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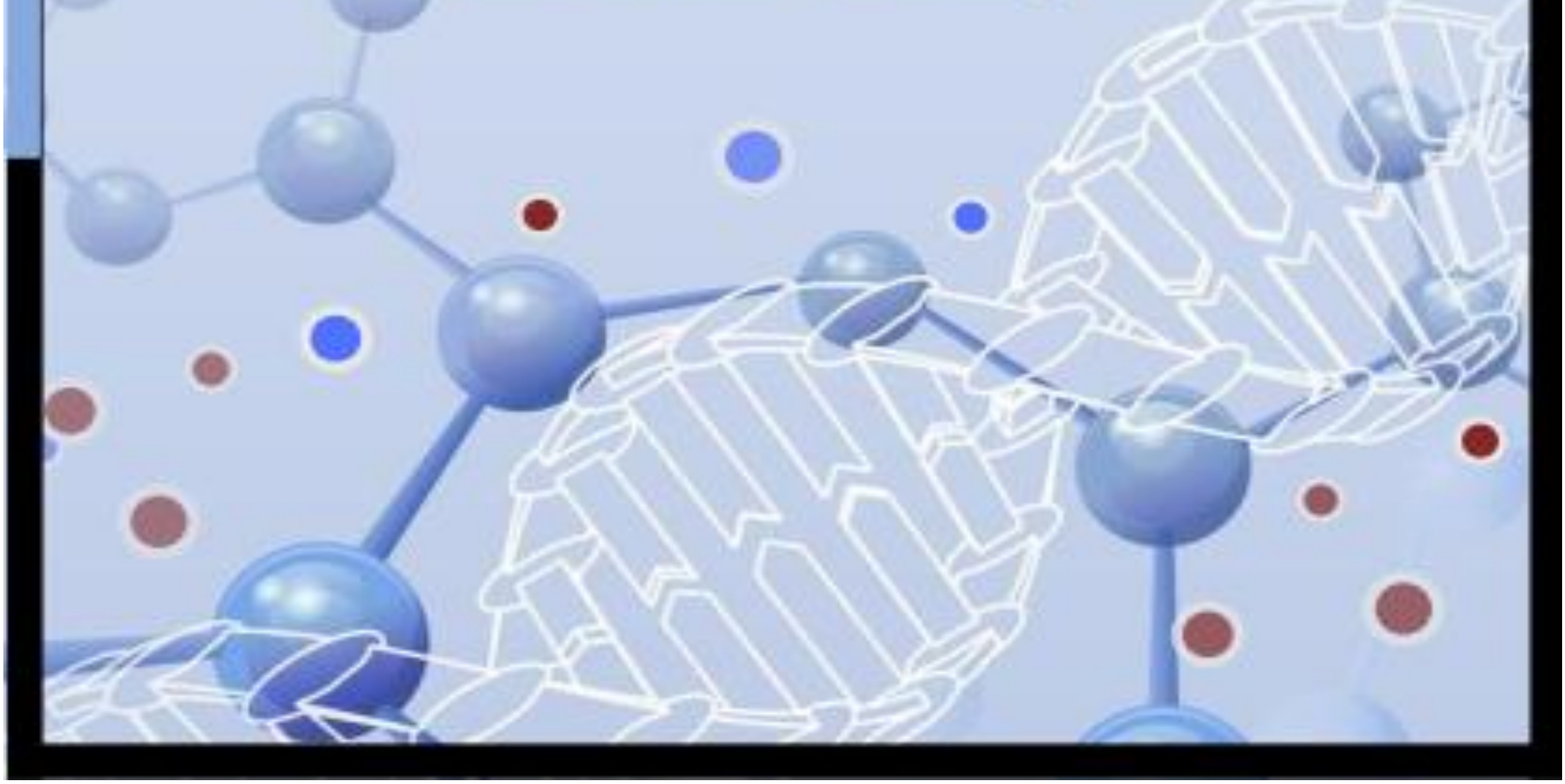

SUDAN MEDICAL LABORATORY JOURNAL

AN OPEN ACCESS PEER REVIEWED SCIENTIFIC JOURNAL

ISSUED BY

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FACULTY OF MEDICAL LABORATORY SCIENCES

SMLJ

﴿وَلَقَدْ آتَيْنَا دَاوُودَ وَسُلَيْمَانَ عِلْماً
وَقَالَا الْحَمْدُ لِلَّهِ الَّذِي
فَضَّلَنَا عَلَى كَثِيرٍ مِّنْ عِبَادِهِ الْمُؤْمِنِينَ﴾
النمل15

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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;

The COVID-19 pandemic has once again emphasized our need to exchange research and knowledge, and as we all know, medical laboratory sciences played a major role during this pandemic. Let us all strive to develop our tools, publish our research, and provide our magazine with everything new, not only in the field of medical laboratories, but in all aspects of the medical field. You will notice that this issue contains original research and a case study.

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.

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And till we hear from you, we remain,

Yours

Dr. Abdulazeem Abdulsalam Ibrahim Alkhidir

Executive Editor

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.

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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*).

Detection of *Candida* spp.in Diabetic Septic Foot in Khartoum State Diabetic Center

Abdulazeem Abdulsalam Ibrahim Alkhidir[1, 2], Amel Abdelazeem[1], Ekhlas Naaman[1], Tagwa NoorEldein[1], Rayyan Ahmed[1], Misoon Abdallah[1] Yousra KamalEldein[1] and Manhal Ahmed Hamza[1]

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2-Omdurman Ahlia University, Department of Medical Microbiology

Abstract

Introduction: Diabetes mellitus is a chronic disorder affects immune system beside other systems and causing premature death or complications within time causing patients suffering. Because of immune system involvement, fungal infection is one of the prominent infections especially if patients presented with wound.

Material and method: 50 DM patients, 20 females and 30 males were enrolled. Double wound swabs were collected from each patient. 1st one for direct microscopy (smear stained with 70% methylene blue). The 2nd one was used for culture in 3 test tubes containing Sabouraud Dextrose Agar, 2 test tubes were incubated aerobically (the 2 tubes used for confirmation).

Result: *Candida* spp. found in 9 (18%) patients. *Candida* spp. found among elder patients.

Conclusion: In this study we were able to isolate *Candida* spp. from diabetic foot ulcer patients.

Key word: Diabetes Mellitus, Candida.

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Introduction

In Sudan, diabetes is now common and major health problem. In North Sudan, in urban areas the evaluated prevalence of diabetes was thought to be around 19% in comparison with 2.5% in rural regions (1, 2). Recently, in Africa the prevalence of diabetes is predictable to increase up to 28 million cases by 2030 in comparison with 14 million in 2011 (3,4). According to the WHO and the IDF expected that the number of adults with diabetes in the world will rise near 629 million by the year 2045 (5, 6).

Diabetes mellitus (DM) is a long-lasting, non-communicable condition that raises blood sugar levels because of insufficient or ineffective insulin secretion (7). In addition, it is quickly emerging as one of the biggest modern threats to public health. (8, 9), Up to 15% of diabetic patients worldwide currently experience diabetic foot ulcers (DFUs), which are still very common among those with the disease (10, 11). The majority of microorganism-causing infections in DFU are bacteria, then few of filamentous fungi and low pathogenic yeast. (12, 13). While, *Candida* spp. is the most commonly isolated yeast from these ulcers with a percentage of 5%–21%. (14). It has previously been studied that the relationship between diabetes and candidiasis (15). due to the increased susceptibility of diabetic patients to fungal infections compared to those non diabetic patients (16, 17). Recent evidence suggests that diabetic persons are more susceptible to periodontal disease , tuberculosis (TB), lung infection by *Legionella pneumoniae*, 'mucormycosis' caused by the *Mucoraceae* family of fungi (18, 19).

It has been described that *Candida spp.* compared to healthy subjects, is more common in patients with type 1 and type 2 DM with poor glycemic control.__*Candida* species are the most frequent cause of fungal infections, which can result in a variety of fatal diseases (20).

(Raiesi.,*et al*) (21) in their study reported that (24.5%) of diabetic patients were affected with fungal infections. In addition, the prevalence of fungus infection was 19.1% in patients with diabetic foot ulcer and 28% of patients with skin and nail lesions. *Candida albicans* and *Aspergillus flavus* were the most common species isolated from thirty patients with fungal infection.

The aim of this study is to detect and estimate fungal infection among diabetic septic foot ulcer in diabetic centers in Khartoum state.

Materials and methods

Sample area and size: The study only performed among diabetic patients with foot ulcers. The study was carried out at Khartoum State diabetic care centers (Zinam hospital and Alarbaein hospital). 50 diabetic patients with foot ulcers, including 30 male and 20 female.

Collection of samples: Under hygienic conditions two sets of swab samples were collected from each patient, the 1st one for direct microscopy (smear stained with 0.1% methylene blue).

The 2nd one was used for culture in 3 test tubes containing Sabouraud Dextrose Agar, 2 test tubes were incubated aerobically (the 2 tubes used for confirmation).

The 3rd tube was incubated at 26^{0}C at room temperature.

Ethical clearance: Ethical approval of this study was taken by Research committee of Omdurman Islamic University.

Methodology

Wet preparation: Specimen was mix well, one drop (10) microliters of the specimen were removed by sterile pipette. One drop (10 µL) of the specimen were placed on a clean microscope slide with the patient's identification number. One drop (10 µl) of 10% Potassium hydroxide (KOH) were added directly to the drop of specimen on the slide. Coverslip was placed on the drops on the slide. Slide was placed on a bright field microscope, low power (10X) focus was used, and high dry power (40X) was scanned. Budding yeast or yeast with pseudohyphae was examine.

Culture preparation:

Sabouraud Dextrose Agar: Suspend 65 g of powder in1L of distilled water, sterilization in autoclave at 121◦c for 15 minutes, then cooled and 1ml chloramphenicol antibiotic (The antibiotic solution is prepared by dissolving 1g of powder in 5ml DW) was added.

Corn Meal Agar: Suspended 17.0 g of powder in 1L distilled water. Sterilization in autoclave at 121◦ C for 15 minutes

Cultivation of samples: Each samples were cultivated on Sabouraud dextrose agar and Corn meal agar. The culture tubes were incubated at 37°C for 48 h under aerobic conditions. Culture tubes with no growth were further incubated for 7 days before declaring them negative for Candida growth. Samples were stained to check out if they were alive or not, but they were dead as the pandemic of corona virus arrested all targets.

Isolation of fungi: Isolation was conducted by comparing morphology of the grown fungal colonies based on size, color, margins, elevation and spreading. The isolated fungi were first identified morphologically using a compound microscope.

Gram staining: Gram stain was done according to method described by Crickmore. *et al.* **(22)** Organisms colored with red were classified as Gram-negative organisms and violet colored were classified as Gram-positive organisms.

Statistical analysis plan: The numerical datawere collected and analyzed by means of statistical package of social science program (**SPSS**) version 26. The data analysis summarized the results in statistical form, providing the means and the ranges of the data collected.

Results: In this study, 50 diabetic patients with foot ulcers, 30 were male and 20 were female, 9 of the patients which was 18% of the study population had fungal infections which revealed to *Candida* .spp. and the rest were 41(82%) with no *Candida* .spp. as shown in Table 1 and Fig 1.

Microscopic examination revealed Gram positive yeast, round and oval yeast, some of them showed budding with purple color appearance. *Candida*. spp. showed oval and consisted of mother cells, daughter cells by using methylene blue. Cultural characteristic of *Candida* spp. on Sabouraud dextrose agar whitish to creamy color and smooth soft colonies, and on Corn meal agar it was clear two forms of chlamydo-spores and blasto-conidia.

Table 1: Types of fungal infections among DM patients

Type of fungal infection	Number	%
Infection with *Candida* spp.	9	18%
No infection with *Candida* spp.	41	82%
Total	50	100%

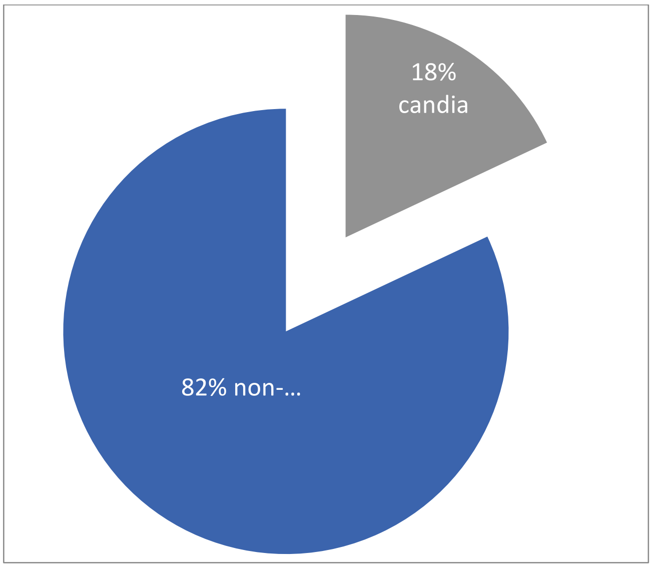

Figure 1: Types of fungal infections among DM patients

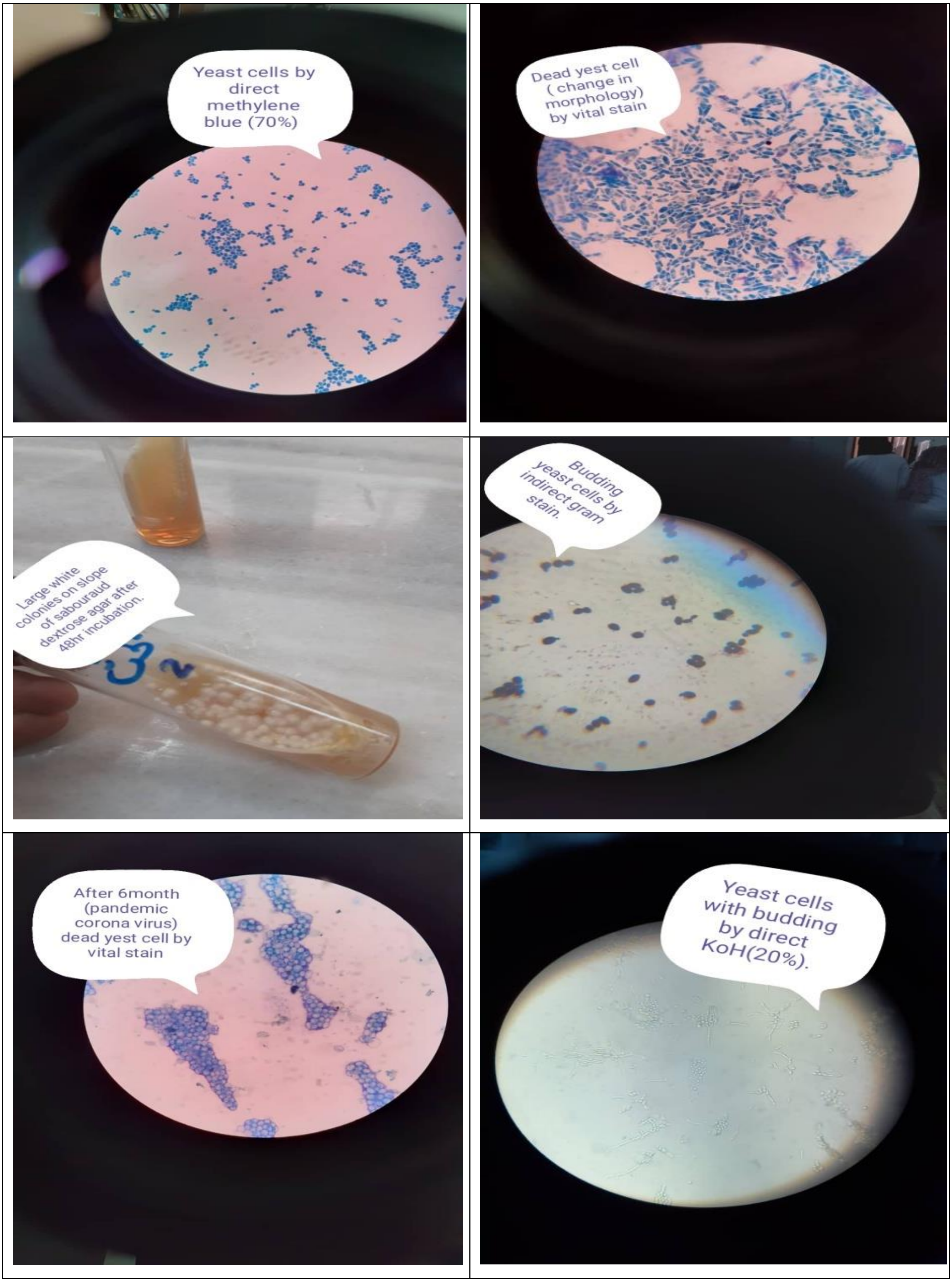

Figure 2: Wet preparation, Gram stain and Culture of samples

According to age, patients were classified into three group's first group from 29-50 years old 2 of them had shown *Candida* spp. infection. Second group 51-70 years old 4 of them shown *Candida* spp. Infection and the last group 71-90 years old 3 of them were recorded as *Candida*. spp. infection As shown in Fig 3.

