SUDAN MEDICAL LABORATORY JOURNAL

AN OPEN ACCESS PEER REVIEWED SCIENTIFIC JOURNAL

ISSUED BY

OMDURMAN ISLAMIC UNIVERSITY

FACULTY OF MEDICAL LABORATORY SCIENCES

Volume 12
Issue 1

SUDAN MEDICAL LABORATORY JOURNAL

AN OPEN ACCESS PEER REVIEWED SCIENTIFIC JOURNAL

ISSUED BY

OMDURMAN ISLAMIC UNIVERSITY

FACULTY OF MEDICAL LABORATORY SCIENCES

SMLJ

وَلَقَدْ آتَيْنَا دَاوُودَ وَسُلَيْمَانَ عِلْمًا

وَقَالَا الْحَمْدُ لِلَّهِ الَّذِي

فَضَّلَنَا عَلَى كَثِيرٍ مِّنْ عِبَادِهِ الْمُؤْمِنِينَ

النمل15

Copyrights

Disclaimer

Editors and authors cannot be held responsible for any errors that have inadvertently occurred before, during or after publication and shall not be liable under any circumstances what-so-ever for any damages suffered as a result of any errors, emissions, or changes.

All articles published represent the opinion of the author and do not reflect the official policy of Sudan Med Lab Journal.

The SML Journal is under the sponsorship of the honorable Director of the University

Table of contents

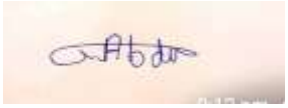
Preface from the Editor-in-Chief

Dear readers and Colleagues,

It gives me a great pleasure to bring before you the first issue, volume 10 of the SMLJ assembled under our joint and articulate editor ship.
Sudan Medical Laboratory Journal (SMLJ) is issued by Omdurman Islamic University, College of Medical Laboratory Sciences. It is a refereed quarterly scientific journal (from 2011 - 2022). Issued it publishes scientific papers in the medical laboratory fields.
The journal can also consider a special issue for the purpose of avoiding delay or to publish a special theme for the conference.
We are continually receiving diverse and novel submissions. We hope that our readers continue to use SMLJ as their primary source for the most up to date knowledge in the field of laboratory sciences and medical researches comprising research papers, short communications, cases studies or reviews.

Aims and scopes

Sudan Medical Laboratory Journal (SMLJ) is issued by Omdurman Islamic University, College of Medical Laboratory Sciences. It publishes scientific papers in the medical laboratory Sciences.
The main objective of this journal is to publish the research papers well in time but with peer review by subject experts. We are confident that our editorial board in different specialties nationally and internationally reputed. The ultimate objective of dissemination of knowledge is to improve patient management and enhance health care delivery. In the process, the scientific work of the authors is viewed by a larger audience and is peer reviewed by global experts. The authors are, in turn, rewarded with appreciation, promotion and acknowledgement by peers.
This is a peer-reviewed journal published yearly. It is aims to reflect medical laboratory scientific research in various aspects of medicine as well as regional and international relevant research. Basic scientific research clinical practice, experiences that help in patient management are also welcome. Review articles, original articles, case reports are welcome. Local research in sciences education and history of laboratory and medicine will be considered for publication.
Thanks to the authors for their contributions to this issue and the editorial staff for their dedication. Thanks to our readers for their continued support and interest in our publications.



Dr. Abdelsamee Elobied Mohamed Elamin

Editor- in-Chief

Editorial

"We are judged by hope"

Arabian poem

Dear SMLJ readers;

"In these difficult times that our country is going through, and after experiencing war in our streets, villages, cities, and inside our homes, we are now, after absorbing the shock, rising again in all fields to work on building our nation and looking far ahead to be better than we were before. We are governed by hope from our faith and upbringing.

We are publishing this issue after a pause due to the shock of the war, we have to continue the journey of scientific publishing through your leading journal. Scientific research must continue to build a nation worthy of it. We move forward together in all fields, and our country rises with knowledge."

.

We received a flood, call it, a streaming progression of papers, requesting urgent publishing services. Our sudden enthusiasm and exuberance were not good enough to suffice. That is why, we are late.

We would like to inform you that your journal available on-line and our URL is http://www.journal.oiu.edu.sd/ojs/index.php/mlj so, just and retrieve your article without the bother to request.
Please now send your next article at the submissions form through the submissions

Webpage: http://www.journal.oiu.edu.sd/ojs/index.php/mlj/submissions
or directly through the email: smlj@oiu.edu.sd and ebade72@oiu.edu.sd
And till we hear from you, we remain,

Yours

Dr. Abdulazeem Abdulsalam Ibrahim Alkhidir

Important Directives

Counsels to contributors

Manuscript submission: We only request the authors to fulfill certain prerequisites on submitting their research work papers. The directives mentioned are meant to ensure security, to facilitate and accelerate the publishing process as well as to eliminate inhospitable conflicts that might probably erupt.

The manuscripts, in the form of software copies, in CDs, flash, or any removable medium, are to be, either manually handed over to the Journal's authorized staff or sent via the journal's e-mail address, accompanied by a covering letter expressing the wish to publish the article in question. The authors are bound to fill, beforehand, an *undersigned* **Transfer** of Copyright Agreement form to be submitted as a scanner image.

Articles for publication always should be prepared in a 1.5cm-spaced typewritten on size A4 paper and with 3.81cm margin on left side and 3 cm on all other sides. *Not to forget*, the alignment (***left to right***) through all the text, is required.

Names of authors should be followed by their official address indicated by the numbered superscript as follows:

Last name + initials i.e. Cornel, C. E[1]. Ford, E. J. H[2]. Evans, J[3].

Articles reviewed by a peer reviewer, are subject to acceptance, rejection or to acceptance after complying with amendments in the enclosed report.

Each author will receive a copy of his/her published submitted article sent via email address. Order for extra issues or articles of other authors will be charged for.

Article template consists of an **abstract** (less than 300 words). It is of high importance to give, *here*, the name and address (*namely email*) of the *Correspondence Address,* followed by an introduction (*a short concise overview of previous relevant research*), **materials and methods** (*a description of the methodology used*), **patient selection** (*inclusion and exclusion criteria*), **results** (*comprising text, tables and figures, avoiding repetition of data*), **discussion** (*of the results obtained*) and **references** (*in Vancouver Style*).

Original Article **Sudan Medical Laboratory Journal**

Association between *Helicobacter pylori* infection and upper gastrointestinal symptoms in Sudanese patients, Khartoum state- Sudan

AbdulAzeem AbdulSalam Ibrahim Alkhidir[1,2], Arwa Algaili[1], Asma Ahmed[2], Baraa Yousif[1], Eslam Ahmed[1], Fatima Alzahra Nsraldeen[1] Alzahra Abdalkhalige[1],.

[1]Department of Microbiology ,Faculty of Medical Laboratory Sciences, Omdurman Islamic University, Sudan.
[2]Department of Microbiology ,Faculty of Medicine, Omdurman Ahlia University, Sudan.

Abstract

Introduction: *Helicobacter pylori* is small, spiral, Gram-negative bacilli that plays a role in the pathogenesis of a number of diseases, is strongly associated with gastric cancer and peptic ulceration. The bacterium highly links to duodenal ulcer, which was classified as a group I carcinogen in 1994 by the WHO.

Objectives-This study aimed to isolate *Helicobacter Pylori* from stomach biopsy, and to assess the correlation between *H.pylori* infection and upper gastrointestinal symptoms (Epigastric pain, and hunger) and Duodenitis.

Material and Methods: This is a descriptive cross-sectional study.Out of 40 participants were included in this study. The antral mucosal biopsy specimens were obtained. According to standard microbiology procedure the specimens were cultured on Modified Brain heart Hemoglobin Urea Agar which is selective and differential media for *H. pylori*, the urease activity of *H.pylori* observed within 24 hours in this media .while, the growth were observed after 3 days following the incubation of cultured plates under microaerophilic condition provided by candle jar.

Result: *H. Pylori* was detected in 2(5%) of 40 stomach biopsy specimens. And this study revealed that there is no association between *H.pylori* infection and Epigastric pain, hunger pain and Duodenitis.

Conclusion-This study concludes that there is no association between *H.pylori* infection and upper gastrointestinal symptoms (epigastric pain and hunger) and duodenitis.

Keywords: *H.pylori***,** Stomach biopsy, Endoscopy

Corresponding author: AbdulAzeem AbdulSalam Ibrahim Alkhidir, ebade73@oiu.edu.sd

Introduction: *H.Pylori (H.pylori)* are a small, spiral, Gram-negative bacillus that plays a role in the pathogenesis of a number of diseases (1). *H.pylori* is strongly associated with gastric cancer and peptic ulceration (2). The bacterium highly links to duodenal ulcer, which was classified as a group I carcinogen in 1994 by the WHO (3). Acute infection can yield an upper gastrointestinal illness with nausea and pain, vomiting and fever may also be present. The acute symptoms may last for less than 1 week or as long as 2 weeks. After colonization, the *H.pylori* infection persists for years and perhaps decades or evens a lifetime (4). Long term carriage can lead to gastric adenocarcinoma and mucosa associated lymphoid tissue (MALT) and gastric lymphoma (5).There is also a growing awareness that chronic *H.pylori* infection may be associated with an increased risk of extra gastric diseases that include host iron deficiency (6), Cardiovascular, neurological, metabolic, autoimmune and dermatological diseases (7). The accurate detection of *H.pylori* is

essential for the management of patients and for the eradication of the bacterium following treatment. Since the discovery of *H.pylori*, several diagnostic methods have become available for determining the presence of *H.pylori* infection. These tests can be assessed by invasive and noninvasive methods (8). invasive (culture, histopathological examination, rapid urease test and molecular tests) (9), which require endoscopy to obtain biopsies of gastric tissues (10) and non-invasive (urea breath test, serological tests, stool culture and stool antigen/nucleic acid tests) methods may be use (9). Culture stills play a major role in the diagnostic spectrum. Culture continues to be the only test allowing for a comprehensive analysis of pathogen characteristics and susceptibility to antibiotics (11). Numerous antibiotic regiments have been evaluated for treating *H.pylori* infections (12). More success has been achieved for treatment of gastric or peptic ulcer by using a combination of bismuth ,a proton pump inhibitor (e.g., omeprazole), and one or more antibiotics (ampicillin, metronidazole, clarithromycin, tetracycline) (13). Due to the increased side effects of the treatment regimens and the development of antimicrobial resistance, a number of natural compounds have been tested as potential alternatives (14). The World Health Organization (WHO) estimated that 80% of the population in developing countries relay on traditional medicine, mostly plant based drugs, for primary health care (15). This study aimed to isolate *Helicobacter Pylori* from stomach biopsy, and to assess the correlation between *H.pylori* infection and upper gastrointestinal symptoms (Epigastric pain, Hunger and duodenitis).

Materials and Methods-This is a descriptive cross sectional study, Specimens were collected from Fedail, Military, Hospitals and ADC (Advanced Diagnostic Center) -Khartoum state Sudan. A total of forty patients were included in this study, samples of endoscopic gastric biopsies were taken from patients attending to Fedail, military hospitals and Advanced Diagnostic Center, department of gastro intestinal tract endoscopy. Data was obtained by self –administrative questionnaire. Approval to conduct this study was obtained from the Research Ethics Committee of Faculty of Medical Laboratory Sciences-Omdurman Islamic University. After explanation the study and its goal, a verbal consent was taken from the participant before proceeding with the study and collecting biopsy and stool samples.

Preparation of Modified brain heart hemoglobin urea agar (MBHUA) media- This media was modified by Alkhidir (16), Modified Brain heart Urea Agar (MBHUA) was prepared by adding 52 gm of brain heart agar (Hi media - India), 20 gm of urea base (Hi media - India), and 0.0012 gm of phenol red as an indicator (Hi media - India) to 740 ml of distilled water and sterilized by autoclaving at 121ºC for 15minutes, then cooled to 50-55ºC. 10 ml of antibiotic solution was added (1 mg of vancomycin, 5 mg of trimethoprim, and 5mg of amphotericin B in 10 ml sterile distilled water), and 3gm of urea crystal (Hi media - India) was added and then mixed thoroughly and poured into sterile disposable Petri dishes (17).

Sample collection and transportation-Stomach biopsy specimens were collected in plain container

contain 2 ml of normal saline and transported in sterile plain container containing sterile normal saline or phosphate buffer saline at 4^0C.

Sample processing and culture-The 40 stomach biopsy specimens were centrifuged in normal saline or phosphate buffer saline at 3000rpm for 2minutes,then supernatant was discarded and two to three drops of sediment was cultured on modified media (MBHUA) using sterile wire loop by ordinary method (primary, secondary, tertiary and zigzag) under a septic condition.

All plates incubated at 37^0C in microaerophilic conditions using candle jar up to 5 days and observed for growth and change in the color of the medium daily, growth was observed after 3 days. Colonies showed change in the color of the medium to pink (indicating rise in the PH of the medium due urease production with subsequent breakdown of urea into ammonia and carbon dioxide).

Then purified colonies were identified based on Gram stain reaction, and biochemical tests (catalase, oxidase, and urease). The data analyzed by using Statistical Package for Social Sciences (SPSS) program**.**

Result: Among 40 stomach biopsy obtained from participants by upper gastrointestinal endoscopy, only two (5%) biopsies showed growth of *H.pylori*, and the rest (95%) showed no growth, (Figure 1). According to the gender equal percentage of growth of *H.pylori* from stomach biopsies was observed, (Table 1).

Out of 40 Endoscopic biopsy enrolled in this study, divided into three age groups; less than 30 years, 30-60 years, and less than 60 years. And growth of *H.pylori* was found in 0%, 2.5%, and 2.5% respectively. Evaluation of culture result according to the age showed statistically insignificance correlation with the age, (*P* value=0.5), (Table 2).

In patients with Epigastric pain the percentage of *H.pylori* growth was 5%, and 70% showed no growth. In those without epigastric pain the percentage of growth was 0%, and no growth was 25%, (Table 3).

In patient with hunger the percentage of growth of *H. pylori* was 2.5%, and no growth was 47.5%, and in those without of hunger showed similar result, (Table 3).

In in patients with Duodenitis frequency of *H. pylori* growth was 0%, and no growth was 25%. And in those without Duodenitis growth percent of *H.* pylori was 5%, and 70% showed no growth.

Growth of *H. pylori* was observed in 5% of patients had past history of *H.pylori* infection, and no growth observed in 42.5% of them. No growth observed patient had not past history of *H. pylori* infection, (Table 4).

In patients had antibiotic treatment the percentage of growth was 5%, and no growth was 40%, and there was no growth observed in those not use antibiotic treatment.

According to P value of different variable there was insignificance when analyzed statistically, (Table 4).

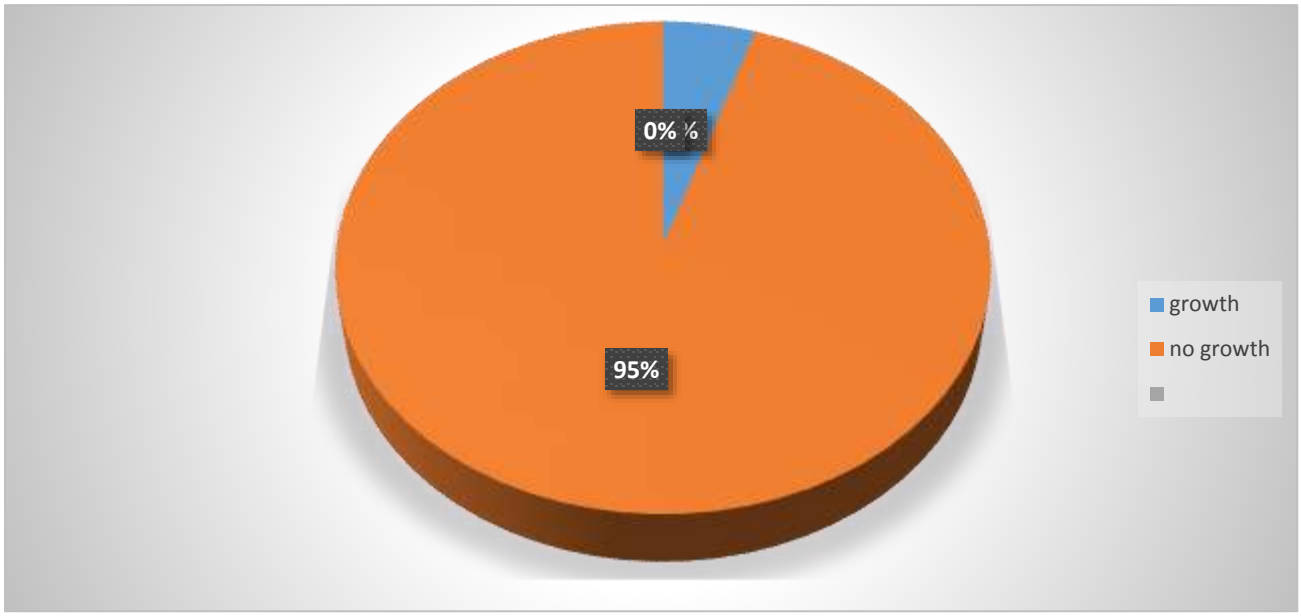


Figure 1: frequency of growth of H.pylori in Stomach biopsy samples

Table 1: frequency of growth of *H.pylori* among gender in Stomach biopsy samples

		culture result		*P* value
		Growth	No growth	
Sex	Male	2.5%	47.5%	1
	Female	2.5%	47.5%	
Total		5%	95 %	

Table 2: comparison of culture result with age group (biopsy)

		culture result		*P* value
		growth	No growth	
Age	less than 30 years	0%	30%	0.5
	30-60 years	2.5%	45%	
	more than 60 years	2.5%	20%	
	Total	5%	95%	

Table 3: comparison of culture result with symptoms

		culture result		*P* value
		Growth	No growth	
Epigastric pain	Yes	5%	70%	0.4
	No	0%	25%	
	Total	5%	95%	
Hunger	Yes	2.5%	47.5%	1
	No	2.5%	47.5%	
	Total	5%	95%	
Duodenitis	Yes	0%	25%	0.4
	No	5%	70%	
	Total	5%	95%	

Table 4: comparison of culture result with Past history of *H.pylori* infection and previous antibiotic use

		Culture result		P value
		Growth	No growth	
Past infection	Yes	5%	42.5%	0.1
	No	0%	52.5%	
	Ttal	5%	955	
Previous antibiotic use	Yes	5%	40%	0.1
	No	0%	55%	
	Ttal	5%	955	

Discussion:

The primary isolation of *H. pylori* from biopsy specimens is difficult process. This may be due to fastidious nature of *H. pylori* and a number of factors that are hard to control (patchy distribution of the organism on gastric mucosa, contamination of biopsy forceps, ingestion of anesthetic, presence of oropharyngeal flora, loss of the viability of the organism during transportation, etc.) (18).

