterol Nutr.* 2005; 41: 445-453.
8. Varadarajulu S, Wilcox CM, Hawes RH, Cotton PB. Technical outcomes and complications of ERCP in children. *Gastrointest Endosc*. 2004; 60: 367-371.
9. Guelrud M, Zambrano V, Jaen D. Endoscopic sphincterotomy and laparoscopic cholecystectomy in a jaundiced infant. *Gastrointest Endosc* 1994; 40: 99–102.
10. Issa H, Al-Haddad A, Al-Salem AH. Diagnostic and therapeutic ERCP in the pediatric age group. *PediatrSurg Int.* 2007; 23: 111-116.
11. Pfau PR, Chelimsky GG, Kinnard MF, Sivak MV Jr, Wong RC, Isenberg GA, et al. Endoscopic retrograde cholangiopancreatography in children and adolescents. *J Pediatr Gastroenterol Nutr* 2002; 35: 619-623.

12. Poddar U, Thapa BR, Bhasin DK, Prasad A, Nagi B, Singh K. Endoscopic retrograde cholangio-pancreatography in the management of pancreaticobiliary disorders in children. *J Gastroenterol Hepatol.* 2001; 16: 927-993.
13. Keil R, Snajdauf J, Stuj J, Kalousova J, Nevolova P, Janik V, et al. Endoscopic retrograde cholangiopancreatography in infants and children. *Indian J Gastroenterol*. 2000; 19(4): 175 – 177.
14. Poddar U, Thapa BR, Chhabra M, Rao KL, Mitra SK, Dilawari JB, et al. Choledochal cysts in infants and children. *Indian Pediatr.* 1998; 35 (7):613-618.
15. Kumar R, Nguyen K, Shun A. Gallstones and common bile duct calculi in infancy and childhood. *Aust N Z J Surg.* 2000; 70(3):188-191.
16. Ohnuma N, Takahashi T, Tanabe M, Yoshida H, Iwai J. The role of ERCP in biliary atresia. *Gastrointest. Endosc* 1997;45 (5): 365 – 370.
17. Taj MA, Leghari A, Qureshi S, Ghazanfar S, Niaz SK, Quraishy MS. Endoscopic retrograde cholangiopancreatography: a therapeutic modality in children and adolescents.*J Pak Med Assoc.* 2012; 62(2):98-101.
18. De Angelis P, Foschia F, Romeo E, Caldaro T, Rea F, di Abriola GF, et al. Role of endoscopic retrograde cholangiopancreatography in diagnosis and management of congenital choledochal cysts: 28 pediatric cases. *Pediatr Surg.* 2012; 47(5): 885-888.
19. Davenport M, Betalli P, D'Antiga L, Cheeseman P, Mieli-Vergani G, Howard, ER. The spectrum of surgical jaundice in infancy. *Journal of Pediatr Surg.* 2003; 38(10): 1471-1479.
20. Reddy DN, Sriram PV, Rao GV. Endoscopic diagnosis and management of tropical parasitic infestations. *Gastrointest Endosc Clin N Am.* 2003; 13(4): 765 – 773.
21. Ozer B, Serin E, Gumurdulu Y, Gur G, Yilmaz U, Boyacioglu S. Endoscopic extraction of living fasciola hepatica: case report. *Turk J Gastroenterol*. 2003; 14(1): 74-77.

Peer Reviewer: Mohamad M. Radwan: Assistant professor of Tropical Medicine and Hepatogastroenterology ,Faculty of Medicine ,Zagazig Universty,Egypt.

Editor:Mohamad Emara:Lecturer of Tropical Medicine and Hepatogastroenterology ,Faculty of Medicine ,Zagazig Universty ,Egypt.

Intestinal Parasitic Infections in Elementary Schools Children at Dilla Town and its Peripheral Villages

Feleke Eriso
Parasitologist, Department of Biology,Dilla University, Dilla, Ethiopia

Corresponding Author
Feleke Eriso

Mobile:
+251916514682

E mail:
feleke.eriso@yahoo.com

Key words:Parasitic; Fatal; Morbidity; Symptomatic; Abscess; Immuno-compromised

Background and study aim: There is no information established about the diversity of human intestinal parasites at Dilla Town & its peripheral villages. The existence of various species of intestinal parasites in tropical and subtropical regions with moist and warm climate is expected to be high. Dilla Town and its peripheral villages is found in the tropics. The objective of this study was to establish a statistically verified status about the diversity of human pathogenic intestinal parasites at Dilla Town and its peripheral villages.

Patients and methods: Stools samples of 710 student children were collected in 0.85% saline solution from elementary schools and observed under microscope in the laboratory of parasitology. Microphoto-graphs of the diagnostic stages of the parasites found were taken with a digital camera from the fields of vision of the microscope.

Results: Out of 710 student children examined 257,225,200,170,107,61,40,9,8, 8 and 4 were positive, the corresponding infection rates being 36%, 32%, 28%, 24%, 15%, 9%, 6%, 1%, 1%, 1%, and 0.6% of *Ascaris lumbricoides, Entameba histolytica, Giardia lamblia, Strongyloides stercoralis, Trichuris trichiura, Hookworms, Schistosoma mansoni,Taenia saginata,Enterobius vermicularis,Hymenolepis nana,* and *Fasciola hepatica* respectively. The infected children were given curative treatment.

Conclusion: Preventive measures against parasitic infections must be implemented by focusing on:

- Blocking transmission of infective stages,
- Delivering public health education,
- Providing the public with clean water supply for domestic use & drinking,
- Improving personal hygiene and environmental sanitation, and
- Identifying and treating infected individuals to prevent the spread of infections.

INTRODUCTION

Intestinal parasitic infections represent one of the most important infections that cause the global health problem of humans [1-4]. The sources of parasitic infections for man are poor personal hygiene, poor sanitation of the environment such as contamination of soil and water supplies with human excrement, wrong methods of sewage disposal, the parasites that adhere to edible vegetables and fruits, fingers, utensils, door handles, money, dust, flies that rest on food materials, reservoir hosts, and contaminated fingernails. It had been demonstrated that in the rural area of Cote d'Ivoire two children were found infected with several intestinal parasites at the same time where one of them was infected with 8 and the other child had been harboring 10 different intestinal parasite species [5-9]. Gastrointestinal parasites of man persist to exist, causing morbidity and mortality in less developed tropical countries of the world. Parasitic infections are enhanced by poor personal hygiene, ignorance, poverty, poor environmental sanitation, and by warm and moist climatic conditions [10-13]. The infective filariform larvae and cercariae have the ability to pierce intact human skin and get into the blood stream [14].

PATIENTS AND METHODS

Student children of 8 different elementary schools at Dilla Town and its peripheral villages were the sites of sample taking. The specific study sites included: -Walame Don Bosco, Meskele Eyesus, Mekane Yesus, Dawit, Kofe, Dilla, Chichu, and Haroresa Elementary Schools. The total sample size of 710 student children was decided to be examined for the suspected intestinal parasites of man. The samples taken from student children of the schools cited above were fresh stools. The task of this survey with a high response rate was executed by way of randomized diagnostic tests. Collection of stools samples and documenting related information from the schools had been carried out repeatedly in 3 different durations from:

- 6/12/2006 to 10/6/2007,
- 10/9/2008 to 25/6/2009, and
- 4/11/2011 to 25/6/2012.

However, the deliberate follow up to be certain about the prevalence and persistence of the intestinal parasites of man at Dilla Town and its peripheral villages, was performed by taking fresh stools samples of ten students from each of the eight different elementary schools once every year from 2000 up to towards the beginning of 2014.

The statistics selected, being relevant to interpret and analyse the results of this study project were:- *histogram* and *correlation coefficient*. The fresh stools sample of each student child was examined with a compound light microscope at three stages:

- Direct wet mount,
- Concentration technique, and
- Permanent stained preparation.

Procedure

Direct wet mount:

- About 2.5 ml of fresh stools sample was taken in a small vial from each student of the schools selected. Immediately after that, 0.85% NaCl solution in distilled water and warmed to 37ºC was added to each vial of fresh stools sample taken. Then, 1 drop of 0.85% warm (37ºC) aqueous NaCl (saline solution) was placed on a clean slide.
- Following that, about 1 drop of the stools specimen (from that of any single student) was added to the slide and mixed with the drop of NaCl solution.
- The saline wet mount was covered with a cover slip and examined under a suitable objective lens. This procedure of using warm saline solution was to allow determining the motility and gross morphology of trophozoites [11,15]. In the mean time, care was taken not to allow the sample on the slide to dry or cool; otherwise, the motility of trophozoites could have ceased. The stools specimen of any particular student child who was positive for the suspected parasites was preserved in 5% formalin (for protozoans) or in10% formalin (for nonprotozoan parasites) to be used in the stages of Concentration Technique and Examination of Permanent Stained Preparation. The stools specimen of each student child was prepared, observed, and preserved exactly in this way.

Concentration technique:

- Involved concentrating the number of the diagnostic stages of the suspected parasites, primarily of cysts, eggs or larvae in the stool specimen that was collected and preserved in 5% or 10% formalin. These concentrated and preserved specimens were part of the complete examination and allowed the detection of small numbers of the parasites that could have been difficult. In order to concentrate the number of the diagnostic stages of the parasites, diethyl-ether had been mixed with the suspension of the stool sample. Then, the speed and concentration time were set at 1000 rpm for 2 minutes.
- Next, the parasites particularly the cysts, eggs, or larvae were expected to sediment at the bottom of the centrifuge tube and the floating stools debris was discarded.

Examination of permanent stained preparations:

- Detection and identification of intestinal protozoan and helminth parasites preserved in 5% or 10% formalin respectively would depend on the examination of a permanently stained smears under the oil immersion objective lens.
- These stained slides would provide a permanent record of the suspected intestinal parasites of man.
- The identifications in the stages (steps) of Direct Wet Mount and Concentration Technique would be tentative until confirmed by the permanent stained slide.
- The staining was with Safranin.
- About 3 drops of Safranin solution was added to the stools specimen suspension preserved in

formalin in a bottle of about 50 ml and waited for about 6 hours to get the diagnostic stages of the parasites stained [16,17].

- A drop of Yetwin Mounting Medium melted at 65^0 C was placed on a clean slide, then on this drop of mounting medium, a drop of the stools specimen preserved in formalin and stained with Safranin was added and mixed well with the tip of a needle [18]. Next, the specimen was covered with a coverslip and left on a table for about 24 hours to let the mounting medium solidify and harden.
- Thereafter, the specimen in the hardened mounting medium was examined under the oil immersion objective lens to check the presence of the suspected intestinal parasites of man.

Additional methods:

- Baermann apparatus technique was employed for the diagnostic tests of *S. stercoralis* [19,20]. Here, the Low or Middle Power objective lens was used in observing under the compound light microscope.
- The suspensions of stools samples were made more diluted and thinner for the diagnostic examination of protozoan parasites than for those of helminths. In this case, High Power objective lens was used in observing under the compound light microscope.
- In all methods of this paper, from all fields of vision (i.e., low power-oil immersion) of the microscope, microphotographs of the parasites diagnosed were taken using a digital camera and transferred to a computer for further processing.