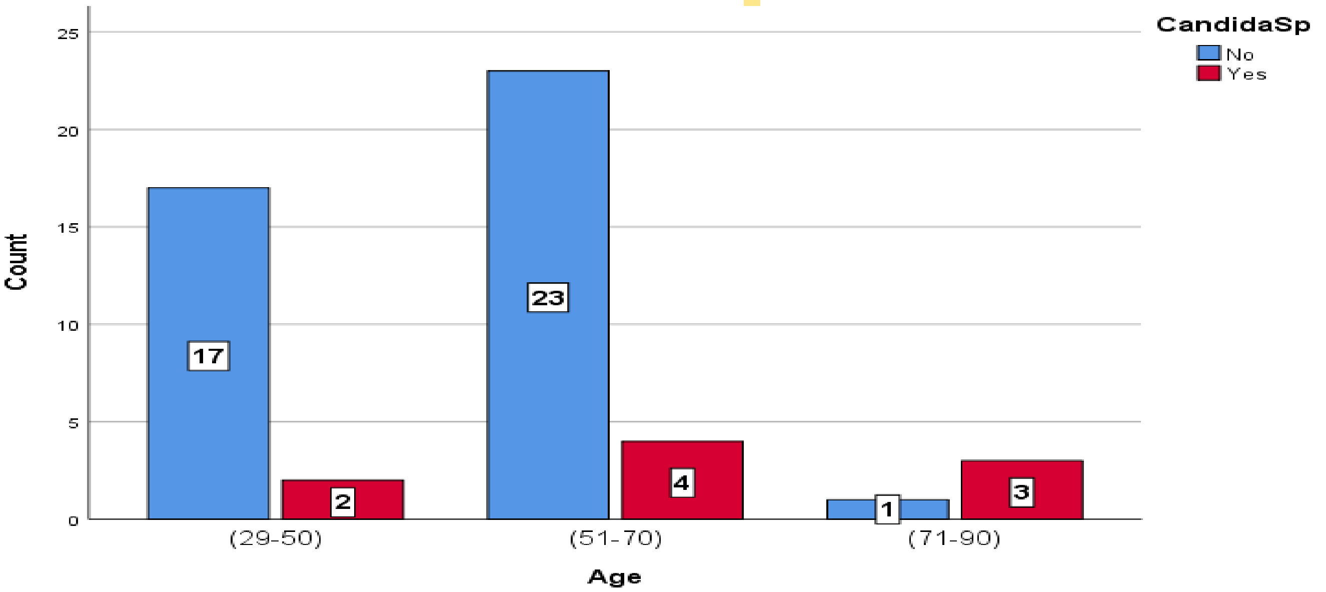

Figure 3: Percentage of infection with *candida spp* among age groups

Considering gender, 23 males presented with non-candida infection, 7 with *Candida*. spp, females 18 with non-*Candida* infection and 2 with *Candida*. Spp. as in Figure 4.

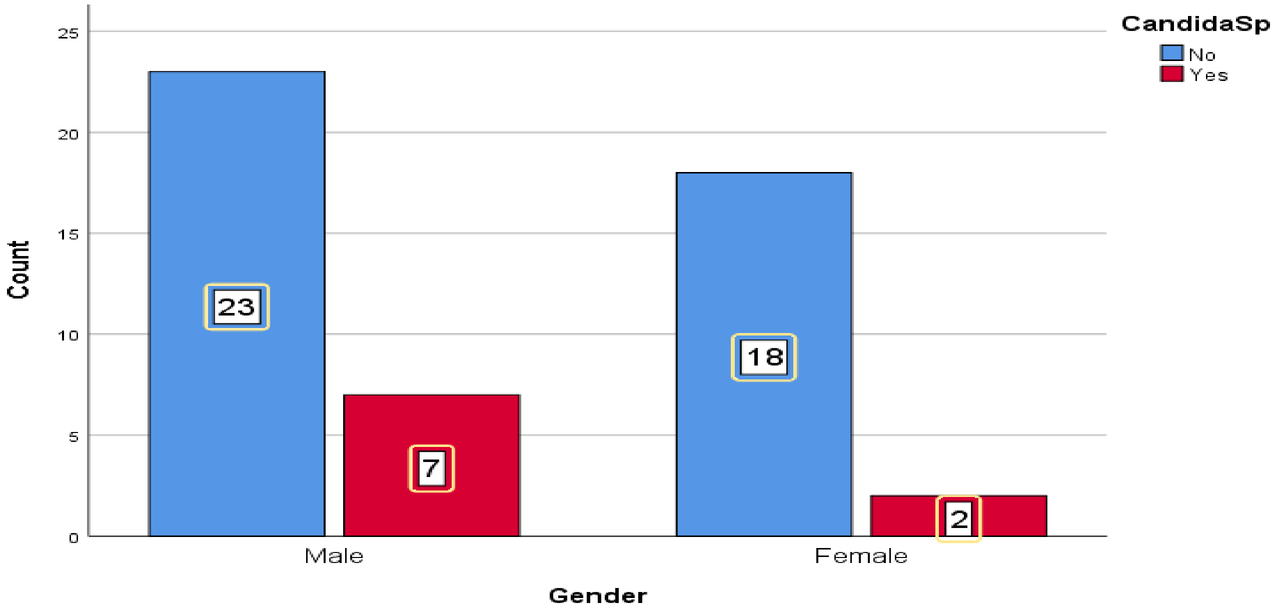

Figure 4: Gender distribution of fungal infection among DM patients

Pearson's correlation with age of diabetic patient's duration of wound infection among diabetic patients, it was negative correlation as in Figure 5

R value -.15

Figure 5: Correlation between age and duration of disease among DM patients

Discussion

We believe that microbiological profile of diabetic foot ulcer patients ranges from chronic bacterial infections to opportunistic fungal infections. Immuno-compromised patients are known to be susceptible for fungal infection and are a major public health concern worldwide

However, fungal infections in diabetic foot ulcers infections have not been studied extensively.

(DFU) diabetic foot ulcer, it is a chronic disease that affect skin and tissue damage in a diabetic patient, which is difficult to heal.

The fungi involved in diabetic foot ulcers are mainly *Candida* spp. Other species commonly isolated are *Trichophyton* spp. and *Epidermophyton Floccosum*.

It was clear that diabetic patients with fungal foot infection were correlated with age, sex and duration of diabetes Eckhard *et al.* (23). In the present study, the majority of patients were males 60% which is similar to that observed in other studies Eckhard., *et al* (23). and Sanniyasi., *et al* (24). Considering age and duration of wound infection, a negative correlation presented as R value was -0.15. The result of this study, indicated fungal infection was (18%) which is higher compared to Sanniyasi., *et al* (25) who recorded 16.2% in diabetic patients, but these percentages are higher than other study conducted by Mehra .,*et al* (26) with (11.43%) . In addition, a previous studies done by both Fata *et al* (27)and Bansal *et al*(14) Reported (52%) and (50%) respectively positivity *Candida* spp. in their cultures. Recently, very high prevalence of *Candida* spp. recorded by Abilash *et al* (28)(88.8%) in diabetic foot ulcer.

Unfortunately our further investigation to determine the Candida species not completed due to Corona virus shutdown.

In conclusion, the results of this study demonstrated that the majority of fungal infections that isolated from diabetic foot ulcer patients were *Candida*. spp.

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Original Article Sudan Medical Laboratory Journal

Vitamin D Receptors Gene Polymorphisms among Sudanese Patients with Prostate Cancer

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Abstract

Background: Prostate cancer has been most frequently diagnosed cancer in men in Sudan regarding to statistics data from the National Cancer Registry. Moreover, it was the second most prevalent cancer in men globally. BsmI, TaqI, FokI, and ApaI restriction enzymes are the most extensively investigated single nucleotide polymorphisms (SNP) in VDR, respectively, they are found in exon 2 (FokI), intron 8 (ApaI and BsmI) (TaqI).

Material and method: Study attempted to investigate the vitamin D receptor gene polymorphisms in Sudanese prostate cancer patients, this case control study included eight seven subjects they were split into two groups, cases group which included 42 prostate cancer patients who were identified by histology and control group which included 45 healthy individual as control. Data were collected by questionnaire and analyzed by statistical package for social sciences version (21) and SNP stat online web-based application program.

Results: Total of 87 individuals were included; 42 as case and 45 as control with age mean and median (71.78±8.04) and (70) respectively, rs731236, rs1544410 and *rs2228570* SNPs has significant association between prostate cancer risk when compared with control group, contrary to this finding *rs7975232* failed to find any associated with prostate cancer, furthermore all four SNP failed to find any associated with stage, Gleason grade, age, BMI, PSA level, and vitamin D levels.

Conclusion: The study concluded that polymorphisms of VDR gene have significant association with prostate cancer except ApaI SNP.

Key Words: VDR SNPs, prostate cancer, vitamin D receptor, Sudan.

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Introduction

Prostate Cancer is most frequently diagnosed cancer and is the second most prevalent cause of death among males (1). Its prevalence is highest in Africans, then Whites, then Mongolians, which may be attributed to genetic, social, and environmental variations (2). The active form of vitamin D (1,25-dihydroxyvitamin D3), which aids in controlling prostate development, is produced by prostate epithelial cells. A synthetic version of vitamin D3 called calcitriol has antiproliferative and prodifferentiation effects in prostate cancer. By attaching to the vitamin D receptor, 1,25-dihydroxyvitamin D3 is able to perform its intended function (VDR).To control the genes that produce vitamin D, the VDR forms a heterodimer, generally with the retinoid X receptor (3). The steroid hormones like testosterone and vitamin D which operate through the androgen receptor (AR) and VDR, respectively, on prostate cell division (4). Multiple nuclear receptors are expressed by prostate epithelial cells, which control cell division and proliferation in the prostate gland. Nuclear hormone receptor VDR controls gene transcription. The relevance of the VDR in prostate cancer is still debatable, despite evidence that its regulation is altered in prostate cancer (5). There was no link found between the frequency of any VDR genotype and prostate cancer in the Taiwanese (Asian) population (6). Other research, on the other hand, suggested that ApaI polymorphism confers vulnerability to sporadic disease (7, 8). A link between the VDR gene's in exon 2 and intron 8 SNPs (rs2228570 and rs1544410), and the risk of cancer and other diseases has been discovered (9 - 11). Only the rs2228570 variation in the VDR gene affects the structure of the protein (12). The identification of genetic variations connected to disease vulnerability, particularly the wide range of cancers, may be the key to advancing preventative medicine. In order to aid in early detection and therapy, polymorphisms in the VDR gene may be helpful in identifying those who are more likely to acquire disease. The relationship between vitamin D levels and VDR polymorphism and prostate cancer has not been previously investigated in Sudan. This observational case-control study aims to association the Vitamin D Receptors Gene Polymorphisms among Sudanese Patient with Prostate Cancer.

Material and Method

Study design

This case control based-hospital study conducted in the National Cancer Institute (NCI), Gezira University, Wad Medani, Sudan. Eight Seven subjects were included they were divided into two groups, cases group which included 42 prostate cancer patients who were identified by histology and control group which included 45 healthy individuals.

Sample collection by using venipuncture from a peripheral vein techniques, 5 mL of whole blood was collected into EDTA containing tubes (Becton Dickinson, USA) and stored at -20 °C until the DNA isolation procedure was performed. The G-spinTM Total DNA Extraction Mini Kit from iNtODEWORLD, Inc. USA was used for DNA extraction from blood samples.

CTPP Technique for mutation detection

In order to genotype a sizable number of samples quickly and affordably, PCR-CTPP (polymerase chain reaction using confronting two-pair primers) was developed. These techniques designed two-pair primers (four primers) into a standard PCR tube to amplify allele-specific DNA products, resulting in variable band sizes that allow differentiation between alleles.

ACTPP primers sequence and product size:

SNP rs:	Primer sequence	Product size
Fok1 (rs2228570)	AP570T: 5- CTGGCCGCCATTGCCTtCA – 3 CP570T: 5- CCAGGCAGCTGATTCCAAG -3 AP570C: 5- GCTTGCTGTTCTTACAGGtAC–3 CP570C: 5- TCACCTGAAGAAGCCTTTGC -3	240 bp (T) 169 bp (C) Common 390 bp
Bsm1 (rs1544410)	AP410A: 5- GCCACAGACAGGCCTaCA – 3 CP410A: 5- GTCAAGGGTCACTGCACATT -3 AP410G:5- AGCCTGAGTATTGGGAAcGC–3 CP410G: 5- CTGGGCAACCTGAAGGGAG -3	180bp (A) 221 bp (G) Common 368 bp
Taq1 (rs731236)	CPXFw: 5 - AGGTGCGCCCATGGAAGGA -3 AP236C: 5- CAGGACGCCGCGCTGCTC -3 AP236T: 5- CAGGACGCCGCGCTGCTC -3 CPXRev: 5 – TGGATAGGGGAGGTGGCAG -3	382 bp (C) 271 bp (T) common 617 bp
Apa1 (rs7975232)	CPXFw: 5 - AGGTGCGCCCATGGAAGGA -3 AP232A: 5- CAGGAGCTCTCAGCTGGTCA -3 AP232C: 5- GTGGGATTGAGCAGTGATGG -3 CPXRev: 5 – TGGATAGGGGAGGTGGCAG -3	192 bp (A) 464 bp (C) common 617 bp

PCR mix

The MAGSYBR qPCR Kit was supplied as a 2X concentrated used to amplification of DNA fragments from (APSLABS India A-8 Surya Terrace, Pratik Nagar, Yerwada, Maharashtra, Pune-411006.Three sets of primers had been provided by Macrogen Inc. (Korea). The PCR carried out with each set of primers on all samples in a 15µl reaction magnitude. Each reaction for Fok1(rs2228570), Taq1 (rs731236) and Apa1 (rs7975232) SNP consisted of 7µl of 2X MAGSYBR qPCR Master Mix (APSLAB, India), 1µl of AP for both alleles, 0.5µl of CP (F and R) and DNA template was 5µl, 6µl and 6µl of for Fok1, Taq1 and Apa1 respectively. For Bsm1(rs1544410) it was 7µl of 2X MAGSYBR qPCR Master Mix (APSLAB, India), 0.8µl of AP for both alleles, 0.4µl of CP (F and R), 5µl of DNA template and 0.6µl of Distil water.

PCR Conditions

For all four SNPs, there were 40 thermal cycles, and the PCR conditions were performing as the following: Taq1 (rs731236) and Apa1 (rs7975232) Denaturation, Annealing and Extension program was ($94^{\circ C}$ / 40 sec, $60^{\circ C}$/ 40 sec and $72^{\circ C}$ / 40 sec) respectively. Fok1 (rs2228570) program was ($94^{\circ C}$/1 min, $55^{\circ C}$/45 sec and $72^{\circ C}$/45 sec) for Denaturation, Annealing and Extension respectively. Bsm1 (rs1544410) Denaturation, Annealing and Extension program was ($95^{\circ C}$/ 40 sec, $58^{\circ C}$/30 sec and $72^{\circ C}$/ 40 sec).

Data collection and analysis

Well-designed questionnaires with all the necessary biographical and diagnostic information were used to collect the data. SPSS software program was used to analyze the data (Chi squire was used to calculated association). Number and percentage of patients or means and standard deviation range of data were used to summarize patient clinic and demographic variablesr. SNP stat online web-based application program was used for analysis of single SNPs: multiple inheritance models (co-dominant, dominant, recessive, over-dominant and log-additive).

Ethical approval

The purpose of the study was explained to each participant, and they all gave their agreement to participate. The National Cancer Institute at the University of Gezira and the Gezira State Health Ministry's ethical committee both gave their approval to this study.

Results

Table (1): Comparison of Fok1 and Bsm1SNPs between case and control group

Fok1 (Total =87)					
Model	**Genotype**	**Cases (42)**	**Controls (45)**	**OR (95% CI)**	**P-value**
Co-dominant	**C/C**	**12 (28.6%)**	**32 (71.1%)**	**1.00**	**0.000**
	C/T	**26 (61.9%)**	**12 (26.7%)**	**0.17 (0.07-0.45)**	
	T/T	**4 (9.5%)**	**1 (2.2%)**	**0.09 (0.01-0.93)**	
Dominant	**C/C**	**12 (28.6%)**	**32 (71.1%)**	**1.00**	**0.000**
	C/T-T/T	**30 (71.4%)**	**13 (28.9%)**	**0.16 (0.06-0.41)**	
Recessive	**C/C-C/T**	**38 (90.5%)**	**44 (97.8%)**	**1.00**	**0.130**
	T/T	**4 (9.5%)**	**1 (2.2%)**	**0.22 (0.02-2.02)**	
Over dominant	**C/C-T/T**	**16 (38.1%)**	**33 (73.3%)**	**1.00**	**0.000**
	C/T	**26 (61.9%)**	**12 (26.7%)**	**0.22 (0.09-0.55)**	
Log-additive	**-**	**-**	**-**	**0.21 (0.09-0.48)**	**0.000**
Bsm1 (Total =87)					
Model	**Genotype**	**Cases (42)**	**Controls (45)**	**OR (95% CI)**	**P-value**
Co-dominant	**A/A**	**8 (19.1%)**	**23 (51.1%)**	**1.00**	**0.004**
	G/A	**18 (42.9%)**	**9 (20%)**	**0.17 (0.06-0.54)**	
	G/G	**16 (38.1%)**	**13 (28.9%)**	**0.28 (0.10-0.84)**	
Dominant	**A/A**	**8 (19.1%)**	**23 (51.1%)**	**1.00**	**0.002**
	G/A-G/G	**34 (81%)**	**22 (48.9%)**	**0.23 (0.09-0.59)**	
Recessive	**A/A-G/A**	**26 (61.9%)**	**32 (71.1%)**	**1.00**	**0.360**
	G/G	**16 (38.1%)**	**13 (28.9%)**	**0.66 (0.27-1.62)**	
Over dominant	**A/A-G/G**	**24 (57.1%)**	**36 (80%)**	**1.00**	**0.020**
	G/A	**18 (42.9%)**	**9 (20%)**	**0.33 (0.13-0.86)**	
Log-additive	**---**	**---**	**---**	**0.54 (0.32-0.92)**	**0.020**