Multiple effort from multiple laboratories have been unsuccessful and the optimal condition to recover *H. pylori* from stool still not known (19). The frequency of *H.pylori* growth among gender is equal.

The patients were classified into 3 age groups, less than 30 years, 30-60 years, more than 60 years. In our study there is no association between age ,gender, and culture result, this result agree with study done by Uszczynska K *et* al in Poland, Akbar DH and Eltahawy ATA in Saudi Arabia, and Petrovic M *et al* in Serbia (20-22), and study done by Maha *et al,* Saudi Arabia, aimed to estimate the prevalence of *H.pylori* among patients suffering from upper gastrointestinal symptoms, *H.pylori* status in patients was determined by histology, rapid urease test, and ELIZA. The study showed that there was a significance correlation between age, gender and *H.pylori* infection (23) this may be due to use of more than one test for detection of *H.pylori* ,which are more sensitive than culture, increasing chance for detection of the organism.

Our study revealed that there is no significance association between symptoms of epigastric pain, hunger, and duodenitis and *H.pylori* infection. In study done by Rosenstock, *et al* in Denmark, that aimed to assess the relation between *H.pylori* infection and gastro-intestinal symptoms and syndromes, random sample size was 3589 and Werdmuller, *et al* in Australia, sample size was 404 reported that there was association between epigastric pain and *H.pylori* infection, this may be due to variation in sample size and geographical area (24, 25).

The present study showed that there is no association between past infection, Previous antibiotic use and positive growth culture.

Patients with past infection with *H.pylori* and use antibiotic treatment showed positive *H.pylori* culture this may be due to patients were not complete the course of treatment, or patients had been recovered from the past infection and this is new infection, or patient was infected with antibiotic resistant *H.pylori* strain.

Conclusion-This study concluded that, there is no

association between upper gastrointestinal symptoms (epigastric pain and hunger), duodenitis, past *H.pylori* infection, previous antibiotic use and *H.pylori* infection.

References

1. Kouitcheu Mabeku LB, Eyoum Bille B, Tchouangueu TF, Nguepi E, Leundji H. Treatment of Helicobacter pylori infected mice with Bryophyllum pinnatum, a medicinal plant with antioxidant and antimicrobial properties, reduces bacterial load. Pharmaceutical biology. 2017;55(1):603-10.

2. Wang YC, Huang TL. High-performance liquid chromatography for quantification of plumbagin, an anti-Helicobacter pylori compound of Plumbago zeylanica L. Journal of chromatography A. 2005;1094(1-2):99-104.

3. Wang YC. Medicinal plant activity on Helicobacter pylori related diseases. World journal of gastroenterology. 2014;20(30):10368-82.

4. Jawetz M, Adelberg, editor. Medical Microbiology. 27 ed: McGraw Hill Education; 2016.

5. Kabir S. Detection of Helicobacter pylori in faeces by culture, PCR and enzyme immunoassay. Journal of medical microbiology. 2001;50(12):1021-9.

6. Flores SE, Aitchison A, Day AS, Keenan JI. Helicobacter pylori infection perturbs iron homeostasis in gastric epithelial cells. PloS one. 2017;12(9):e0184026.

7. Lewinska A, Wnuk M. Helicobacter pylori-induced premature senescence of extragastric cells may contribute to chronic skin diseases. Biogerontology. 2017;18(2):293-9.

8. Aktepe OC, Ciftci IH, Safak B, Uslan I, Dilek FH. Five methods for detection of Helicobacter pylori in the Turkish population. World journal of gastroenterology. 2011;17(47):5172-6.

9. Cagdas U, Otag F, Tezcan S, Sezgin O, Aslan G, Emekdas G. [Detection of Helicobacter pylori and antimicrobial resistance in gastric biopsy specimens]. Mikrobiyoloji bulteni. 2012;46(3):398-409.

10. Russmann H, Kempf VA, Koletzko S, Heesemann J, Autenrieth IB. Comparison of fluorescent in situ hybridization and conventional culturing for detection of Helicobacter pylori in gastric biopsy specimens. Journal of clinical microbiology. 2001;39(1):304-8.

11. Gonzalez-Vazquez R, Herrera-Gonzalez S, Cordova-Espinoza MG, Zuniga G, Giono-Cerezo S, Hernandez-Hernandez JM, et al. Helicobacter pylori: detection of iceA1 and iceA2 genes in the same strain in Mexican isolates. Archives of medical research. 2012;43(5):339-46.

12. Patrick R. Murray P, Ken S. Rosenthal P, George S. Kobayashi P, Micheal A. Pfaller M. Medical Microbiology. 2002(Four).

13. Parija SC. Text book of Microbiology and Immunology. 2012.

14. Mandalari G, Bisignano C, Cirmi S, Navarra M. Effectiveness of Citrus Fruits on Helicobacter pylori. Evidence-based complementary and alternative medicine : eCAM. 2017;2017:8379262.

15. Yagoub SO. Anti-Microbial Activity of *Tamaridus indica* and *Adansonia digitata* Extracts Against E. coli Isolated from Urine and Water Specimens. Research Journal of Microbiology. 2008;3(3):193-7.

16. Abdulazeem Abdulsalam Ibrahim Alkhidir, Esraa Hassan Mohammed Ibrahium, Fatima Suliman Ali Ahmed, Lubaba Bashieer Alameen Bashieer, Mashaer Hassan Mohammed Hessen, Manal Bashieer Alameen Bashieer, et al. Detection of Helicobacter Pylori from Stomach Biopsies: A Rapid Culture Method. Med Forum. 2022;33(10):10.

17. Alkhidir AAI, Abusalif KO, Nasseir MEO, Elhussain KMK. Helicobacter pylori: Rabid culture method and The antibacterial effect of Moringa and Ginger against H. pylori. 2017:5.

18. Piccolomini R, Di Bonaventura G, Festi D, Catamo G, Laterza F, Neri M. Optimal combination of media for primary isolation of Helicobacter pylori from gastric biopsy specimens. Journal of clinical microbiology. 1997;35(6):1541-4.

19. Dore MP, Osato MS, Malaty HM, Graham DY. Characterization of a culture method to recover Helicobacter pylori from the feces of infected patients. Helicobacter. 2000;5(3):165-8.

20. Leszczynska K, Namiot A, Namiot Z, Leszczynska JK, Jakoniuk P, Chilewicz M, et al. Patient factors affecting culture of Helicobacter pylori isolated from gastric mucosal specimens. Advances in medical sciences. 2010;55(2):161-6.

21. Petrovic M, Artiko V, Novosel S, Ille T, Sobic-Saranovic D, Pavlovic S, et al. Relationship between Helicobacter pylori infection estimated by 14C-urea breath test and gender, blood groups and Rhesus factor. Hellenic journal of nuclear medicine. 2011;14(1):21-4.

22. Akbar DH, Eltahawy AT. Helicobacter pylori infection at a university hospital in Saudi Arabia: prevalence, comparison of diagnostic modalities and endoscopic findings. Indian journal of pathology & microbiology. 2005;48(2):181-5.

23. Abo-Shadi MA, El-Shazly TA, Al-Johani MS. Clinical,Endoscopic,Pathological and Serological finding ofH.pylori Infection in Saudi Patients with UpperGastrointestinal Diseases. British Journal of Medicine & Medical Research. 2013;3(4):1109-24.

24. Rosenstock S, Kay L, Rosenstock C, Andersen LP, Bonnevie O, Jorgensen T. Relation between Helicobacter pylori infection and gastrointestinal symptoms and syndromes. Gut. 1997;41(2):169-76.

25. Zipser RD, Parikh MV. Is repeat testing needed for Helicobacter pylori? The Journal of the American Board of Family Practice. 2000;13(6):449-50.

Original Article Sudan Medical Laboratory Journal

Molecular Detection of Occult Hepatitis B Virus among Human Immunodeficiency Virus Patients in Khartoum

Hassan Babieker Said[1], Waleed Abdelateif Hussein[1,2]

[1]Department of Medical Microbiology, Faculty of Medical Laboratory Sciences, Al Neelain University.
[2]Immunology deparment, Faculty of Medical Laboratory Sciences, Al Neelain University.

Abstract

Background: Hepatitis B, a potentially life-threatening liver infection caused by the hepatitis B virus (HBV) is a major global health problem. Of the two billion people infected with the virus, more than 296 million are chronic carriers and more than 686,000 die annually from HBV-related complications, including cirrhosis and hepatocellular carcinoma.

Aim: This study was carried out to determine the prevalence of Occult Hepatitis B Virus among Human Immunodeficiency Virus patients in order to establish basic knowledge for future HIV Patient care.

Method: A total number of 88 Serum samples from Human Immunodeficiency Virus patient was collected and tested to determine the HBV exposure rate and the presence of HBsAg and detection of Occult Hepatitis B Virus .

Results: in This study , the mean age of them was 35.98 years, 64.7% (n= 57) were male and 35.3% (n= 31) were female, the exposure rate of HBV was 39.7%,and we found high rate of exposure in male (63%) and We found the high rate of exposure in age group (25-35 years) (45.7%) and The prevalence of HBsAg in study population was 6.82% we found high prevalence rate in female (66.7%) and high prevalence rate in age group (25 – 35years) (66.7%) in the other hand the prevalence of OHB was 2.4% and We found high rate in male 100% and we found similar distribution to infection in just two age group; group (25-35)years(50%) and age group (less than 15 years) (50%) .

Conclusion: This study highlights the urgent need for continued fully screening and education about Occult HBV infection and strategies that ensure future HIV Patient care.

Key words: Occult hepatitis B Virus, HIV, HBV DNA, HBsAg, Anti-HBc.
Corresponding author: **Waleed Abdelateif Email: Waleed.86h@gmail.com**

Introduction: Hepatitis B, a potentially life-threatening liver infection caused by The hepatitis B virus (HBV) is a major global health problem, Of the two billion people infected with the virus, more than 240 million are chronic carriers (1) , WHO estimates that 296 million people were living with chronic hepatitis B infection in 2019, with 1.5 million new infections each year (2), and more than 686,000 die annually from HBV-related complications, including cirrhosis and hepatocellular carcinoma (3).

A growing body of evidence is emerging showing that the prevalence of HBV is significantly higher amongst HIV-positive individuals, presumably because of the shared transmission risks and risk factors (4). HIV generally accelerates the natural course of HBV infection and facilitates faster progression of liver disease to cirrhosis and hepatocellular carcinoma (HCC) (5).

Traditionally, HBV is diagnosed by serological techniques to detect antigens or antibodies. The hepatitis B surface antigen (HBsAg) is often used for routine diagnosis since it is considered as the hallmark of infection. During acute infection, antibodies to HBV core antigens (anti-HBc) (initially both IgM and IgG) appear 1–2 weeks after the appearance of HBsAg, while IgG persists during chronic infection. The presence of antibodies to HBsAg (anti-HBs) represents immunity to HBV infection (6).

Occult hepatitis B (OHB) has been increasingly recognized over the last 2 decades as a public health concern. It is characterized by the presence of Hepatitis B virus (HBV) DNA in plasma ,liver, and/or peripheral blood mononuclear cells (PBMC) of patients with no detectable hepatitis B surface antigen (HBsAg) in serum,Occult hepatitis B infection is common in HIV infected patients (7) .

A study by Coffin et al., for instance, has shown 17.8% and 40% prevalence rates of OHB in serum and PBMC of HIV infected patients respectively (8). Similar study in a cohort of HIV infected people reported 47% prevalence rate of OBH ,In Africa, about 100 million individuals are estimated to be infected with HBV or HCV (9), Also, HBV and HCV infections are highly endemic in Africa and are responsible for 80% of liver cirrhosis and HCC, with HBV being the main cause of end-stage liver disease (10).

HIV-infected people are three to six times more likely to develop chronic or long-term hepatitis B infection because of their suppressed immune systems than individuals without HIV (11).

The global prevalence of HBV/HIV co-infection varies from 1.13% to 59% . In the United States of America [USA], the prevalence of HIV/HBV in children is 2.6% and 4.9% in China (12) .

Reports from Africa have revealed that the prevalence of HBV/HIV co-infection is between 10% and 20% as many countries in sub-Saharan Africa are typically classified as endemic, high, or intermediate countries with HBV infections (13).

In Tanzania, a prevalence of 1.2% was documented in children aged 18 months to17 years while 12.1% was documented by Route et al in Cote d' Ivoire in West Africa (14,15).

Methods: Study design and setting: This was Descriptive Cross-sectional study confined on patient infected with of Human immunodeficiency Virus already Study done in Khartoum state, Sudan at Khartoum North Teaching Hospital and Omdurman Teaching Hospital during April -2021 to August - 2021.

Sample collection and Data: 88 Serum sample that we were collected it from lab that already have samples of patients who follow up in at Khartoum North Teaching Hospital lab and Omdurman Teaching Hospital lab and kept frozen at -18^{o}C and Personal data (Gender /Age) obtained from the investigation request or files of HIV patient using datasheet.

Statistical analysis: Statistical analysis of database was prepared and processed by using Statistical Package for Social Science (SPSS.) program version 20.X2 and T test was used when appropriate p value of less than 0.05 was considered statistically significant.

Serological testing: Initially, all samples were screened for HBsAg using a commercially available enzyme-linked immunosorbent assay (ELISA) kit from RecombiLISA™ by (CTK , USA ,lot ; E0 531S2f00) and samples that were HBsAg negative were further screened for anti-HBc Ab using ELISA commercial kit for the qualitative detection of antibodies to hepatitis B core antigen (anti-HBc) in human serum, using kits for (Fortress Diagnostics Limited,ref; bxe0761a,LOT;HBc-2202-1).

DNA extraction: DNA from HBsAg negative samples was extracted from 200 µl from serum using the (PowerPrep™ Viral DNA / RNA Extraction Kit **(**CatNo.E 0 0 0 7 / Kogenebiotec /Seoul, Korea 08507**))**was used for the purification of viral DNA in serum,The Kit includes all reagents necessary for purification of viral nucleic acids from serum according to the manufacturer's instructions The extracted DNA was amplified immedia- tely after extraction or stored at −20 °C awaiting subsequent amplification.

Polymerase chain reaction(PCR) for HBV: The PCR was performed by processing the extracted DNA from serum with primers were detected the specific portion of surface gene, the primers used consisted of**:-**

Forwardprimer**:**5'ATCCTGCTGCTATGCCTCATCT -3'

Reverseprimer:3GAACCACTGAACAAATGGCACT-5' (15).

The reaction was performed in 20 µl volume using Maxime PCR blue mix master mix (Intron Co, Korea).The volume included:,(total 20 µl): 1 µl forward primer, 1 µl reverse primer, 4 µl extracted DNA and 14µl distilled water as show in table The DNA was amplified in thermocycling conditions using PCR machine (Techne co, Japan) as follow: initial denaturation at 94°C for 2 min, followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 60.5°C for 30 sec and extension at 72°C for 1 min, with a final extension 72°C for 7 min as show in table (2-2),5 µ l of the amplified product was subjected to direct analysis by gel electrophoresis in 2% Agarose.the PCR products will be electrophoresed in 2% agarose gel prepare in1 X TBE buffer Boiled for 30 sec in microwave oven and cool to50°C), adding ethidium bromide (3 µl) stain and evaluate under ultraviolet light, The specific DNA product for HBV was 294 bp of each sample will determine by identifying the bp amplified DNA bands in comparison with the100-bp DNA ladder will be used as DNA size marker .

Results: Description of the Study Population: A total of eighty eight Human Immunodeficiency Virus were enrolled in this study. Four samples (4.54%) of the total subjects were aged less than 15 years, 8 (9.09%) samples were found to be between (15-24 years) while 34(38.6%) samples were found to be between (25-35years).on the other hand we found 27(30.6%) samples in age group (36-45 years) also we found 15(17.04) samples in age group more than 45 years the distribution of the gender among study population showed 57(64.7%) was male and 31(35.3%) was female.

Prevalence of Anti-HBc among HIV patient: among the 88 samples there was 35(39.7%)

samples tested positive for Anti-HBc as showed in Table (1)

Table (1) prevalence of anti-HBc among HIV patient:

Variables	Patients (n=88)	Percent(%)
positive	35	39.7%
negative	53	60.3%

Distribution of Anti-HBc among the Gender And Age Group: Among 35 Anti–HBc positive samples, we found two samples among Age group (Less than 15 years) and three samples among age group (15-24years)**,**we also found 16 samples among age group(25-35years) and 9 sample among age group (36-45years),also five samples among age group (more than 45years) there is no significant association between Anti-HBc positive samples and Age group (P.value:0.799).

Variables	Subjects	Percentage (%)
Age : (years)		
Less than 15	2	5.8%
15 – 24	3	8.6%
25 – 35	16	45.7%
36 – 45	9	25.7%
More than 45	5	14.2%
Gender :		
Male	22	63%
Female	13	37%

Among 35 Anti–HBc positive samples, **22** (63%) were male and **13**(37%) were female, there is no significant association between Anti-HBc positive samples and gender (P.value:0.239), as shows in table (2).

Table (2):Description of the Anti-HBc patients Population:

Prevalence of "Overt" HBV infection among HIV patient: Among the 88 samples there was 6 samples tested positive for HBsAg as shows in table (3) .

Table (3) prevalence of "Overt" HBV infection among HIV patient:

Variables	Patients (n=88)	Percent (%)
HBsAg :		
positive	6	6.82%
negative	82	93.18%

Distribution of "Overt" HBV infection among the Age Group And Gender: Among six HBs Ag positive samples, we found four samples among Age group (25-35years) and two samples among age group (36-45 years)**,** there is no significant association between overt HBV infection and Age group (P.value:0.509).Also among HBs Ag positive samples, **2** (33.3%) were male and **4**(66.7%) were female, there is no significant association between overt HBV infection and gender (P.value:0.095),as shows in table (4).