Treatment:

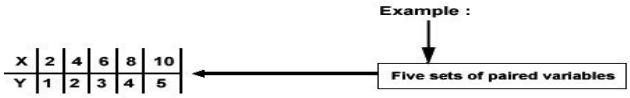
- Prescriptions and clinical supervision in the treatment of infected student children had been performed by an authorized medical doctor (i.e., Dr. Corazon B. JACA, FMA).
- Student children of 9 years and above in age were provided with and advised to take metronidazole 250 mg 1 capsule 3 times, i.e., after breakfast, lunch, and dinner daily for 7 consecutive days against *E. histolytica* or /and *G. lamblia* infections. On the other hand, student children of 8 years & below in age were provided with and advised to take metronidazole oral suspension 5 ml (equivalent to metronidazole 250 mg 1 capsule) 3 times, i.e., 5 ml after each of the three meals, daily for 7 consecutive days against *E. histolytica* or/ and *G. lamblia* infections.
- Albendazole 400 mg 1 tablet single dose on empty stomach was prescribed against infections with intestinal parasitic helminths excluding *S. stercoralis.*
- Student children infected with *S. stercoralis* were given and advised to take albendazole 400 mg 1 tablet once immediately before going to bed for night sleep daily for 3 consecutive days.

RESULTS

The list of the most important diagnostic stages of intestinal parasites represented the actual parasitic protozoan and helminth species that were experimentally observed and recorded. This was done by way of randomized diagnostic tests. Out of 710 student children examined, the exact numbers of infected ones and the corresponding infection rates with each specific species of the pathogenic intestinal parasites was directly recorded. The analysis by the statistic of *correlation coefficient* had proved that the relation among the intestinal parasites investigated, due to various factors, in this study was very strong. The relation was nearly a perfect correlation, i. e., ± 1. In this particular study the computed value of correlation coefficient was almost a perfect positive correlation (+1), because it was 0.99984, whereas the tabulated critical values are 0.754 & 0.874 at $\alpha = 0.05$, and $\alpha = 0.01$ levels of significance respectively for 5 degrees of freedom. The unprocessed raw data in the form of variables "X" and "Y" for Fig. 2 was that of Table 1.

Meaningfulness of applying the statistic of correlation can be imparted by citing several pairs or sets of paired variables that are appropriate to be explained using *correlation coefficient* as follows:

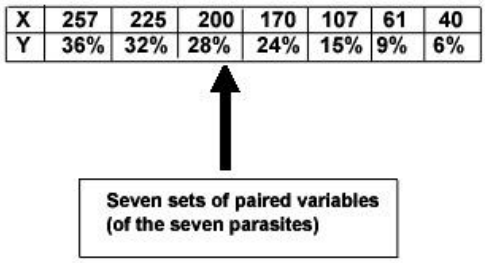
- educational qualification and salary, = stands for one pair of variables.
- money in the bank and interest,
- family size and weekly expenditures,
- property owned and taxes paid,
- hours of study and grades obtained,
- the time it takes a body to fall and the distance it falls,
- the price of steel and the quantity of it sold,
- married males and their incomes,
- carrot lengths and their maximum diameters,
- average industrial stocks and money supply, etc.



The paired variables used in the statistic of **correlation coefficient** employed in this paper were:

- The number of students positive for *A. lumbricoides* and the infection rate of *A. lumbricoides*,
- The number of students positive for *E. histolytica* and the infection rate of *E. histolytica*,
- The number of students positive for *G. lamblia* and the infection rate of *G. lamblia*,
- The number of students positive for *S. stercoralis* and the infection rate of *S. stercoralis*,
- The number of students positive for *T. trichiura* and the infection rate of *T. trichiura*,
- The number of students positive for hookworms and the infection rate of hookworms,
- The number of students positive for *S. mansoni* and the infection rate of *S. mansoni*.

What were measured in the correlation coefficient were the relationship among the paired variables of the parasites and not the relationship of the number of infected students and the quantity of percentages. Each of the seven parasites produced only one pair. For example, the pair produced by *G. lamblia* consisted of the variables 200 and 28%.



The percentile infection rate of each species of the intestinal parasites in the population of student children, were depicted in the statistic of histogram for impartive and practically meaningful impression.

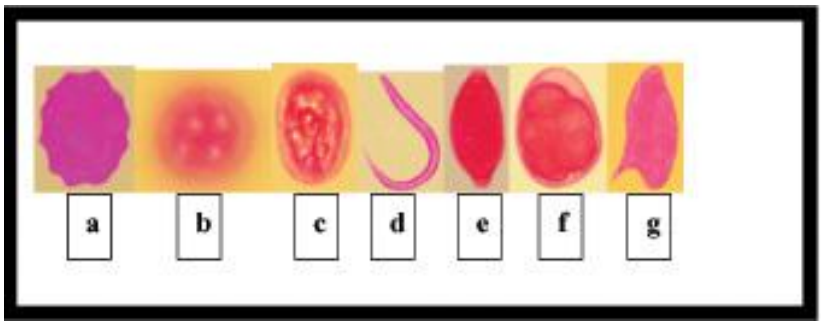


Figure (1): The list of of the most important diagnostic stages of intestinal parasites isolated from fresh stools samples of children at Dilla Town and its peripheral villages.

(a) *Ascaris lumbricoides* egg, magn†. X640; (b) *Entameba histolytica* cyst, magn. X640; (c) *Giardia lamblia* cyst, magn. X1600; (d) *Strongyloides stercoralis* rhabditiform larva, magn. X640; (e) *Trichuris trichiura* egg, magn. X640; (f) Hookworm egg, magn. X640; (g) *Schistosoma mansoni* egg, mag. X640.
Each of these seven pictures was colored using a computer Adobe Photoshop
▪CS and transformed from its original magnified size to the resolution of 1200 pixels/inch with the quality of 12 (maximum) and large file compatible with A4 page format.

†magn. Stands for the term magnification that gives the size of an image and serves to explain how many times the actual size of the specimen was magnified to get that particular size of the image.

Table (1): The list of human intestinal parasites isolated from fresh stools samples of children at Dilla Town and its peripheral villages.

The parasites found, as a sample size n	No. of student children positive for the parasite out of 710 students examined, as a variable X	Infection rate in percentage, as a variable Y
1. *Ascaris lumbricoides*	257	36%
2. *Entameba histolytica*	225	32%
3. *Giardia lamblia*	200	28%
4. *Strongyloides stercoralis*	170	24%
5. *Trichuris trichiura*	107	15%
6. Hookworms	61	9%
7. *Schistosoma mansoni*	40	6%

$$r = \frac{n\Sigma XY - \Sigma X\Sigma Y}{\sqrt{n\Sigma X^2 - (\Sigma X)^2}\sqrt{n\Sigma Y^2 - (\Sigma Y)^2}}$$

$$= \frac{7(28526) - 159000}{\sqrt{7(202344) - 1123600}\sqrt{7(4022) - 22500}}$$

$$= \frac{199682 - 159000}{\sqrt{1416408 - 1123600}\sqrt{28154 - 22500}}$$

$$= \frac{40682}{\sqrt{292808}\sqrt{5654}}$$

$$= \frac{40682}{541.12 x 75.193}$$

$$= \frac{40682}{40688.44}$$

$$= \underline{\underline{0.99984}}$$

Figure (2): *Correlation coefficient*, $r^{\neq}$, depicting the same environment of Dilla Town and its peripheral villages being favorable for the variety of human intestinal parasites.
≠ represents or stands as a symbol for the term "correlation coefficient".

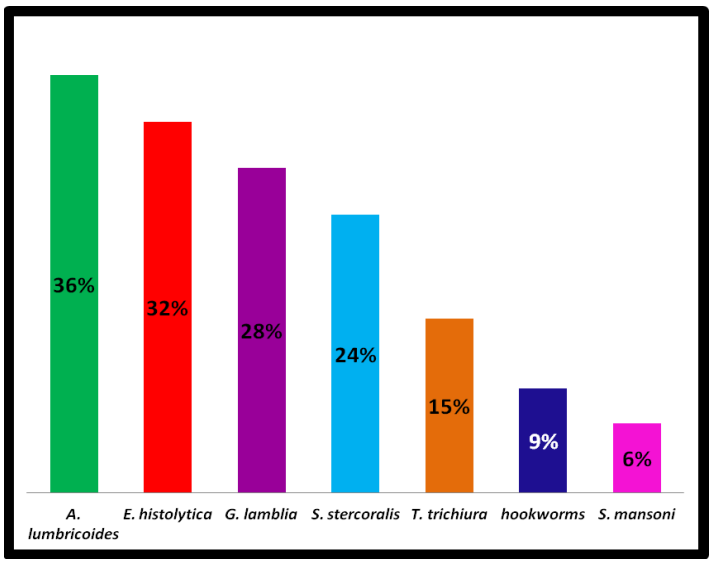


Figure (3): The **Statistic of Histogram**, demonstrating the list of different species of the intestinal parasites isolated from fresh stools samples of student children and the percentile infection rate in the children with each of these parasitic species.

DISCUSSION

The calculated *correlation coefficient*, or "r" value was greater than the critical values at both $\alpha = 0.05$ and $\alpha = 0.01$ levels of significance. Thus, the computed value was significant and demonstrated a very strong evidence for the fact that the same environment, i.e., Dilla Town & its peripheral villages was favorable for the variety of human intestinal parasites mentioned above as the climate of this very region is warm and moist. That was so because the computed value of r was 0.99984, being almost +1, or nearly a perfect positive correlation. The other major factors observed, by the researcher of this study project, to be responsible for the very strong correlation among the different species of intestinal parasites recorded include:- poor environmental sanitation, poor personal hygiene of the target children, lack of clean water for domestic use or drinking, and several poverty related problems. Multiple infections as many as 5 different species of pathogenic intestinal parasites were practically observed harbored in a single individual child.

The list of the most important diagnostic stages of intestinal parasites represents the first hand and pure identifying original data to be interpreted and analyzed. The set of data in Fig. 1 was a substantiated evidence for the existence of various species of intestinal parasites and multiple infections in the population of student children participated in this study project.

The data of Table 1 was an excellent set as an unprocessed raw data for statistical analysis and interpretation in Figs. 2 and 3. In brief, Table 1 was a complete statistical set to meet the need of a scholar reader about the planned study executed.

The infection rates in percentage with each specific species of intestinal parasites in the population of student children was imperatively devised and forwarded in the statistic of histogram.

In 710 student children examined the number of species of the intestinal parasites were 11; however, four of them were scanty in frequency and not in the rate that can be a threat for the health of human population of the area. The four species of intestinal parasites that had been observed to be rare in prevalence were *Taenia saginata*, *Enterobius vermicularis*, *Hymenolepis nana*, and *Fasciola hepatica* with the corresponding infection rates of 1%, 1%, 1%, and 0.6% respectively. These four species of parasites were not included in the statistical analyses of Table 1 and Figs.1-3, because they were with negligible frequency in prevalence compared to the other 7 species of parasites.