Table (2): Comparison of Apa1 and Taq1 SNPs between case and control group

Apa1 (Total =87)					
Model	**Genotype**	**Cases (42)**	**Controls (45)**	**OR (95% CI)**	**P-value**
Co-dominant	**A/A**	**18 (42.9%)**	**25 (55.6%)**	**1.00**	**0.180**
	A/C	**21 (50%)**	**14 (31.1%)**	**0.48 (0.19-1.19)**	
	C/C	**3 (7.1%)**	**6 (13.3%)**	**1.44 (0.32-6.54)**	
Dominant	**A/A**	**18 (42.9%)**	**25 (55.6%)**	**1.00**	**0.240**
	A/C-C/C	**24 (57.1%)**	**20 (44.4%)**	**0.60 (0.26-1.40)**	
Recessive	**A/A-A/C**	**39 (92.9%)**	**39 (86.7%)**	**1.00**	**0.340**
	C/C	**3 (7.1%)**	**6 (13.3%)**	**2.00 (0.47-8.57)**	
Over dominant	**A/A-C/C**	**21 (50%)**	**31 (68.9%)**	**1.00**	**0.072**
	A/C	**21 (50%)**	**14 (31.1%)**	**0.45 (0.19-1.08)**	
Log-additive	**---**	**---**	**---**	**0.86 (0.46-1.62)**	**0.650**
Taq1 (Total =87)					
Model	**Genotype**	**Cases (42)**	**Controls (45)**	**OR (95% CI)**	**P-value**
Co-dominant	**C/C**	**6 (14.3%)**	**20 (44.4%)**	**1.00**	**0.007**
	T/C	**21 (50%)**	**16 (35.6%)**	**0.23 (0.07-0.70)**	
	T/T	**15 (35.7%)**	**9 (20%)**	**0.18 (0.05-0.62)**	
Dominant	**C/C**	**6 (14.3%)**	**20 (44.4%)**	**1.00**	**0.002**
	T/C-T/T	**36 (85.7%)**	**25 (55.6%)**	**0.21 (0.07-0.59)**	
Recessive	**C/C-T/C**	**27 (64.3%)**	**36 (80%)**	**1.00**	**0.100**
	T/T	**15 (35.7%)**	**9 (20%)**	**0.45 (0.17-1.18)**	
Over dominant	**C/C-T/T**	**21 (50%)**	**29 (64.4%)**	**1.00**	**0.170**
	T/C	**21 (50%)**	**16 (35.6%)**	**0.55 (0.23-1.30)**	
Log-additive	**---**	**---**	**---**	**0.43 (0.23-0.79)**	**0.004**

Table (3): Association of Fok1, Bsm1, Apa1 and Taq1 polymorphism with vitamin D level status among prostate cancer patients

Fok1 (Total = 42)					
Model	**Genotype**	**Abnormal VD**	**Normal VD**	**OR (95% CI)**	**P-value**
Co-dominant	**C/C**	**2 (14.3%)**	**10 (35.7%)**	**1.00**	**0.200**
	C/T	**11 (78.6%)**	**15 (53.6%)**	**0.23 (0.04-1.35)**	
	T/T	**1 (7.1%)**	**3 (10.7%)**	**0.39 (0.02-6.85)**	
Dominant	**C/C**	**2 (14.3%)**	**10 (35.7%)**	**1.00**	**0.084**
	C/T-T/T	**12 (85.7%)**	**18 (64.3%)**	**0.24 (0.04-1.40)**	
Bsm1 (Total = 42)					
Co-dominant	**G/G**	**5 (35.7%)**	**11 (39.3%)**	**1.00**	**0.008**
	G/A	**9 (64.3%)**	**9 (32.1%)**	**0.39 (0.09-1.71)**	
	A/A	**0 (0%)**	**8 (28.6%)**	**NA (0.00-NA)**	
Dominant	**A/A**	**0 (0%)**	**8 (28.6%)**	**NA (0.00-NA**	**0.004**
	G/A-G/G	**14 (100%)**	**20 (71.4%)**	**1.00**	
Apa1 (Total = 42)					
Co-dominant	**A/A**	**8 (57.1%)**	**10 (35.7%)**	**1.00**	**0.430**
	A/C	**5 (35.7%)**	**16 (57.1%)**	**2.47 (0.62-9.83)**	
	C/C	**1 (7.1%)**	**2 (7.1%)**	**1.38 (0.10-18.99)**	
Dominant	**A/A**	**8 (57.1%)**	**10 (35.7%)**	**1.00**	**0.220**
	A/C-C/C	**6 (42.9%)**	**18 (64.3%)**	**2.28 (0.61-8.60**	
Taq1 (Total = 42)					
Co-dominant	**T/T**	**5 (35.7%)**	**10 (35.7%)**	**1.00**	**0.690**
	T/C	**6 (42.9%)**	**15 (53.6%)**	**1.47 (0.33-6.55)**	
	C/C	**3 (21.4%)**	**3 (10.7%)**	**0.67 (0.09-5.15)**	
Dominant	**T/T**	**5 (35.7%)**	**10 (35.7%)**	**1.00**	**0.770**
	T/C-C/C	**9 (64.3%)**	**18 (64.3%)**	**1.24 (0.30-5.12)**	

Table (4): Association of Fok1, Bsm1, Apa1 and Taq1 polymorphism with Total PSA levels among prostate cancer patients

Fok1 (Total = 42)					
Model	**Genotype**	**TPSA < 100**	**TPSA >100**	**OR (95% CI)**	**P-value**
Co-dominant	C/C	5 (25%)	7 (31.8%)	1.00	0.680
	C/T	12 (60%)	14 (63.6%)	0.95 (0.23-3.96)	
	T/T	3 (15%)	1 (4.5%)	0.35 (0.03-4.78)	
Dominant	C/C	5 (25%)	7 (31.8%)	1.00	0.820
	C/T-T/T	15 (75%)	15 (68.2%)	0.85 (0.21-3.47)	
Bsm1 (Total = 42)					
Co-dominant	G/G	9 (45%)	7 (31.8%)	1.00	0.600
	G/A	8 (40%)	10 (45.5%)	1.85 (0.45-7.65)	
	A/A	3 (15%)	5 (22.7%)	2.17 (0.36-13.24)	
Dominant	A/A	3 (15%)	5 (22.7%)	1.57 (0.31-8.08)	0.320
	G/A-G/G	17 (85%)	17 (77.3%)	1.00	
Apa1 (Total = 42)					
Co-dominant	A/A	12 (60%)	6 (27.3%)	1.00	0.046
	A/C	7 (35%)	14 (63.6%)	5.41 (1.23-23.89)	
	C/C	7 (35%)	14 (63.6%)	5.41 (1.23-23.89)	
Dominant	A/A	12 (60%)	6 (27.3%)	1.00	0.014
	A/C-C/C	8 (40%)	16 (72.7%)	5.57 (1.30-23.93)	
Taq1 (Total = 42)					
Co-dominant	T/T	11 (55%)	4 (18.2%)	1.00	0.092
	T/C	7 (35%)	14 (63.6%)	4.83 (1.09-21.41)	
	C/C	2 (10%)	4 (18.2%)	3.97 (0.47-33.47)	
Dominant	T/T	11 (55%)	4 (18.2%)	1.00	0.030
	T/C-C/C	9 (45%)	18 (81.8%)	4.64 (1.11-19.46)	

Table (5) Distribution of Fok1, Bsm1, Apa1 and Taq1 polymorphism According to body weight of prostate cancer patients

Model	Genotype	Abnormal Weight	Normal Weight	OR (95% CI)	P-value
Fok1 (Total = 42)					
Co-dominant	C/C	6 (26.1%)	6 (31.6%)	1.00	0.760
	C/T	14 (60.9%)	12 (63.2%)	0.91 (0.23-3.65)	
	T/T	3 (13%)	1 (5.3%)	0.40 (0.03-5.39)	
Dominant	C/C	6 (26.1%)	6 (31.6%)	1.00	0.800
	C/T-T/T	17 (73.9%)	13 (68.4%)	0.84 (0.21-3.29	
Bsm1 (Total = 42)					
Co-dominant	G/G	10 (43.5%)	6 (31.6%)	10 (43.5%)	0.520
	G/A	10 (43.5%)	8 (42.1%)	1.41 (0.35-5.66)	
	A/A	3 (13%)	5 (26.3%)	2.76 (0.47-16.18)	
Dominant	A/A	3 (13%)	5 (26.3%)	2.30 (0.46-11.40)	0.300
	G/A-G/G	20 (87%)	14 (73.7%)	1.00	
Apa1 (Total = 42)					
Co-dominant	A/A	8 (34.8%)	10 (52.6%)	1.00	0.280
	A/C	14 (60.9%)	7 (36.8%)	0.41 (0.11-1.53)	
	C/C	1 (4.3%)	2 (10.5%)	1.87 (0.14-25.92)	
Dominant	A/A	8 (34.8%)	10 (52.6%)	8 (34.8%)	0.280
	A/C-C/C	15 (65.2%)	9 (47.4%)	0.50 (0.14-1.76)	
Taq1 (Total = 42)					
Co-dominant	T/T	8 (34.8%)	7 (36.8%)	1.00	0.100
	T/C	14 (60.9%)	7 (36.8%)	0.51 (0.12-2.12)	
	C/C	1 (4.3%)	5 (26.3%)	4.72 (0.41-54.75)	
Dominant	T/T	8 (34.8%)	7 (36.8%)	1.00	0.690
	T/C-C/C	15 (65.2%)	12 (63.2%)	0.76 (0.20-2.91)	

Table (6): Association of Fok1, Bsm1, Apa1 and Taq1 polymorphism with Gleason stage among prostate cancer patients

Fok1 (Total = 42)					
Model	**Genotype**	**G. Stage< 7**	**G. Stage< 7**	**OR (95% CI)**	**P-value**
Co-dominant	**C/C**	**5 (27.8%)**	**7 (29.2%)**	**1.00**	**0.970**
	C/T	**11 (61.1%)**	**15 (62.5%)**	**1.00 (0.25-4.06)**	
	T/T	**2 (11.1%)**	**2 (8.3%)**	**0.78 (0.07-8.09**	
Dominant	**C/C**	**5 (27.8%)**	**7 (29.2%)**	**1.00**	**0.970**
	C/T-T/T	**13 (72.2%)**	**17 (70.8%)**	**0.97 (0.25-3.85)**	
Bsm1 (Total = 42)					
Co-dominant	**G/G**	**4 (22.2%)**	**12 (50%)**	**1.00**	**0.026**
	G/A	**12 (66.7%)**	**6 (25%)**	**0.17 (0.04-0.75)**	
	A/A	**2 (11.1%)**	**6 (25%)**	**1.00 (0.14-7.09)**	
Dominant	**A/A**	**2 (11.1%)**	**6 (25%)**	**2.63 (0.46-14.97)**	**0.250**
	G/A-G/G	**16 (88.9%)**	**18 (75%)**	**1.00**	
Apa1 (Total = 42)					
Co-dominant	**A/A**	**10 (55.6%)**	**8 (33.3%)**	**1.00**	**0.330**
	A/C	**7 (38.9%)**	**14 (58.3%)**	**2.59 (0.70-9.63)**	
	C/C	**1 (5.6%)**	**2 (8.3%)**	**2.76 (0.20-37.62)**	
Dominant	**A/A**	**10 (55.6%)**	**8 (33.3%)**	**1.00**	**0.140**
	A/C-C/C	**8 (44.4%)**	**16 (66.7%)**	**2.61 (0.73-9.36)**	
Taq1 (Total = 42)					
Co-dominant	**T/T**	**7 (38.9%)**	**8 (33.3%)**	**1.00**	**0.300**
	T/C	**7 (38.9%)**	**14 (58.3%)**	**1.61 (0.40-6.51)**	
	C/C	**4 (22.2%)**	**2 (8.3%)**	**0.37 (0.05-2.97)**	
Dominant	**T/T**	**7 (38.9%)**	**8 (33.3%)**	**1.00**	**0.780**
	T/C-C/C	**11 (61.1%)**	**16 (66.7%)**	**1.21 (0.32-4.55)**	

Table (7): Distribution of Fok1, Bsm1, Apa1 and Taq1 polymorphism according to prostate cancer patient's age

Fok1 (Total = 42)					
Model	**Genotype**	**> 70 Years**	**< 70 Years**	**OR (95% CI)**	**P-value**
Co-dominant	**C/C**	**2 (14.3%)**	**10 (35.7%)**	**1.00**	**0.035**
	C/T	**12 (85.7%)**	**14 (50%)**	**0.22 (0.04-1.24)**	
	T/T	**0 (0%)**	**4 (14.3%)**	**NA (0.00-NA)**	
Dominant	**C/C**	**2 (14.3%)**	**10 (35.7%)**	**1.00**	**0.100**
	C/T-T/T	**12 (85.7%)**	**18 (64.3%)**	**0.27 (0.05-1.50)**	
Bsm1 (Total = 42)					
Co-dominant	**G/G**	**4 (28.6%)**	**12 (42.9%)**	**1.00**	**0.640**
	G/A	**7 (50%)**	**11 (39.3%)**	**0.50 (0.11-2.23**	
	A/A	**3 (21.4%)**	**5 (17.9%)**	**0.56 (0.09-3.52)**	
Dominant	**A/A**	**3 (21.4%)**	**5 (17.9%)**	**0.82 (0.16-4.12)**	**0.810**
	G/A-G/G	**11 (78.6%)**	**23 (82.1%)**	**1.00**	
Apa1 (Total = 42)					
Co-dominant	**A/A**	**7 (50%)**	**11 (39.3%)**	**1.00**	**0.810**
	A/C	**6 (42.9%)**	**15 (53.6%)**	**1.55 (0.40-5.97)**	
	C/C	**1 (7.1%)**	**2 (7.1%)**	**1.18 (0.09-15.99)**	
Dominant	**A/A**	**7 (50%)**	**11 (39.3%)**	**1.00**	**0.540**
	A/C-C/C	**7 (50%)**	**17 (60.7%)**	**1.50 (0.41-5.52)**	
Taq1 (Total = 42)					
Co-dominant	**T/T**	**5 (35.7%)**	**10 (35.7%)**	**1.00**	**0.990**
	T/C	**7 (50%)**	**14 (50%)**	**1.09 (0.26-4.66)**	
	C/C	**2 (14.3%)**	**4 (14.3%)**	**1.19 (0.14-9.87)**	
Dominant	**T/T**	**5 (35.7%)**	**10 (35.7%)**	**1.00**	**0.88**
	T/C-C/C	**9 (64.3%)**	**18 (64.3%)**	**1.11 (0.27-4.52)**	

Discussion:

This case control study examined 4 SNPs of the VDR gene included rs731236 (*TaqI*), rs7975232 (*ApaI*), rs1544410 (*BsmI*) and rs2228570 (*Fok1*) in prostate cancer. In (rs731236 (*TaqI*): Overall C and T allele frequencies were almost similar in proportions but there was mild increased in C, the majority of C allele was in control group. Regarding the genotype frequencies, the TC was common and extensive of this increased in prostate cancer group, this finding agreed with several previous studies reported for the same SNP polymorphisms among prostate cancer patients in Japanese population and Piedmont region USA (4, 5). In contrast Oakley-Girvan, working with African American and White populations, they did not find any association among TaqI with PCa risk in the family or case control data. Similar results were reported by Chen *et al* (2009) (10) when they investigated British individuals of European origin. These findings might strength our conclusion that TaqI has no link with different races in the study area. It was observed that the link between TaqI polymorphism and risk factors for prostate cancer was not fully investigated. This study did not record any association between these risk factors (stage, age, BMI, PSA level, and vitamin D level) and prostate cancer. Study showed a higher frequency of T allele gene and TC genotype increased in the Gleason >7 stage, PCa compared with Gleason < 7, suggesting that PCa patients carrying T allele and TC genotype are most probably more prone to entering an advanced stage. On the other hand, for rs7975232 (*ApaI*): The C allele frequencies was the most frequent overall study population, with mild proportion increased in control group, furthermore AA genotype frequency was the most frequent overall population and it has same proportion of mutant genotype frequencies (A/C-C/C). One of our standing finding is that *ApaI (*rs7975232) SNP failed to find any associated with prostate cancer under the additive, dominant, or recessive genetic models. Other studies conducted elsewhere showed the same findings (13, 14), on the other hand; (7, 12, 15) reported an association between *ApaI (rs7975232)* and prostate cancer risk. This could be attributed to heterogeneity of studies populations. The *ApaI (*rs7975232) SNP failed to find any associated with stage, Gleason grade, age, BMI, PSA level, and vitamin D level. This in line with Suzuki who didn’t observe a relationship between clinic and pathologic parameters and genotype distributions of the ApaI (4). Whereas in rs1544410 (*BsmI*): A and G allele, they appear in similar proportions frequencies overall population, C allele was more common in control group while G alle common in prostate cancer, A/G – G/G mutant showed predominated genotype frequencies overall population. Moreover, it was common in prostate cancer. This finding is supported by several previous studies among Japanese population, Piedmont region USA, and Caucasians population (4, 5, 16). In our study, we found an association between *BsmI* genotypes and high Gleason score. The findings are directly in line with previous findings (12).

Contrary to this finding several studies done in Brazil by Sarah Braga Rodrigues Nunes, a meta-analysis of 27 published studies and Mahesh B. Keitheri Cheteri study in USA which find no association between *BsmI* genotypes and high Gleason score (17, 18). It seems that A/G – G/G genotype alleles have strong tendency for expression regarding to development of prostate cancer among population under current study. The *BsmI(rs1544410)* SNP failed to find any associated with other risk factor such as stage, age, BMI, PSA level, and vitamin D level. Finally in rs2228570 (Fok1): C allele frequencies was the most frequent among study population majority of them were in control group, C/C genotype frequencies was the major and vast of them were appear in control group. Furthermore, (C/T-T/T) the mutant genotype was major in prostate cancer group .This is an important finding in the *Fok1* (*rs2228570*) SNP which has strong association with prostate cancer and was agree with some previous studies (19 - 22).

Conclusion

The study was concluded to the (Fok1, Bsm1 and TaqI) SNPS showed significant association with risk to prostate cancer, whereas ApaI SNP was not crrelated with risk to prostate cancer.