Table (4): Description of the Overt HBV infection Population:

Variables	Subjects	Percentage (%)
Age :(years)		
Less than 15	0	0%
15 – 24	0	0%
25 – 35	4	66.7%
36 – 45	2	33.3%
More than45	0	0%
Gender :		
Male	2	33.3%
Female	4	66.7%

Prevalence of Occult HBV infection among HIV patient: Table (5) shows that among the 82 samples there was already tested negative for HBsAg we found 2(2.4%) sample positive for HBV DNA .

Table (5) Prevalence of Occult HBV infection among HIV patient:

Variables	Patients (n=82)	Percent (%)
HBVDNA		
positive	2	2.4%
negative	80	97.6%

Distribution of the OBI among Gender And Age : Among the two HBV-DNA positive samples , we found One samples among age group (less than 15 years), one sample among Age group (25-35years) and there is no significant association between OBI infection and Age group (P.value:0.087).Among the two HBV-DNA positive samples, we found two male (100%) there is no significant association between OBI infection and gender (P.value:0.125). as shows in table (6).

Table (6): Description of the Occult HBV infection Population:

Variables	Subjects	Percentage (%)
Age :(years)		
Less than 15	1	50%
15 – 24	0	0%
25 – 35	1	50%
36 – 45	0	0%
More than45	0	0%
Gender :		
Male	2	100%
Female	0	0%

Discussion: Laboratory detection of Hepatitis B virus infection is crucial for global control and prevention of HBV disease. Among HIV infected individuals under HAART, the increased longevity may facilitate emergence of chronic liver disease which is often a cause of increased morbidity and mortality, a significant proportion of this burden may be attributed to occult hepatitis B virus infection since it has been shown to have hepatopathogenic potential (17).

In this study, HBc total Ab or the exposure rate was 39.7%, which is lower than The exposure rate reported in Sudan and South Florianópolis (62.8%, 71.2%) respectively (18, 19).) Also we found the exposure rate was similar to studies done in Ethiopia, São Paulo and Southeast (Campinas) (39.5%, 38.6% and 44.0%) respectively (19, 21).Also we found the exposure was higher than studies done in Southern Brazil(27.3%) (21).

In this study, we found high rate of exposure in male (63%) our findings was agree with study done by hatim mudawi in sudan (61.8%)(17), also We found the high rate of exposure in age group (25-35) years(45.7%) .

The prevalence of HBsAg in study population was 6.82%, which is lower than study done by Hatim mudawi in sudan and study done by Rui in South Florianópolis in regions of Brazil (11.7%, 24.3%) respectively (18,19) and we found Slightly similar to study done in Ethiopia (5.5%) (21).

Also we found the our prevalence was higher than studies done in Southern Brazil (2.3%) (20). we found high prevalence rate in female (66.7%) our findings was disagree with study done by hatim mudawi in sudan (57.1%)was male.(18),we also found high prevalence rate in age group (25 – 35)years (66.7%) .

In this study, the prevalence of OHB was 2.4%, OBI the observed prevalence it lower than study done by Yousif in in Khartoum and study done by

hatim mudawi in sudan (9.8%,26.8%) respectively (22,18), and slightly similar to study done by George in Cameroon and study done in India by Saha and study done by Salpini in Yaoundé, Cameroon (5.9%,6.3,6.9%) respectively (22,24,25). Also we found the prevalence was higher than studies done by Cohen in Netherlands (0%) (26),we found high rate in male (100%)it is disagree with study done by George in Cameroon (85.%) was female and agree with study done by hatim mudawi in sudan (61.5%) was male (18),we found similar distribution to infection in just two age group; group (25-35) years (50%) and age group (less than 15) years (50%)was disagree with study done by George in Cameroon rate in age group (25-35)years(30%) and age group (36-45) years (23).

It should be noted however that the prevalence of OBI is dependent on the Sensitivity of the DNA assay used, demography and the population studied (27),Thus, in the Sudan study that used a more sensitive real time PCR method compared to the conventional PCR method used in this study. We propose that the difference in prevalence with our study and the Yaoundé study may be due to demographics and sensitivity of method.among HIV patients, several studies conducted worldwide have reported prevalence of OBI ranging from 0% to more than 90% (26, 28).

Conclusion: Our current study was done to detect the prevalence of occult hepatitis B infection among HIV patient. The exposure rate to HBV infection was 39.7% and the prevalence of HBsAg was 6.82%, and the OBI was (2.4%). In the present study there is no significant association between our findings and demographic data.

References:

1. WHO.Hepatitis Bfact sheet. (2016). http://www.who.int/mediacentre/factsheets/fs204/en/. Accessed 20 Sept 2016.2014; 385 (9963): 117171.doi:10.1016/S01406736 (14)61682-2.

2.WHO.HepatitisBfactsheet.(2021).https://www. who.int/news-room/fact sheets /de tail / hepatitis-b. Accessed 27 July 2021.

3. Lancet.(2013).Global regional, and national age–sex specific all-cause and cause-specific mortality for 240 causes of death, 1990–2013: a systematic analysis.for the global burden of disease study.

4. Diop-Ndiaye H, Toure-Kane C, Etard JF, Lo G, Diaw P, Ngom-Gueye NF.(2008).

5. Vallet-Pichard A, Pol S.(2005). Natural history and predictors of severity of chronic hepatitis C virus (HCV) and human immunodeficiency virus (HIV) co-infection. J Hepatol .;44 (1Suppl) : S28–34 .doi:10.1016 / j. j hep .2005.11.008.

6. TrepoC,Chan HL, Lok A.(2014).Hepatitis B virus infection Lancet ;384(9959):2053–63. doi:10.1016/S0140-6736(14)60220-8.1-6-camron.

7. Raimondo G, Pollicino T, Cacciola I, Squadrito G.(2007). Occult hepatitis B Virus infection .J Hepatol; 46:160–170. https: //doi.org/ 10.1016 /j.jhep .2006 .10.007 PMID: 17112622.

8. Witt MD, Lewis R J, Rieg G,Seaberg EC, Rinaldo CR, Thio CL.(2013). Predictors of the isolated hepatitis B core antibody pattern in HIV-infected and -uninfected men in the multicenter

AIDS cohort study. Clin Infect Dis; 56:606612.h ttps://doi.org/10.1093/cid/cis908PMID:2309092 7.7–9 ethoipia.14(3):120–6.

9. Coffin C, Mulrooney-Cousins P, Osiowy C, van der Meer F, Nishikawa S,Michalak T.(2014). Virological characteristics of occult hepatitis B virus in a NorthAmerican cohort of human immunodeficiency virus type 1-positivepatients on dual active anti-HBV/HIV therapy. J Clin Virol. 60:347–353 https://doi. org/10.1016/j.jcv. 2014.04.021 PMID: 24881491

10. Lar PM, Pam VK, Christopher PB, Gwamzhi L, Mawak JD.(2013). Prevalence and immune status of HIV/HBV co-infected pregnant women. Afr J Clin Exper Microbiol.; Hepatitis B, C seroprevalence and delta viruses in HIV-1 Senegalese patients at HAART initiation (retrospective study). J Med Virol.;80(8):1332–6. doi:10.1002/jmv.21236. PMID: 18171303.

11. Lemoine M, Eholie´ S, Lacombe K . (2015). Reducing the neglected burden of viral hepatitis in Africa: Strategies for a global approach. J Hepatol.;62(2):469–76. https://doi.org/10.1016/j. jhep.2014.10.008 PMID: 25457207.

12. Zhou S, Zhao Y, He Y, Li H, Bulterys M, Sun X, (2010). Hepatitis B and hepatitis C seroprevalence in children receiving antiretroviral therapy for human immunodeficiency virus-1 infection in China, 2005–2009. J Acquir Immune Defic Syndr.;54(2):191–6.https: //doi. org/ 10.1097 /QAI.0b01 3e3181c9 9226 PMID: 20032784.

13. Thio CL.(2009).Hepatitis B and human immunodeficiency virus co-infection.Hepat Med Evid and Research.; 49(S5):138–45.

14. Telatela SP, Matee MI, Munubhi EK.(2007). Seroprevalence of hepatitis B and C viral co-infections among children infected with human immunodeficiency virus attending the paediatric HIV care and treatment centreat Muhimbili National Hospital in Dar-es-Salaam, Tanzania. BMC Public Health.; 7(1):338.

15. Rouet F, Chaix ML, Inwoley A, Anaky MF, Fassinou P, Kpozehouen A,.(2008). Frequent occurrence of chronic hepatitis B virus infection among West African HIV type-1-infected children. Clin Infect Dis.; 46(3):361–6. https://doi.org/10.1086/525531PMID: 18171303.

16. Muhammad Nasir Riaz, Muhammad Faheem, Muhammad Ayaz Anwar, Ummar Raheel, Yasmeen Badshah, Hashaam Akhtar, Kosar Tamanna, Muhammad Tahir,Najam us Sahar Sadaf Zaidi, and Ishtiaq Qadri.(2016).PCR-Based Molecular Diagnosis of Hepatitis Virus (HBV and HDV) in HCV Infected Patients and Their Biochemical Study, Journal of Pathogens Volume 2016, Article ID 3219793, 8 pages http://dx.doi.org/10 .1155 / 2016 /3219793.

17. Shire NJ,Rouster SD, Stanford SD, Blackard JT, Martin CM, Fichtenbaum CJ,.(2007).The prevalence and significance of occult hepatitis B virus in a prospective cohort of HIV-infected patients. JAIDS J Acquir Immune Defic Syndr. ; 44(3):309–14. Doi :10. 1097 /QAI.0b0 13e318 0 2e29a9.Am J Trop Med Hyg ; 40:200 –6.

18. Hatim Mudawi, Waleed Hussein, Maowia Mukhtar , Mukhlid Yousif , Omer Nemeri ,Dieter Glebe, Anna Kramvis (2014).Overt and occult hepatitis B virus infection in adult Sudanese HIV

patients; International Journal of Infectious Diseases; 29 (2014) 65–70

19. Rui Alberto Roldão de Almeida Pereira, Aparecida Duarte Hg Mussi,Vergínia Correa de Azevedo e Silva and Francisco José Dutra Souto.(2006).Hepatitis B Virus infection in HIV-positive population in Brazil: results of a survey in the state of Mato Grosso and a comparative analysis with other regions of Brazil; BMC Infectious Diseases 2006, 6:34 doi:10.1186/1471-2334-6-34

20. Saulo Martins, Andréa do Livramento, Michelle Andrigueti, Iara Fabricia Kretzer, Marcos José Machado, Celso Spada, Aricio Treitinger.(2014).The prevalence of hepatitis B virus infection markers and socio-demographic risk factors in HIV-infected patients in Southern Brazil. Revista da Sociedade Brasileira de Medicina Tropical 47(5):552-558, Sep-Oct, 2014, http://dx.doi.org/10.1590/0037-8682-0109-2014 .

21. Nishi H. PatelID, Vanessa Meier-StephensonID , Meaza Genetu, Debasu DamtieID,Ebba Abate, Shitaye Alemu, Yetework Aleka, Guido Van MarleID, Kevin Fonseca,Carla S. Coffin, Tekalign DeressaID.(2020).Prevalence and genetic variability of occult hepatitis B virus in a human immunodeficiency virus positive patient cohort in Gondar, Ethiopia PLoS ONE 15(11):e0242577.https://doi.org/10.1371/journal. pone.0 242577.

22. Yousif M, Mudawi H, Hussein W, Mukhtar M, Nemeri O, Glebe D,(2014).Genotyping and virological characteristics of hepatitis B virus in HIV-infected individuals in Sudan. Int J Infect Dis. 2014;29:125–32.doi:10.1016/j.ijid..07.002.

23. George Gachara , Tshifhiwa Magoro, Lufuno Mavhandu, Emmaculate Lum, Helen K. Kimbi, Roland N. Ndip, and Pascal O. Bessong. (2017). Characterization of occult hepatitis B virus infection among HIV positive patientsin Cameroon. AIDS Research and Therapy, Gachara *et al. AIDS Res Ther*14:11 DOI 10.1186/s12981-017-0136-0.

24. Saha D, Pal A, Sarkar N, Das D, Blackard JT, Guha SK, Saha B, Runu Chakravarty. (2017). Occult hepatitis B virus infection in HIV positive patients at a tertiary healthcare unit in eastern India. PLoS ONE 12(6):e0179035. https://doi.org /10.1371/journal. pone.0179035.

25. Salpini R, Forkam J, Ceccareilli L, Santoro MM, Nanfack A, Sosso SM, (2016).High burden of HBV-infection and atypical HBV strains among HIV infected Cameroonians. Curr HIV Res.;14(2):165–71.

26. Cohen Stuart JW, Velema M, Schuurman R, Boucher CA, Hoepelman AI. (2009). Occult hepatitis B in persons infected with HIV is associated with low CD4 counts and resolves during antiretroviral therapy. J Med Virol. ;81(3):441–5.doi:10.1002/jmv.21422.

27. Chemin I, Trepo C.(2005).Clinical impact of occult HBV infections. J Clin Virol. ;34(Suppl 1):S15–21.doi:10.1016/S1386-6532(05)80005-8.

28. Gupta S, Singh S. (2010). Occult hepatitis B virus infection in ART-naive HIVinfected patients seen at a tertiary care centre in north India. BMC Infect Dis.;10:53. doi:10.1186/1471-2334-10-53.

Original Article **Sudan Medical Laboratory Journal**

Methylenetetrahydrofolate Reductase (MTHFR C677T) Gene Polymorphism in Sudanese Patients with Acute Lymphocytic Leukemia

Ghaidah Fathy Alrahman Mohamed[1*], Mahdi H.A. Abdalla[2]

[1]Department of Hematology, Faculty of Medical Laboratory Sciences, Alneelain University, Sudan
[2]Department of Hematology, Faculty of Medical Laboratory Sciences, Omdurman Ahlia University, Sudan

Abstract

Background: Acute lymphocytic leukemia is one of the major types of leukemia that are found in Sudan. This study aimed to examine the associations of MTHFR c677T polymorphism with the risk of acute lymphocytic leukemia among patients in Sudan.

Methods: A total of 50 patients with myocardial infarction and 50 healthy controls who were matched by age and gender were included in the study. MTHFR C677T polymorphism was studied in both cases and healthy controls, 2 ml of blood samples were collected from both patients and controls into EDTA anticoagulated tubes, the extraction of DNA was done using the salting out approach, The primers, were used to amplify the MTHFR C677T fragment, the amplified fragment was detected on an agarose gel electrophoresis. And count the blood cells.

Results: low frequency of the mutants MTHFR C677T genotype with CT genotype being present in 6.7% of the patients and CC genotype representing 93.3% of the population. Among healthy controls, the frequency of CT genotype was 8% while that of CC genotype was 90%. There was no significant association between MTHFR gene polymorphism and the risk of ALL.

Conclusion: Our data suggest a low impact of MTHFRC677T gene polymorphism on the of developing ALL.

Key words: ALL, Acute Lymphoblastic Leukemia, Sudan, MTFHR gene.

Corresponding author: Ghaidah Fathy Alrahman Mohamed Email: mahdi_razig@hotmail.com

Introduction: Acute lymphoblastic leukemia (ALL) is heterogeneous a group of hematological malignancies that arise from clonal proliferation of immature lymphoid cells in the bone marrow, peripheral blood and other organs [1]. ALL is the most common pediatric malignancy as it represents about 25% of childhood Cancers and approximately 75% of all pediatric leukemias [2].

There has been a gradual Increase in the incidence of ALL in the past 25 years with the peak incidence of ALL being at 2 years of age [3][4]. Many genetic polymorphisms such as TEL-AML1 genes fusion [5], E2A-PBX (PBX1) genes fusion [6], BCR-ABL genes fusion [7], MLL-AF4 genes fusion [8], and IGH-MYC genes fusion [9] have been reported to be associated with ALL. MTHFR gene polymorphism was considered as a risk factor for ALL that is also associated with the disease outcome [10][11].

The MTHFR gene is located on the short (p) arm of chromosome 1 at position 36.3;

it spans approximately 2.2Kb and consists of 11 exons [12]. The MTHFR gene encodes for MTHFR enzyme which plays a central role in folate metabolism by irreversibly converting 5,10-methylenetetrahydrofolate to 5-methylenetetra-

hydrofolate, the predominant circulating form of folate. 5- Methylenetetrahydrofolate donates a methyl group to homocysteine in order to be converted further to methionine [13]. Two common single nucleotide polymorphisms in MTHFR have been reported, a C→T transition at nucleotide 677 in exon 4 and an A→C transition in exon 7 at position 1298. In C677T, the polymorphism occurs at nucleotide 677 (C- T) in the human MTHFR gene and subsequently results in an alanine to valine substitution at position 222 in the amino acid structure of the MTHFR protein. Individuals with two copies of 677C (677CC) have the "normal" or "wild-type" genotype while 677TT individuals (homozygous) are reported to have mild MTHFR deficiency. 677CT individuals (heterozygotes) are almost like normal individuals with normal enzymatic activity [14].

Studying the effect of different gene polymorphisms on developing ALL is important as it may provide data regarding the exact risk of developing the disease, disease outcomes, and possible treatment options. There is no enough and conclusive data regarding the role of MTHFR gene C677T polymorphism on the risk of developing AL and this study aim to provide evidence-based data regarding this issue in Sudan.

Materials and methods: This was a prospective case-control study that was conducted on 60 ALA patients and 50 healthy controls. ALL patients were recruited from Radiation and Isotopes Center-Khartoum (RICK) covering all patients who presented to the hospital with ALL during the period from 1st of January to 31st of September, 2015. Control samples were taken from the blood bank and matching by age and gender was applied. 5ml of venous blood samples were collected from patients in EDTA tubes and complete blood count test was performed using an automated cell counter (Sysmex-kx21) at Radiation and Isotopes Center-Khartoum (RICK). DNA analysis was performed at the department of hematology, faculty of medical laboratory sciences, Alneelain University. The DNA was extracted by salting out method. MTHFR C677T fragment was amplified using the forward primer:5`-TGAAGGAGAAGGTGTCTGCGGGA -3` and the reverse primer: 5`-AGGACGGTGCGG TGAGAGTG- 3`. The amplification was carried out in thermo-cycler (Techne TC-412, UK). An initial 5-minutes denaturation step that at 94°C was performed followed by 40 Cycles of 3 steps: 30-seconds denaturation step at 94°, 1-minute annealing step at 59°C, and 1-minute extension step at 72°C. Then a final 7-minutes extension step at 72 °C was done. The PCR reactions was performed in a final 20 µl solution containing 4 µl of premixed, ready to use, 5x FIRE Polmaster mix (Solis BioDyne, Russian), 11 µl of DNAase free DW, 3µl of genomic DNA and 1 µl of each primer). The amplified fragment was then digested with a 10 U Hinf1endonuclease (New England Biolab, UK) over night and then was visualized using agarose gel electrophoresis. Thin blood film was prepared and stained by lishman stain. Then it was examined under microscope cell morphology was determined.