CONCLUSION

In conclusion, the major preventive measures against parasitic infections in humans in the area where the research work has been executed must be by focusing on:

- Blocking transmission of infective stages,
- Delivering public health education,
- Providing the public with clean water supply for domestic use and drinking,
- Improving personal hygiene and environmental sanitation, and
- Identifying and treating infected individuals to prevent the spread of infections.

Funding:

The financial support to cover the cost of this research project was given by the Research and Dissemination Office of Dilla University.

Conflict of interest:

I confirm that I don't have any competitive conflict of interest with any body.

Ethical approval:

Ethical permission /clearance to perform the research work for the well-being of human subjects was obtained from:- Dilla University, the Office of Gedeo-Zone Administration, and the Directors of the schools involved in the study. The demands for the continuity of this study project and involvement by the participant student children & their parents was unusually high.

ACKNOWLEDGEMENTS

Dilla Don Bosco, i. e., Dilla Ethiopian Catholic Church was very kind and quick to accept the request forwarded by the researcher of this study project for medical prescription and supervision assistance in favor of the infected student children. Dilla Don Bosco participated in this study project by assigning a medical doctor [Dr. Corazon B. Jaca FMA] for purchasing relevant medicines as well as for prescription and clinical supervision in the process of medication.

I am very grateful to Dilla University for its providing me with the necessary fund to perform this study. I am also thankful to Dilla University & the main Administrator Office of Gedeo-Zone for their writing letters to the Directors of elementary schools, requesting them to be cooperative for my research activities with their students.

I am deeply thankful to Ato Tilahun Kebede and Ato Mingizem Birhan who are computer science teachers and technicians in Dilla University for their providing me with computer tools such as Adobe Photoshop and Equation Editor and installing them in my laptop.

REFERENCES

1. Narango RM, Aloo PA, Kabiru EW, Nyanchongi BO. The risk of pathogenic intestinal parasitic infections in Kisii Municipality, Kenya. *BMC Public Health* [serial on the Internet]. 2008 Jul 14 [cited 2012 Dec 20.]; 8(27): [about 6 p]. Available from:http://www.biomedcentral.com/1471-2458/8/237.
2. Mordi RM, Okaka CE. Prevalence of intestinal parasites in Edo State. *Int J Health Res* [serial on the Internet]. 2009 Sep [cited 2012 Dec 20]; 2(3): [about 3 p.]. Available from: http: //www.ijhr.org.
3. Coulibaly JT, Furst T, Silue KD, Knopp S, Hauri D, Ouattara M, et al. Intestinal parasitic infections in school children in different settings of Cote d'Ivoire: effect of diagnostic approach and implications for control. *J Med Lab Prof* [serial on the Internet]. 2012 Jul 6 [cited 2012 Dec 20];5(135): [about 4 p].Available from: http: // www.parasitesandvectors.com/content /5/1/ 135.
4. Kurpad AV, Regan MM, Nzareth D, Nagarai S, Gnanou J, Young VR. Intestinal parasites increase the dietary lysine requirement in chronically undernourished Indian men.*Am J Clin Nutr* [serial on the Internet]. 2003Jun 13 [cited 2012 Dec 20]; 78(6): [about 4 p.]. Available from: http: //www.ncbi.nlm.nih.gov/pubmed/14668277.
5. Assefa S, Erko B, Medhin G, Assef Z, Shimelis T. Intestinal parasitic infections in relation to HIV/AIDS status, diarrhea and CD4 T-cell count. *Infect Dis* [serial on the Internet]. 2009 Sep 18 [cited 2012 Dec 20]; 9(155): [about 4 p.]. Available from: http: //www.biomedcentral.com/1471-2334/9/155.
6. Al-Naemy BS, Al-Kalak S, Rahemo ZIF. The intestinal parasites of Bashiqa District, Nineveh Governorate, Iraq. *Int J Mol Zool* [serial on the Internet]. 2012 Oct 22 [cited 2012 Dec 20]; 2(6): [about 3p]. Available from:http: //bio.sophia-publisher. com/html-475-25-ijmz.
7. Singh A, Houpt E, Petri WA. Rapid diagnosis of intestinal parasitic protozoa,with a focus on *Entamoeba histolytica*. *Interdiscip Perspect Infect Dis* [serial on the Internet]. 2009 Mar 30 [cited 2012 Dec 20]; 2009(2009): [about 3 p.]. Available from:http://www.hindawi.com/journals/ipid/2009/5470 90/.
8. Molina CD, Molina MM, Molina JM. Intestinal parasites in Southeast Asian refugees two years after immigration. *West J Med* [serial on the Internet]. 1988 Oct [cited 2012 Dec 20]; 149(4): [about 3 p.]. Available from: http: //www.ncbi. nlm.nih.gov>…>West J Med>.149(4);Oct 1988.
9. Garg PK, Perry S, Dorn M, Hardcastle L, Parsonnel J. Risk of intestinal helminth and protozoan infection in a refugee population. *Am J Trop Med Hyg* [serial on the Internet]. 2005 Mar 20 [cited 2012 Dec 20]; 83(3): [about 4p.]. Available from: http: //www. Ajtm.org/content/73/2/386.
10. Estridge BH, Reynolds AP, Walters NJ. Basic medical laboratory techniques. 4th ed. USA; Delmar Division of Thomson Learning 2000; 551,553.
11. Cheesbrough M. District laboratory practice in tropical countries, part 1. USA; Cambridge University Press1998; 193, 200-2.
12. Paniker CKJ. Textbook of medical parasitology. 6th ed. New Delhi; Jaypee Brothers Medical Publishers 2007; 113-26,165-94.
13. Bogitsh BT,Carter CE, Oeltmann TN. Human parasitology. 3rd ed. India; Elsevier Inc 2005; 324-27, 334-61.
14. Gilles HM. Soil-transmitted helminths (geohelminths). In: Cook GC. editor. Manson's tropical diseases. 20th ed. London; WB Saunders 1996; 1369-1402, 1413-1447.
15. Estridge BH, Reynolds Ap, Walters NJ. Basic medical laboratory techniques. 4th ed. Asia: Thomson Learning. 2000; 463.
16. Merck KGA. Microscopy: Safranin 0 (C.I.50240) Certistain. [serial on the Internet]. 2011; [about 1 p.]. Available from: www.merck-chemcals. com/ microscopy.
17. Boyd PF. Basic medical microbiology. 5th ed. USA: Little, Brown and Co. (Inc.). 1995; 557.
18. Beadle GW, Emerson R, Whitaker DM, Lawson CA, Lewis RW, Biirmester MA et al. Full text of "Animal tissue techniques". [serial on the Internet]. USA: W.H. Freeman and Co. 1962; [about 1 p.]. Available from: http://archive.org/stream/ animal-tissuech00hurr.
19. Lok JB. *Strongyloides stercoralis*: a model for translational research on parasitic nematode biology. Worm Book. [serial on the Internet]. 2007; [about 2 p.].Available from: http:/www. wormbook.org.

20. Rosenblatt JE. Clinical importance of adequately performed stools ova and parasite examinations. *Clin Infect Dis*. [serial on the Internet]. 2006; 42(7): [about 3p.]. Available from: cid.oxford journals.org/content/42/7/979.full ▼Clinical Infect ious Diseases.

Peer reviewer : Mohieddin Elbaboly: Professor of Parasitology, Faculty of Medicine, Zagazig University, Egypt.

Editor: Tarik Zaher: Professor of Tropical Medicine,Faculty oF Medicine, Zagazig University, Egypt.

Circulating Resistin and Visfatin Levels in Patients with Inflammatory Bowel Disease as Predictors of Treatment Response

Jihan A. Shawky[1], Hosam M. Dawod[1], Hesham A. Atia[2], Hany A. Labib[3]

[1] *Department of Tropical Medicine, Faculty of Medicine, Zagazig University, Egypt*
[2] *Department of Internal Medicine Faculty of Medicine, Zagazig University, Egypt*
[3] *Department of Clinical Pathology Faculty of Medicine, Zagazig University, Egypt*

Corresponding Author
Jihan A. Shawky

Mobile:
+20111950071

E mail:
jihan.shawky2013@yahoo.com

Key word: Resistin; Visfatin; inflammatory bowel disease

Background and study aim: Ulcerative colitis (UC) and Crohn's disease (CD) are the common forms of inflammatory bowel disease (IBD). The etiology and pathogenesis are not fully understood yet. Many studies have been focused on adipokines in the pathogenesis of IBD. IBD is associated with alteration in fat distribution and development of white adipose tissue (WAT) hypertrophy in the mesentery. Most of adepokines are formed in WAT or in immune cells play an important role in IBD manifestations. The aim of this study was to evaluate the levels of resistin and visfatin in IBD patients before and after treatment.

Patients and Methods: 40 patients with IBD (15 patients with CD and 25 patients with UC) attended to Tropical Medicine and Internal Medicine Departments; Zagazig University Hospitals from March 2012 till December 2013 were included in this study. Serum levels of resistin and visfatin were measured before treatment and 3 months after treatment.

Results: The mean serum levels of resistin in Crohn's disease ranged from 12.2+2 ng/ml to 9.0 + 4.0 (P= 0.1) and the mean serum levels of visfatin in Crohn's disease ranged from 5.6+4.6 ng/ml to 3.4+4.1 ng/ml (P= 0.04) before and after treatment respectively, and the mean serum levels of resistin in ulcerative colitis ranged from 11.2+2 ng/ml to 7.5+ 3.1 ng/ml (P= 0.039) and the mean serum levels of visfatin in ulcerative colitis ranged from 3.7+1.2 to 2.5+1.1 ng/ml (P= 0.004) before and after treatment respectively.

Conclusion: The serum levels of resistin and visfatin decreased significantly after treatment induction for IBD so can be used as a marker for treatment success.

INTRODUCTION

Inflammatory bowed disease (IBD) is inflammatory conditions of small intestine and the colon. Ulcerative colitis (UC) and Crohn's disease (CD) are the most common two forms [1,2,3].

The pathogenesis of IBD still not clear, it is thought that it is a result of genetic predisposition or immune response of the gut to its commensal bacteria [4], also the role of lifestyle, other factors as non steroidal anti-inflammatory drugs [5], smoking [6] or recent appendectomy [7]. Recent studies have been focused on pro-inflammatory cytokines and circulating adipokine levels [8,9].

Inflammatory bowel disease (IBD) is associated with changes in fat distribution and fat mass as increasing visceral fat mass and development of white adipose tissue (WAT) [10] and subcutaneous adipose tissue [11].

Most of adipokines are formed in WAT or in immune cells play and important role in IBD pathogenesis [12,13].