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Original Article Sudan Medical Laboratory Journal

Association of Serum Calcium Level and Sodium Channel *SCN1A* Mutations with Idiopathic Epilepsy among Sudanese Patients

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Abstract

Background: Genetics research of humans has established that a genetic basis contributes to the susceptibility to epilepsy for a majority of the cases. Al- though many epilepsies are secondary to injury or another illness, approximately 40% are idiopathic, meaning that the original cause is unknown. It is presumed that most idiopathic epilepsies result from genetic abnormalities, with the majority likely caused by mutations in multiple currently un- identified genes. However, research has revealed a growing number of single gene mutations that cause epilepsy.

Objective: To detect some of the genetic mutations which may cause idiopathic epilepsy.

Methods: The current study is a cross-sectional study that had been performed at Sheikh Mohamed Khair center, Banat, Omdurman, and National Centre for Neurological Sciences (NCNS) Khartoum state, during the period 2016 to 2019. Ninety-nine participants were enrolled in this study. Demographic data were collected in a pre- designed questionnaire blood samples were analyzed for biochemical and molecular tests.

Results: Ninety-nine patients diagnosed with idiopathic epilepsy were recruited in this study. The most affected age group was 18 - 40 years accounted for 55% of patients. Females were the majority with 53%. Fifty percent of the patients had the first seizure at age less than 5 years. Ninety percent of the patients have no Family history with epilepsy. All sequenced samples showed genetic mutations, deletion mutation was detected in 71% of the samples. Bioinformatics tools detected a frame shift mutation in the chain of the amino acids.

Conclusion: The current study detected deletion mutations in SCN1A gene (frame shift) can cause epilepsy by changing some amino acids with residues that can affect neuronal stability indirectly.

Keywords: Epilepsy, National Centre for Neurological Science, Sudan, SCN1A

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Introduction

Epilepsy is one of the most frequent neurological disorders; the prevalence estimation for active epilepsy ranges from 0.2% to 4.1% (1). It is a pathological condition characterized by repeated, unprovoked, epileptic seizures (2). Diverse causes of epilepsy are commonly being recognized, such as structural, metabolic, or genetic causes; yet, the majority of epilepsies have unknown etiology (3). It is not surprising that alterations in the ion channels may be apathogenetic cause, and indeed, at least for some syndromes, epilepsies are increasing thought to be “channelopathies” either due to genetic mutations or as the endpoint of hitherto unknown pathological processes (4). The voltage-gated Na^+ channels' primary role is the initiation of action potentials, making them critical determinants of neuronal excitability (5); it consists of large α subunits which connect with further polypeptides, for instance, β subunits to outline efficient voltage-gated ion channels (6). Defects in sodium channels subunits make them susceptible to slow inactivation, *i.e.*, the membrane stays depolarized for a longer time, as a result, it can cause epileptogenesis and increase of **seiz**ures (7, 8). It should be mentioned that changes in sodium channels had been detected in brain tissues of epileptic patients, suggestive of a potential role for sodium channels in the pathophysiology of epilepsy (9).

SCN1A encodes the NaV1.1 subunit expressed mostly in inhibitory GABAergic neurons and is enriched at the axon initial segment, implicating a role in the beginning and the spread of action potentials in these cells (10, 11). It was first implicated in epilepsy in 2000 (12). Several hundred mutations of SCN1A have been reported in epilepsy since first discovered, making it the most frequently known epilepsy gene (13).

This study aims to detect some of the possible causes of idiopathic epilepsy by analyzing a sequence of SCN1A gene in addition to calcium levels.

Materials and Methods

This study is a cross-sectional study that was performed at Sheikh Mohamed Khair centre, Khartoum state. This center is a primary health care center how- ever idiopathic epilepsy diagnosis was provided by neurology physician depending essentially on the description of eyewitness to the seizure attack. Ninety-nine participants were enrolled in this study starting November 2016 to February 2019. Only Patients who were not diagnosed with idiopathic epilepsy and those who refused to patients were excluded from this study. Patients' demographic, and clinical data including age, gender, ethnicity, class of seizure, the onset of seizure, frequency of seizure, seizure classification, precipitating factor, and modality of treatment psychosocial aspects, concomitant illness, and family history were recorded in a predesigned questionnaire. Analyses were performed using Mi- crosoft Office Excel 2010, and Statistical Package for Social Science Program (SPSS version 25). Blood samples were collected from each patient in two con- tainers, EDTA container and LI-heparin, the EDTA samples were processed for DNA extraction, and the heparinized samples were used for calcium estimation.

Biochemical Analysis

Plasma was separated by centrifuge (Hettich Zenterfuge EBA200, Kirchlengern, Germany) at 3000 RPM for 5 minutes and then stored at −20°C. Calcium con- centrations were obtained by semi-automated chemistry Analyzer (Mindary BA-88A).

Molecular Genetic Analysis

Extraction of deoxyribonucleic acid (DNA) was performed from whole blood samples using QIAGEN® DNA extraction kits (vacuum protocol).

SCN1A gene was amplified using polymerase chain reaction (PCR), in PCR tube 20 µL of readymade Master Mix (4 µL of 5× Firepol® Master Mix (Solis Bio-Dyne, Tartu, Estonia) was added to 1 µL forward primer, 1 µL reverse primer, and 14 µL distilled water), followed by 2 µL of DNA.

Primers used for amplification were 5 TACCCTGTTCCGAGTGATCC 3 forward primer and5'GCTGTTGCCAAAGGTCTCAA3' reverse primer, then the amplified PCR products were separated using 2% gel electrophoresis. Then, se- parated DNA was visualized using UV light.

DNA Sequencing

Fifteen PCR products were sent to China for sequencing (BGI solutions co.LTD).

Data Analysis

Sequencing Analysis

The sequencing results were analyzed using multiple bioinformatics software and tools. The nucleotides sequences of SCN1A gene were searched for sequences similarity using nucleotide BLAST NCBI (https://www.ncbi.nlm.nih.gov/) then subjected to multiple sequence alignment using Bio-Edit software. The detected mutations were analyzed using bioinformatics tools, the information regarding the detected SNPs were obtained from National Center for Biological Information (NCBI), as for predicting damaging amino acid substitutions, mutation taster was used in addition to HOPE.

Statistical Analysis

Demographic, clinical, and sequencing findings were performed by Microsoft Office Excel, and Statistical Package for Social Science Program 2010 SPSS version 25, which included frequencies, cross-tabulation.

Results

Demographic Results

Ninety-nine patients diagnosed with idiopathic epilepsy were recruited in this study, Highest age group category was 18 - 40 years accounted for 55% of Patients, followed by less than 18 years group (32%), then 41 - 65 years (11%) whilemore than 65 years group were only 2% (**Table 1**).

Table 1. The age groups in idiopathic epileptic patients.

Age	No of patients	%	P-value
More than 65 years	2	2.0%	
Less than 18 years	32	32.0%	<0.001
18 - 40 years	55	55.0%	

Regarding gender, females were the majority with 53% and 47% males (**Table2**).

Table 2. The gender domination in idiopathic epileptic patients.

Gender	No of patients	%	P-value
Female	53	53.0%	0.549
Male	47	47.0%	

Fifty percent of the patients had the first seizure at age less than 5 yrs, 28% of them had it in age between 5 - 10 years 22% had the onset of seizure at age more than 10 years (**Table 3**).

Table 3. The onset of seizure in age groups among idiopathic epileptic patients.

Onset of seizure	No of patients	%	P-value
Less than 5 years	50	50.0%	0.001
More than 10 years	22	22.0%	
5 - 10 years	28	28.0%	

Ninety percent of the patients have no Family history with epilepsy while the rest of them (10%) declared to have a family history (**Table 4**).

Table 4. The family history among idiopathic epileptic patients.

Family history	No of patients	%	P-value
No	90	90.0%	
Yes	10	10.0%	

Biochemical Results

Cross-tabulation of calcium among patients revealed calcium level < 8.5 mg/dL was found in 54.4% of the patients followed by 39.4% with normal calcium level (8.5 - 10.5) (**Table 5**).

Table 5. Calcium levels in idiopathic epileptic patients.

Calcium (mg/dL)	Frequency	Percentage %
Less than 8.5	54	54.4%
8.5 to 10.5	39	39.4%
More than 10.5	6	6.1%

Molecular and Sequencing Results

In the current study, the sequencing findings of the SCN1A gene showed that, at chr2:166848853 C>G was detected in (57%) of the samples, while C>T was de- tected in 14%, both mutations showed splice site effect. Deletion AT was de- tected at chr2:166848848-49 in 71% of samples. Also C>T on chr2:166848847 was detected (71%) with splice site effect, T>A single base exchange was detected in (14%) on chr2:166848841 with splice site effect, in addition, Deletion A at po- sitions chr2:166848832 and chr2:166848824, were observed in 71% of the sam- ples which appear to have a splice site effect (**Figure 1**).

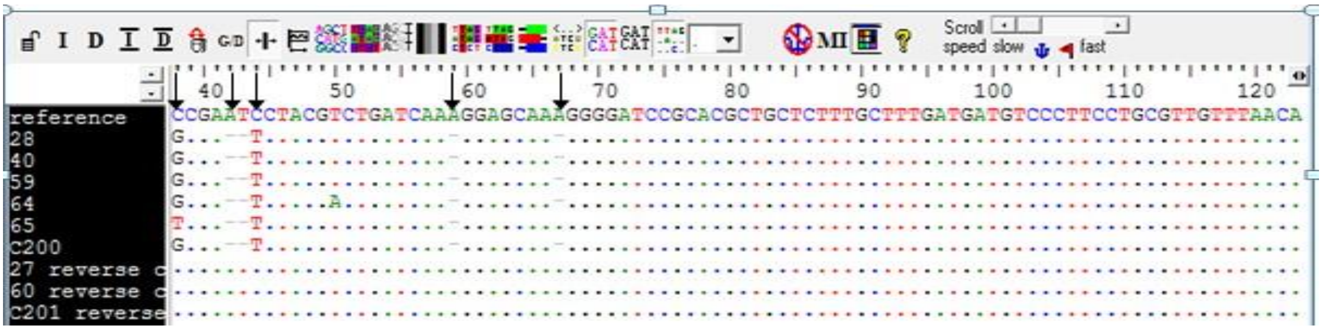
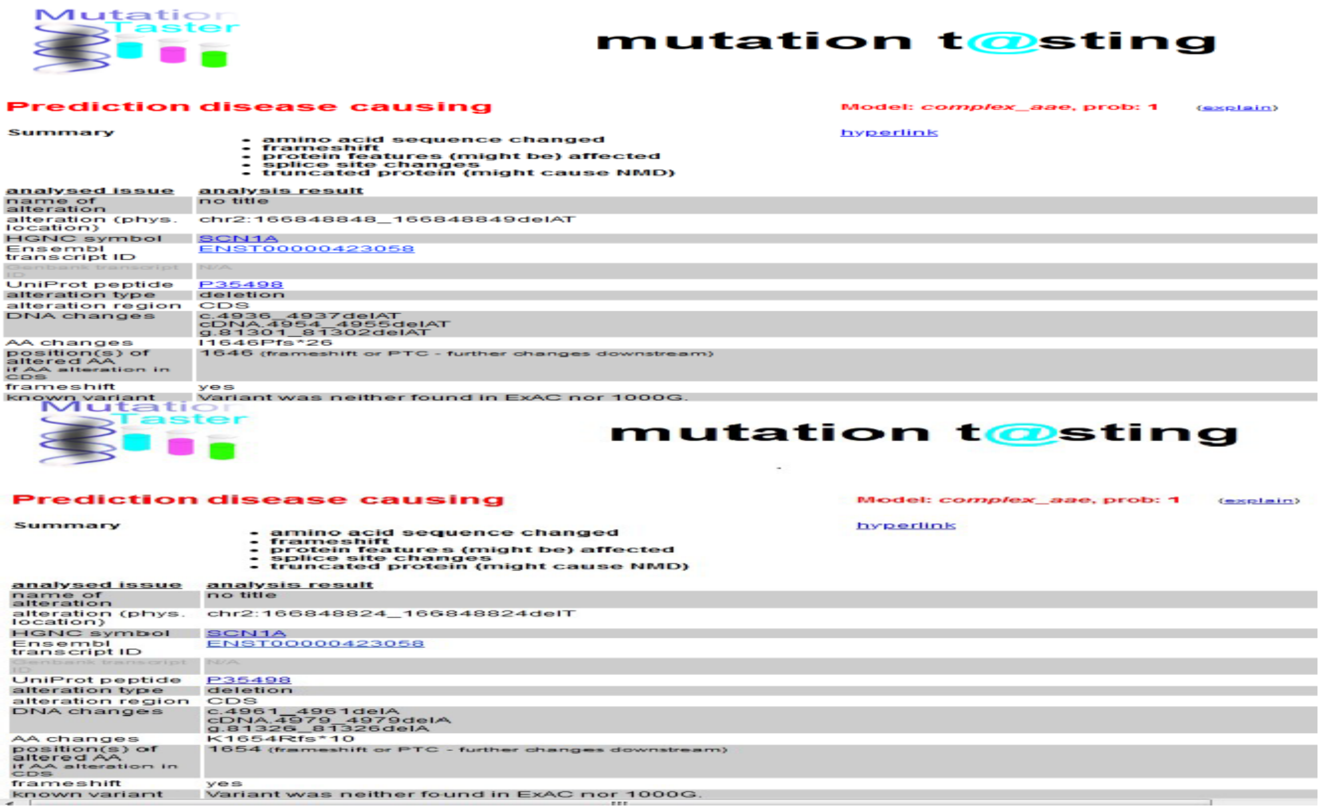


Figure 1. The alignment of SCN1A gene sequence obtained from NCBI database with our samples, using BioEdit program. Arrows indicates mutations sites.

Bioinformatics Results

Furthermore, SCN1A gene mutations were analyzed using mutation taster pro- gram (http://www.mutationtaster.org/) to obtain the type and location of muta-tions, while Hope database (https://www3.cmbi.umcn.nl/hope/) was used to detect the mutation impact upon the protein. The findings of mutation taster program showed that deletion AT and A created frame shift mutations with amino acid change at positions I1646P and G1652E respectively, **Figures 2-5**.



mutation t@sting

Prediction disease causing

Model: *complex_aae*, prob: 1 (explain)

hyperlink

Summary
- amino acid sequence changed
- frameshift
- protein features (might be) affected
- splice site changes
- truncated protein (might cause NMD)

analysed issue	analysis result
name of alteration	no title
alteration (phys. location)	chr2:166848848_166848849delAT
HGNC symbol	SCN1A
Ensembl transcript ID	ENST00000423058
Genbank transcript ID	N/A
UniProt peptide	P35498
alteration type	deletion
alteration region	CDS
DNA changes	c.4936_4937delAT cDNA.4954_4955delAT g.81301_81302delAT
AA changes	I1646Pfs*26
position(s) of altered AA if AA alteration in CDS	1646 (frameshift or PTC - further changes downstream)
frameshift	yes
known variant	Variant was neither found in ExAC nor 1000G.

mutation t@sting

Prediction disease causing

Model: *complex_aae*, prob: 1 (explain)

hyperlink

Summary
- amino acid sequence changed
- frameshift
- protein features (might be) affected
- splice site changes
- truncated protein (might cause NMD)

analysed issue	analysis result
name of alteration	no title
alteration (phys. location)	chr2:166848824_166848824delT
HGNC symbol	SCN1A
Ensembl transcript ID	ENST00000423058
Genbank transcript ID	N/A
UniProt peptide	P35498
alteration type	deletion
alteration region	CDS
DNA changes	c.4961_4961delA cDNA.4979_4979delA g.81326_81326delA
AA changes	K1654Rfs*10
position(s) of altered AA if AA alteration in CDS	1654 (frameshift or PTC - further changes downstream)
frameshift	yes
known variant	Variant was neither found in ExAC nor 1000G.

Figure 2. Deletion AT mutation at chr2:166848848-166848849 using Mutation taster program.