Statistical analysis was performed using statistical package for social science (SPSS) software. Evaluation of patient´s data was performed using the t-test. Comparison of frequency distribution

between groups was made by means of the *X*2 test. All tests are two-sided and P-value less than 0.05 have been considered as statistically significant. Crude odds ratios (OR) were also calculated and given with 95% confidence intervals (CI).

Result: A total of 60 patients and 50 controls were included in the study. The median age of ALL patients was 5 years with males representing 50% (n = 30) and female representing the other 50% (n = 30). Similarly, 50% (n = 25) of the controls were male and 50% (n = 25) were females with a median age of 6 years.

Complete Blood Cells count was calculated for all ALL patients and control subjects and MTHFR C677T genotype status was determined. The mean hemoglobin (Hb) level for ALL patients was 8.42 ±1.99 g/dl Vs 12.45 ±1.26 g/dl, the meanTWBCs count was 62.8± 40.7×10^9/L Vs 7.95 ±1.94×10^9/L, and the mean platelets count was 44.3 ±34.4×109/L Vs 318.65 ±89.0×109/L, respectively (Table 1).

Table 1: Comparison of hematological characteristics between ALL patients and control subjects.

Parameters	Cases	Control	P value
Hb mean ±SD (g/L)	8.426 ±1.998g/L	12.454 ±1.26g/L	000
TWBCs mean±SD (*109/L)	62.8150 ± 40.72*109/L	7.952 ±1.945*109/L	
Platelets mean ±SD (*109/L)	44.333 ±34.426*109/L	318.65 ±89.01*109/L	
Plasts	12.7833 ±18.657		

Regarding MTHFR 677 polymorphism, 93.3% (n = 56) of ALL patients had MTHFR 677CC genotype while 6.7% (n = 4) had MTHFR 677CT genotype. On the other side, 92% (n = 46) of control subjects had MTHFR 677CC genotype while 8% (n = 4) had MTHFR 677CT genotype. However there was nog significant difference in genotype distribution between the two groups (OR = 0.8214, 95%CI = 0.194-3.4662, P =0.088) (Table 2).

Table 2: Comparison of MTHFR C677T Polymorphism between ALL patients and control subjects.

Genotype	ALL patients	Controls	OR	95% CI	*P* value
CC	56	46	0.8214	0.194 -3.4662	0.088
CT	4	4			

Discussion: This was a case-control study that aimed to evaluate the association between MTHFR C677T polymorphism and acute lymphocytic leukemia among a Sudanese population. In this study we found that the frequency of MTHFR C677T genotype was slightly higher among ALL patients (93.3% Vs 92%) with a 0.821 fold increase in the risk of developing ALL. However, this difference in frequency was not statistically significant (P = 0.088). Similarly, a marginal protection (OR = 0.90) from ALL has been reported in the presence of MTHFR C677T

polymorphism in a previous study (15). In a previous meta analysis of 51 case-control studies, no significant association was found between MTHFR C677T polymorphism and the risk of ALL (16), however, in another meta analysis of 37 case-control studies, a significant association between the two was reported among Caucasians (17).

This study was limited by the small number of sample size and inclusion of patients from only one centre, however, the lack of financial abilities and human resources was the reason for that.

Conclusion: In conclusion, we investigated whether the MTHFR C677T polymorphism and the risk of ALL were related. Our findings showed that the study group had a low prevalence of MTHFR C677T mutant genotypes with little effect on the risk of ALL. More studies are needed to formulate evidence-bases, conclusive data with this regard.

References:

1. Pathak P, Hess R, and Weiss MA. Liposomal vincristine for relapsed or refractory Ph-negative acute lymphoblastic leukemia: a review of literature. Ther Adv Hematol. 2014; 5(1): 18–24.

2. Greenlee RT, Murray T, Bolden S, Wingo PA. Cancer Statistics, CA Cancer J Clin 2000;50:7–33.

3. McNally RJ, Rowland D, Roman E, Cartwright RA. Age and Sex distribution of Hematological Malignancies in the U.K. Hematol Oncol 1997;15:173–89.

4. Shah A, Coleman MP. Increasing Inciudence of Childhood Leukemia: a controversy Re-examined. Br J Cancer 2007;97:1009–12.

5. Stams WA, den Boer ML, Beverloo HB, et al. Expression levels of TEL, AML1, and the fusion products TEL-AML1 and AML1-TEL versus drug sensitivity and clinical outcome in t(12;21)-positive pediatric acute lymphoblastic leukemia. Clin. Cancer Res. 2005;11 (8): 2974–80.

6. McWhirter JR, Neuteboom ST, Wancewicz EV, Monia BP, Downing JR, Murre C. Oncogenic homeodomain transcription factor E2A-Pbx1 activates a novel WNT gene in pre-B acute lymphoblastoid leukemia. Proc. Natl. Acad. Sci. U.S.A. 1999;96 (20): 11464–9.

7. Rudolph C, Hegazy AN, von Neuhoff N, et al. Cytogenetic characterization of a BCR-ABL transduced mouse cell line. Cancer Genet Cytogenet. 2005;161(1):51–6.

8. Caslini C, Serna A, Rossi V, Introna M, Biondi A. Modulation of cell cycle by graded expression of MLLAF4 fusion oncoprotein. Leukemia. 2004;18 (6): 1064–71.

9. Martín-Subero JI, Odero MD, Hernandez R, et al. Amplification of IGH/MYC fusion in clinically aggressive IGH/BCL2-positive germinal center B-cell lymphomas. Genes Chromosomes Cancer. 2005 Aug;43(4):414-23. doi: 10.1002/gcc.20187.

10. Sazawal S, Chaubey R, Kaur P, Chikkara S, Kumar B, Bakshi S, Arya LS, Raina V, Das Gupta A, Saxena R. MTHFR Gene Polymorphisms and the Risk of Acute Lymphoblastic Leukemia in Adults and Children: A Case Control Study in India. Indian J Hematol Blood Transfus. 2014 Dec;30(4):219-25. doi: 10.1007/s12288-013-0295-7. Epub 2013 Aug 28.

11. Umerez M, Gutierrez-Camino Á, Muñoz-Maldonado C, Martin-Guerrero I, Garcia-Orad A. MTHFR polymorphisms in childhood acute lymphoblastic leukemia: influence on methotrexate therapy. Pharmgenomics Pers Med. 2017 Mar

27;10:69-78. doi: 10.2147/PGPM.S107047.

12. Goyette P, Sumner JS, Milos R, Duncan AM, Rosenblatt DS, Matthews RG, Rosen R. Human methylenetetrahydrofolate reductase: isolation of cDNA mapping and mutation identification. Nat. Genet. 1994; 7: 551.

13. Fodinger M, Hörl WH, Sunder-Plassmann G. Molecular biology of 5, 10-methylenetetrahydrofolate reductase. J Nephrol.2000; 13: 20–33.

14. Schneider JA, Rees DC, Liu YT, Clegg JB. Worldwide distribution of a common methylenetetrahydrofolate reductase mutation. Am. J. Hum. Genet. 1998; 62: 1258–60.

15. Roy Moulik, N., Parveen, F., Kumar, A. et al. MTHFR gene polymorphism in acute lymphoblastic leukemia among North Indian children: a case‐control study and meta-analysis updated from 2011. J Hum Genet 59, 397‐404 (2014).

16. Li SY, Ye JY, Liang EY, Zhou LX, Yang M. Association between MTHFR C677T polymorphism and risk of acute lymphoblastic leukemia: a meta-analysis based on 51 case-control studies. Med Sci Monit. 2015 Mar 12;21:740-8. doi: 10.12659/MSM.892835.

17. Jiang Y, Hou J, Zhang Q, Jia S-T, Wang B-Y, Zhang J-H, et al. The MTHFR C677T Polymorphism and Risk of Acute Lymphoblastic Leukemia: an Updated Meta-analysis Based on 37 Case-control Studies [Internet]. Vol. 14, Asian Pacific Journal of Cancer Prevention. Asian Pacific Organization for Cancer Prevention; 2013. p. 6357‐62.

Original Article **Sudan Medical Laboratory Journal**

Detection of NOTCH1 Mutation among Chronic Lymphocytic Leukemia in Sudanese Population

Ebtihal Ahmed Babekir [1*], Ameen Abdulaziz Basabaeen[1, 2], Ibrahim Khider Ibrahim[1] Gamal. M. Elimairi [3]

[1] Department of Haematology, Faculty of Medical Laboratory Sciences, Al Neelain University, Khartoum, Sudan.
[2] Ministry of Health & Population, Hadhramout, Yemen.
[3]Department of Pathology, Faculty of Medicine, Elrazi University.

Abstract

Introduction: The proto-oncogene NOTCH1 is frequently mutated in around 10% of patients with chronic lymphocytic leukemia (CLL). The NOTCH signaling pathway in CLL cells serves a role in survival and resistance to apoptosis. The most common mutation of NOTCH1 is C.7544-7545delCT, which accounts for ~80% of all NOTCH1 mutations.

Objectives: The aim of this study was to detect the prevalence the NOTCH1 c.7541_7542delCT mutation in Sudanese patients with B- cell lymphocytic leukemia (B-CLL).

Materials and Methods: A Case-control study was conducted in Khartoum state, Sudan, during the period from April 2017 to April 2018, involved 110 CLL patients. Physical examination, complete blood count, and Immunophenotyping were performed in all patients to confirm the diagnosis. Clinical staging such as Rai and Binet were studied. Blood samples were collected from all participants; DNA was extracted by using ANALYTIKJENA Blood DNA Extraction Kit. Detection of NOTCH1 c.7544_7545delCT mutation was performed using conventional PCR-based amplification refractory mutation system (ARMS) method.

Results: The NOTCH1 c.7544-7545CT mutation was detected by AS-PCR in **46** out of **110** CLL Sudanese patients (**41.8%**).The distribution of T allele among the cases was 93.6% while the negative cases were 6.4% in cases and controls, No significant association of NOTCH1 mutation with the age and gender. Also the distribution of G allele among cases was 91% while the negative was 9%, in compare to control in which 80.9% was positive and 19.1% were negative with no significant association.

Conclusion: NOTCH1 mutations were frequently detected in B cell CLL Sudanese patients.

Keywords: B- CLL- NOTCH1- mutations- prognosis - Sudanese
Corresponding author: **Ebtihal Ahmed Babekir**

Introduction: Chronic lymphocytic leukemia (CLL) is a heterogeneous disease with highly variable clinical courses and survivals ranging from months to decades. In particular, a subset of CLL patients is known to experience a progressive symptomatic disease poorly responsive to the common immunochemotherapeutic regimens (1,2). A fraction of these high-risk CLL, overall accounting for 5%–10% of cases, can be identified by screening for TP53 mutation/ deletion,1,2 whereas an additional fraction of cases has been recently shown to bear mutations involving the NOTCH1, SF3B1 and BIRC3 genes. Overall, alterations of these genes occur in~ 20% of CLL patients at diagnosis and have significant correlations with survival in consecutive series from independent institutions (3–7). The NOTCH signaling pathway in CLL cells serves a role in

survival and resistance to apoptosis (8). The most common mutation of NOTCH1 is C.7544-7545delCT, which accounts for ~80% of all NOTCH1 mutations(8). This mutation is identified in ~10% of patients with CLL during diagnosis(9). This mutation frequently occurs in patients without immunoglobulin heavy-chain variable region (IGHV) mutations and with trisomy(10). The C.7544-7545delCT NOTCH1 mutation is a 2-bp frame shift deletion within exon 34 that produces a premature stop codon in the PEST domain, a peptide sequence rich in proline, glutamic acid, serine and threonine, which acts as a signal for protein degradation and typically limits the intensity and duration of NOTCH1 signaling(10).

The presence of this mutation has been associated with an intermediate risk of CLL and transformation to high grade lymphoma.

Mutations in the NOTCH1 gene have recently been identified as new genetic alterations associated with shorter time to- first-treatment and progression-free survival (PFS) (5,11–13). Furthermore, clinical resistance to the anti-CD20 monoclonal antibodies in CLL patients with mutated NOTCH1 was found in some clinical trials, which manifested as a lack of benefit from the addition of rituximab to fludarabine cyclophosphamide,or ofatumumab to chlorambucil (14–17). The data about the clinical impact of NOTCH1 mutations among Sudanese B – cell chronic lymphocytic patients is not previously identified, so the aim of this study was to detect the prevalence the NOTCH1 c.7541_7542delCT mutation in Sudanese patients with B- cell lymphocytic leukemia (B-CLL)., as well as, it's relation to the disease clinical impact and biological features and patient outcome.

Methods: Study Population: This study was a cross-sectional study, conducted in Khartoum state, Sudan, in the period from April 2017 to April 2018, a total of 110 patients with Chronic Lymphocytic Leukemia. Patients were obtained at Flow Cytometry Laboratory for Leukemia & Lymphoma Diagnosis, they were referred for Immunophenotype diagnosis.

Patient's diagnosis was done based on clinical history, physical examination and complete blood count. The peripheral blood is important to demonstrate morphological abnormalities and immunophenotypic criteria. Nevertheless, B lymphocyte ≥5000×109 /l, considered as a positive in our diagnosis according to International Workshop on Chronic Lymphocytic Leukemia (1) . The stage of the Chronic Lymphocytic Leukemia was assessed by Rai and Binet (18,19), classification . All patients were newly diagnosed without any previous B-CLL treatment; As explained in our previous work (20).

Sample collection: Four ml of peripheral Blood samples were collected from all patients and divided equally in two tubes; one tube for Complete blood count and Immunophenotype and another tube for molecular analysis. Also, 2ml of whole blood from control group in (EDTA) for molecular analysis.

Determination of Blood Count: Two ml of peripheral blood (PB) were withdrawn from each patient; these samples were collected in EDTA containing tubes for Complete blood count. All results such total WBC, Absolute lymphocyte

count, Hemoglobin level, RBC and platelets were recorded. And a blood smear stained by May Grunwald Giemsa was obtained for all patients.

DNA extraction: After confirmed immunopheno typing of patients, genomic DNA was extracted from all blood samples collected from patients and controls by using *ANALYTIKJENA* Blood DNA Extraction Kit (Germany) (REF-845-KS-1020050), according to the manufacturer's instructions. After DNA extraction DNA quality was evaluated, the β-globin gene amplification was used to assess the quality of DNA in all extracted samples, as previously described (21). All specimens for β-globin gene were Successful amplification, [Primers shown in Supplementary Table-1]. DNA quantification was done after DNA extraction by using a NanoDrop spectrophotometer. Then DNA samples were routinely stored at -20℃.

NOTCH1 c.7544_7545delCT mutation analysis:

The presence of NOTCH1 c.7544_7545delCT mutation was detected by ARMS using primers and PCR parameters developed by Fabbri et al (3) with litile modifications .

Two separated PCR reaction mixtures of 20 µl were prepared for each sample (one for detection of the wild type allele and the other for the detection of mutant-type allele). PCR was performed by using 4µl 5× HOT FIREPol Blend (2,14–16) Master Mix, (Solis BioDyne, Estonia), Cat. No. 04-25-00125), 2µl of genomic DNA, 0.5 µl of each primer, and 13 µl distilled water.

The thermocycling condition for the wild-type allele including 95 °C for 2 min followed by 30 cycles at 95 °C for 30 s, 57.4 °C for 30 s, 72 °C for 30 s with a final extension at 72 °C for 5 min in TECHNE Tc-412-UK PCR Thermal Cycler 96 well. A touchdown PCR was developed to detect the mutant -type allele. The initial denaturation step was 2 min in duration at 95°C, followed by 15 cycles of 30 s at 95°C, 30 s at 62°C (decreasing 0.5 °C per cycle), and 20 s at 72°C, followed by 20 cycles of 30 s at 95°C, 30 s at 55.6°C, and 20 s at 72°C, with a final extension step of 5 min at 72°C,.Amplified PCR products and 50 bp DNA ladder (iNtRON BIOTECHNOLOGY, KOREA), were separated on 2% agarose gel and visualized after staining with ethidium bromide. Amplicon 283 bp indicates the wild-type allele was observed, Amplicon of size 183 bp indicates the mutant-type allele was observed.

Statistical analysis: Patient's data was analyzed using the statistical package for social sciences (SPSS) version 16.0 software (Chicago, IL, USA). Numerical data was summarized as mean and stander deviation and N (%) of study participants, respectively. Chi Square test was used for analyzing qualitative data. Calculation of odds ratio (OR) with confidence interval (CI) for risk estimation was done by Logistic regression analysis.

Table-1: The primers sequence for NOTCH1 c.7544_7545delCT mutation

Primers	Sequence
ForMUT	5′-TCCTCACCCCGTCCCGA-3
ForC	5′-GTGACCG- CAGCCCAGTT-3′’
Rev	5′-AAGGCTTGGGAAAGGAAGC-3
β globin-GH20 (Forward)	5’-GAAGAGCCAAGGACAGGTAC-3’
β globin-PC04 (Reverse)	5’-CAACTTCATCCACGTTCACC-3’

*For MUT: Forward specific for the mutant allele, ForC: Forward common for both mutant and wild-type alleles, Rev: common reverse primer

Results: Mutational Analysis: The NOTCH1 c.7544-7545CT mutation was detected by AS-PCR in 46 out of 110 CLL Patients (41.8%) figure (1). No significant association of NOTCH1 mutation with the age and gender respectively), No significant association of NOTCH1 mutation with the age and gender (P.value=0.133, 0.203 respectively) (Table-2). On the other side regarding organomegally; there was a significant association between NOTCH1 mutation and hepatomegaly (P.value=0.005) (Table-3).