Resistin is one of the cysteine-rich proteins family, it was described as adipocyte-derived mediator of hepatic insulin resistance [14], it is produced by mononuclear cells [15], and minimal amount produced by visceral adipose tissue [16]. Resistin has pro-inflammatory properities as it induces the production of IL-6, IL-1B and TNF-α from monocyte [17,18], it is observed to be elevated in patients with IBD [19].

Visfatin is adipokine identified in visceral adipose tissue, its structure is identical to pre-B-cell colony-enhancing factor (PBEF) [20], its levels is higher in obese women compared to normal weight [21], the main source of visfatin is WAT-derived macrophages and stromal vasculature [22,23]. Visfatin has pro-inflammatory properties as

induction of TNF-α, IL-6, IL-8 by peripheral mononuclear cells [24,25]. Visfatin elevation has been observed in IBD and can be attributed to be a causative factor of decreased bone mass density in IBD [26,27].

The aim of this study was to evaluate the levels of resistin and visfatin in IBD patients before and after treatment.

PATIENTS AND METHODS

Forty patients with active IBD admitted to Tropical Medicine and Internal Medicine Departments, Zagazig University Hospitals, Egypt (age range from 20 to 43 years old) were enrolled.

Activity of Crohn's disease evaluated by CD activity index score [28], while activity in UC according to Robert et al [29].

Exclusion criteria:

Hypothyroidism or hyperthyroidism

Diabetes mellitus

Adrenal failure

Hyperlipidemia

COPD

Autoimmune disease

All patients were subjected to the following:

- Full history taking.
- Thorough clinical examination.
- Complete blood picture.
- Liver function test.
- C-reactive protein (CRP).
- Erythrocyte sedimentation rate.
- Colonoscopy and biopsies for histopathology.
- Serum level of visfatin and resistin were measured by ELISA using a commercially available kit (BioVision Research Products, Mountain View, USA) And (Linco Research, St. Charles, MO,USA) respectively before induction of treatment and 3 months after treatment .
- Informed consent was obtained from all patients and the study was approved by our Institutional Review Board.

Statistical analysis:

All data were expressed as mean and standard deviation. For quantitative data (normally distributed) comparison between two groups was done using student t-test ($p < 0.05$; significant). All statistical calculations were done using SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 15 for Microsoft Windows.

RESULTS

Patients were matched for age, sex. Colonoscopy and biopsies divided the patients to 10 patients with CD and 25 patients with UC with follow up loss of 4 cases and one case need surgical intervention.

The mean serum levels of resistin in Crohn's disease ranged from 12.2+2 ng/ml to 9.0 + 4.0 (P= 0.1) and the mean serum levels of visfatin in Crohn's disease ranged from 5.6+4.6 ng/ml to 3.4+4.1 ng/ml (P= 0.04) before and after treatment respectively, and the mean serum levels of resistin in ulcerative colitis ranged from 11.2+2 ng/ml to 7.5+ 3.1 ng/ml (P= 0.039) and the mean serum levels of visfatin in ulcerative colitis ranged from 3.7+1.2 to 2.5+1.1 ng/ml (P= 0.004) before and after treatment respectively.

Table (1): Patients and disease characteristics

	CD (N= 10)	UC (N= 25)
Age (years) **Mean ± SD**	35 ± 9	40 ± 4
Gender		
Male	3	18
Female	7	6
Treatment		
5 ASN	8	25
N steroid	7	24
Azathioporine	8	8
Infliximab	1	0

CD, Crohn's disease**; UC**, Ulcerative colitis.

Table (2): Serum level of resistin and visfatin in CD before and after treatment

CD	Before treatment	After treatment	P
S. Resistin	12.2 ± 2 ng/ml	9.0 ± 4.0 ng/ml	0.1
S. Visfatin	5.6 ± 4.6 ng/ml	3.4 ± 4.1 ng/ml	0.04
C. reactive protein	6 ± 1.5 mg/dl	0.5 ± 0.4 mg/dl	<0.01

Significant decrease in serum levels of visfatin with decrease in resistin and C. reactive protein in Crohn's disease after treatment.

Table (3) : Serum level of resistin and visfatin in UC before and after treatment

UC	Before treatment	After treatment	P
S. Resistin	11.2 ± 2 ng/ml	7.5 ± 3.1 ng/ml	0.039
S. Visfatin	3.7 ± 1.2 ng/ml	2.5 ± 1.1 ng/ml	0.004
C. reactive protein	4.5 ± 0.5 mg/dl	0.5 ± 0.2 mg/dl	<0.05

Significant decrease in serum levels of visfatin with decrease in resistin and C. reactive protein in ulcerative colitis after treatment.

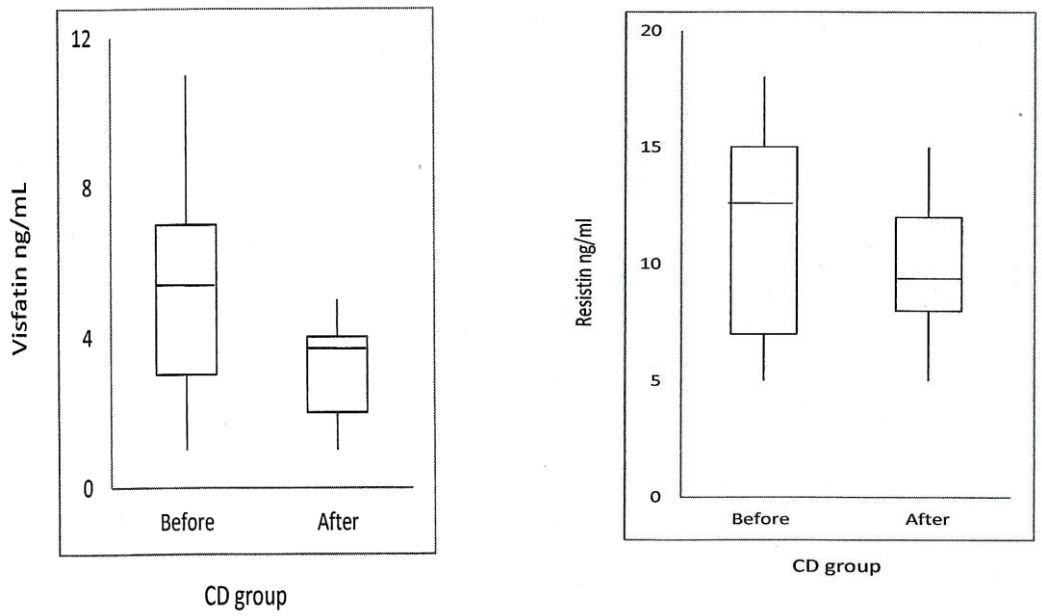


Figure (1): Serum levels of visfatin and resisitin in Crohn's disease before and after treatment.

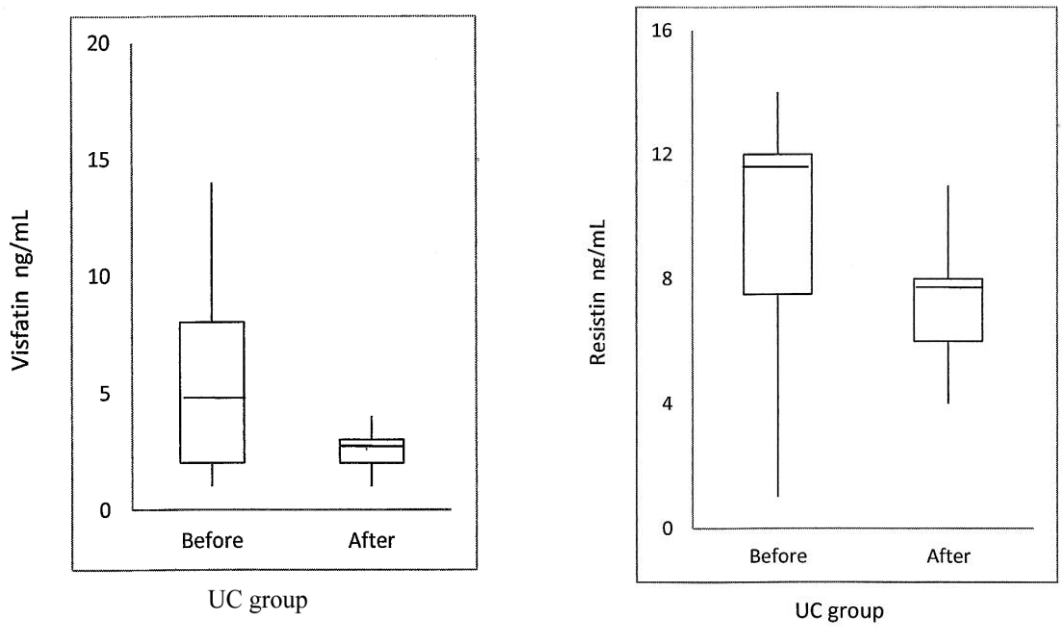


Figure (2): Serum levels of visfatin and resisitin in Ulcerative Colitis before and after treatment.

DISCUSSION

In the current study, we measured the circulating levels of two adipocytokines in patients with active IBD before and after induction of treatment by three months, which are produced by WAT, are closely related to chronic inflammation, a fact that may implicate them in pathogenesis and follow up of the response to medical treatment.

In our study, both resistin and visfatin have a significantly lower circulating levels after 3 months of induction of treatment of active UC and CD patients as compared with levels before treatment (P= 0.1 and P= 0.04 respectively in CD patients and P= 0.039 and P= 0.004 respectively in UC patients, these results are inconsistence with Young et al. [30] who found that its serum level decreased significantly (P= 0.046) after induction therapy suggesting as a marker of successful therapy, whereas, the serum level of resistin showed no significant alteration after treatment or significant correlation with changes in CRP or clinical indices and this may be explained by shorter duration in their study 10 weeks than in this study (12w) which may give more time for obvious significant changes.

In other study, Valenkin et al. [19] found that both resistin and visfatin were increased in active disease group not in those in remission denoting the effect of treatment and the mean serum resistin and visfatin were 12.2$\pm$2 ng/ml in CD patients and 11.2$\pm$2 ng/ml in UC patients before treatment and this was not in concordance with results obtained by Kostantinos et al. [31] who showed higher levels and this could be explained by larger number of patients and the importance of value of decrease not the one time value itself.

The CRP showed significant decrease after treatment in both CD and UC patients ($P<0.01$ and $P<0.02$ respectively) and this result is matching with that of Young et al. [30] and these results of resistin, visfatin and CRD increase their importance as possible marker for treatment response and for follow up after induction treatment.

CONCLUSION

The serum levels of resistin and visfatin decreased significantly after treatment induction for IBD, so can be used as a marker for treatments.

Funding: None.

Conflicts of interest: The authors declare no conflict of interest.

Ethical approval: Approved.