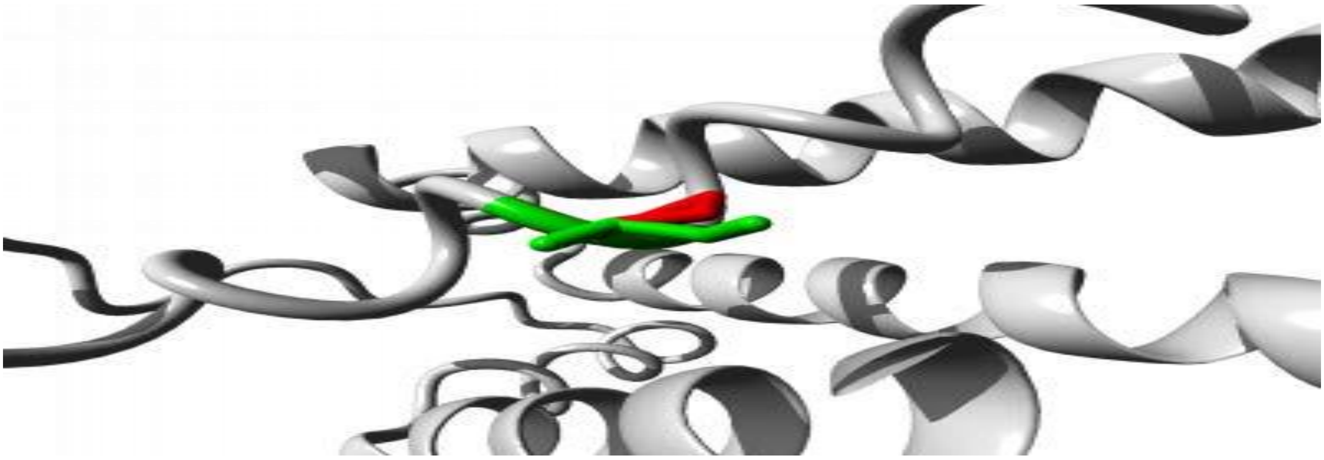
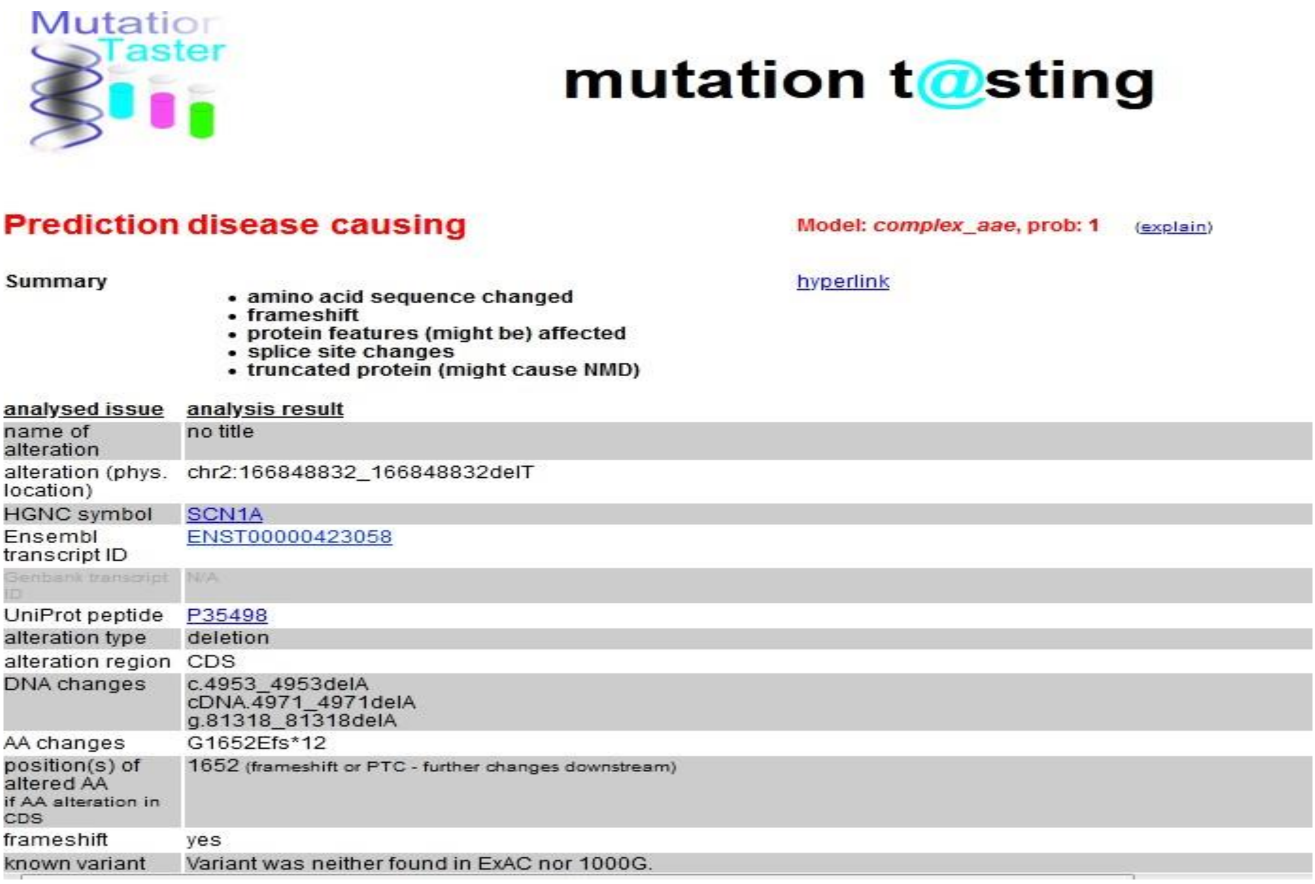


Figure 3. The wild and mutant aa residues at position (I1646P) using Hope program(PDB:6AGF). The wild type is in green and the mutant in red color.



mutation t@sting

Prediction disease causing

Model: *complex_aae*, prob: 1 (explain)

Summary

- **amino acid sequence changed**
- **frameshift**
- **protein features (might be) affected**
- **splice site changes**
- **truncated protein (might cause NMD)**

hyperlink

analysed issue	analysis result
name of alteration	no title
alteration (phys. location)	chr2:166848832_166848832delT
HGNC symbol	SCN1A
Ensembl transcript ID	ENST00000423058
Genbank transcript ID	N/A
UniProt peptide	P35498
alteration type	deletion
alteration region	CDS
DNA changes	c.4953_4953delA cDNA.4971_4971delA g.81318_81318delA
AA changes	G1652Efs*12
position(s) of altered AA if AA alteration in CDS	1652 (frameshift or PTC - further changes downstream)
frameshift	yes
known variant	Variant was neither found in ExAC nor 1000G.

Figure 4. Deletion A mutation at chr2:166848832-166848832 using Mutation taster program.

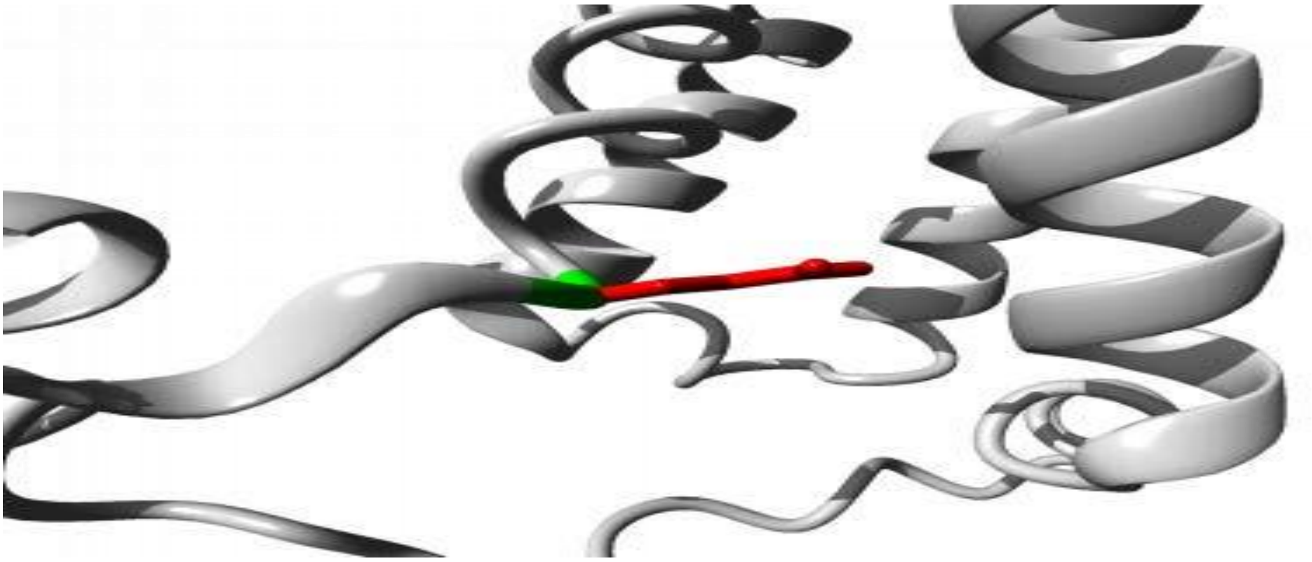
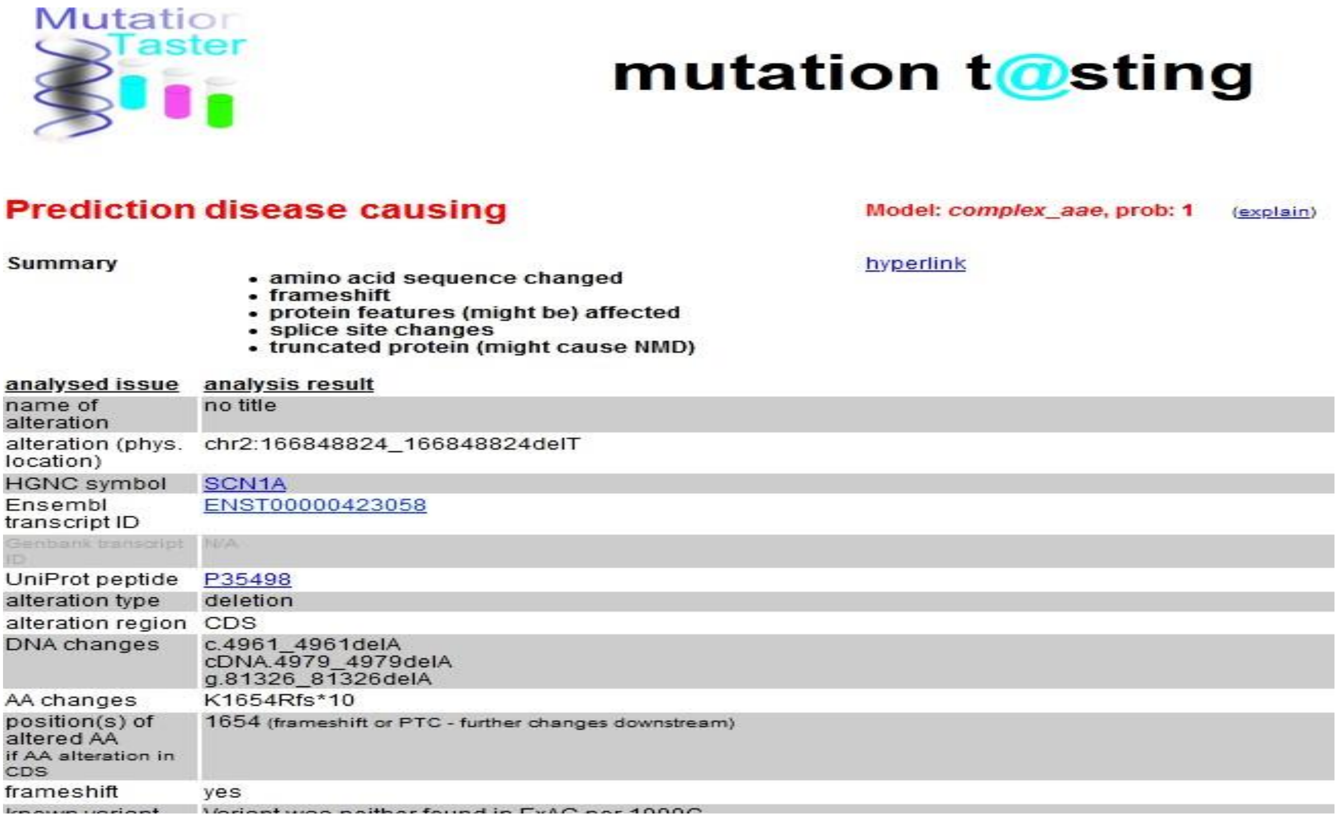


Figure 5. The wild and mutant aa residues at position (G1652E) using Hope program(PDB:6AGF). The wild type is in green and the mutant in red color.

In addition tothat, deletion A showed amino acid change at position 1654 from Lysine to Ar-ginine (**Figure 6**, **Figure 7**).



Prediction disease causing

Model: *complex_aae*, prob: 1 (explain)

Summary

- amino acid sequence changed
- frameshift
- protein features (might be) affected
- splice site changes
- truncated protein (might cause NMD)

hyperlink

analysed issue	analysis result
name of alteration	no title
alteration (phys. location)	chr2:166848824_166848824delT
HGNC symbol	SCN1A
Ensembl transcript ID	ENST00000423058
Genbank transcript ID	N/A
UniProt peptide	P35498
alteration type	deletion
alteration region	CDS
DNA changes	c.4961_4961delA cDNA.4979_4979delA g.81326_81326delA
AA changes	K1654Rfs*10
position(s) of altered AA if AA alteration in CDS	1654 (frameshift or PTC - further changes downstream)
frameshift	yes

Figure 6. Deletion A mutation at chr2:166848824-166848824 using Mutation taster program.

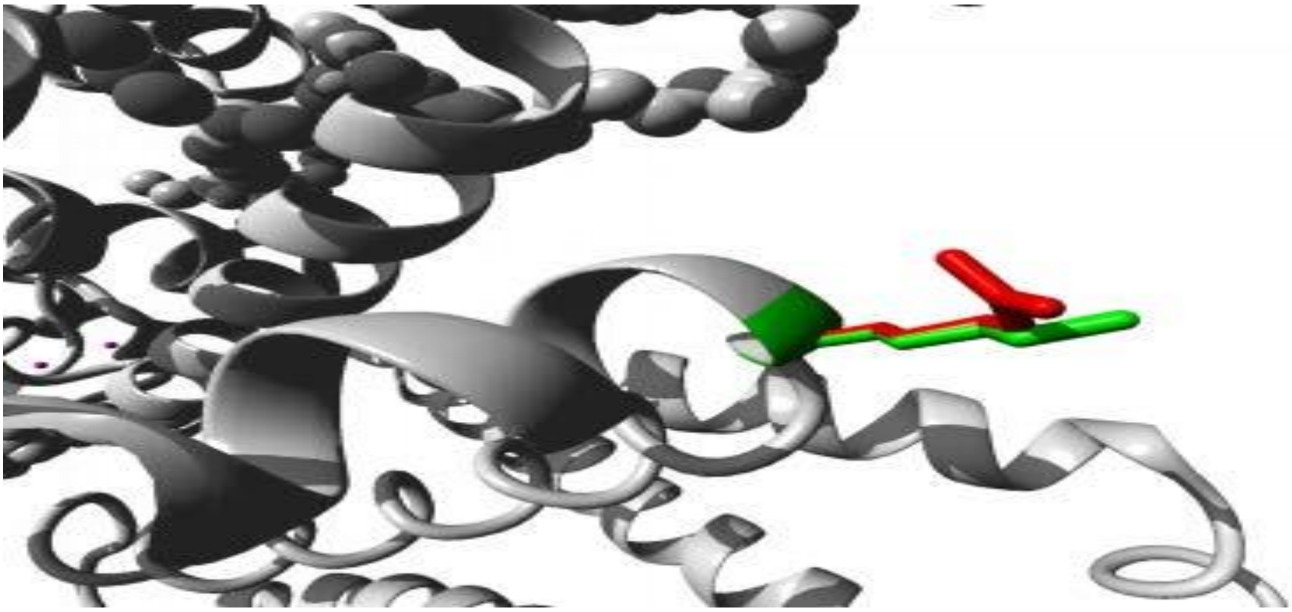


Figure 7. The wild and mutant aa residues at position (K1654R) using Hope program (PDB: 6AGF). The wild type is in green and the mutant in red color.

Discussion

Epilepsy is one of the most frequent neurological disorders; the pooled incidence rate of epilepsy was 61.4 per 100,000 person-years (95% CI 50.7 - 74.4) (14) with a 3% risk of developing epilepsy across all ages (15).

The current study was performed on 99 patients with idiopathic epilepsy; it showed that 50% of the participants had onset of the disease in an age less than 5 years old. Though according to Marini Cet (16) there is a shred of increasing evidence for the existence of IGE beginning beyond the third decade.

Regarding gender, males were less than females 53% females, 47% male. Simi- lar findings were conducted in Asadi study (17) females 57%, 43% were males. Outnumbering of females over males is expected as Studies showed that female sex steroid hormones have repeatedly been shown to affect neuronal excitability (18).

The diagnosis of idiopathic epilepsy starts with a questionnaire establishing the Family History of Epilepsy (FHE) on these patients (19). In this study, 90% of the patients have no Family history with epilepsy while the rest of them (10%) declared to have a family history. Unlike Babtain FA study results (20) which showed a significant association between idiopathic epilepsy and the presence of FHE.

Calcium level was normal (mean 8.32 mg/dl and SD 1.25), while A prospec- tive case-control study done by Oladipo O (21) showed that calcium levels are lower in children with epilepsy compared to the controls during the seizure-free periods.

Nearly 70% of epileptic patients lack an obvious pathogenetic cause and genetics are believed to play a vital role in its causation (22) Voltage-gated sodium channels are an important group of ion channels, which play an important role in generating action potential and depolarization of the neurons. Mutation in the SCN1A gene causes defective gating in the sodium channel and thus causes hyper excitability and seizures (23). More recent studies have indicated that common variants in the SCN1A gene may be risk factors for common epilepsies like temporal lobe epilepsy and idiopathic/genetic generalized epilepsy (GGE/IGE) (24). Factors that do not necessarily change the structure of the gene however do affect the progression of transcription and translation is recognized as epigenetic factors (25). Any perturbation in the transcription, translation, or epigenetic mechanisms can generate defective proteins which lead to diseases. Seizures occur as a result of a complex interplay of altered gene expressions, increased neuronal excitability, and disturbed intrinsic neuronal properties (26, 27) Defects in epilepsy genes give a critical insight into the patho-mechanisms of seizure generation and propagation, which has an impact on the management of the patients (28).

In this study C > T on chr2:166848847 mutation was detected with single Base Exchange alteration type and splice site effect, similar mutation was reported earlier in NCBI (rs777853016).

Also several deletions were detected on SCN1A gene which is located in chromosome 2, it was found that AT deletion at chr2: 166848848_166848849 changed the amino acid sequence at position 1646, protein features (might be) affected and the splice site had changed. As a result, isoleucine muted into a pro- line, with the size difference, mutation caused an empty space in the core of the protein. L proline is a GABA-analogue, its accumulation in the cytosol of GA- BAergic neurons causes competitive inhibition of Glutamic Acid Decarboxylase (GAD) leading to deficient gamma-aminobutyric acid (GABA) production (29).

GABA is the main inhibitory neurotransmitter in the cerebral cortex, it main- tains the inhibitory tone that counterbalances neuronal excitation. When this balance is disturbed, seizures may arise (30).

A deletion at chr2: 166848832_166848832 (frame-shift mutation) made a change in the amino acid sequence with glycine mutation into a glutamic Acid at posi- tion 1652. The most flexible of all residues is glycine. This flexibility might be required for the protein's function. Mutation of this glycine can eliminate this function.