Hematological analysis: Concerning hematological parameters there is no significant differences between B cell CLL patients with NOTCH1 mutations and those without (P.value>0.05 for all) (Table-4).

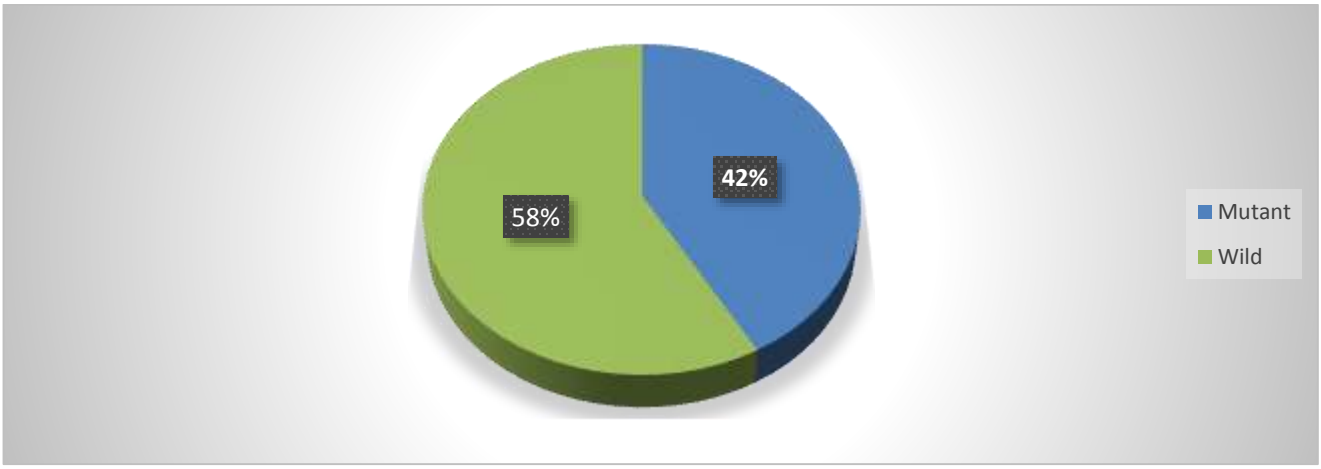


Figure 1-: Distribution of Genotype NOTCH1

Table-2: Association of Genotype NOTCH1 with gender

		Genotype NOTCH1		Total	P. value	Odd ratio (95%CI)
		Mutant	Wild			
Gender	Male	36 (32.7%)	43 (39.1%)	79 (71.8%)	0.203	1.758 (0.734 to 4.212)
	Female	10 (9.1%)	21 (19.1%)	31 (28.2%)		
Total		46 (41.8%)	64 (58.2%)	110 (100.0%)		

Table-3: Association of NOTCH1 with the Organomegally

		Genotype NOTCH1		Total	P. value
		Mutant	Wild		
Lymphadenopathy	Absent	10 (9.1%)	22 (20.0%)	32 (29.1%)	**0.150**
	Present	36 (32.7%)	42 (38.2%)	78 (70.9%)	
Splenomegaly	Absent	24 (21.8%)	32 (29.1%)	56 (50.9%)	**0.822**
	Present	22 (20.0%)	32 (29.1%)	54 (49.1%)	
Hepatomegaly	Absent	45 (40.9%)	51 (46.4%)	96 (87.3%)	**0.005***
	Present	1 (0.9%)	13 (11.8%)	14 (12.7%)	

Table-4: Comparisons of patient's hematological data according to NOTCH1 mutation

Parameter	Genotype NOTCH1		P. value
	Mutant (n=46)	Wild (n=64)	
TWBCs	90.8 ± 73.1	94.4 ± 77.6	0.808
RBCs	3.7 ± 0.9	3.7 ± 0.9	0.909
PLTs	185.3 ± 93.0	192.1 ± 113.3	0.739
HB	11.3 ± 2.6	11.0 ± 2.4	0.550
Granulocytes	12.2 ± 7.1	12.4 ± 7.9	0.889
Monocytes	2.9 ± 1.9	2.4 ± 1.9	0.130
Lymphocytes	84.9 ± 8.3	85.2 ± 9.0	0.819
Absolute Lymphocyte x103/ul	79.4 ± 68.2	84.3 ± 73.2	0.724
Absolute B Lymphocyte x103/ul	71.1 ± 65.3	74.9 ± 68.4	0.765

Discussion: Chronic lymphocytic leukemia (CLL) has a high incidence in Europe and North America, intermediate in Africa and low in Asia (24). Chronic lymphocytic leukemia (CLL) is a disease that affects older people in particular, and it is often determined by the elderly, Young people rarely experience clinical symptoms that are very heterogeneous, Leukemia originates initially through changes or mutations. In the genetic material, it affects the programmed cell death of blood cells. Diagnosis: made by blood counts, blood spots, and immunoglobulin enlarged B-lymphocytes that define cloned B-cell clusters that carry CD5 antigen as well as typical B-cell markers. (23)

Certain gene mutations, including Notch homolog1, translocation-associated (Drosophila) (NOTCH1) are known biomarkers for CLL prognosis. (8)

NOTCH1 mutations were detected in 41.8% of B cell CLL cases; and this figure is higher than was previously reported 24% (Van Vlierberghe et al., 2013), 10% of another study (Sportoletti P., et al 2014). There was No statistically significant differences in age and gender in relation to NOTCH1 mutations in B cell CLL cases, this may be due to difference in race and in clinical conditions of our Sudanese patients which may have more progressive disease due to the suggested difference in the molecular and biological behaviors.In consistence with data reported by Villamor et al., (2013) there was No other significant variations in the hematologic & clinical data in the relation to NOTCH1 mutation were detected; Except for organomegally in which revealed a significant association between NOTCH1 mutation and hepatomegaly(***P.valu =0.005***) .

Conclusion: NOTCH1 mutations were frequently detected in B cell CLL Sudanese patients. NOTCH1 mutations may had harmful effect on patient outcome in B- CLL patients; so identification of NOTCH1 mutations in B-CLL patients at diagnosis is recommended for better stratification and may aid in patient management and treatment.

References

1. Hallek M, Cheson BD, Catovsky D, Caligaris-cappio F, Dighiero G, Do H. D 5446. 2008; 111(12) :5446–57.

2. Hallek M. Chronic lymphocytic leukemia: 2017 update on diagnosis, risk stratification, and treatment. Am J Hematol. 2017;92(9):946–65.

3. Fabbri G, Rasi S, Rossi D, Trifonov V, Khiabanian H, Ma J, et al. Analysis of the chronic lymphocytic leukemia coding genome: role of NOTCH1 mutational activation . J Exp Med. 2011; 208(7):1389–401.

4. Puente XS, Pinyol M, Quesada V, Conde L, Ordóñez GR, Villamor N, et al. Europe PMC Funders Group Whole-genome sequencing identifies recurrent mutations in chronic lymphocytic leukaemia. 2012;475(7354):101–5.

5. Rossi D, Rasi S, Fabbri G, Spina V, Fangazio M, Forconi F, et al. Mutations of NOTCH1 are an independent predictor of survival in chronic lymphocytic leukemia. Blood 2012;119(2):521–9. availablewww.ncbi.nlm.nih.gov/pubmed/2207706

6. Quesada V, Conde L, Villamor N, Ordóñez GR, Jares P, Bassaganyas L, et al. Exome sequencing identifies recurrent mutations of the splicing factor SF3B1 gene in chronic lymphocytic leukemia. Nat Genet.2012;44(1):47–52. Available from: http://dx.doi.org/10.1038/ng.1032

7. Wang L, Lawrence MS, Wan Y, Stojanov P, Sougnez C, Stevenson K, et al. SF3B1 and Other Novel Cancer Genes in Chronic Lymphocytic Leukemia. N Engl J Med. 2011;365(26):2497–506.

8. Shahjahani M, Mohammadiasl J, Noroozi F, Seghatoleslami M, Shahrabi S, Saba F, et al. Molecular basis of chronic lymphocytic leukemia diagnosis and prognosis. Cell Oncol. 2015;38(2):93–109.

9. Sportoletti P, Baldoni S, Del Papa B, Aureli P, Dorillo E, Ruggeri L, et al. A revised NOTCH1 mutation frequency still impacts survival while the allele burden predicts early progression in chronic lymphocytic leukemia.Leukemia.2014;28(2):436.

10. Landau D, Wu CJ. CLL : molecular heterogeneity revealed by high-throughput genomics. 2013;1–13.

11. Villamor N, Conde L, Martínez-Trillos A, Cazorla M, Navarro A, Beà S, et al. NOTCH1 mutations identify a genetic subgroup of chronic lymphocytic leukemia patients with high risk of transformation and poor outcome. Leukemia. 2013;27(5):1100–6.

12. Lionetti M, Fabris S, Cutrona G, Agnelli L, Ciardullo C, Matis S, et al. High-throughput sequencing for the identification of NOTCH1 mutations in early stage chronic lymphocytic leukaemia: Biological and clinical implications. Br J Haematol. 2014;165(5):629–39.

13. Baliakas P, Hadzidimitriou A, Sutton LA, Rossi D, Minga E, Villamor N, et al. Recurrent mutations refine prognosis in chronic lymphocytic leukemia. Leukemia. 2015;29(2):329–36.

14. Stilgenbauer S, Schnaiter A, Paschka P, Zenz

T, Rossi M, Döhner K, et al. Gene Mutations and Treatment Outcome in Chronic Lymphocytic Leukemia. Blood. 2014;123(21):3247–55.

15. Bilous NI, Abramenko I V., Chumak AA, Dyagil IS, Martina Z V. Detection of notch1 c.7544-7545delct mutation in chronic lymphocytic leukemia using conventional and real- Time polymerase chain reaction. Exp Oncol. 2016 Jun 1;38(2):112–6.

16. Tausch E, Mertens D, Stilgenbauer S. Advances in treating chronic lymphocytic leukemia. F1000Prime Rep. 2014;6(August).

17. Pozzo F, Bittolo T, Arruga F, Bulian P, MacOr P, Tissino E, et al. NOTCH1 mutations associate with low CD20 level in chronic lymphocytic leukemia: Evidence for a NOTCH1 mutationdriven epigenetic dysregulation. Leukemia. 2016;30 (1) :182–9.

18.Nieman PE. of Chronic Randomized. 1975 ; 45 (2):197–203.

19. Binet JL, Auquier A, Dighiero G, Chastang C, Piguet H, Goasguen J, et al. A new prognostic classification of chronic lymphocytic leukemia derived from a multivariate survival analysis. Cancer. 1981;48(1):198–206.

20. Basabaeen AA, Abdelgader EA, Babekir EA, Eltayeb NH, Altayeb OA, Fadul EA, et al. Clinical presentation and hematological profile among young and old chronic lymphocytic leukemia patients in Sudan. BMC Res Notes [Internet]. 2019;12(1):12–7.https://doi.org/10.1186/s13104-019-4239-7

21. Kerr JR, Al-Khattaf A, Barson AJ, Burnie JP. An association between sudden infant death syndrome (SIDS) and Helicobacter pylori infection. Arch Dis Child. 2000;83(5):429–34.

22. Borggrefe T, Oswald F. The Notch signaling pathway: transcriptional regulation at Notch target genes. Cell Mol Life Sci 2009;66:1631–46. https://doi.org/10.1007/ s00018-009-8668-7.

23. Hallek, M. (2019). Chronic lymphocytic leukemia: 2020 update on diagnosis, risk stratification and treatment, annual clinical up dates in hematological malignancies, amj hematol. 2019;94:1266–1287.

24. Shenoy PJ, Malik N, Sinha R, et al. Racial Differences in the Presentation and Outcomes of Chronic Lymphocytic Leukemia and Variants in the United States. Clinical Lymphoma Myeloma & Leukemia. 2011; 11(6): 498-506

Original Article Sudan Medical Laboratory Journal

Seroprevalence of *Toxoplasma gondii* Infection among Pregnant and Aborted Women in Khartoum State, Sudan

Adam Ahmed Mohamed[*1], Shirehan M.Ibrahim[2], Nasr M.Nsar Ahmed[3], Sahar M. Seedahmed[4]

[1*]Department of Medical Microbiology, Faculty of Medical Laboratories, National University, Khartoum, Sudan
[2]Department of Medical Microbiology, Faculty of Medical Laboratories, Ibn Sina University, Khartoum, Sudan
[3]Department of Medical Microbiology, Faculty of Medical Laboratories, Omdurman Ahlia University, Sudan
[4]Department of Medical Microbiology, Faculty of Medical Laboratories, International University of Africa, Sudan

Abstract

Introduction: Congenital toxoplasmosis occurs in foetuses upon a primo infection of a pregnant woman with *T. gondii*. According to the trimester of infection, it does only cause miscarriage or stillbirth but also serious and progressive visual, hearing, motor, and cognitive complications in a born child. A crucial step in antenatal care is the detection of *T. gondii* infection in the pregnant mother and her fetes.

Objective: To determine the sero-prevalence of *Toxoplasma gondii* infection among pregnant and aborted women and other virile of risk factors studied.

Methods: One hundred pregnant women attending antenatal care in Khartoum State, Sudan collection of 3 ml ,centrifugation, save at 4c can be detected by Enzyme Linked Immunosorbent Assay test for IgM study was conducted from June 2022 to July 2022 screened for immunoglobulin (IgM) antitoxoplasma antibodies using ELISA technique.

Results: Total of 100 pregnant women test were seropositive for IgM was 47 (47%) and while 53 (53%) seronegative. None of the examined women had IgM antitoxoplasma antibodies. The highest rate of infection (25%) was detected among women aged 26-30 years. No statistically significant relation was observed between *T.gondii* sero-prevalence and other virile of risk factors studied(gestational age, aborted and non-aborted pregnant women, group age, educational level , occupation , demographic data) S (p.value > 0.05).

Conclusions: This study concluded a high seropositivity for *Toxopalsma gondii* indicating potential for abortion and congenital transmission. Women living in Khartoum higher risks for *T. gondii* infection. The higher prevalent was in age group (26-30) years. Educational level , occupation , demographic data was statistically significant.

Keywords: Toxoplasma gondii, pregnant women, abortion, Enzyme linked Immune Sorbent assay, IGM, seropositive ,seronegative, seroprevelance.

***Corresponding Author: Adam Ahmed Mohamed** Sahersedahmed@gmail.com

Introduction: *Toxoplasma gondii* is an obligate intracellular protozoan parasite with a complex life cycle. This agent causes toxoplasmosis between humans and animals and is one of the most prevalent chronic infections, infecting one third of the globe population (1). It's an important cause of reproductive failure in humans and farm animals leading to significant socio-economic losses worldwide (2). Human infection with *T.gondii* causes toxoplasmosis postnatal toxoplasmosis is usually an asymptomatic disease, but often takes a severe course in immune compromised hosts (3). Congenital toxoplasmosis is acquired through vertical transmission of *T.gondii* to the fetus by transplacental transfer from the mother usually following acute maternal infection. If congenital

toxoplasmosis occurs early in pregnancy, it may lead to severe damage or abortion. (4)

Though the infection is frequently asymptomatic or mild and self-limiting (fever, agitation, lymphadenopathy), infection happening throughout pregnancy leads vertical transmission to the fetus (5). Earlier research has revealed that toxoplasmosis infection was more frequent between those with history of close contact with cats, raw meat and vegetable consumption, and low learning level. The most significant benefit in the serology of Toxoplasma is to detect whether the pregnant lady has acute infection or not, and if so, whether it happened before pregnancy (6). Primary infection with *T. gondii* during the third trimester of pregnancy carries a higher risk of congenital transmission than that acquired during the first trimester. (7)

In Sudan, the first report of human toxoplasmosis was dated back to 1966, with different prevalence rates according to the regions and the people's habits.(8). Around 65% of Sudanese domestic animal was infected with toxoplasmosis. (9)

Materials and Methods:

Study Design and Setting: The study was experimental study, descriptive study was conducted in antenatal care hospitals in Khartoum State, Sudan in between June 2022 to july2022, from Pregnant and Aborted women in Khartoum State.

Specimen collection: Three ml of blood sample were collected in sterile container from each participant through venepuncture, the obtained blood samples were collected into plain vacationer. Sera were separated after centrifugation for 15 minutes in 3000 rpm. Then were stored in -20 ◌̊C till used. (10)

ELISA for detection of *T.gondii* IgM Abs: Serological diagnosis was done following the ELISA technique (enzyme-linked immunosorbent assay) The ELISA test kit provides a semi quantitative in vitro assay for human antibodies of the immunoglobulin class IgM against *Toxoplasma gondii* in serum or plasma for the diagnosis of toxoplasmosis..

Procedure: Test performance using fully automated analysis devices Sample dilution and test performance are carried out fully automatically using an analysis device.

Pipetting protocol:

	1	2	3	4	5	6	7	8	9	10	11	12
A	**C**	**p6**	**p14**	**p22**								
B	**pos**	**P7**	**P15**	**P23**								
C	**nag**	**P8**	**P16**	**P24**								
D	**P1**	**P9**	**P17**									
E	**P2**	**P10**	**P18**									
F	**P3**	**P11**	**P19**									
G	**P4**	**P12**	**P20**									
H	**P5**	**P13**	**P21**									

The above pipetting protocol is an example of the semi quantitative analysis of antibodies in 24 patient samples (P 1 to P 24). Calibrator (C), positive (pos.) and negative (neg.) control as well as the patient samples have been incubated in one well each. The reliability of the ELISA test can be improved by duplicate determinations of each sample. The wells can be broken off individually from the strips. This makes it possible to adjust the number of test substrates used to the number of samples to be examined and minimizes reagent wastage. Both positive and negative controls serve as internal controls for the reliability of the test procedure. They should be assayed with each test run.

Calculation of results: The extinction value of the calibrator defines the upper limit of the reference range of non-infected persons (cut-off) recommended by EUROIMMUN.

Semi quantitative**:** Results can be evaluated semi quantitatively by calculating a ratio of the extinction value of the control or patient sample over the extinction value of the calibrator. Use the following formula to calculate the ratio:

Extinction of the control or patient sample ÷ Extinction of calibrator = Ratio

EUROIMMUN recommends interpreting results as follows:

Ratio <0.8: negative

Ratio ≥1.1: positive

Statistical Analysis: Statistical analysis was performed using the statistical package for social sciences (SPSS version 20). Demographic data were categorized and means were compared using the student T test. Chi square test was used to test the significance of risk factors associated with seropositive patients; results considered to be statistically significant if P value < 0.05.