REFERENCES

1. Baumgart DC, Carding SR, IBD : Cause and immunobiology.. *Lancet* 2007; 369 : 1627-40.
2. Baumgart DC, Carding SR. IBD, clinical aspect and established and evolving therapies. *Lancet* 2007; 369 (9573): 1641-57.
3. Xavier RJ, Podolsky DK. Unraveling the pathogenesis of IBD. *Nature* 2007; 448 (7152):427-34.
4. Heap Ga, Van Heel DA. The genetic of chronic inflammatory diseases. *Hum Mol Genet*. 2009; 18 : R101-106.
5. Bjarnson I, Zanelli G, Smith T, Prouse P, Williams P, Smethurst P et al. Non-steroidal anti-inflammatory drug-induced intestinal inflammation in humans. *Gastroenterology* 1987; 93: 480-89.
6. Odes HS, Pich A, Reif S, Halak A, Lavy A, Keter D et al. Effects of current cigarette smoking on clinical course of Crohn's disease and ulcerative colitis. *Dig Dis Sci* 2001,46 : 1717-21.
7. Frisch M, Johansen C, Mellemkiear L, Engels EA, Gridley G, Biggar RJ et al. Appendectomy and subsequent risk of inflammatory bowel diseases. *Surgery* 2001;130 : 36-43.
8. Olszanecka-Clinanowics M, Chudek J, Kocelak P, Szromek A, Zahorska-Markiewicz B. Body fact changes and activity of tumor necrosis factor system – a 5 year follow up study. *Metabolism* 2011;60 : 531-36.
9. Olszanecka-Glinianowicz M, Zahorska-Markiewicz B, Janowska J, Zurakowski A. Serum concentrations of nitric oxide, tumor necrosis factor (INF)-alpha and TNF-soluble receptors in women with overweight and obesity. *Metabolism* 2004;53: 1268-73.
10. Desreumaux P, Ernst O, Geboes K, Gambies L, Berrebi D, Muller-Alouf H et al. Inflammatory alterations in mesenteric adipose tissue in Chrons disease. *Gastroentrology* 1999;117(1): 73-81
11. Valentini L, Buning C, Hengstermann S, Schaper L, Koernicke T, Norman K et al. Malnutrition and impaired muscle strength in patients with Crohns disease and ulcerative colitis in remission. *Nutrition* 2008;24(7-8):694-702
12. Mattacks CA, Sadler D, Pond CM. The cellular structure and lipid/ protein composition of adipose tissue surrounding chronically stimulated lymph nodes in rats. *J Anat* 2003;202:551-61.

13. Gambero A, Marostica M, Abdalla Saad MJ Pedrazzoli J Jr. Mesenteric adipose tissue alterations resulting from experimental reactivated colitis. *Inflamm Bowel Dis* 2007;13: 1357-64.
14. Steppan CM, Bailey ST, Bhat S, Brown EJ, Banerjee RR, Wright CM et al. The hormone resistin links obesity to diabetes. *Nature* 2001;409 : 307-12.
15. Patel L, Buckels AC, Kinghorn IJ, Murdock PR, Holbrook JD, Plumpton C et al. Resistin is expressed in human macrophages and directly regulated by PPAR gamma activators. *Biochem Biophys Res Commun* 2003;300: 472-76.
16. Savage DB, Sewter CP, Klenk ES, Segal DG, Vidal-Puig A, Considine RV et al. Resistin/Fizz 3 expression in relation to obesity and peroxisome proliferators-activated receptor-gamma action in humans. *Diabetes* 2001;50:2199-202.
17. Bokarewa M, Nagaey I, Dahlberg L, Smith U, Tarkowski A. Resistin, an adipokine with potent proinflammatory properties. *J Immunol* 2005;174 : 5789-95.
18. Steppan CM, Lazar MA.The current biology of resistin. *J Intern Med* 2004;255 : 439-47.
19. Valentini L, Wirth EK, Schweizer U, Hengstermann S, Schaper L, Körnicke T, et al. Circulating adipokines and the protective effects of hyper-insulinemia in inflammatory bowel disease. *Nutrition* 2009;25: 172-81.
20. Piestrzeniewics K, Luczak K, Komorowski J. Obesity and adiponectin in acute myocardial infarction. *Cardiol* 2007;14 : 29-36.
21. Zahorska-Markiewicz B, Olszanecka-Glinianowicz M, Janowska J, Kocełak P, Semik-Grabarczyk E, Holecki M, et al. Serum concentration of visfatin in obese women. *Metabolism* 2007; 56: 1131-34.
22. Cural Ca, Wegner V, Sengenes C, Miranville A, Tonus C, Busse R, et al. Macrophages in human visceral adipose tissue: increased accumulation in obesity and a source of resistin and visfatin. *Diabetologia* 2006;49: 744-47.
23. Varma V, Yao-Borengasser A, Rasouli N, Bodles AM, Phanavanh B, Lee MJ, et al. Human visfatin expression relationship to insulin sensitivity, intramyocellular lipids and inflammation. *J Clin Endocrinol Metab* 2007;92: 666-72.
24. Chang YC, Chang TJ, Lee WJ, Chuang LM. The relationship of visfatin/ pre-B-cell colony-enhancing factor/ nicotinamide phosphoribosyl transferase in adipose tissue with inflammation, insulin resistance, and plasma lipids. *Metabolism* 2010;59: 93-99.
25. Dahl TB, Yndestad A, Skjelland M, Øie E, Dahl A, Michelsen A, et al. Increased expression of visfatin in macrophages of human unstable carotid and coronary athero-sclerosis possible role in inflammation and plaque destabilization. *Circulation* 2007;115: 972-80.
26. Moschen AR, Kaser A, Enrich B, Mosheimer B, Theurl M, Niederegger H, et al. Visfatin an adipocytokine with proinflammatory and immunomodulating properties. *J Immunol* 2007;178: 1748-58.
27. Moschen AR, Geiger S, Gerner R, Tilg H. Pre-B-cell colony enhancing factor/NAMPT/ visfatin and its role in inflammation related bone disease. *Mutat Res* 2011;690: 95-101.
28. Best WR, Becktel JM, Singleton JW, Kern F Jr. Development of a Crohn's disease activity index. National cooperative Crohn's Disease study.*Gastroentrology*.1976:70:439-444.
29. Langan RC, Gotsch PB, Krafczyk MA, Skillinge DD. Ulcerative Colitis: Diagnosis and Treatment. *Am FamPhysician* 2007; 76(9):1323-1330.
30. Hwangbo Y, Kim HJ, Shim J, Jang JY, Dong SH, Hokim BU, Chang YW. Change of Circulating Leptin, Adiponectin, Resistin, and Visfatin Level after Treatment of Patients with Active Inflammatory Bowel Disease. *Intest Res.* 2010;8(2): 151-61
31. Karmiris K1, Koutroubakis IE, Xidakis C, Polychronaki M, Voudouri T, Kouroumalis EA. Circulating levels of liptin, adeponectin, resistin and ghrelin in inflammatory bowel disease *Inflamm Bowel Dis*. 2006;12: 100-105.

Peer reviewers: Salah Elgamal: Professor and Head of Internal Medicine Departments, Hepatogastroenterology Unit, Mansoura Faculty of Medicine, Egypt. **Ahmad Abdel-Razik** MD, Tropical Medicine Department, Faculty of Medicine, Mansoura University, Egypt.
Editor: Mohamad Emara, Lecturer of Tropical Medicine and Hepatogastroenterology, Faculty of Medicine, Zagazig University, Egypt.

Serum and CSF Nitric Oxide in Cases of Acute Meningitis

Rashed M. Hassan[1], Soha, E. Khorshed[1], Seham M. Zaki[2], Hoda A. Ibraheem[2]

[1] *Tropical Medicine Department, Faculty of Medicine, Zagazig University, Egypt*

[2] *Clinical Pathology Department, Faculty of Medicine, Zagazig University, Egypt*

Corresponding Author
Soha E. Khorshed

E mail: sohaesmat@hotmail.com

Key words:Serum;CSF;Meningitis,Nitric oxide

Background and study aim: In several cases of meningitis ; routinely used diagnostic procedures are unable to identify the cause of this disease. The aim of the present study was to differentiate between acute bacterial and viral meningitis as it is very important matter.**Patients and Methods:** Fifty patients who admitted to Fever Hospital were included in this study. They were classified into 2 groups according to CSF criteria, Group I (22 patients) with viral meningitis, Group II (18 patients) with acute bacterial meningitis. In addition 10 subjects were included as control group (GroupIII) .All subjects were subjected to clinical examination, routine laboratory investigation, lumbar Puncture, CSF analysis (bacteriological, cytological and chemical). Also, Nitric Oxide (NO) levels in serum and CSF were measured.

Results: The study found that serum and CSF NO levels were more highly significantly elevated in patients with bacterial meningitis than in patients with viral meningitis and controls. While there is no significant difference between patients of viral meningitis and controls. The rise of NO levels was significantly associated with protein, WBC and C-reactive protein levels.**Conclusion:** Patients with bacterial meningitis had highly significant elevation in the serum and CSF levels of NO. Their levels were correlated with markers of inflammation as CRP, WBC and protein level of CSF. Serum NO examination is an easy, rapid and cheap test. It can be measured in acute meningitis without waiting the results of culture to determine the nature of infection either viral or bacterial.

INTRODUCTION

Meningitis is an inflammation of the cerebrospinal membranes that may develop from infectious or non-infectious etiology. Although the etiologies are different, clinical symptoms and signs are similar in meningitis [1]. Acute meningitis is a medical emergency mainly caused by infection with viruses or bacteria [2]. Viral meningitis is usually relatively mild and clears up within a week or two without specific treatment [3]. By contrast, acute bacterial meningitis (ABM) has characteristics of rapid progression and a high mortality rate as well as having the potential of forming permanent neurological or audiological sequelae [4].

Routine laboratory analysis of cerebrospinal fluid (CSF) is not efficient enough to discriminate between etiologies especially in the early phase of the disease [5,6]. Therefore several studies suggest that various inflammatory mediators including cytokines, platelet activating factor, and reactive oxygen species contribute to the pathological process of meningitis, and the development of neuronal injury [7,8]. Nitric oxide (NO) may have some important pathophysiological effect during bactrial meningitis [9].

Nitric oxide (NO) is an uncharged molecule composed of seven electrons from nitrogen and eight electrons from oxygen. NO exhibits the potential to act as either an oxidizing or reducing agents [10]. NO is produced by the oxidation of the terminal guanidine group of L-arginine in the presence of oxygen and nicotinamide adenine dinucleotide phosephate (NADPH) by a family of enzymes Known as nitric oxide synthase (NOS2) [11].NO is not a stable substance, and it converts into

nitrite and nitrate in 5-10 seconds. NO may play role in microwaves cellular injury, cerebral edema, C.S.F pleocytosis, neurological injury and necrotic cell death in oligodendroglias [9-12].

The mechanisms responsible for damage in the central nervous system are not yet completely understood. In some experimental studies, it was observed that NO destroyed the blood-brain barrier either directly or indirectly by the effect of TNF-alpha [13]. Another possible scenario is that bacterial DNA stimulates the inflammatory response and activates genes connected with the anti-inflammatory response and cell differentiation. Toll-like receptors (TLRs) play a significant role in this response; during a bacterial infection, these receptors activate the migration of microglia and leucocytes to the CNS [14].