The wild-type residue charge was neutral, while the mutant residue charge is negative, this can cause repulsion between the mutant residue and neighboring residues. Also, glycine location is on the surface of the protein, alteration of this residue can disturb interactions with other molecules or other parts of the pro- tein. It should be mentioned that the torsion angles for glycine are unusual .only glycine is flexible enough to make these torsion angles, so any mutation will de- velop an incorrect confirmation which will disturb the local structure (31). glu- tamic acid is considered to be a major excitatory neurotransmitter in the verte- brate CNS (32). Studies conducted in the past few decades are evidence for the role of glutamic acid in epilepsy (33). A>- was identified at 166848824_166848824, with a frame-shift mutation it caused a change in the amino acid sequence with mutation of a Lysine into an arginine at position 1654. Arginine, also known as l-arginine (symbol Arg or R), is an α-amino acid that is used in the biosynthesis of proteins (34). It is a pre- cursor for the synthesis of Nitric Oxide (NO) (35). Nitric Oxide (NO) is a short- lived, gaseous signaling molecule that is produced endogenously by a family of enzymes called the Nitric Oxide Synthases (NOS), which catalyze the synthesis of NO from the amino acid arginine (36) it have been pointed out as potential neurotransmitters or retrograde messengers [37] linked to synaptic plasticity (38) and regulation of brain excitability, including the triggering of seizure activity (39) (40). The involvement of NO in epileptic disorders has been shown in experiments with systemic injection of NOS inhibitors (39, 41).

The study was limited by two factors, first, the sequencing analysis was done to selected samples, this is due to the limited financial source, this research was self-funded and no any other institutional or governmental funded given. Secondly, the sample size is not like other published articles in the same field, that is because the majority of the patients refused to be included in the study and we didn't find enough collaboration with the co-patients and the patients.

Conclusion

A deletion mutation in SCN1A gene (frameshift) can cause epilepsy by changing some amino acids with residues that can affect neuronal stability indirectly.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Sero-pattern and Occupational Risk Factors Associated with Hepatitis B Virus Infection Among Health Care Workers at Wad Medani Teaching Hospital, Sudan

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Abstract:

Hepatitis B virus (HBV) infection is a major public health problem worldwide. This cross-sectional study was conducted to detect the rate of HBV and risk factors among health care workers (HCWs) using different serological markers. Two hundred and fifty HCWs comprised physicians, lab specialists, nurses, employers, and wash workers. The HBV serological markers were analyzed using ELISA and Cobas411e. Out of 250 HCWs, 103 (41.2%) were males and 147 (58.8%) were females. HBsAg, HBeAg, HBeAb, and total HBcAb were detected in 2.8%, 2.0%, 4.4%, and 37.6% of study participants, respectively. Wash workers are the most vulnerable group, followed by employers and nurses. Furthermore, 115 (47.3%) HCWs were vaccinated and produced no HBsAg. The study spotlights to the positive impact of vaccination in reducing the prevalence of HBV infection, and also recommends the implementation of vaccination and hygiene policies to ensure a higher level of infection control.

Keywords: HBV serological markers, Health Care Workers, Vaccination, Good Hygiene policies

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Introduction:

Hepatitis B virus (HBV) infection is a major public health problem worldwide. More than two billion people have been infected with HBV, and 400 million are considered carriers of the virus (1-3). The World Health Organization (WHO) estimated that, the prevalence of hepatitis B virus infection is more than 10% (4), that there are 360 million people in the world chronically infected with HBV in sub-Saharan Africa and East Asia (5), and that 65 million are found in Africa. Thus, Africa, carries approximately 18% of the global burden of HBV infection (6). Regions of the world with high or intermediate prevalence of Hepatitis B surface antigen (HBsAg) include much of Eastern Europe, Asia, Africa, the Middle East, and the Pacific Islands. The majority of new infections occur in countries with low endemicity (i.e., HBsAg prevalence of less than 2%) (7,8).

Health practitioners or health care workers (HCWs) are at high risk for exposure to HBV from infected patients, and when accidentally stuck with a contaminated needle or other sharp medically used instrument, they may potentially transmit HBV to patients too (4,5,9). The frequencies of exposure to infected body fluids or blood-contaminated sharps, as well as the duration of employment in an occupational risk category, have been shown to be associated with the risk of HCWs acquiring occupationally related HBV infection. Sudan is classified among countries with high HBsAg endemicity (Mudawi, 2008). Exposure to HBV infection ranges from 47% to 78% HBsAg, with sero-prevalence ranging from as low as 6.8% in central Sudan to as high as 26% in southern Sudan (10).In previous studies, the occupational risk of HBV infection among the HCWs was high among nurses in Khartoum State (11), and among nurses and cleaning staff in Omdurman State (12). Serologic markers for HBV were detected in 843 HCWs in Al-shaab Teaching Hospitals in Khartoum State, and the prevalence of anti-HBc, HBsAg, HBsAb, and HBeAg was found to be 57%, 6%, 37%, and 9%, respectively (13). Chronic hepatitis B infection was found in 2.9 percent of tertiary hospital employees in Rwanda (14), 8.1 percent in Uganda [4], and 13 percent in Nigeria (15). Apparently there are considerable differences in the rate of chronic HBV-infection among HCWs in sub-Saharan Africa (5). Whereas the literature on hepatitis B virus infection in Sudan is growing, there is still a paucity of information on HBV among HCWs, particularly in the Sudan outskirts. This study contributes to this discourse by presenting and determining the prevalence and occupational risk factors of HBV infection among HCWs and also providing updated guidelines for the most informative marker in screening policies.

Method

From March to May 2022, a cross-sectional study was carried out at Wad Medani Teaching Hospital, Sudan's largest referral hospital. The study subjects were divided into five groups, each with 50 participants. These groups include physicians, laboratory specialists, nurses, employers, and washing workers. All the participants were informed of the study objectives. Ethical approval was obtained from the ethical review board of the University of Gezira, the Faculty of Medical Laboratory Sciences, and the Ministry of Health in Gezira State. Two types of data were collected and analyzed for each participant: (1) Demographic and risk factors were collected using a structured questionnaire and analyzed using a computer-based software program. (2) Clinical data (blood samples) were collected according to standard instructions for blood sample collection, then carried out and analyzed at the Blood Bank Department of the same hospital, the Prime Design Center for Training and Human Resources Development, and the Department of Medical Microbiology, Faculty of Medical Laboratory Sciences, University of Gezira. Five ml of venous blood were collected from each participant in a plain tube and were transported immediately to the laboratory, where serum was separated and tested for HBsAg using an enzyme-linked immunosorbent assay (ELISA), which performed according to manufacturer instructions (Fortress Diagnostics; Batch: hhbs-1407-1; Code: UK). HBeAg, HBeAb, and HBcAb were measured based on the protocol provided with the automated Cobas411e machine (Roche Diagnostics GmbH, Germany).

Statistical analysis: A comparative statistical analysis was performed using the statistical program SPSS version 20 for the variables. Descriptive statistics were calculated in the form of frequency with percentages for categorical variables. Associations between seropositivity and risk factors were estimated and tested by the chi-square test. P value less than 0.05 was considered significant. The ICT's sensitivity, specificity, and predictive values were also calculated.

Results:

In this study, 250 HCWs were recruited to participate. The study subjects were randomly selected but equally distributed (50 cases each) according to their profession: physicians, lab specialists, nurses, employees, and wash workers. Among the total, 41.2% (103/250) were males and 58.8% (147/250) were females. Table 1 showed that the largest group (38.8%) is between the ages of 26 and 35. Table 2 showed that 2.8% (7/250) were positive for HBsAg when using ELISA. 4.4% and 2.0% of all participants were positive for HBeAb and HBeAg, respectively. Moreover, 37.6% of participants tested positive for IgG HBcAb.

Table1. Demographic characteristics of 250 HCWs participated in this study (N=250).

Characteristics	Frequency (%)
Gender	
Male	103 (41.2%)
Female	147 (58.8%)
Total	250 (100%)
Age group (years)	
15-25	74 (29.6%)
26-35	97 (38.8%)
36-45	55 (22.0%)
46-55	21 (8.4%)
56 and more	3 (1.2%)
Total	250 (100%)

Table 2. Frequency and percentages of HBV serological markers among study subjects.

Serological Markers	Positive (%)	Negative (%)	Total
HBsAg (ELISA)	7 (2.8%)	243 (97.2%)	250 (100%)
HBeAb	11 (4.4%)	239 (95.6%)	250 (100%)
HBeAg	5 (2.0%)	245 (98.0%)	250 (100%)
HBcAb IgG	94 (37.6%)	156 (62.4%)	250 (100%)
HBcAb IgM	0 (0.0%)	250 (100%)	250 (100%)

Table 3. Percentage and frequency of HBV serological markers among HCWs (N= 250)

HCWs	Serological Markers	Positive (%)	Negative (%)
Physicians	HBsAg	0 (0%)	50 (100%)
	HBeAb	0 (0%)	50 (100%)
	HBeAg	0 (0%)	50 (100%)
	HBcAb IgG	5 (10%)	45 (90%)
	HBcAb IgM	0 (0%)	50 (100%)
Total		**5 (2.0)**	**245 (98.0)**
Lab. Specialist	HBsAg	0 (0%)	50 (100%)
	HBeAb	0 (0%)	50 (100%)
	HBeAg	0 (0%)	50 (100%)
	HBcAb IgG	12 (24%)	38 (76%)
	HBcAb IgM	0 (0%)	50 (100%)
Total		**12 (4.8)**	**238 (95.2)**
Nurse	HBsAg	1 (2%)	49 (98%)
	HBeAb	1 (2%)	49 (98%)
	HBeAg	1 (2%)	49 (98%)
	HBcAb IgG	20 (40%)	30 (60%)
	HBcAb IgM	0	50 (100%)
Total		**23 (9.2)**	**227 (90.8)**
Employers	HBsAg	4 (8%)	46 (92%)
	HBeAb	3 (6%)	47 (94%)
	HBeAg	0	50 (100%)
	HBcAb IgG	23 (46%)	27 (54%)
	HBcAb IgM	0	50 (100%)
Total		**30 (12.0)**	**220 (88.0)**
Wash-worker	HBsAg	2 (4%)	48 (96%)
	HBeAb	7 (14%)	43 (86%)
	HBeAg	4 (8%)	46 (92%)
	HBcAb IgG	34 (68%)	16 (32%)
	HBcAb IgM	0	50 (100%)
Total		**47 (18.8)**	**203 (81.2)**

Table 4: Association between HBsAg infection and possible risk factors.

Risk factor		HBsAg using ELISA		P value
		Positive (N=7)	**Negative (N=243)**	
Occupations	Physicians	0 (0.0%)	50 (20.6%)	0.03
	Lab Specialists	0 (0.0%)	50 (20.6%)	
	Nurse	1 (14.3%)	49 (20.2%)	
	Employers	4 (57.1%)	46 (18.9%)	
	Wash Workers	2 (28.6%)	48 (19.7%)	
Age groups/year	15-25	0 (0.0%)	74 (30.4%)	0.221
	26-35	4 (57.1%)	93 (38.2%)	
	36-45	2 (28.6%)	53 (21.8%)	
	46-55	1 (14.3%)	20 (8.3%)	
	56<	0 (0.0%)	3 (1.3%)	
Gender	Male	5 (71.4%)	98 (40.3%)	0.099
	Female	2 (28.6%)	145 (59.7%)	
Job duration/hours	3-6	1 (14.3%)	15 (6.2%)	0.299
	7-10	2 (28.6%)	166 (68.3%)	
	11-15	3 (42.8%)	40 (16.4%)	
	16-24	1 (14.3%)	22 (9.1%)	
Surgery exposure	Exposed	3 (42.9%)	73 (30.0%)	0.467
	Not exposed	4 (57.1%)	170 (70.0%)	
Blood transfusion	Yes	0 (0.0%)	23 (9.5%)	0.393
	No	7 (100%)	220 (90.5%)	
Sprocket took off	Exposed	4 (57.1%)	126 (51.9%)	0.782
	Not exposed	3 (42.9%)	117 (48.1%)	
Vaccination	Yes	0 (0.0%)	115 (47.3%)	0.013
	No	7 (100%)	128 (52.7%)	
Infected family members	Yes	0 (0.0%)	15 (6.2%)	0.498
	No	7 (100%)	228 (93.8%)	
Cigarette smoking	Yes	1 (14.3%)	23 (9.5%)	0.669
	No	6 (85.7%)	220 (90.5%)	

Discussion:

In this study, health-care workers (HCWs) are defined as those who have direct or indirect contact with patients and/or clinical samples while working at Wad Madani Teaching Hospital. This is the first study conducted in Wad Madani city to detect the hepatitis B virus among HCWs. The prevalence of HBsAg was 2.8% in the current study, while a higher prevalence (6%) was identified by Elmokashfi in 2012 in Al-Shaab Teaching Hospital in Khartoum State (13). This finding was almost identical to a study conducted in Nigeria last year, which found the prevalence of hepatitis B among HCWs to be 2.3% [8]. It was also found to be lower than the prevalence reported in Tanzania (5.7%) (16), Nigeria (17%) (17), Uganda (8.1%) [4], and Najran in Saudi Arabia (8.7%) (18). An exposure that might place HCWs at risk for infection may be a percutaneous injury, contact of the mucous membrane and nonintact skin with blood, tissue, or other body fluids that are potentially infectious (19). According to Bond and his colleague, at health care centers, when there are no regulations for safe practice during dealing with patients, daily hygiene, and medical waste, the wash workers and nurses will be at high risk for infections. The employers can also get infected by means of contaminated surfaces. HBV has been demonstrated to survive in dried blood at room temperature on environmental surfaces for weeks (20), and this may likely cause HCWs to be more vulnerable toward infection than the general population. HBV is more infectious than other blood-borne viral pathogens and is about 100 times more infectious than HIV (21). This infectiousness could be explained by its higher viral load in the blood, longer viability in the environment (more than 7 days at room temperature), and transmissibility in the absence of visible blood [21].HBsAg was found in 8% of employers and 4% of wash-workers. This finding is nearly in agreement with a study conducted in Addis Ababa (6.3%) (22), and this may be different from what was obtained by Shao and his colleagues in Tanzania, who detected that physicians were the leaders, followed by nurses and laboratory specialists (16). It was also reported by U.S. Public Health Service Guidelines that wash workers are more exposed to HBV infection than other HCW (21). Indirect inoculation through improperly collected and/or segregated sharp materials is considered an occupational hazard for wash workers (21, 23). The difference within HCWs might be explained by the different levels of both the risk of exposure to infectious materials and awareness of the safe practice of daily hygiene. The vaccination against HBV has had a significant impact on reducing the number of people infected with HBV. It remains the most important strategy for the protection of HCWs from HBV infection. Despite the fact that all HCWs should be vaccinated against HBV, only 47.3% of study participants were. The non-vaccinated worker may present themselves as a risk for HBV infection. Beside this study, several studies found that other factors such as job duration, surgery exposure, infected family members, and sprocket takeoff may not increase the risk of HBV infection (24, 25). The serologic markers of HBV are varied and complex. HBsAg is used as a routine diagnostic serological marker for HBV infection at Wad Madani Teaching Hospital [26]. And there are two reasons for using HBsAg and the ELISA technique: firstly, the ease, availability, and cost of the surface antigen test kit. Secondly, the cost, reliability, and confidence related to the ELISA technique are relevant to the area of study.The normal sequence of serological events in the course of hepatitis B infection is now well characterized. Signs of active virus replication, including HBsAg supported by the presence of HBeAg, were observed among the nurses and wash workers. They may transmit the infection to other workers, patients, and their family members. In contrast, none of the physicians and laboratory specialists presented the outcome of surface or envelope antigen; instead, a minority of them produces HBcAb-IgG only, which indicates previous infection or exposure to the hepatitis B virus in an undefined time frame.These findings highlight the need to continue improving the working environment of HCWs by providing hepatitis B vaccination and following hygienic policies in the hospital. The vaccination program for HCWs should continue to be implemented within the framework. It is mandatory to expand this study to include the remaining health care workers in order to identify carriers and chronic cases in the hospital and formulate evidence-based data for action. To reduce the prevalence of HBV among HCWs, a new strategy is needed to reduce occupational exposure to blood and body fluids by maintaining adequate personal protective equipment supported by regulations, and mandatory vaccination against hepatitis B is required for HCWs as they are among the risky groups in the community. In addition, continuous training on infection prevention procedures should be provided for all HCWs.

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Original Article Sudan Medical Laboratory Journal

The Utility of Serum Total and Placental Alkaline Phosphatase Activity as Predictive Markers for Preeclampsia among Sudanese Pregnant Women

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Abstract

Background: Serum total alkaline phosphatase (TALP) and placenta alkaline phosphatase (PALP) measurement can afford a simple, reliable and economical adjunctive laboratory parameter in hypertensive disorders of pregnancy.

Objective: To determine the utility of serum TALP and PALP as predictive markers for preeclampsia among Sudanese pregnant females.

Materials and Methods: Cross-sectional comparative hospital-based study conducted in Khartoum state atOmdurman Maternity Hospital from March to June 2022. Hundred Sudanese pregnant women age ranged between (20 to 45 years), 50 pregnant women with preeclampsia and 50 normal pregnant women as control group. The serum TALP, PALP were measured using full automated analyzer (response® 910), while serum urea, uric acid by enzymatic method and creatinine by chemical method. The data obtained was analyzed by using SPSS version (26).

Results: Preeclamptic women showed significant increase in means of serum uric acid (7.4± 2.3 mg/dL VS 3.6± 1.0 mg/dL), urea (33.8± 4.0 mg/dL Vs 17.5 ± 6.2 mg/dL), PALP (151.5± 76.1IU/L VS 111.1±65.4 IU/L, P-value < 0.05) and the ratio of PALP/TALP (0.66 ±0.2 IU/L VS 0.52±0.1 IU/L, P-value< 0.05) compared to normal pregnant women. The Receiver operating characteristics curve (ROC) showed optimum cut-off value of PALP >133 IU/L and ratio >0.589 as predictor of preeclampsia among Sudanese pregnant women.