Results: This study was conducted on 100 Pregnant and Aborted women in every stage of pregnancy the present study was conducted in Khartoum state. The overall prevalence rate of *Toxoplasma gondii* among case study groups the results by direct enzyme-linked immunosorbent assay (ELISA) technique, test were seropositive for IgM was 47 (47.0%) and while 53 (53.0%) seronegative .

The age of the subjects ranged between 20-39 years old; the age groups were divided into three groups, 20-25 years, 26-30 years and 31-39 years. The frequency of each groups as follow 20-25 years 17 sample, (17.0%), 26-30 years 58 sample, (58.0%) and 31-39 years is 25 sample, (25.0%), total 100 (100.0%) . The prevalence of *T. gondii* in different stage of pregnancy gestational age into three groups. First trimester, Second trimester and Third trimester, The frequency of each groups as follow First trimester 11 sample, (11.0%).Second trimester 15 sample, (15.0%) and Third trimester 74 sample, (74.0%) total 100 (100.0%) .Frequency of educational level among study population into three groups primary school. secondary school and university. The frequency of each groups as follow primary school 41 sample,(41.0%). secondary school 48 sample,(48.0%) and university 11 sample,(11.0%) total 100 (100.0%) .Frequency of occupation among study population into four groups house wife. student, teacher and nurse , The frequency of each groups as follow house wife 93 sample,(93.0%), student 3 sample, (3.0%), teacher

3 sample,(3,0%) and nurse 1 sample (1.0%) total 100 (100.0%) .

The frequency of each groups as follow 20-25 years seropositive with IgM 7 while seronegative 10 , 26-30 years seropositive with IgM 25while seronegative 33 , 31-39 years seropositive with IgM 15 while seronegative 10, p.value 0.190 .

The frequency of each groups as follow First trimester seropositive with IgM 4 while seronegative 7, Second trimester seropositive with IgM 8 while seronegative 7 and Third trimester seropositive with IgM 35 while seronegative 39, p.value 0.683 .

frequency of educational level among study population primary school seropostive with IgM 22,while seronegative 19, secondary school seropostive IgM 21,while seronegative 27 and university seropostive IgM 4 ,while seronegative 7, P.value 0.238 .

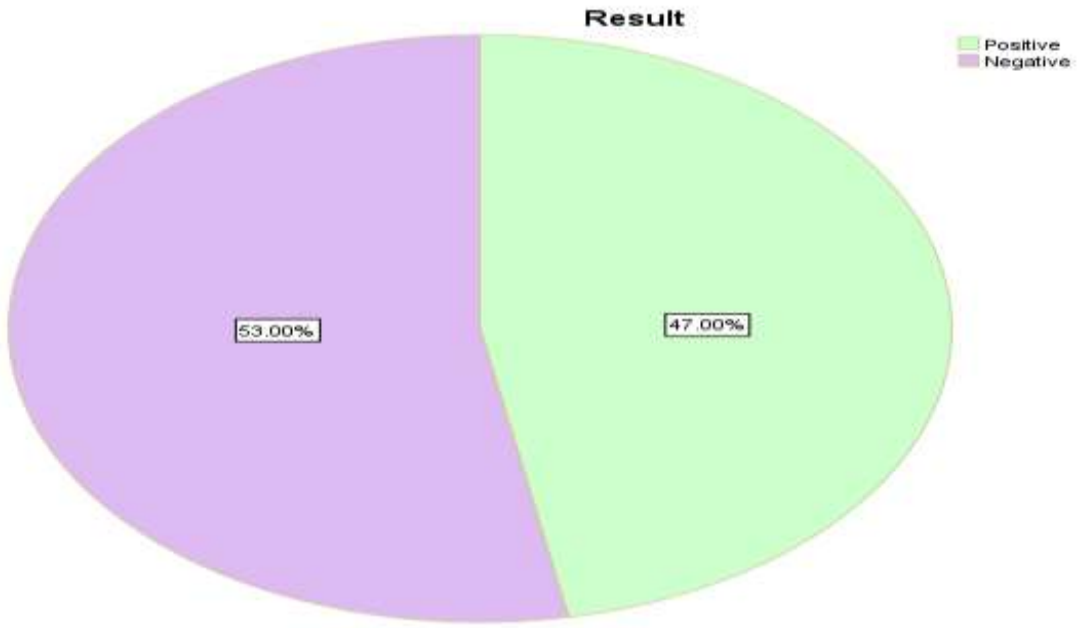
Frequency of occupation among study population house wife seropostive IgM 45, while seronegative 48, student seropostive IgM 1,while seronegative 2, teacher seropostive IgM 0, while seronegative 3, and nurse seropostive IgM 1while seronegative 0, P.vaule 0.494

Table 1: frequency of age group among study population

Age	Frequency	Percent
20-25	17	17.0
26-30	58	58.0
31-39	25	25.0
Total	100	100.0

Table 2: frequency of gestational age among study population

Gestational age	Frequency	Percent
First trimester	11	11.0
Second trimester	15	15.0
Third trimester	74	74.0
Total	100	100.0



Figur1: frequency of Toxoplasma gondii results among study population

Discussion: *Toxoplasma gondii* cause severe impairment and death to fetuses or to newborns through congenital infection. The current study is one of few studies in Sudan to explore the seroprevalence of *T. gondii* infection among one of the most important clinical categories of toxoplasmosis in immunocompetent hosts who are pregnant and aborted women. The study revolts that out one-handed sample they was seropositive of IgM (47%) and while sero-negative (53%), this study was agree with the study conducted by (Majda and his colleagues) in *Toxoplasma gondii* sero-prevalence among pregnant women in Rabat, Morocco, the sample size was 677sample, IgM anti-toxoplasma antibodies seropositive was rate in this study (43%),(13). and also agree with another study conducted by (Rayan and his colleagues) Seroprevalence of toxoplasmosis between aborted ladies in Atbara district, Sudan,the sample size was 152 sample ,while seropostive IgM (35%),(14) . This study was disagree with study conducted by (Joy Nkain and his colleagues*)*, Seroprevalence of Gestational and Neonatal Toxoplasmosis aswell as Risk Factors in Yaoundé, Cameroon, the sample size was 300 sample, IgM anti-toxoplasma antibodies seropositive was rate in this study (8%), (15), and This result was disagree with the study conducted by(Nijem and his colleagues),in Seroprevalence and associated aisk factor of toxoplsmosis in pregnant women in Hebron district, Palestine, the sample size was 204 sample, IgM anti-toxoplasma antibodies seropositive was rate in this study (17%) (16). comparisons with reports from different countries have to be interpreted cautiously this variation has been attributed to climate, cultural differences regarding hygienic and feeding habits.

This result was anti-IgM seroprevalence of *Toxoplasma gondii* increased within the age group of (26-30) years while (25%), this study was agree with the study conducted by (Majda and his colleagues) increased within the age in(16-25)

years was 25% and increased within the gestational age in third trimester while (35%) This in our opinion is highly risky, as it is the most fertile period of childbearing age and this also highlights the need to continue to educate women of child-bearing age on prevention of toxoplasmosis. However, different studies reported an increase in seropositivity of anti-*T. gondii* antibodies with increasing age.

Acknowledgements:

We are grateful to antenatal care hospitals in Khartoum State to perform this study. This work was partly *of M.SC. Degree in Microbiology and Infection Control* under supervision Dr: Sahar Mohammed Seed Ahmed at *National University.*

Authors declare:

No conflicts of interest regarding the publication of this paper.

References:

1. Galvan Ramirez ML, Troyo R, Roman S, et al. A systematic review and Meta -analysis of *Toxoplasma gondii* infection among the Mexican population. *Parasit Vectors*. 2012; 26; 5:271.

2. Hamad MNM. Prevalence of toxoplasmosis among selected group of unmarried volunteers Sudanese females. *MOJ Womens Health.* 2018; 7(6):202–203.

3. Koskiniemi M, Lappalainen M, Heman L (1989) Toxoplasmosis needs evaluation. An Overview and Proposals. AJDC 143:724-728.

4. Field L, Guerina G (1997) Toxoplasmosis. Pediatrics in Review 18(3).

5. Daryani A, Sarvi S, Aarabi M, et al. Seroprevalence of *Toxoplasma gondii* in the Iranian general population: a systematic review and meta-analysis. *Acta Trop.* 2014; 137(1):185–194

6. Golkar M, Rafati S, Abdel-Latif MS, et al. The dense granule protein GRA2, a new marker for the serodiagnosis of acute Toxoplasma infection: comparison of sera collected in both France and Iran from pregnant women. *Diagn Microbiol Infect Dis.* 2007; 58(4):419–26.

7. Vaz RS, Thomaz-Soccol V, Sumikawa E, Guimarães AT. Serological prevalence of *Toxoplasma gondii* antibodies in pregnant women from southern Brazil. Parasitol Res 2010; 106: 661-665.

8. Carter FS, Fleck DG. The incidence of Toxoplasma antibodies in the Sudanese. Trans R Soc Trop Med Hyg 1966; 60: 539-543.

9. Elamin EA, Elias S, Daugschies A, Rommel M. Prevalence of *Toxoplasma gondii* antibodies in Pastrol camels, in the Butana plains, Mid-Eastern Sudan. Vet Parasitol 1992; 43: 171-175.

10. Laboudi M, Taghy Z, Duieb O, Peyron F, Sadak A. Toxoplasma gondii seroprevalence among pregnant women in Rabat, Morocco. Tropical Medicine and Health. 2021 Dec;49(1):1-8.

11. Elhag BK, Elturabi SE, Bahaeldin K, Elturabi SE. Seroprevalence of toxoplasmosis among women with abortion in Khartoum State. Journal of Coastal Life Medicine. 2015;3(7):551-4.

12. Taha RK, Hamad MN, Taha KM, Hamad AE. Seroprevalence of toxoplasmosis between aborted ladies in Atbara district, Sudan.

13. Dubey *JP* (2009) History of the discovery of the life cycle of Toxoplasma Gondii. International *Journal* for Parasitology 39 (8):877-882.

14. Prtersen E, Dubey JP (1998) Biology of

Toxoplasmosis. IN: A Comprehensive clinical guide edited by david hm, joynson tim g, wreghitt excerpt, Cambridge University Press, UK.

15. Ayeah JN, Oladokun A, Sumbele IU, Ilesanmi AO, Bekindaka ON. Seroprevalence of Gestational and Neonatal Toxoplasmosis as well as Risk Factors in Yaoundé, Cameroon. Journal of Parasitology Research. 2022 Mar 21;2022.

16. Al Amleh S, Nijem KI. Seroprevalence and associated risk factors of Toxoplasmosis among pregnant women in Hebron district, Palestine.

Original Article **Sudan Medical Laboratory Journal**

Cytolytic Vaginosis in Reproductive-age Sudanese Women, Wad Medani, Gezira State, Sudan, 2023

Rayan Sidig Adam Abdelgalil[1]*, Esraa Elshaikh Eltayeb Osman[1], Mohamed Siddig M.Elbashir[1], Yasmin Elsamani Elwasila[1], Mohamed Elsanousi Mohamed[2], Elhadi Ibrahim Miskeen[3], Mai Abdulrahman Mohammed Masri[4] and Bakri Yousif M.Nour[5]

[1] Department of Histopathology and Cytopathology, Faculty of Medical Laboratory Sciences, University of Gezira, Sudan
[2] Department of Obstetrics and Gynecology, Faculty of Medicine, University of Gezira, Gezira, Sudan
[3] Department of Obstetrics and Gynecology, University of Bisha, Bisha, Saudi Arabia
[4] Department of Zoology, Faculty of Science, University of Khartoum, Khartoum, Sudan
[5] Department of Medical Parasitology, Faculty of Medical Laboratory Sciences, University of Gezira, Gezira, Sudan

Abstract

Introduction: Cytolytic vaginosis is a condition that defined by the presence of a large number of lactobacilli, along with cytolysis of the intermediate cells of the vaginal epithelium.

Aims: The study aimed to investigate the occurrence of cytolytic vaginosis (CV) in cervical smears of Sudanese females at reproductive age attended to Wad Medani Obstetrics and Gynecology Teaching Hospital and some private clinics.

Methods: A total of 134 cervical Papanicolaou PAP smears were collected from Sudanese married women during the period from February 2020 to January 2023. Collected PAP smears were evaluated according to the 2014 Bethesda System for Reporting Cervical Cytology.

Result: Out of the 134 cervical smears, 78 (58.2%) had normal growth of lactobacilli, 36 (26.8%) had lactobacillus overgrowth, and 20 (14.9%) classified as cytolytic vaginosis. 50% the participants with CV were in the age group 26-36 years, 60% belonged to rural communities and vaginal discharge was the main complaint in 60% of them. And all CV smears were negative for intraepithelial lesions or malignancy (NILM).

Conclusion: The study highlights the importance of accurately diagnosing vaginal discharge, with a specific focus on considering Cytolytic Vaginosis (CV) as a potential cause. CV is an important player in cervical infection or microbial dysbiosis. Women with vaginal discharges should undergo a routine cervical screening examinations.

Key words: Cytolytic Vaginosis, Lactobacilli, Cervical smear, Sudan
Corresponding author: **Rayan Sidig Adam Abdelgalil: reorayona@gmail.com**

Introduction: Albert Doderlein discovered the lactobacillus-dominant configuration in 1892, when he detected a long, thick, motile, rod-shaped, gram-positive, non-spore-producing bacteria in the vaginal fluid. Originally described as Doderlein's bacillus, this bacterium was subsequently called lactobacillus due to its ability to produce lactic acid (1,2) as a result of carbohydrate metabolism(3). Beside the formation of acid, many lactobacilli strain produce lipoteichoic acid, biosurfactants and possess fimbriae, flagella and surface layer proteins (Slps) outside the cell wall (4,5), all of which are proven to promote and improve tissue adhesion capabilities (6). Lactobacilli also produce antimicrobial compounds such as bacteriocins and hydrogen peroxide (H_2O_2) (7). All mentioned

lactobacilli properties function as crucial parts of the female mucosal genital tract's protection barriers against a wide range of microbial illnesses through an efficient natural way to reduce inflammatory responses (8). Lactobacilli colonization of the human cervix epithelial cells was reported with a range of 29-52% (9–11). The properties of such lactobacilli play an important role as defensive mechanisms against the growth of potentially harmful pathogens (7). The cervical epithelium might exhibit histological abnormalities as a result of physiochemical changes brought on by an imbalance in these defenses (12). In several cases, this might result in cervical lesions or even tumor development (13,14).

On the other side, lactobacilli overgrowth can lead to a common condition known as Cytolytic vaginosis (CV), which was originally characterized by Cibley and Cibley in 1991 (15). To sustain this balance lactobacilli increase their acid-producing capabilities, resulting in a decrease in vaginal pH (3.5-4.0) and subsequent promotion of epithelial damage(16). In the published literature, the most often used method for diagnosing CV is wet mount microscopy to reveal lactobacilli overgrowth/ cytolysis, and to exclude Candidiasis, where yeast culture is essential (17). The differences in commensal microbiota in sub-Saharan Africa are thought to contribute to the prevalence and transmission of cervicovaginal infection and dysbiosis in the region (18).Thus, health systems are in urgent need of better data most importantly, data and research specific to women's health in Sudan. This study focuses on the identification of Cytolytic Vaginosis in reproductive-age women using the Papanicolaou (PAP) cervical smear test among a panel of Sudanese females at reproductive age.

Materials and methods:_Study design: This descriptive cross-sectional study included a total of 134 married Sudanese women, who were referred to Wad Medani Obstetrics and Gynecology Teaching Hospital and some private clinics during the period from February 2020 to January 2023.

Sample collection: After inserting a medium-sized, sterile, disposable plastic speculum that allowed complete visibility of the cervical os and ectocervix, consultant gynecologists acquired cytological samples by scraping the cervix's surface with a cervical brush. The samples were prepared into direct conventional smears, and they were then immediately fixed for 15 minutes in 95% ethyl alcohol.

Sample processing: Ethyl alcohol fixed smears were hydrated by using descending concentrations of 95% alcohol through 70% alcohol to distilled water. Smears were then stained with Papanicolaou stain using standard PAP stain procedure and evaluated according to the 2014 Bethesda System for Reporting Cervical Cytology. Lactobacilli growth on smears was classified into three categories. Group one had a few lactobacilli in the background and an aberrantly normal growth pattern. Group 2 was presented with an overgrowth of lactobacilli (a large number of lactobacilli in the background). Group 3 displayed additional characteristics of epithelial cell cytolysis; mainly the presence of fragmented epithelial cells, bare nucleoli with prevalent lactobacilli overgrowth in the background. Group 3 represents the CV criteria

as described by The International Society for the Vulvovaginal Disease (ISSVD).

Data analysis: Descriptive statistics, such as frequency distributions, were examined using IBM Statistical Package for Social Sciences (SPSS) version 25 (data were presented as percentages).

Ethical consideration: Each participant was asked to sign a written informed consent before specimen collection. The study was approved by the Ministry of Health in Gezira State, Sudan.

Results: In the present study 134, randomly selected Sudanese married women at reproductive age were enrolled. The socio-demographic characteristics of the participating women are shown in Table1. The ages of the participants ranged from 15 to 49 years. The predominant population of the subjects in this study falls into the age group 26-36 years (47.8%) with more than half (58.2%) residing in rural areas. The majority (70.1%) of participants were undergraduates, 27.6% were graduates and only 2.2% were postgraduates (Table 1).

Cytomorphologically, 78) 58.2%) of smears appeared to be having normal growth of lactobacilli, 36 (26.8%) had lactobacillus overgrowth, and 20 (14.9) had noticeable Cytolytic vaginosis (Table1, Figure 1).

Half of the participants with CV were between the ages of 26 and 36 years (50%, n = 10/20), the majority (60%) belonged to rural communities, and more than half of them were undergraduates (55%) (Table 1).

The smears with CV displayed marked epithelial cytolysis with prominent bare nuclei, such as the presence of fragmented epithelial cells, bare nucleoli due to the prominent lysis of the cytoplasm and lactobacillus overgrowth without pathognomonic findings of bacterial vaginosis, trichomonas vaginalis or candida infection (Figure 1 (C)).