Aim of the study:

In this study, we aimed to measure the level of nitric oxide (nitrite) in serum and CSF of the patients with acute meningitis to assess its role in the differentiation between bacterial and viral meningitis.

PATIENTS AND METHODS

The present study included 50 patients (27 males and 23 females), their age ranged from 15 to 55 years. All patients were admitted to Fever Hospital with picture suggesting of acute meningitis.

Patients were excluded if they had :

1- Antibiotic treatment before admission.
2- Administration of corticosteroid before admission.
3- Traumatic C.S.F punctures.
4- Duration of symptoms more than 72 hours.

Patients were classified into three groups according to CSF analysis :

Group I: composed of 22 patients with viral meningitis.

Group II: composed of 18 patients with bacterial meningitis.

Group III: (control group): In addition 10 subjects were included as control group (GIII). They were well matched with age and sex of patients. In this group the lumbar punctures were done for surgical purposes as hernial or testicular operations.

Immediately after admission, patients were subjected to the followings:

- Through clinical examination with great attention directed towards the nervous system.
- Lumber puncture was done using sterile lumber puncture needles. Samples of C.S.F and blood samples were collected for the following investigations:
- CSF total leucocytic count (ILC) and
- CSF total protein by tubidimetric method using tricholoroacetic acid [15].
- CSF and Serum glucose using ADIVA 1650 autoanalyzer (siemens medical solution Diagnostics).
- CSF gram stain and bacterial culture.
- Serum high sensitivity CRP (hs CRP) was determined by particle enhanced immuno-nephelometry using BN Prospec (siemens medical solutions Diagnostics). The intra – assay precision ranges from 2.3 to 4.47. CV, inter- assay precision ranges from 2.1 to 5.77. CV and analytical sensitivity is 0.175 mg/l. CRP levels >3 mg/l. was considered positive [16].
- Blood: E.S.R, C.R.P, protein, total leucoctic count.
- Nitric oxide (nitrite) in C.S.F and serum was performed by using the nitric colorimetric method (Biodiagnostie Nitrite Assay Kit).

Statistical analysis:

Data were entered, checked and analyzed using SPSS version 10.0 (Statistical Package for the Social Sciences, Chicago, IL). Data were expressed as number and parentage for qualitative variables, mean ± standard deviation for quantitative ones. Independent sample t test and paired sample t test were used as appropriate for analysis of results. $P<0.05$ was considered significant.

RESULTS

Fifty patients (27 males, 23 females) were included in this study. Twenty two patients had viral meningitis and eighteen patients had bacterial meningitis according to CSF parameters . As regard the bacteriological results of CSF examination of patients with bacterial meningitis, the causative organism could be identified in 16 of the 18 patients. The diagnosis of the other 2 patients was based on the ground of typical CSF findings of bacterial meningitis. Staining of the CSF deposit with gram stain was positive in 100% of patients with bacterial meningitis, while

the culture of CSF was positive in 88.8% of patients.

The CSF nitrite, protein, glucose, lymphocyte and WBC values of the patients and control group are shown in Table 3.

Nitrite level in the CSF samples of patients with bacterial meningitis were significantly elevated in comparison to viral meningitis group and control group (P<0.01, P<0.01, respectively). However, no significant elevations were found in patients of viral meningitis (P>0.05) compared to control.

There was significant differences of protein and glucose between groups (P< 0.01).

We investigated the correlations between nitrite levels and WBC count; protein ;glucose level of C.S.F. In bacterial meningitis a positive correlation was found between nitrite levels and WBC count (r=0.834, P<0.001), protein (r= 0.866, P<0.001). A negative correlation was found between nitrite and glucose levels (r= 0.312, P<0.05). There was a positive correlation between serum CRP and serum nitrite level (r=0.964-P< 0.001).

Table (1): The demographic data of different groups

	Viral meningitis (n = 22)	**Bacterial meningitis (n = 18)**	**Control (n = 10)**
Age			
X ± SD	18.02±15.19	12.2 ± 11.82	26±7.12
range	(15. – 55 y.)	(15 – 65 y.)	(18- 40 y.)
Sex			
Male	12	9	6
Female	10	9	4

Table (2): Different clinical parameters in the viral and the bacterial groups

Parameter	**Viral meningitis (n=22)**	**Bacterial meningitis (n=18)**	**P value**
Fever.	20(90.9%)	18 (100%)	0.55
Headache.	16 (72.7%)	15 (83.3%)	0.67
Photophobia.	2 (9.1%)	6 (33.3%)	0.13
Vomiting.	16 (72.7)	12 (66.6)	0.94
Neck stiffness.	15 (68.2%)	14 (77.7%)	0.75
Kernig's sign.	10 (45.4%)	12 (66.6%)	0.30
Brudziniski's sign.	7 (31.8%)	11 (61.1%)	0.12
Convulsions.	3 (13.6%)	5 (27.7%)	0.47
Confusion.	13 (59.1%)	10 (55.5%)	0.92
Rash.	2 (9.1%)	3 (16.6%)	0.81

Table (3): Laboratory finding of CSF and serum in bacterial and viral compared to control groups

CSF / serum Parameters	Viral group (n=22)	Bacterial group (n=18)	Control group (n=10)	P1	P2	P3
CSF						
Nitric oxide (umol/l)	12.6 ± 0.64	17.078± 1.78	3.38±0.39	>0.5	<0.01	<0.01
WBCs (per ml)	125.68±46.35	2949.28±1497.7	4.7±2.22	<0.001	<0.01	<0.01
Protein (mg/dl)	41.91±5.73	152.33±55.713	28.8±7.2	<0.001	<0.01	<0.01
Glucose (mg/dl)	68.5±8.20	42.39±7.12	61.5±5.4	>0.05	<0.01	<0.01
Serum						
Nitric oxide (umol/l)	3.59±0.51	10.64±0.89	11 ± 0.7	<0.001	<0.001	<0.001
WBCs (cell/cmm)	6146.9± 1946.85	17438.05 ± 5548.16	6146.9 ± 1434.22	<0.001	<0.001	<0.001
Platelets (cell/cmm)	118323.691 ± 4173.25	104751.93 ± 20136.8	175140.91 ± 164.31	<0.001	<0.001	<0.001
CRP (mg/dl)	14.77 ± 4.74	95.17 ± 26.60	1.14 ± 0.359798	<0.001	<0.001	<0.001

P1: viral vs control p2: bacterial vs control p3: viral vs bacterial

Table (4): Correlation between viral CSF nitric oxide (nitrite) and other viral CSF parameters

Parameter	Viral CSF nitrite (m mol / L)	
	R	P
Viral CSF WBCs (per ml)	0.251	>0.05
Viral CSF protein (mg/dl)	0.374	>0.05
Viral CSF glucose (mg/dl)	0.221	> 0.05

Table (5): Correlation between viral serum nitric oxide (nitrite) and viral serum CRP

Parameter	Viral serum nitrite (m mol / L)	
	R	P
Viral serum CRP (mg/dl)	0.310	>0.05

Table (6): Correlation between bacterial CSF nitric oxide (nitrite) and other bacterial CSF Parameters

Parameter	Bacterial CSF nitrite (m mol/L)	
	R	P
Bacterial CSF protein (mg/dl)	0.866	<0.001
Bacterial CSF WBCs (per u)	0.834	<0.001
Bacterial CSF glucose (mg/dl)	0.312	> 0.05

Table (7): Correlation between bacterial serum nitric oxide (nitrite) and bacterial serum CRP

Parameter	Bacterial serum nitrite (m mol / L)	
	R	P
Bacterial serum CRP (mg/dl)	0.946	<0.001

DISCUSSION

Meningitis is a severe infectious disease that results from inflammation of the membranes surrounding the brain and the spinal cord. It may be caused by several microorganisms. Meningitis usually results from a viral infection which is the main cause of aseptic meningitis. Moreover, the main cause of septic meningitis is bacterial meningitis (BM). Less commonly, a fungal infection may result in fungal meningitis i.e. also, a part of aseptic disease [17].

Currently, interest in the role of NO in the pathogensis of bacterial meningitis responsible for cell death [14,18]. A study of the murine model of meningitis indicated that *Escheichia coli* stimulate iNOs expression in the brains of investigated animals and that the resulting NO may influence the survival of bacteria inside the macrophages [19] Furthermore during the course viral infections.

An increased level of radicals (reactive oxygen species, ROS) has been observed. The published data from a murine model investigation of herpetic encephalitis identified the role of oxidative stress in neuronal injury [20].

This study was conducted in Fever Hospital to measure the level of nitric oxide (nitrite) in serum and CSF of the patients with acute meningitis and to analyze the correlation between its level and both the clinical and the laboratory parameters in a trial to differentiate between bacterial and viral meningitis.

In this study we found that the CSF NO level in the bacterial group was significantly higher than that of the viral group. These results indicate that the bacterial meningitis leads to increased in production of NO in the CSF. In contrast, patients with viral meningitis did not show high CSF NO concentrations, suggesting that viral factors do not cause substantial increase of NO production in CSF compartments. These observations were in agreement with those of Murawska-Ciałowicz et al. [21] and Mahmoud et al. [22] who reported that there was an increase in the levels CSF NO metabolites (nitrite) in patients with bacterial meningitis than in those with viral meningitis. Also, Murawska et al. [23] and Qureshi et al. [24] found that an increase in level of CSF NO was not observed in viral meningitis.

In this study, we found that bacterial CSF WBCs count was higher than that of viral CSF WBCs count (mean of 2949.28 for bacterial cases and mean of 125.68 for viral cases). Also, we found that the type of CSF WBCs was different in the 2 types of meningitis (it was found to be mainly polymorphs (PMNLs) in the bacterial meningitis, but it was found to be mainly lymphocytes in the viral type). The same results were reported by Abro et al. [25] who noted that, in bacterial meningitis, CSF WBCs, usually polymorphs, increases significantly with mild increase in CSF WBCs count, mostly lymphocytes in the viral type.

In the present study, we found that bacterial meningitis CSF protein content was higher than that of viral cases (a mean of 152.33 for bacterial cases and a mean of 41.91 for viral cases). Also, we found that higher levels of CSF protein parallel the severity of the cases. The same results were reported by Feigin and Pearlman et al. [26]. This could be explained by that the meningeal inflammation increase the flow of the proteins into the CSF. Also, tissue destruction may increase the CSF proteins.

In our study, we found that bacterial meningitis CSF glucose content was lower than that of viral cases (a mean of 42.39 for bacterial cases and a mean of 68.5 for viral cases). Similar result was reported by Tunkle et al. [27], who reported that CNS bacterial infections can cause lowered CSF glucose levels, although CSF glucose levels are usually normal in CNS viral infections.