Conclusion: Sudanese women with preeclampsia had an elevated serum uric acid, urea, PALP and ratio of PALP/TALP. Moreover, PALP and ratio of PALP/TALP can be used as predictive marker of preeclampsia in pregnancy.

Key words: Preeclampsia, Total ALP, PALP, urea, uric acid

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Introduction

Preeclampsia is a complex and serious multi-system disorder of human pregnancy with a worldwide incidence of 5–7% and contributes significantly to maternal and perinatal morbidity and mortality (1). It is characterized by hypertension, proteinuria, and a generalized systemic vasoconstriction arising from circulatory disturbances secondary to a generalized endothelial dysfunction caused by inflammation (2). It is associated with defective endovascular trophoblastic invasion and insufficient remodeling of the uterine spiral arteries (3,4) Altered renal function is an essential component of the pathophysiological process in pre-eclampsia and close monitoring of renal function is important to ensure a timely delivery before serious renal damage occurs.

Several Alkaline phosphatase (ALP) isoenzymes are elaborated in human tissues including placental isoform and as independent genetic loci (5,6). The existence of four human isoenzymes, each encoded by independent genetic loci, has been well documented and includes an intestinal isoenzyme, localized in the brush border of the mucus membrane. A second isoenzyme, derived from placental syncytiotrophoblasts, appears in the second trimester. Placental like ALP (tissue nonspecific), includes enzymes originating from bone, liver, lung, and leukocytes (1).The primary source of human placental Alkaline Phosphatase (PLAP) is the syncytiotrophoblastic plasma membranes, during the second & third trimesters of pregnancy. As gestation progresses, the concentration of PLAP rises till term and this can be caused by the detachment of ALP from the membrane into the maternal circulation (7,8). In normal pregnant women it rises to a level 2-3 times higher than that of non-pregnant women and do possess a long half-life (seven days postpartum) (9). The placental-type human ALP, which often occurs in human non-trophoblastic tumors, has been considered a marker for malignant transformation. Yet, the appearance of this heat-stable enzyme is not universal for human tumors, many of which may produce heat-labile ALP enzymes (10). The objective of present study was to determine the utility of serum TALP and PALP as predictive markers for preeclampsia among Sudanese pregnant females**.**

Materials and Methods

Study population: The study was a hospital-based cross sectional comparative study; carried out after obtaining an ethical clearance from Sudan university of Science and Technology ethical committee and informed consent from each study subject. The study was carried out in the Omdurman Maternity Hospital from March to June 2022. 100 pregnant women (50 hypertensive women and 50 normotensive women) were recruited into the study. Inclusion criteria for preeclamptic: pregnant women with a blood pressure of ≥ 140/90 mmHg, which included patients in all the category of HDP according to the National High Blood Pressure Education Program classification (11).

Exclusion and Inclusion criteria: Inclusion criteria for comparative group: 50 healthy pregnant women with no history of pregnancy related complications, diabetes mellitus or any other chronic medical illness. The age of the study group was between 18 and 35 years and their gestational ages were between 36 and 40 weeks (peak PLAP rise is between 34 and 40 week of gestation). Pregnant women with diabetes mellitus, jaundice, chronic liver disorders, anemia and other pregnancy associated disorder like gestational diabetes, complicated pregnancies (vaginal bleeding after 28-week, fetal distress and congenital abnormalities) were excluded.

Blood sample and data collection: Five ml of blood sample (from both normal and preeclamptic pregnant women) was collected in a plain vacutainer. The individual samples were centrifuged at 1200 rpm for 10 min and the separated serum was used for the analysis of total alkaline phosphatase (TALP), placental alkaline phosphatase - (PLAP), creatinine and uric acid. The biochemical assays were carried out using procedures approved by the IFCC. The analyses were essentially carried out on the same day within four hours so as to minimize the inactivation of ALP by denaturation. Alkaline phosphatase (12) and uric acid (13) in serum was estimated based on a spectrophotometric method by a fully automated random access chemistry analyzer. The initial values obtained without heat inactivation pertained to the serum total ALP activity. Serum PLAP (heat stable fraction of ALP) was measured by the thermal inactivation method (14). 0.5 ml of sera samples were added into small thin-walled glass tubes placed in thermostatically controlled water bath stabilized at 65^0C. The water level was at least 3 cm above the samples. Exactly following 30 minutes, the serum tubes were rapidly removed and placed in an ice bath for 3 min before returning it to room temperature. The ALP activities of the processed samples were determined similar to that of total ALP and this represented the heat stable fraction of ALP (PLAP).

Statistical analysis: The data was analyzed using the SPSS software, version 26. The values were expressed as mean and standard deviation. The independent Students t-test was performed to compare the mean values in hypertensive and normotensive pregnant women. Pearson's correlation analysis was performed to determine the association between various test parameters. A p-value of < 0.05 was considered to be significant. Receiver operating curves (ROCs) were drawn to elicit the optimum sensitivity, specificity and cut-off values.

Results

One hundred Sudanese pregnant women were included in this study. 50% (n=50) with hypertension disorders of pregnancy and 50% (n=50) were normotensive pregnant women. Their gestation age between 34 and 40 weeks of gestation. Study revealed that there was significant increase in mean of serum PALP, uric acid levels and PALP/TALP ratio in pregnant women with hypertension disorder of pregnancy when compared with counterparts. However; there were insignificant difference in the level of serum creatinine and TALP activity among women with hypertension disorder of pregnancy when compared with normotensive pregnant women (Table1).

In Spearman correlation study revealed that PALP/TALP ratio correlated positively with serum creatinine (Table 2). The Receiver operating characteristic curve (ROC) of PLAP/ALP ratio showed an optimum cut-off at 0.589 with 66% sensitivity and 64% specificity, with a significant area under the curve (AUC=0.695). The PLAP showed an optimum cut-off at 133 with 56% sensitivity and 72% specificity, with a significant area under the curve (AUC = 0.659) (Figure 1).

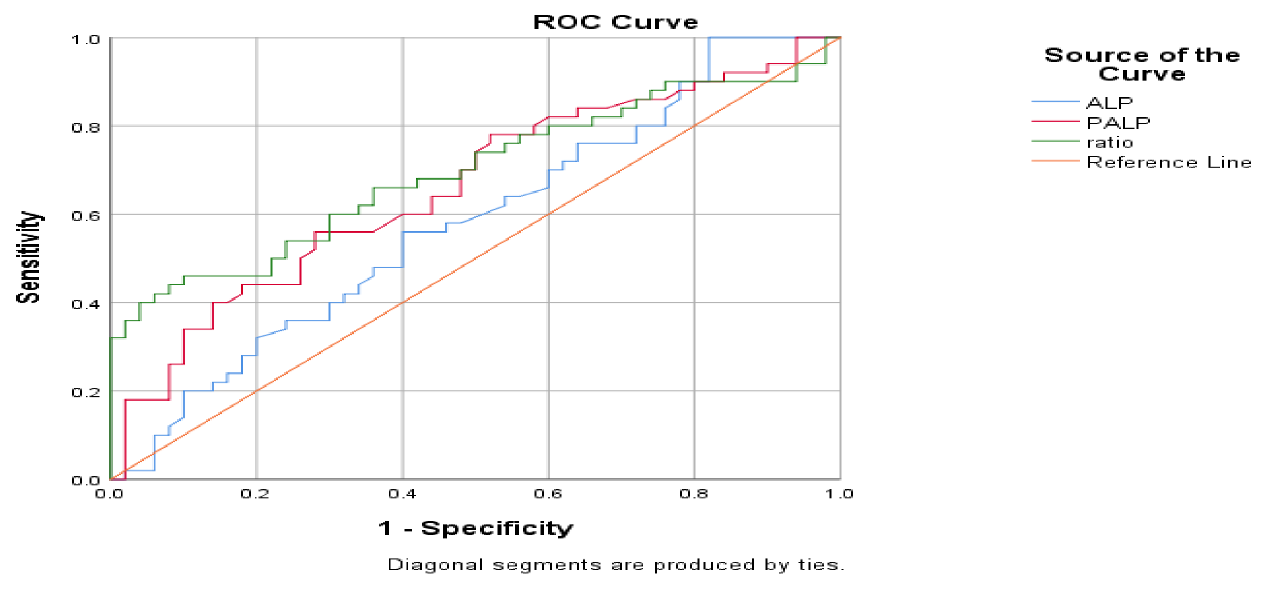
Table 1: Comparison of age, SBP, DBP, urea, creatinine, total and placental alkaline phosphatase in preeclamptic and normal pregnant women.

Parameters	preeclamptic (n =50)	normal pregnant (n= 50)	P- value
Age (year)	27.6 ±7.6	27.6 ±5.2	0.987
SBP mmHg	163.7 ±27.9	108.3 ±10.4	0.000
DSP mmHg	104.2 ±17.8	74.8 ±7.7	0.000
Urea (mg/dL)	33.8 ±4.0	17.5 ±6.2	0.008*
Creatinine(mg/dL)	1.5 ±0.3	0.6 ± 0.1	0.076
Uric acid (mg/dL)	7.4 ±2.3	3.6 ±1.0	0.000*
TALP IU/L	221.8 ± 76.4	204.06 ± 93.2	0.300
PALP (IU/L)	151.5 ± 76.1	111.1 ± 65.4	0.005 *
PALP/TALP ratio	0.66 ± 0.2	0.52 ± 0.1	0.000*

Independent sample t-test was used to compare between means.
P-value considered significant at 0.05.

Table 2: Spearman correlation between age blood pressure urea and creatinine with TALP, PALP and ratio among women with hypertension disorder of pregnancy.

		Age	SBP	DBP	Urea	creatinine	UA
ALP	**r**	-0.131	0.029	0.019	0.119	0.073	0.231
	P	0.363	0.840	0.898	0.426	0.623	0.122
PALP	**r**	-0.108	0.039	0.048	0.139	0.220	0.061
	P	0.456	0.788	0.742	0.120	0.137	0.686
PALP/ALP ratio	**r**	-0.096	0.042	0.087	0.218	0.330	0.216
	P	0.505	0.773	0.550	0.141	0.024*	0.184



Variables	95%: Confidence Interval	AUC	P- value	% Sensitivity	% Specificity	Cut-off
ALP IU/L	0.470 - 0.694	0.582	0.159	56	60	212.0
PALP IU/L	0.552- 0.766	0.659	0.006	56	72	133.0
Ratio PALP/ALP	0.591-0.800	0.695	0.001	66	64	0.589

Figure 1: Receiver operating characteristics curve of total, placental ALP and ratio.

Discussion

The present study showed significant increase of serum PALP in preeclamptic pregnant women when compared to normal pregnant group. This finding agreed with study done by Mishra and his colleagues which found that about 2-fold increased ALP in hypertensive pregnant indicative of placental dysfunction and may be a warning to impending fetal health ***(15).*** Moreover; study by Hutchinson and his group found that the elevated levels of serum PALP in hypertensive pregnant women may be attributed to placental dysfunction, which results in increased serum levels of these enzymes. Shedding of syncytiotrophoblast into the maternal circulation is a normal part of pregnancy, but is increased during pre-eclampsia. In pre-eclampsia, this process of syncytio-trophoblast renewal is overactive and complicated by necrosis and apo necrosis of the syncytio-trophoblast particles (***16).*** Also, study showed significant increase in ratio of serum PALP/ALP in case compared to control group. This finding in agreement with study done by Rajagambeeram and his colleagues which found that the ratio of PLAP/ALP was significantly higher in hypertensive disorders of pregnancy with p-value of <0.001.(***17).*** The other aspect in this study is raised serum uric acid level in hypertensive pregnant women to normotensive pregnant group. Bainbridge and Roberts suggested that hyperuricemia in hypertensive pregnant women is multifactorial (18). In hypertensive pregnant women, elevated levels of uric acid are not only attributed to decreased renal excretion but also to increased oxidative stress resulting from placental ischemia and increased activity of xanthine oxidase enzyme (19). Uric acid is a potent inhibitor of endothelial function, found to induce systemic and glomerular hypertension in animals (20). Also, increased tubular reabsorption and decreased tubular secretion of uric acid in tubules or diminished renal blood flow, similar to the physiologic response to hypovolemia may be the cause for hyperuricemia. Serum creatinine is widely used as an indicator for GFR. Our study revealed that there was a statistically insignificant difference in the mean values of serum creatinine level in hypertensive pregnant women to normotensive pregnant group; these was consistent with the previous study (21). Moreover; study pointed that PLAP/ALP ratio with an optimum cut-off at 0.589 and PLAP with an optimum cut-off at 133 IU/L have diagnostic value in preeclampsia. Rajagambeeram and his group showed a better and practical approach towards the assessment of hypertensive disorders of pregnancy would be to utilize heat stable ALP isoenzyme (PALP) and PALP/ALP ratio as an adjuvant marker in the armamentarium of biochemical tests, especially since the same is simple to assay and reliable as well as economical and sensitive (17).However, the future outcome of preeclamptic pregnant women, which could have provided more information regarding both maternal and fetal outcome could be the scope of future studies.

Our finding considered first study among preeclamptic Sudanese women who assess the cut-off value of PALP and TALP/PALP ratio. However, the limitation of this study is that the outcome of preeclampsia and it is impact on both maternal and fetus was not observed.

Conclusion

The study concluded that Sudanese women with preeclampsia had elevated serum uric acid, PALP and ratio of PALP/TALP. Moreover, PALP and ratio of PALP/TALP can be used as predictive markers of preeclampsia in pregnancy.

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Original Article Sudan Medical Laboratory Journal

The Effects of Different Drugs Regimens in the Disease Activity Index and Uric Acid Levels of Sudanese Patients with Rheumatoid Arthritis.

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Abstract.

Background: In Sudan, rheumatoid arthritis is treated by steroids and disease-modifying anti-rheumatic drugs (DMARDs). The typically used DMARDs include methotrexate, hydroxychloroquine (HCQ), sulfasalazine, and leflunomide.
Objective: In this study, authors assess the different drug regimens used in Sudan and their role in affecting the clinical disease activity index (CDAI) and serum uric acid (UA) of the patients.
Methodology: This was a comparative analytical case control study enrolling 192 participants, (96) rheumatoid patients as cases and (96) non- rheumatoid as controls. Clinical disease activity index (CDAI) was performed to the patients, and venous blood samples, analyzed by automated spectrometry, were taken to measure the two groups uric acid. Results were analyzed using Microsoft Excel 2013. T-test was performed to assess the difference between two numerical means, and Analysis of variance (Anova) was used to assess the difference between three means or more. For categorical data, Chi-square test was used.
Results: Only four patients were males (5%0) and the remaining 95% were females. Mean UA in males was 5.6 while in females it was 4.45, and the difference was significant (P=0.047).The difference between genders concerning the CDAI grading was insignificant (P=0.51). Lefulonodmide gave the highest rate of CDAI remission (67%) and the lowest serum UA mean (4.26.), while HCQ gave the least percent of severe-high CDAI score (20%). The last mentioned results were all statistically insignificant (P>0.05).
Conclusion: CDAI score was not correlated to specific drug regimen in Sudan. Further studies are needed to assess the drug regimens effect in CDAI levels in Sudan.

Key Words: Rheumatoid arthritis, Uric acid, CDAI, Sudanese, DMARDs.
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Introduction.

Inflammatory arthritis and extra-articular involvement are hallmarks of the systemic autoimmune illness rheumatoid arthritis (RA). It is a chronic inflammatory condition that predominantly affects synovial joints and has an unclear cause (1). It often begins in small peripheral joints, frequently symmetric, and proceeds to affect proximal joints if left untreated (1). Joint deterioration caused by cartilage and bone loss over time because of joint inflammation. Early RA is described as having symptoms that have been present for less than six months, while established RA is defined as having symptoms that have been present for more than six months (2).About 0.24% of world population have RA, and, until now, there is no known cure for this disease[ ]. First-line therapy, which consists of the two classes of non-steroidal anti-inflammatory medications (NSAIDs) and corticosteroids, aims to reduce inflammation and alleviate pain (2). NSAIDs are fast-acting and include acetylsalicylate (Aspirin), naproxen, ibuprofen, and etodolac.NSAIDs actthroughthe inhibition of cyclooxygenase pathway,hindering prostagla- ndins, prostacyclin, and thromboxanes formation. Corticosteroids are a more potent anti-inflammatory medication than NSAIDs, but they come with greater side effects. For this reason, they are only indicated for a short period of time at low doses, during exacerbations or flares of RA (2)

Today, the standard-second line- of care is early treatment with disease-modifying anti-rheumatic drugs (DMARDs). The drugs typically used in treating RA include methotrexate, hydroxychloroquine (HCQ), sulfasalazine, and leflunomide (1).

Leflunomide is an oral medication that is converted to malononitrilamide, which inhibits the synthesis of ribonucleotideuridine monophosphat pyrimidine (2). A study by Choe and Kim published in 2015 mentioned that leflunomide reduced serum uric acid concentrations through increased urinary excretion of uric acid, which might not reflect changes in disease activity status in RA. This implies that uric acid may not influence systemic inflammation in RA (2).

In Sudan, the commonly prescribed drug regimens are steroid alone,steroid with a combination with other drug (mainly a DMARD), HCQ, Methotrexate, and, recently Lefulonomide. The long-term effect of these drugs in disease progression and patients' activity still not reported in literature. In this study, authors meant to assess the different drug regimens used in Sudan and their role in affecting the clinical disease activity index CDAI of the patients. Serum uric acid also was measured, concerning different combination of drugs.

Materials and Methods.