Cytopathological diagnosis was also used for identifying infectious microorganisms and cytomorphological changes associated with various infections. Data regarding some other infection was available for comparison in our previous study in which reported microorganisms in the studied cases were *Gardnerella vaginalis* (n=11), *Candida* species (n=9), *Trichomonas vaginalis* (n=2) and Koilocytic changes of Human Papilloma Virus in only one sample. The presence of theses pathogens tend to associate with a decrease or absence of Lactobacilli.

The cytology findings for the smears of study participants showed that 92% of them were negative for intraepithelial lesions or malignancy (NILM), 2.2% of them had atrophy, 2.2% had atypical squamous cells of undetermined significance (ASC-US), 0.7% had atypical glandular cells (AGC), and 0.7% had adenocarcinomas. All CV smears were detected in the NILM group.

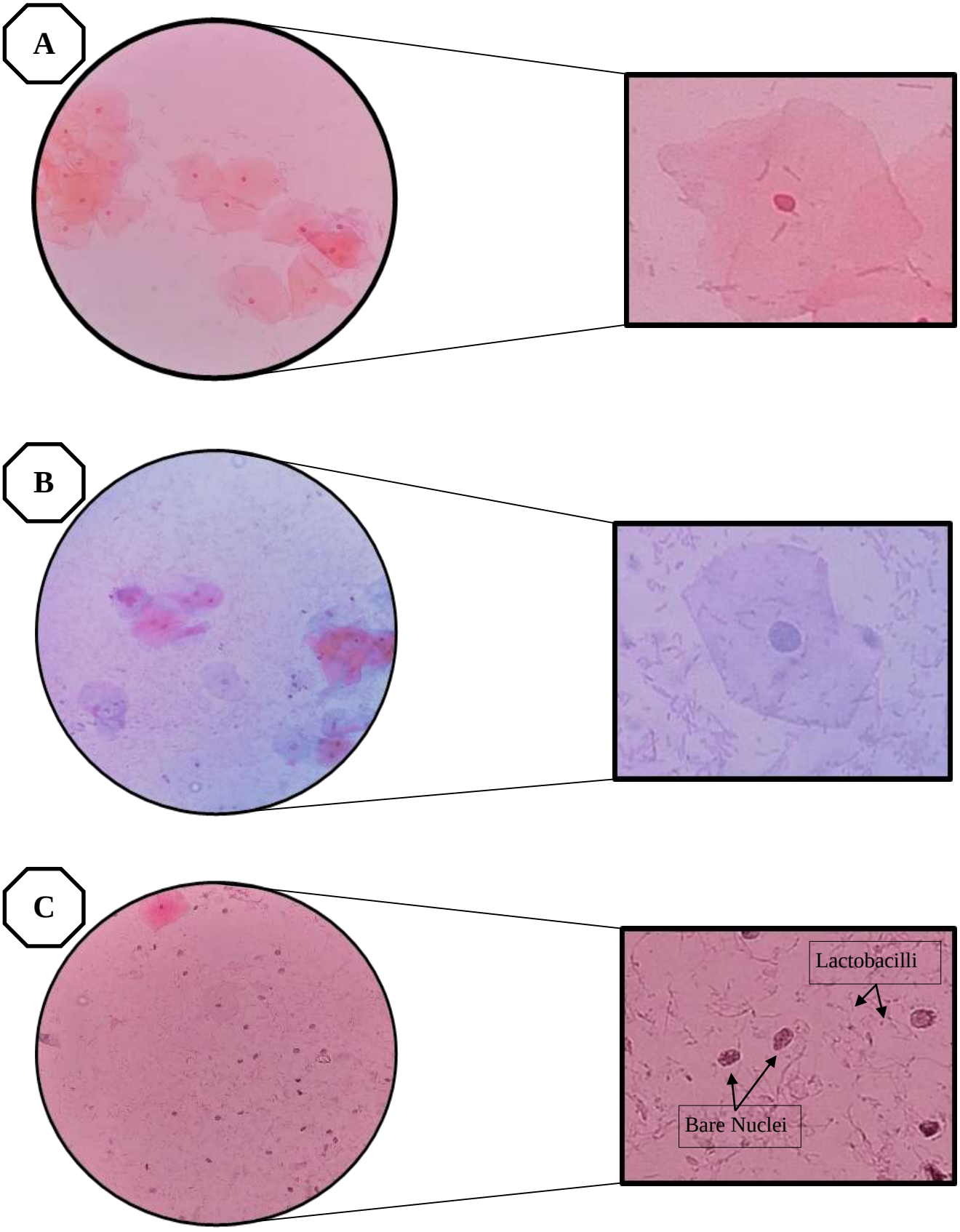


Figure 1: Microscopic observation of the cervical PAP smears (lactobacilli distribution). A, Apparently normal growth of lactobacilli (presence of normal growth of lactobacillus). B, Overgrowth of lactobacilli (presence of a large number of lactobacilli and normal epithelial cells). C, Cytolytic vaginosis (presence of a large number of lactobacilli, and naked nuclei of intermediate cell).

Table 1. Demographic and cytomorphological presentation of study participants

Characteristics	Apparently normal lactobacillus growth (n = 78)	lactobacillus overgrowth (n = 36)	Cytolytic vaginosis (n = 20)	Total (n = 134)
Age at presentation				
15 – 25 years	16 (11.9%)	3 (2.2%)	4 (3.0%)	23 (17.2%)
26 – 36 years	37 (27.6%)	17 (12.7%)	10 (7.5%)	64 (47.8%)
37 – 49 years	25 (18.7%)	16 (11.9%)	6 (4.5%)	47 (35.1%)
Residency				
Rural	46 (34.0%)	20 (14.9%)	12 (9.0%)	78 (58.2%)
Urban	32 (23.9%)	16 (11.9%)	8 (6.0%)	56 (41.8%)
Level of education				
Undergraduate	56 (41.8%)	27 (20.1%)	11 (8.2%)	94 (70.1%)
Graduate	19 (14.2%)	9 (6.7%)	9 (6.7%)	37 (27.6%)
Postgraduate	3 (2.2%)	0 (0.0%)	0 (0.0%)	3 (2.2%)

Discussion: A balanced microbiota shields healthy women against illnesses and premature birth. Among women who are of reproductive age, a small percentage may develop lactobacilli overgrowth leading to Cytolytic vaginosis (CV), which refers to vaginal intermediate epithelium destruction by lactobacilli or in combination with other bacteria. The prevalence and incidence of CV is unclear due to frequent misdiagnosis with candidiasis, as indicated by their lack of response to repeated antifungal medication (15).

In the present study, a total of 134 samples of cervical smears were evaluated. The result of the cytomorphological PAP smears indicated CV in 14.9% of smears out of 134 smears evaluated as satisfactory according to the 2014 Bethesda System for Reporting Cervical Cytology. Lower frequencies were reported by Cerikcioglu and Beksac (19), who described that CV was identified in 7.1% of 210 women with vaginal signs or symptoms indicative of vulvovaginal candidiasis (VVC). Only 3.9% of those in a different study's sample of 1,152 people with vulvovaginal symptoms had CV (20). Contrarily, Puri (21) reported a relevant CV prevalence of 16.3% in 190 women who underwent a year of outpatient gynecological care and had cervical smears that showed signs of inflammation. Higher incidences were observed in other studies, such as Yang *et al* (22) finding that 143 (26.7%) women were diagnosed with CV and had vaginal discharge.

The most common complaint among women of reproductive age is vaginal discharge, which is a clinical manifestation of cytolytic vaginosis(23). 60% of participants with CV in the current study experienced vaginal discharge, while all other CV subjects reported other problems or none at all. Indicating that a wide range of ineffective antifungal and antibacterial medications would be used to treat a great majority of women with CV (i.e., 10% of all individuals with vaginal discharge in the total group). These treatments have the potential to change the pH balance in the vagina, leading to an excessive proliferation of lactobacilli

(24).

Numerous factors can affect the risk factors of vaginal infections. In contrast to non-tropical regions, the hot climate and its impact on specific hygiene practices may have an impact on instances in Sudan. Sociodemographic traits, other clinical conditions, inadequate access to healthcare, inadequate health education, and a lack of attention to other prevalent vaginal diseases like bacterial vaginosis and Sexually transmitted diseases (STDs) are also contributing factors (25,26). Finding the cause of any vaginal discharge is crucial since it accounts for a significant portion of gynecological consultations, which in turn contributes significantly to health care costs.

Cytolytic vaginosis (CV) is a little-understood gynaecological condition. The cervicovaginal microbiome encompasses a wide variety of microorganisms found in the cervix, such as bacteria, viruses, fungi, and other microbes. Conversely, women who have a dominant presence of *Lactobacillus* species (except for L. iners-dominant) can facilitate the clearance of human papillomavirus (HPV) and prevent cervical lesions (27). Several studies propose key factors in the diagnosing of CV, which include the absence of *Trichomonas* spp., *Gardnerella vaginalis*, *Atopobium vaginae*, *Megasphaera* spp., *Sneathia* spp., and *Prevotella* spp., as well as an increase in Lactobacillus spp, (19,20,28). This correlates with a decrease in the number of Lactobacilli, when other pathogenic organisms were identified in our previous study (29).

The prevalence of cervical cancer in sub-Saharan Africa was supposed to partially attribute to increased cervical inflammation resulting from higher likelihood of cervical infection and/or microbial dysbiosis. According to cervicovaginal microbiome reports, major health concerns like human immunodeficiency virus (HIV) and human papillomavirus (HPV) alter the microenvironment of the cervical epithelium, which in turn puts some selective pressure on the bacterial populations that live there (18). Therefore, it is important to carefully consider the intricate and dynamic interactions in the vaginal microbiota as well as the crucial elements maintaining balance. Cervical disorders can be caused by a number of different factors, any of which that significantly affect the vaginal microbiota and upset this equilibrium.

To improve the prevention, diagnosis, and treatment of serious health concerns, it is imperative to have a better understanding of the particular elements specific to the region that cause cervical disorders. CV development at the moment.

Conclusion: The study highlights the importance of accurately diagnosing vaginal discharge, with a specific focus on considering Cytolytic Vaginosis (CV) as a potential cause. CV is less prevalent than candidiasis or bacterial vaginosis (BV), yet it might be confused with both. Women experiencing CV constitute significant portion of gynecological problems affecting female's health. CV is an important player in cervical infection or microbial dysbiosis. Women with vaginal discharges should undergo a routine cervical screening examinations.

Acknowledgments: We would like to express our thanks to the German Academic Exchange Service (DAAD) and Ministry of Higher Education in Sudan for funding this study. Also we acknowledge

the staff of obstetrics and gynecology in Wad Medani Obstetrics and Gynecology Teaching Hospital and Gezira Center for Fertility and Infertility Researches. Our thanks are extended to the staff of histopathology and cytopathology laboratory in Faculty of Medical Laboratory Sciences, University of Gezira for their technical assistance.

Conflict of interest: Authors have nothing to declare.

References:

1. Lash AF, Kaplan B. A Study of Döderlein's Vaginal Bacillus. J Infect Dis. 1926 Apr 1;38(4):333–40.

2. Thomas S. Döderlein's Bacillus: Lactobacillus Acidophilus.J Infect Dis. 1928 Sep 1; 43(3):218–2

3. Hammes WP, Vogel RF. The genus Lactobacillus. In: Wood BJB, Holzapfel WH, editors. The Genera of Lactic Acid Bacteria [Internet]. Boston, MA: Springer US; 1995 [cited 2023 Mar 17]. p. 19–54. Available from: http://link.springer.com/10.1007/978-1-4615-5817-0_3

4. Avall-Jääskeläinen S, Palva A. Lactobacillus surface layers and their applications. FEMS Microbiol Rev. 2005 Aug;29(3):511–29.

5. Kc JH, Ke H, M G, Ta T, Pm S. Surface-layer protein extracts from Lactobacillus helveticus inhibit enterohaemorrhagic Escherichia coli O157:H7 adhesion to epithelial cells. Cell Microbiol. 2007 Feb [cited 2023 Mar 18];9(2). https://pubmed.ncbi.nlm.nih.gov/16925785

6. Granato D, Perotti F, Masserey I, Rouvet M, Golliard M, Servin A, et al. Cell surface-associated lipoteichoic acid acts as an adhesion factor for attachment of Lactobacillus johnsonii La1 to human enterocyte-like Caco-2 cells. Appl Environ Microbiol. 1999 Mar;65(3):1071–7.

7. Aroutcheva A, Gariti D, Simon M, Shott S, Faro J, Simoes JA, et al. Defense factors of vaginal lactobacilli. Am J Obstet Gynecol. 2001 Aug;185(2):375–9.

8. Valenti P, Rosa L, Capobianco D, Lepanto MS, Schiavi E, Cutone A, et al. Role of Lactobacilli and Lactoferrin in the Mucosal Cervicovaginal Defense. Front Immunol. 2018 Mar 1;9:376.

9. Corbishley CM. Microbial flora of the vagina and cervix. J Clin Pathol. 1977 Aug 1;30(8):745–8.

10. Moberg P, Eneroth P, Harlin J, Ljung-Wadström A, Nord CE. Cervical bacterial flora in infertile and pregnant women. Med Microbiol Immunol (Berl). 1978 Oct 20;165(3):139–45.

11. Vielfort K, Sjölinder H, Roos S, Jonsson H, Aro H. Adherence of clinically isolated lactobacilli to human cervical cells in competition with Neisseria gonorrhoeae. Microbes Infect. 2008 Oct;10(12–13):1325–34.

12. Audirac-Chalifour A, Torres-Poveda K,Bahena -Román M, Téllez-Sosa J, Martínez-Barnetche J, Cortina-Ceballos B, et al. Cervical Microbiome and Cytokine Profile at Various Stages of Cervical Cancer: A Pilot Study. PLoS ONE. 2016 Apr 26;11(4):e0153274.

13. Wu S, Ding X, Kong Y, Acharya S, Wu H, Huang C, et al. The feature of cervical microbiota associated with the progression of cervical cancer among reproductive females. Gynecol Oncol. 2021 Nov;163(2):348–57.

14. Zheng JJ, Miao JR, Wu Q, Yu CX, Mu L, Song JH. Correlation between HPV-negative cervical

lesions and cervical microenvironment. Taiwan J Obstet Gynecol. 2020 Nov;59(6):855–61.

15. Cibley LJ, Cibley LJ. Cytolytic vaginosis. Am J Obstet Gynecol. 1991 Oct 1;165(4):1245–9.

16. Yang S, Liu Y, Wang J, Li S, Zhang Y. Variation of the Vaginal Lactobacillus Microbiome in Cytolytic Vaginosis. J Low Genit Tract Dis. 2020 Oct;24(4):417–20.

17. Voytik M, Nyirjesy P. Cytolytic Vaginosis: a Critical Appraisal of a Controversial Condition. Curr Infect Dis Rep. 2020 Oct;22(10):26.

18. Klein C, Kahesa C, Mwaiselage J, West JT, Wood C, Angeletti PC. How the Cervical Microbiota Contributes to Cervical Cancer Risk in Sub-Saharan Africa. Front Cell Infect Microbiol. 2020 Feb 12;10:23.

19. Cerikcioglu N, Beksac MS. Cytolytic vaginosis: misdiagnosed as candidal vaginitis. Infect Dis Obstet Gynecol. 2004;12(1):13–6.

20. Batashki I, Markova D, Milchev N. [Frequency of cytolytic vaginosis examination of 1152 patients]. Akush Ginekol (Sofiia). 2009;48(5):15–6.

21. Puri S. Cytolytic vaginosis: A common yet underdiagnosed entity. Ann Trop Pathol. 2020;11 (1):29.

22. Yang S, Zhang Y, Liu Y, Wang J, Chen S, Li S. Clinical Significance and Characteristic Clinical Differences of Cytolytic Vaginosis in Recurrent Vulvovaginitis. Gynecol Obstet Invest. 2017;82 (2) :137–43.

23. Majigo MV, Kashindye P, Mtulo Z, Joachim A. Bacterial vaginosis, the leading cause of genital discharge among women presenting with vaginal infection in Dar es Salaam, Tanzania. Afr Health Sci. 2021 Jun;21(2):531–7.

24. Lin YP, Chen WC, Cheng CM, Shen CJ. Vaginal pH Value for Clinical Diagnosis and Treatment of Common Vaginitis. Diagnostics. 2021 Oct 27;11(11):1996.

25. Husain NE, Burhan A, Ahmed IAI, Mohammed SI, Hammad N. Women's cancers in Sudan with a focus on cervical cancer: turmoil, geopolitics and opportunities.2022 [cited 2023 Aug 9].http://ecancer.org/en/journal/article/1433-

26. Elsheikh M, Altom E, Idris A, Osman M, Yousif Elemam I, Alobaid AE. The role of cytological smears in identification of bacterial infection and abnormalities in vaginal epithelium cells among apparently health married Sudanese Women. Microbes Infect Dis. 2021 Oct 19;0(0):0

27. Curty G, de Carvalho PS, Soares MA. The Role of the Cervicovaginal Microbiome on the Genesis and as a Biomarker of Premalignant Cervical Intraepithelial Neoplasia and Invasive Cervical Cancer. Int J Mol Sci. 2019 Dec 28;21(1):222.

28. Hu Z, Zhou W, Mu L, Kuang L, Su M, Jiang Y. Identification of Cytolytic Vaginosis Versus Vulvo vaginal Candidiasis. J Low Genit Tract Dis. 2015 Apr;19(2):152–5.

29. Osman EEE, Abdelgalil RSA, Elbashir MSM, Elwasila YE, Mohamed ME, Miskeen E. Identification of cervico-vaginal pathogenic microorganisms in pap smear among reproductive women, Wad MedanI, Gezira State, Sudan, International Journal of Science Academic Resear ch 2023 July; 04(07): 5955-5958.

Original Article Sudan Medical Laboratory Journal

Assessment of Procalcitonin (PCT) Level Among Type-2 Diabetic Mellitus with septic foot

Mohammad A S[1], Alradia M A[1], Muram M A1, Amaar M I[2]. Abdelsamee Elobied Mohamed Elamin[3], Ahmed Mahdi Fadallah[3]

[1]Department of clinical chemistry, faculty of medical laboratory sciences, Sudan international university -Sudan
[2]Department of biochemistry, faculty of sciences and technology, Neelen University - Sudan.
[3]Department of clinical chemistry, faculty of medical laboratory sciences, Omdurman Islamic University –Sudan

Abstract

Introduction: Procalcitonin (PCT) is marker of inflammatory response, stimulated by bacteria products endotoxins and cytokines [IL.1, IL.2, IL.6 and TNF alpha].