The low CSF glucose levels may be explained by the inhibition of mitochondrial respiration that enhance the anaerobic glycolysis through excessive NO production [28,29].

In this work, nitric oxide (nitrite) level, generally, was found to be higher in serum than in CSF of all groups. Also, we found that there was a highly significant difference ($P<0.001$) between serum NO (nitrite) and CSF NO in all groups. It was also found that levels of both serum NO and CSF NO were higher in bacterial meningitis. These nitric oxide CSF/serum ratios and indices are suggestive of its local production in the CNS as well as its passage through the disturbed blood brain barrier. The same result was detected by Hamed et al. [30].

In this work, in the bacterial group, the serum level of the nitric oxide (nitrite) was found to be higher in patients with Gram positive CSF cultures than in patients with negative CSF cultures. This could be explained by that the

nitric oxide (NO) production is related to the presence of the organism. The same result was reported by Bell and McCromick [31]. In this work, we found that bacterial blood WBCs count was higher than that of viral cases (a mean of 17438.05 for bacterial cases and a mean of 6146.9 for viral cases). However, Bell and McCormick [31] reported that leucocytosis does not necessarily differentiate bacterial from aseptic meningitis since the later can be also associated with significant leucocytosis.

In this study, we found that, there was a positive highly significant correlation between CSF NO (nitrite) and CSF protein in both bacterial ($r=0.866$, $p<0.001$) and viral meningitis ($r=0.97$, $p<0.001$). Also, a positive highly significant correlation was found between CSF NO (nitrite) and CSF WBCs in both bacterial (r 0.834, $p<0.001$) and viral meningitis ($r=0.922$, $P<0.001$). van Furth et al. [32] Murawska-Ciałowicz et al. [21] and Cetin et al. [33] reported similar results in their studies.

However, Mahmoud et al. [22] found no correlation between CSF nitrite levels and CSF white blood cells count or protein levels. Also, Kornelisse et al. [34] and Duke et al. [35] did not detect a correlation between CSF nitrite level and leukocyte count. In these studies, it was emphasized that a "major source of nitrite was not inflammatory cells".

These positive correlations between CSF nitrite-leukocytes and nitrite-protein levels in meningitis could be explained by that during episodes of meningitis especially the bacterial, TNF-alpha in CSF induces NO syntheses and consequently the production of NO, which in turn, mediates the increase in the permeability of the blood-brain barrier. This, in turn, will increase the entry of proteins and inflammatory cells (leukocytes) into the CSF compartments leading to the observed positive correlations between CSF nitrite-leukocytes and nitrite-protein levels in meningitis. The same explanation was assumed by Cetin [33].

In conclusion, the higher level of nitric oxide (nitrite) in both serum and CSF of cases of bacterial meningitis represents a rapid, easy and cheap method for the differentiation between viral and bacterial causes of acute meningitis.

Funding: Non.
Conflict of interst: Non.
Ethical approval: Approved.

REFERENCES

1. Allan R, Tunkel W ,Scheld M . Acute meingitis. Princeiples and Practice of infections Disease (Eds. Mandell Gl, Bannet JE, Dolin R) Charchill Livigstone, New York 2000; PP: 956-996.
2. Rosskl . Acute bacterial meningitis. *Semin Neurol* 2000; 20-293-306.
3. Sormunen P, Kallio Ml, Kilpi , Pettola H . C–reactive protein is useful in distinguishing gram stain–negative bacterial meningitis from viral meningitis in children. *J Med* 1999; 328, 21-28.
4. Leib SL, Tauber MG . Pathogenesis of bacterial meningitis. *Infect Dis Clin North Am*1990; 13: 527 -48.
5. Spano A, Harrell F , Durack DI . Differential diagnosis of acute meningitis. Analysis of the predictive value of initial observation. *JAMA* 1989; 262 : 2700-2707.
6. Lindquist L, Linné T, Hansson LO, Kalin M, Axelsson G. Value of cerebrospinal fluid analysis in the differential diagnosis of meningitis: a study in 710 patients with suspected central nervous system infection. *Eur J Clin Microbiol Infect Dis*. 1988 ;7(3):374-80.
7. Braun JS, Tuomanen E . Molecular mechanisms of brain damage in bacterial meningitis. *Adv Pediatr Infect Dis* 1999;14: 49-71.
8. Tureen J . Effect of recombianant human tumour necrosis factor- alpha on cerebral oxygen uptake. Cerebrospinal fluid lactate, and cerebral blood flow in the rabbit. Role of nitric oxide. *J Clin Invest*1995; 95 : 1086-91.
9. Komellsse RF, Hoekman K, Visser JJ, Hop Wc, Huijmans JG, van der straiten, de Groote R . the role of nitric oxide in bacterial meningitis in children. *J Infect Dis*1996; 174 : 120-6.
10. Beckman Js , Koppenol WH . Nitric oxide, Superoxide, and Peroxynitrite: the good, the bad, and the ugly. *Am J Physiol* 1996; 271: c1424-c1437.
11. Arzumanian V, Stankevicius E, Kevelaitis E, Kevelaitis E . Mechanisms of nitric oxide synthesis and action in cells. *Medicine* 2003; 39(6): 535-41.
12. Pifster Hw, Bematowiz A, Kodel U, Wick M . Nitric oxide production in bacterial meningitis. *J Neurol Neurosurg Psychiatry* 1995; 58: 384-5.
13. Freyer D, Manz R, Ziegenhorn A, Weih M, Angstwurm K, Döcke WD et al. : Cerebral endothelial cells release TNF-alpha after stimulation with cell wall of streptococcus pneumonia and regulate inducible nitric oxide synthase and ICAM-1 expression via outocrin loops. *J Immunol*1999; 63:4308-14.

14. Gerber J , Nau R . Mechanisms of injury in bacterial meningitis. *Curr Opin Neurol* 2010; 23(3): 312-8.
15. Shahanigan S, Brown PI , ASH Ko . Turbidimetric Measurement of total urinary Proteins. *Am J Clin Pathol* 1984; 81: 651.
16. Chenillot O, Henny J, Steinmetz J, Herbeth B, Wagner C, Siest G. high sensitivity c-reactive protein : Biological variations and reference limits. *Clin Chem Lab Meal* 2000; 38-1011.
17. Ryan K , Ray G . Local system infection. Sherries. Medical Microbiology, In Ryan K (ed.). 4th ed., 2004; PP:867-879. McGrow Hill, Edmical Publishing Division.
18. Weber JR , Tuomanen EI . Cellular damage in bacterial meningitis: an interplay of bacterial and host driven toxicity. *J Neuroimmunol* 2007; 184(1-2):45-52
19. Mittal R, Gonzalez-Gomez I, Goth KA, Prasadarao . NV. Inhibition of inducible nitric oxide controls pathogen load and brain damage by enhancing phagocytosis of Escherichia coli K1 in neonatal meningitis. *Am J Pathol* 2010; 176(3): 1292-305
20. Schachtele SJ, Hu S, Little MR, Lokensgard JR . Herpes simplex virus induces neural oxidative damage via microglial cell Toll-like receptor-2. *J Neuroinflammation* 2010; 28;7:35.
21. Murawska-Ciałowicz E, Szychowska Z, Tr busiewicz B. Nitric oxide production during bacterial and viral meningitis in children. *Int J Clin Lab Res* 2000; 30:127-31
22. Mahmoud MK, Fawy G, Salah K. Recent advances in the pathophysiology of meningitis in children. *Alexandria journal of pediatrics*2005*;* 19(1):13- 16.
23. Murawska E, Szychowska Z, Trebusiewicz B.Nitric oxide (NO) in children with meningitis. *Przegl Epidemiol* 1997; 51(3):309-15.
24. Qureshi GA, Baig SM, Bednar I, Halawa A, Parvez SH. The neurochemical markers in cerebspinal fluid to differentiate between aseptic and tuberculous meningitis. *Neurochem Int* 1998;32:197-203.
25. Abro A, Saleh A, Haseb H . Cerebro-pinal fluid analysis acute bacterial versus viral meningitis. *Pak J Med Sci* 2008; 24 (5):645-650.
26. Feigin RD , Pearlman E . Bacterial meningitis beyond the neonatal period. In: Textbox of pediatric Infectious Diseases. Edited by: Feigin RD and Cherry JD 4th eds.1998; W.B Saunders Company, Philadelphia.
27. Tunkel AR, Hartman BJ, Kaplan SL, Kaufman BA, Roos KL, Scheld WM, Whitley RJ. "practice guidelines for the mangment of bacterial meningitis". *Clinical Infectious Diseases*2004; 39 (9):1267-84.
28. Albina JE , Mastrofrancesco B . Modulation of glucose metabolism in macrophages by products of nitric oxide synthase. *Am J Physiol*1993; 264:C1 594-9
29. Amaee FR, Comis SD, Osborne MP, Drew S, Tarlow MJ. Possible invovlment of nitric oxide in the sensorrineural hearing loss of bacterial meningitis. *Acta Otolaryngol* (Stohk)1997;117:329-36
30. Hamed SA, Hamed EA, Zakary MM. Oxidative stress and S100B protein in children with bacterial meningitis. *BMC neurology* 2009;8(9):51.
31. Bell E , McCromick F . Bacterial meningitis. In: Neurological infection in children, 2nd Ed 1981.
32. van Furth AM, Seijmonsbergen EM, Groeneveld PH, van Furth R, Langermans JA. Levels of nitric oxide correlate with high levels of tumor necrosis factor alpha in cerebrospinal fluid samples from children with bacterial meningitis. *Clin infect Dis* 1996 ;22:876-8.
33. Cetin K, Erol S, Aksoy H, Tasyaran MA.Relation of cerebrospinal fluid nitric oxide levels to prognosis and differential diagnosis of meningitis. *Turk J Med Sci* 2002;32:385-390.
34. Kornelisse RF, Hoekman K, Visser JJ, Hop WC, Huijmans JG, van der Straaten PJ. The role of nitric oxide in bacterial meningitis in children. *J Infect Dis* 1996; 174-120-6.
35. Duke T, South M, Stewart A. Activation of the L-arginine nitric acid pathway in severe sepsis. *Arch Dis* 1997; 76:203.

Peer Reviewer: Eman Elshamy:Professor of Tropical Medicine and Hepatogastroenterology ,Faculty of Medicine ,Zagazig Universty,Egypt.

Editor:Tarik Zaher:Professor of Tropical Medicine and Hepatogastroenterology ,Faculty of Medicine ,zagazig Universty ,Egypt.

Video Case 1: Extraction of Impacted Coin in Upper Esophagus of a 5- Years Old Boy

Tarik Zaher
Tropical Medicine Department, Faculty of Medicine, Zagazig University, Egypt

A 5-years old Egyptian boy presented by mild dysphagia for solids for one year with repeated chest infections and fever. On plain chest X ray impacted coin was found in upper esophagus. After sedation by 2.5 mg I.M. midazolam and oral chloral hydrate solution ; the coin was extracted endoscopically by shark tooth forceps.