Aim: to assess PCT level and to investigate diagnostic value as an early marker for septic foot among type 2 DM.

Material and Methods: In cross-sectional hospital based study (n 40) septic food patients (age ranged from 41 to 78 years old) and (n 40) DM without septic foot as control group were enrolled. Specimen were collected from Zeenam Specialized center, Khartoum State during the period from June to July 2017. Serum PCT levels were measured using ICROMA® instrument.

Results: Analyses of frequency showed that, septic food was common in female 58% than male 42%, while the majority are obese 70% followed by 13% overweight and 17% normal weight. Moreover poor control septic food patients account 1.5:1 fold. Comparison revealed significant increase of PCT in diabetic septic foot when compared with control (p-value 0.000). Dotblot regression showed PCT correlate positively with age R=0.153 P=0347, while correlated with HbA1c R=0.368, P=0.020. Moreover, inverse correlation was observed between PCT, duration of disease and BMI (R=0.413, P=0.008 and R=0.458, P=0.003) respectively.

Conclusions: The data that, septic food is common in obese DM Sudanese female. PCT is significantly higher in DM septic food patients. Thus could be useful an early diagnostic marker for septic food.

Key words: PCT, septic foot, type-2 DM, obese, Sudan.
Corresponding author: Mohammad A S

Introduction: Diabetes mellitus is a defect on carbohydrate metabolism some patients may experience acute life threatening hyperglycemic episodes such as sepsis and septic shock (1). Prevalence of diabetic mellitus is unknown because of missing non-diagnosed subject, but the diagnosed cases according to world health organization (WHO) in 2017 are 422 million adult. Diabetes are also at risk of developing wounds and sores that do not heal well, they are at high risk of developing infection. Moreover, again because of the diabetes, the infections can get severe quickly. When infection overwhelms the body, the body can respond by developing sepsis and going into septic shock. Sometimes called blood poisoning, sepsis is the body's often-deadly response to infection or injury. Sepsis kills and disables millions and requires early suspicion and rapid treatment for survival. Sepsis is a life-threatening condition that arises when the body's response to infection injures its own tissues and organs (2). It is from the micro vascular complications, the loss of a limb or foot is

he most feared complications of diabetes and still, the foot problems is neglecting and remain one of the commonest reasons for diabetic patients to hospitalize. Diabetic foot ulcers are common in diabetic patients has incidence rate 25% of patient with diabetic became infected. These minor routine lesions can be the harbinger of infections and diabetic foot ulcers and eventually lead to complications. Diabetic foot ulcers precede almost 85% of amputations, (3).

Procalcitonin (PCT) is acute phase reactant that stimulated by bacteria endotoxins and cytokines [IL.1, IL.2, IL.6 and TNF alpha, PCT 116 amino acid, produce by C. cell of thyroid gland. Where PCT can be produced by several other cell types from wide range of organs in response to inflammation. The level of PCT in the blood stream of healthy individual is blow the limit of detection (0.01ng/l) of clinical assays (4). PCT was raised in response to proinflammtory stimulus especially of bacterial origin. PCT has half-life 25 to 30 hours and increase in plasma 3 to 6 hours of stimulus (5).situation in Sudan, aim of this study.

Materials and Methods: This is analytical cross-sectional hospital based study carried out in Khartoum state [Zeenam specialized center] during the period from June to July 2017. Eighty DM type 2 patients were enrolled in this study, (n 40) DM with septic foot as case and (n 40) DM without septic foot as control group.

Ethical Consideration: Ethical clearance approved from local Faculty committee of Sudan international university. Verbal inform consent was obtained from each participant before enrollment in the study.

Sampling collection and processing:

Venipuncture (3 ml) was collected under septic conditions from each participants. Serum has obtained after centrifugation at 3000-4000 RPM for 10 min, then serum separated in new containers and stored at –20^{o}C until analyzed. Serum PCT level measured by using ICROMA® instrument. In addition, HbA1c measured by using MISBA-i2 instrument.

Data analysis: Data was analyzed using SPSS version 21. Mean±SD of all the variables was determined. Independent *t*-test was applied to compare the significance of difference of parameters between two groups. Pearson's correlation coefficient was determined to evaluate correlation between different parameters *p*-value ≤ 0.05 was considered as significant.

Results: Distribution of the gender among the case group shows that female 1.4 and male 1 fold, (Figure 1).

Frequency of Wight was 70% were obese, 13% over weight and 17% are normal, (Figure 2).

The result show that poor control was 60% and good control 40% 1.6 to 1 fold of control, (Figure 4).

The result of independent T-test show that, the mean of PCT levels significantly increased in septic foot (1.49±0.96ng/ml) as compared to control (0.18±0.11ng/ml) with p-value 0.000, presented in (Table1).

Correlation showed that, the HA1c levels in septic foot was correlated with PCT levels (R=0.368, P=0.020), (figure 4).

PCT levels was inversely correlated with duration of the septic foot (R=0.413.P=0.008), (Figure 5).

PCT level was inversely correlated with BMI (R=0.458, P=0.003) (Figure 3.6). In addition, positively correlated with age (R=0.153 P=0347) (Table 3.2).

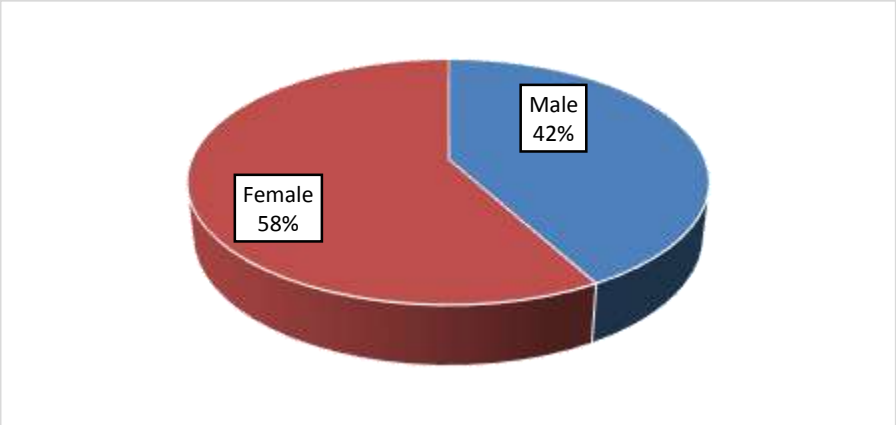


Figure1: Shows the gender distribution among case group.

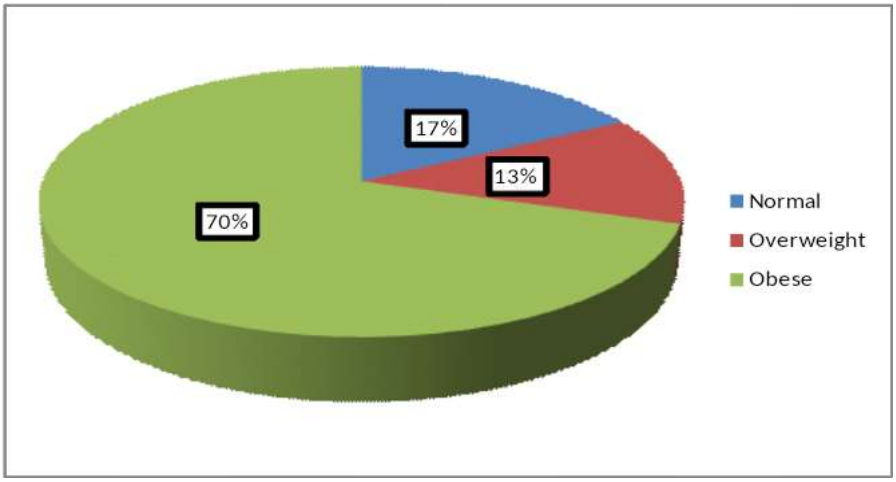


Figure2: Shows the frequency of the septic foot among BMI.

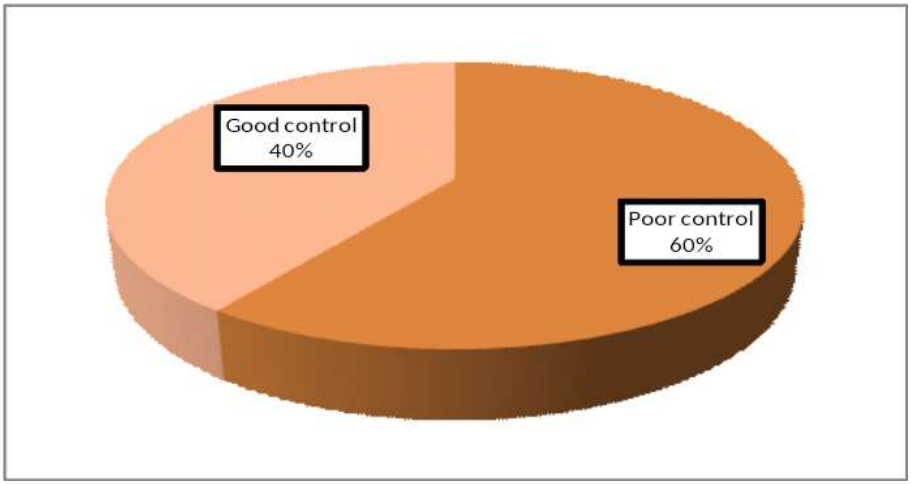


Figure3: Shows the frequency of diabetic good control and poor control.

Table 1: Mean concentration comparison of PCT levels among the study groups:

Parameters	Case (Mean ±SD)	Control (Mean ±SD)	*P-value*
PCT	1.49±0.96	0.18±0.11	0.000
HBA1c (%)	8.03±1.45	8.99±1.63	0.031
BMI	34.6±7.20	34.6±7.57	0.988
Age	58.8±10.2	55.8±11.1	0.215

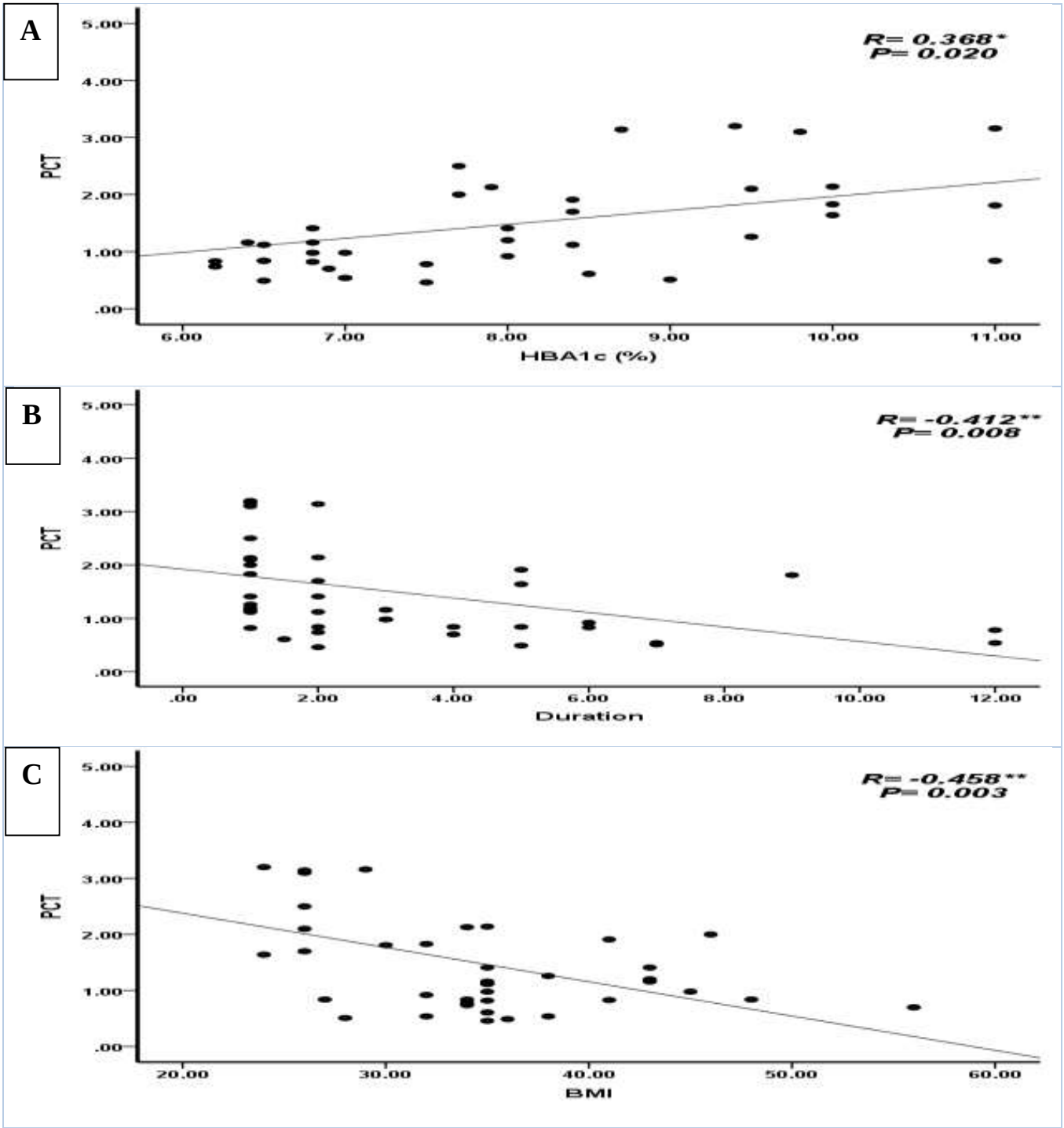


Figure 4. Dotblot regression showed A: correlation between PCT and HbA1c, B: PCT and duration and C: PCT and BMI. R= positive or negative correlation, P= strength of correlation.

Discussion: Diabetes mellitus is diseases that affect glucose metabolism that can cause serious complications, such as septic foot. Previously researchers investigate the association of PCT and DM septic foot as inflammatory marker.

In fact that, age and sex have globally identified as risk factors for diabetic mellitus (6, 7). Therefore, the present study revealed that, the percentage of diabetes septic foot is more common in female 58% than male 42%. Therefore, in agreement with and that previous finding that, there are more women with diabetic than male (6, 7), thus justified by men were more physically active than women were, and this probably could have enhanced the improved insulin sensitivity in men than women of the same age group advantage (8).

Frequency analyses also found that, more of our population were obese 70% followed by 13% over weight and 17% normal weight. Indeed obesity increase risk to develop DM that fat is deposit on the cell membrane that affect receptor sensitivity to insulin (1). Therefore obese DM patient susceptible to septic more than non-obese DM. In addition to that, 1.5:1 fold of our population was poorly control 60% and 40% was good control. Since concurrent with previous state that, poor control are more susceptible to devolved diabetic septic foot (9).

Independent t-test analyses provide experimental evidence that, mean PCT level was significantly increased in DM septic foot patients when compared with control p= 0.000. This finding in agreement with study noted that, PCT increase in septic foot diabetic disease (10). In fact that, PCT level is marker of bacterial infection in septic foot ulcer, due to inflammatory response (11). Indeed the diagnosis of sepsis is challenge thus assessment is unreliable because many culture sample do not yield microorganisms in these patients (10).

Interestingly our results revealed positive correlation between PCT and HbA1c R= 0.368, P= 0.020), which indicate that, poor control are more vulnerable to septic foot. Moreover dot blot regression showed that, PCT levels inversely correlated with duration of sepsis R= 0.413, P=0.008. Researchers reported that, PCT is acute phase hormone that appear in early infection in response of various cytokines such as IL-1, IL-6, and TNF, and it became detectable within 3-4 horse and peak 6-24 hours (12,13). In addition PCT level was inversely correlated with MBI R= 0.458, P= 0.003.

Conclusions: The study concludes that, septic food is common in obese DM Sudanese female. PCT is significantly higher in DM septic food patients and positively correlate with HbA1c. Thus could be useful predictor and early diagnostic marker for septic food.

References:

1. Tietz textbook of clinical and molecular diagnostics. Carbohydrates, 1986, 25, 837-864.

2. Meisner M, T Schakowsky K, palmers T, Schmid t J.] Comparison of procalcitonin and C-reactive protein plasma concentration at deferent SOFA source during the course of sepsis and MODS .1994; 3 [1] 45-50.

3. Fassil W, Gemechu M D. Diabetic foot infections. American family physician, 2013; 88(3):177-184.

4. Akpan U P, John OI, Daniel E N. gender and age specific prevalence and associated risk factor of

type 2 diabetic mellitus in uyo metropolis ,2017; 14[1]:17-24.

5. Dandona p N, Wilson M F. Procalcetetonin increase after endotoxin in normal subject. Journal of clinical endocrinology and metaboli.sm, 1998; 79[6]:1605 -1608.

6. Mantfh M T, June M j .acute phase reactant in infection .open forum infection disease, 2015; 7[2] 1-5.

7. Agbogli H K, Annan R, Agyeman M Mensah E E. Prevalence and Risk Factors of Diabetes Mellitus Among the Inhabitants of Kumasi Metropolis , Journal of Clinical Diabetes Research , 2017;1[1] 1-5.

8. Fundamental of clinical chemistry. Carbohydrate, 2008; 7, 373-381.

9. William J J, Jeffcott M R, Prof K G.Diabtic foot ulcer. ScenceDirect, 2003; 361[9386]:1545-1551.

10. Charalampos P, Jean L V .sepsis biomarker .critical care biomed center, 2012; 31[37],1-19.

11. Nematollah J J, Mahdi S F, Amin S M. can procalctonin be an accurate diagnostic marker for the classification of diabetic foot ulcer .international journal of endocrinology and metabolism,2013;12[1]:e13376.

12. Karzai W M, Oberhoffer M J, Meier H A, anew indicator of systematic response to sever infection, critical care biomed center, 2016; 3 [1] 45-55.

13. Lady H J, S S K .managing hyperglycemia in diabetic foot .diabetic management, 2016; 4 [3]1-5.