Video Case 2: Polypectomy in Ulcerative Colitis Patient

Tarik Zaher
Tropical Medicine Department, Faculty of Medicine, Zagazig University, Egypt

A 58-years old Egyptian women presented by bleeding per rectum for 4 monthes was examined by colonoscopy which revealed inflammation and friability of the mucosa of rectum , sigmoid and descending colon with single pedunculated sigmoid colon polyp.Histopathology of mucosal biopsies confirmed the diagnosis of ulcerative colitis.Histopathology of biopsies from the polyp revealed hyperplastic nature of the polyp.The patient was treated by oral mesalamin (3 gm per day) and oral azathioprin(100 mg per day) with successful clinical remission. One month later the patient was prepared for polypectomy(see the video) which was followed by mild blood ooze which was secured by band ligation of the stump.This polyp was different from the multiple sessile pseudo-polyps of ulcerative colitis which do not need polypectomy.

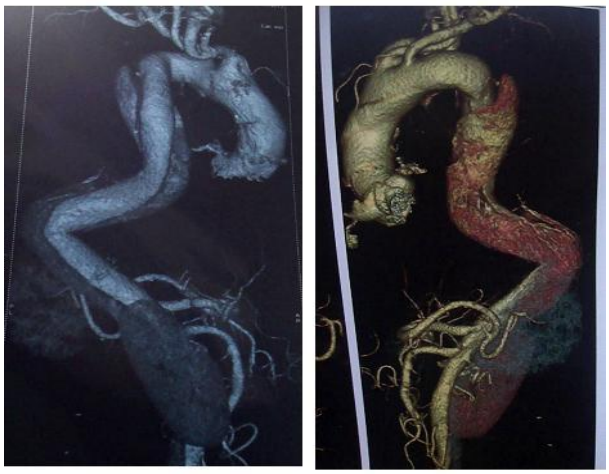
Image Case: Aortic Dissection: Uncommon Cause of Agonizing Abdominal Pain

Mohamad H. Emara[1], Emad H. Emara[2]
[1] *Tropical Medicine Department, Faculty of Medicine, Zagazig University, Egypt*
[2] *Radiodiagnosis Department, Faculty of Medicine , Zagazig University, Egypt*
emara_20007 @yahoo.com

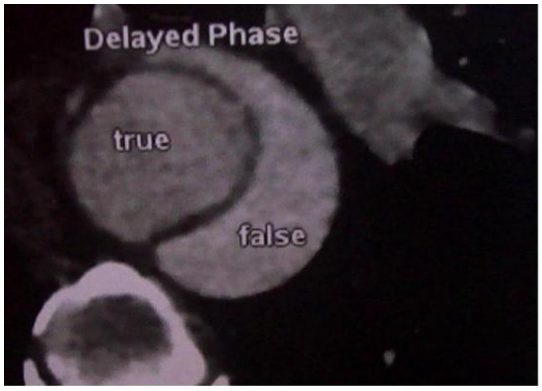
We reported a 59 years old male presented with severe agonizing abdominal pain just to the left side of the umbilicus and referred to the left shoulder. The patient was hypertensive on bisoprolol but no history of acute coronary syndrome. He was misdiagnosed at a primary health care 5 days before diagnosis. When presented he was hemodynamically stable with blood pressure 130/80. On abdominal examination mildly tender oblong mass was felt to the side of the umbilicus. The ECG examination was irrelevant. On gray scale abdominal ultrasonographgy a double channel abdominal aorta was seen that was later confirmed by Doppler study. CT angiography showed large intimal dissection sparing the aortic arch distal to the left subclavian artery and extends all through the descending thoracic and abdominal aorta till the bifurcation with small extension to the left common iliac artery (Figure 1). The true lumen (Figure 2) was seen along the right of the false lumen (well opacified along the left side of the true lumen). Fortunately, the celiac, superior mesenteric and right renal arteries were seen originating from the true lumen. The left renal artery was involved by the false lumen and it was patent (Type 3 DeBakey- Type B Stanford aortic dissection). The patient was operated upon with endoprothesis replacement. The reported rate of primary abdominal aortic dissection is less than 2%, compared with that of ascending aortic dissection (70%), descending aortic dissection (20%), and aortic arch dissection (7%) [1]. Abdominal aortic dissection presented with acute and severe abdominal pain is not common [2] and this entity of caused should not be overlooked particularly in elderly and hypertensive patients.

REFERENCES

1. Roberts CS, Roberts WC. Aortic dissection with the entrance tear in abdominal aorta. *Am Heart J* 1991;121:1834–5.
2. Borioni R, Garofalo M, De Paulis R, Nardi P, Scaffa R, Chiariello L. Abdominal Aortic Dissections: Anatomic and Clinical Features and Therapeutic Options**.***Tex Heart Inst J*. 2005; 32(1): 70–73.



Fig(1): Showed large intimal dissection sparing the aortic arch distal to the left subclavian artery and extends all through the descending thoracic and abdominal aorta.



Fig(2): The true lumen was seen along the right of the false lumen (well opacified along the left side of the true lumen).

Liver Disease and Fasting during the Month of Ramadan

Staff Members of Tropical Medicine Department, Faculty of Medicine, Zagazig Universty,Egypt

BACKGROUND

The fasting during the month of Ramadan is an integral part of the Islamic believes. During this month Moslems begins daily fasting from the time of dawn to the time sunset in a continuum. This month is a lunar month that rotates throughout the seasons of the year. Although Ramadan fasting is safe for all healthy individuals with no adverse effects on the heart, lung, liver, kidney, eyes, hematologic profile, endocrine and neuropsychiatric functions, those with various diseases should consult specialist for the possibility of fasting [1]. This year fasting in Ramadan bears peculiars including the very long day time of about 16 hours, its occurrence in the summer time and during the month of July. This hot atmosphere exposes not only patients with chronic liver diseases but also the general populations to excessive sweating and possibly the problems of electrolytes disturbances.

The spectrum of liver diseases in the Egyptian community is wide ranging from self limited acute hepatitis to liver cirrhosis and hepatocellular carcinoma (HCC). We commonly see many patients with liver diseases who willing and sometimes insist to fast.

The staff members in the Department of Tropical Medicine, Zagazig University, Egypt through a panel discussion shaded the light on this issue. We knew that the level of evidence regarding the following recommendations is very weak because the scarcity of the studies focusing this subject, but it is necessary to formulate a roadmap to help clinicians as well as patients to decide who can fast without harm.

ACUTE HEPATITIS

The panelists recommended that all patients of acute hepatitis should never fast. Some experts reported delayed recovery of serum bilirubin and liver enzymes in some patients who insisted to fast; indeed rapid recovery occurred when the oral diet resumed. These patients needs frequent light diet and they should avoid fasting.

CHRONIC HEPATITIS

There is a consensus among our staff that all patients with chronic hepatitis of any etiology without associated co-morbidities and with good liver functions can tolerate fasting. The evidence comes from previous studies [2] and also from the personal experience. Furthermore, patients with chronic viral hepatitis under interferon (IFN) therapy should be separately evaluated. Patients under IFN therapy but without side effects can tolerate fasting although are advised to avoid fasting in the day of IFN injection to avoid exhaustion and dehydration. Whereas, patients under IFN therapy but with major side effects should avoid fasting.

BILHARZIAL HEPATIC FIBROSIS

There is a consensus among our staff that all patients with bilharzial hepatic fibrosis without esophageal varices can tolerate fasting.

LIVER CIRRHOSIS

This category of liver diseases is of particular importance not only because it's high prevalence in Egypt, but mainly because patients with liver cirrhosis are prone to rapid deterioration and development of many complications. Although, the patients may feel better due improvement of their dyspeptic symptoms with fasting; their liver became exhausted.

Compensated cirrhosis

Patients with Child A cirrhosis who have good liver function can fast. Some staff recommends that patients of this category should adhere to certain precautions including but not limited to: they should receive good amounts of fluids containing electrolytes and sugar throughout the night time, should avoid direct exposure to sun light, should practice moderate activity jobs, should never delay Iftar and should delay Sohoor to the time dawn. Others recommend that patients may be re-evaluated after 10 days from fasting for both clinical and laboratory parameters. Patients who are reported as liver functions Child A but with history of any previous decompensation e.g. ascites, encephalopathy, upper GIT bleeding....etc should avoid fasting. The issue of upper GIT bleeding was evaluated in many studies. It

seems that Fasting during Ramadan increase bleeding from peptic ulcer [3-5]. Variceal bleeding and fasting in Ramadan was recently studied, it seems that fasting cirrhotics have less frequency of variceal bleeding when compared with non-fasting [2,3] and this may be explained by the increased venous flow with the regular diet [6].

Decompensated cirrhosis

Patients of liver cirrhosis with Child B and C class, should never fast. Indeed, several reports of adverse events had been reported including the increasing ascites, edema, hepatic encephalopathy, muscle cramps, and variceal bleeding [1].

HEPATOCELLULAR CARCINOMA

There is a consensus among our staff that all patients with HCC should never fast. This is applied for patients with hepatic solitary or multiple focal lesions as well as advanced HCC. In fact, those patients are prone to hypoglycemia due to many reasons including the demand for glucose by an enormous tumour mass, reduced level of glucose-6-phosphatase and phosphorylase, and increased levels of insulin-like growth factor II than in normal liver [7].

FUTURE PERSPECTIVE

We aim to conduct prospective studies to evaluate the impact of continuous fasting on different types of liver diseases. We also will operate with other colleagues from different Egyptian institutions to update these recommendations and strengthen the level of evidence regarding each recommendation.

REFERENCES

1- Azizi F. Islamic Fasting and Health. *Ann Nutr Metab* 2010;56:273-82

2-Elnadry MH, Nigm IA, Abdel Aziz IM, Elshafee AM, Elazhary SS, Abdel Hafeez MA, et al. Effect of Ramadan fasting on Muslim patients with chronic liver diseases. *J Egypt Soc Parasitol* 2011;41:337-46.

3- Amine ElM, Kaoutar S, Ihssane M, Adil I, Dafr-Allah B. Effect of Ramadan fasting on acute upper gastrointestinal bleeding. *J Res Med Sci* 2013;18:230-33.

4- Hosseini-Asl K, Rafieian-Kopaei M. Can patients with active duodenal ulcer fast Ramadan? *Am J Gastroenterol* 2002;97:2471-2.

5- Emami M, Rahimi H. Effects of Ramadan fasting on acute upper gastrointestinal bleeding due to peptic ulcer. *J Res Med Sci* 2006;11:170-5.

6- Sugano S, Yamamoto K, Takamura N, Momiyama K, Watanabe M, Ishii K. Azygos venous blood flow while fasting, postprandially, and after endoscopic variceal ligation, measured by magnetic resonance imaging. *J Gastroenterol* 1999;34:310-4.

7- Sherlock S, Dooley J. Malignant Liver Tumours. In Diseases of the Liver and Biliary System, 11th edition, Blackwell Science Ltd, 2002:537.