This study was conducted at two referred outpatient (ROP) clinics at Khartoum-Sudan. Cases that met the1987 revised criteria of American college of rheumatology (ACR) for rheumatoid arthritis enrolled as cases and non-rheumatoid patients as control subjects. The patients' sample size was120 patients.100 patients had been enrolled in the study after 20 quit due to financial limitations. Only 96 patients continue because three samples were missed and one sample was distorted, then 96 control subjects matched according to age and sex with the 96 cases and the total number was 192 participants including (cases and controls). 2ml of venous blood sample was withdrawn from cases and control subjects under aseptic conditions. The sample underwent centrifugation and then analyzed and calculated by automated spectrometry at the central lab of Omdurman Military Hospital.

Clinical disease activity index (CDAI) to assess the disease activity that depends mainly on clinical presentation (symptoms and signs). A recoded pre tested questionnaire and a written consent for data collection filled from the participants by the research doctor. The study results analyzed by Microsoft Excel 2013. Data were presented in the results section in tables and figures: Table 1 exhibits general descriptive data and the significance level as P value is considered significant at 0.05 or less. Figures represents mean levels of specific parameters with p value highlighted. T-test was performed when in need to assess the difference between two means, and Analysis of variance (Anova) was used to assess the difference between two means. For categorical data, Chi-square test was used to determine the P value.

Ethical clearance obtained from IRB of Sudan Medical Specialization Board.

Results.

General descriptive data:

In this study, 7% of the patients were in the age between 21 and 40, 52% were between 41 and 60, 33% were between 61 and 80, and 8% were above 80 years of age. Only four patients were males (5%0) and the remaining 95% were females. Mean UA in males was 5.6 while in females it was 4.45, and the difference was significant (P=0.047). The difference between genders concerning the CDAI grading was insignificant (P=0.51). Figure 1.

Nine patients were using Steroid alone, while 65 were using steroid with other drug combination. Four patients used methotrexate alone and four with combination. Four patients used to take Lefulonomide and 14 in combination with other drugs, and seven patient were receiving HCQ alone for RA management, one patient was on Sulphaslalzine. Considering Patients on Steroids only, none of the patients (0%) was on remission, three (33.4%) scored a low CDAI, one (11.1%) scored moderate, and five (55.6%) had a high CDAI. In the patients who use Steroid combined with other drugs, Six (9.2%) were on remission CDAI, 11(16.5%) had low CDAI, 31(47.7%) had moderate CDAI, and 17(26.2%) had high CDAI.

In the Patients group using HCQ, Three (30%) had CDAI remission, 1(10%) scored low CDAI, 4(40%) had a moderate CDAI, and two (20%) were with a high CDAI.

In the patients group that uses Methotrexate, One (12.5%) had remission, 3(37.5%) scored a low CDAI, 1(12.5%) had a moderate CDAI and three (37.5%) were with a high CDAI.

In patients group using Lefulonomide only, two patients (67%) were on remission, and one (33%) has a high CDAI, and in the Lefulonomide using group in combination with steroid, one patient (6%) was on remission while eight (50%) had a high CDAI. There were five (31%) having a moderate CDAI, and two (13%) with a low CDAI. However, the P value of the difference between groups in CDAI grade was insignificant (P=0.1).

Different drug regimen and their effects in serum Uric Acid: Mean Serum UA in the patient using Lefulonomide was the lowest compared with other groups: 4.26±1.5mg/dl. UA was 4.54±1.17 mg/dl in the steroid using patients, 4.74±1.11mg/dl in the patient using methotrexate, and 4.6±1.7 mg/dl for patient on HCQ, and the overall mean UA was 4.51±1.26 mg/dl. The result, however, was statistically insignificant (P=0.58); table 1.

The differences between UA levels in the groups: steroid alone and in combinations, Methotrxate alone and in combination, and Lefulonomide alone and in combination, was not significant (P values=0.91; 0.84, and 0.33, respectively). Figure (3).

Table 1: Descriptive statistics of serum UA overall and in the different patients 'groups.

Variable/UA	*Overall*	*Steroids*	*Meth.*	*HCQ*	*Lef.*
Mean	4.51	4.54	4.74	4.6	4.26
Median	4.35	4.4	4.45	4.9	3.5
Mode	3.3	3.3	#N/A	N/A	3.5
Standard Deviation	1.260867	1.169179	1.110904	1.731088	1.541938
Range	5.1	5.1	3.1	4.8	4.8
Minimum	2.6	2.6	3.6	2.6	2.6
Maximum	7.7	7.7	6.7	7.4	7.4
Count	96	63	8	7	17
Confidence Level (95.0%)	0.255475	0.294454	0.928739	1.60099	0.792791
P value of the difference	0.58				

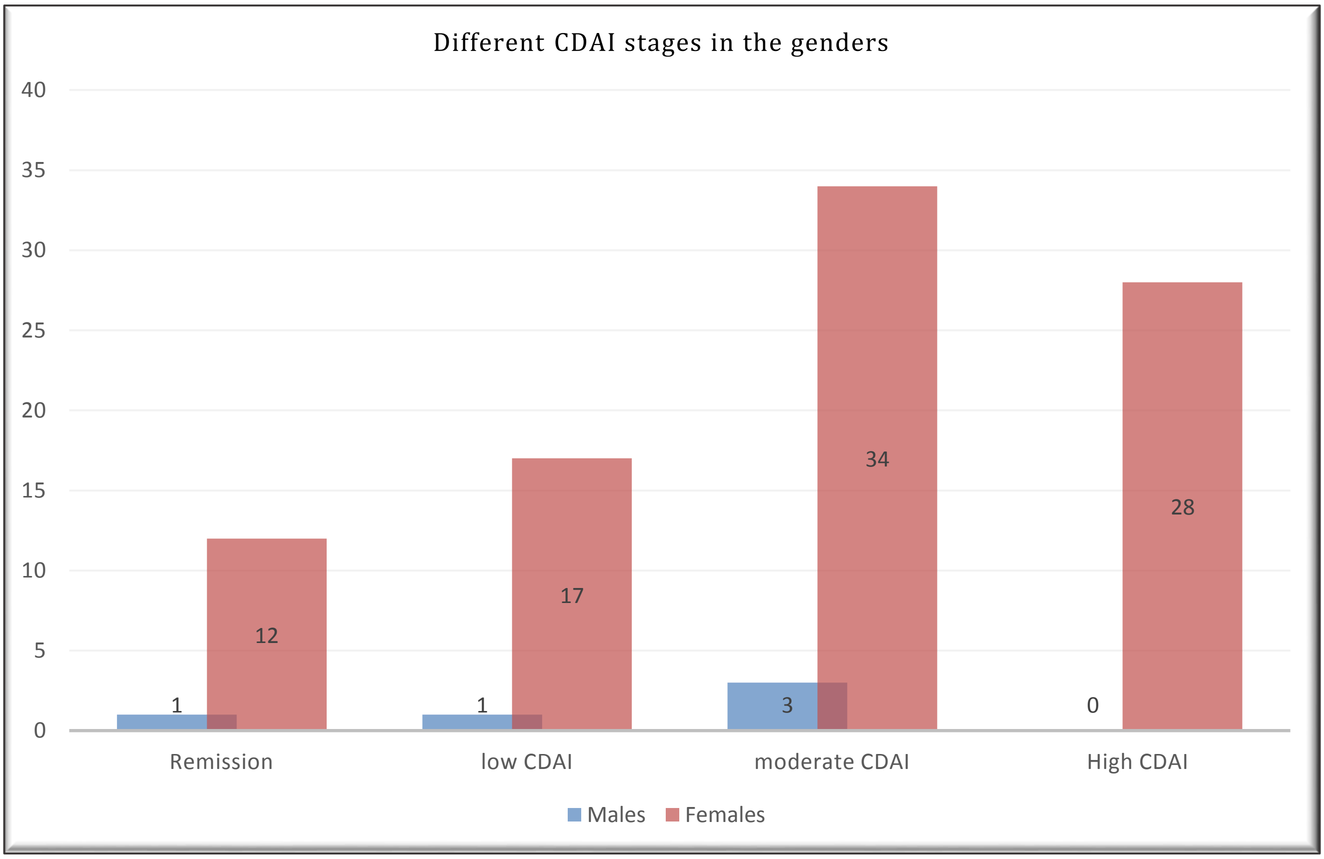

Figure 1: Difference in CDAI scores between male and female patients (P=0.047).

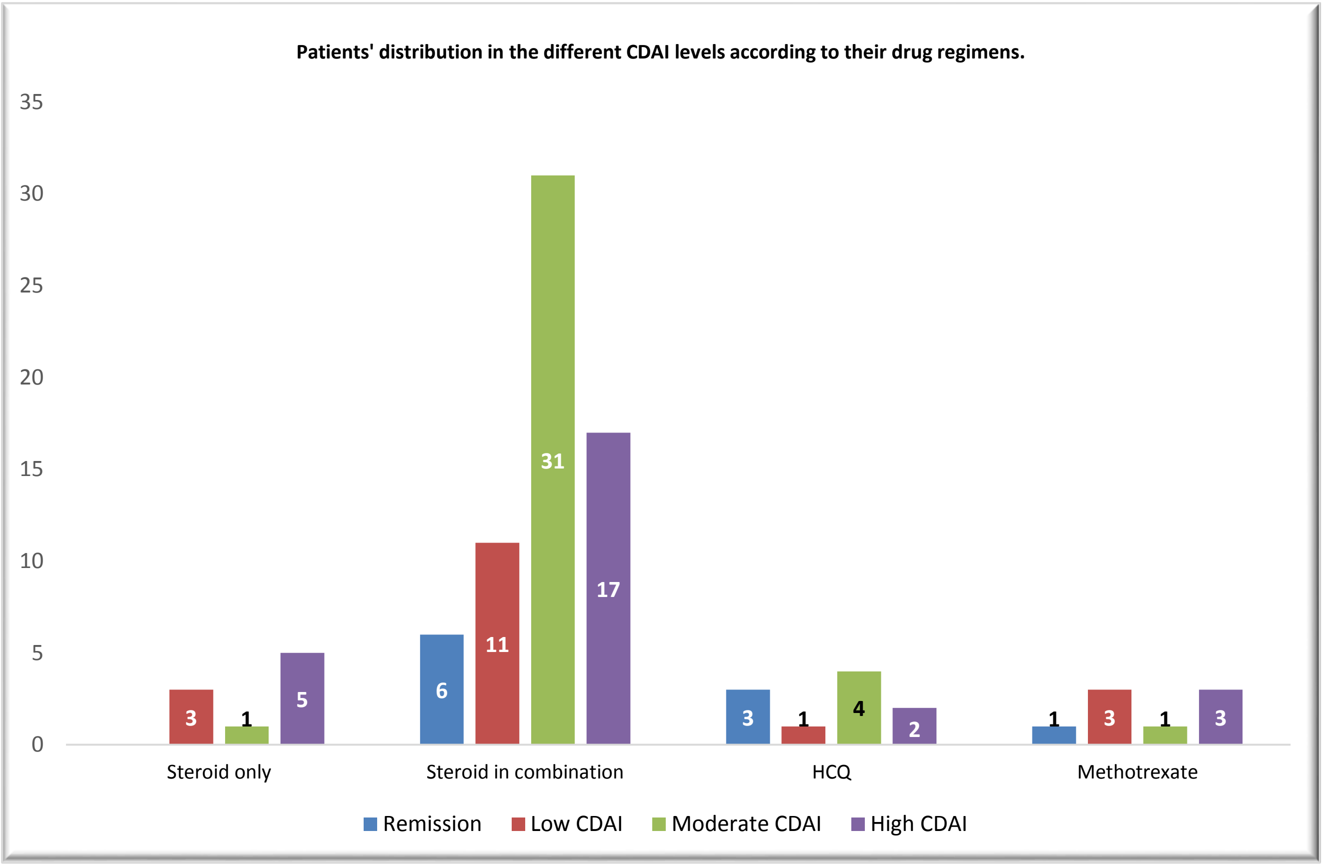

Figure 2: CDAI score for major group classes. Numbers of patients only exhibited.

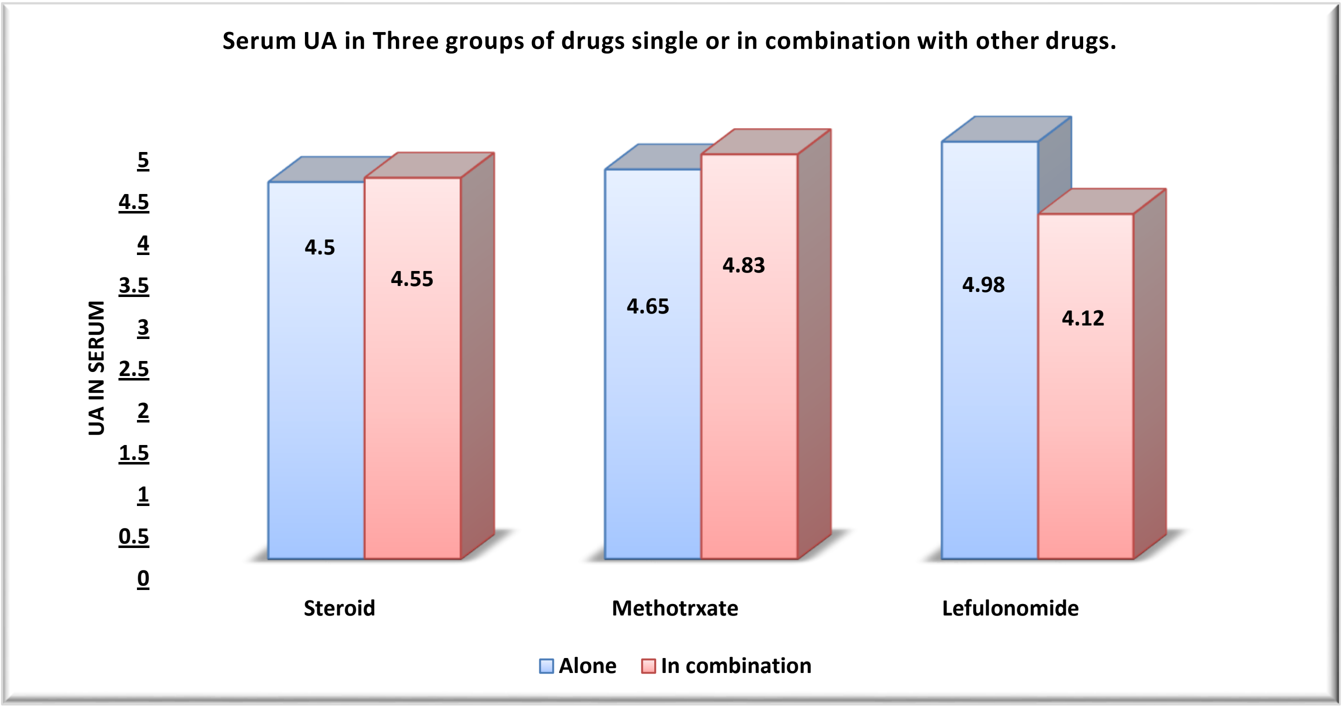

Figure 3: comparing serum UA levels in three main drugs; Steroids, Methotrexaye, and lefulonomide, either alone or combined with other drugs. Diffrence was insignificant.

Discussion.

CDAI is a composite index for quantifying disease activity in RA. It has been developed from the disease activity index DAS, and it is preferable in the account that it does not require any laboratory assessment (4). CDAI utilizes four clinical parameters namely, swollen and tender joints- out of 28 joint, global assessment of the patient and assessor on a visual analogue scale (5).The CDAI is the only composite index that does not incorporate an acute phase response and can therefore be used to conduct a disease activity evaluation essentially anytime and anywhere (6). It is one of five measures that has been recommended by the American college of rheumatology, the others are: 28-joint Disease Activity Score (DAS28-ESR/CRP), Simplified Disease Activity Index (SDAI), Routine Assessment of Patient Index Data 3 (RAPID3), and Patient Activity Scale-II (PAS-II) (7). In CDAI, the lower the value, the better is the prognosis. Two disadvantages of CDAI is the non-involvement of the ankle and feet in assessment, and the absence of investigation. The score interpretation adopted in our research was: ≤2.8 as a remission; >2.8 and ≤10 as a low Disease Activity; >10 and ≤22 as a moderate Disease Activity; and >22 as a high Disease Activity. In this current study, the use of steroid in combination, particularly with HCQ, gave higher remission rate and lower percent of patients were in high index than those using steroid only. In the eight patients taking Methotrexate, one (12.5%) was on remission and three (37.5%) were with a high CDAI, and the remaining three groups were inferior when compared to HCQ group. As mentioned by Choe and Kim in 2015, Lefulonomide has a potential to lower serum UA. This may attribute to the reduction of inflammatory status and hence improvement of CDAI: an explanation that Choe and Kim had rejected (4). In our current study, Lefulonomide caused lowering in serum UA in the patients only when used in combination with Steroid or HCQ, and the result was statistically insignificant. See figure 3.

In Kirwan et al review of 2005, all studies except one showed a numerical treatment effect in favor of steroids. Their beneficial effects were generally achieved when used in conjunction with other DMARD treatment. Steroids given in addition to standard therapy can substantially reduce the rate of erosion progression in rheumatoid arthritis is convincing. There remains concern about potential long-term adverse reactions to glucocorticoid therapy, such as increased cardiovascular risk, and this issue requires further research (8), and, as remarked by Gotzsche and Johansen, the steroid Prednisolone in low doses (not exceeding 15 mg daily) may be used intermittently in patients with rheumatoid arthritis, particularly if the disease cannot be controlled by other means (9). As seen in our current study, Steroids in combination gave a better results, putting more patients in remission and at the same time, less percent was in the high disease index (see the results text and figure 2).

In 2014, Smolen et al classified DMARDs into Biological and Synthetic, the former is sub classified into original biological or bio-similar, while the synthetic is further r divided into conventional or targeted synthetic (10). In our current study, there were only conventional synthetic DMARDs as they are the common prescriptions in Sudan.

Methotrexate is a well-known DMARD and very common to be used in a combination for the management of RA. According to 2015 NICE (11) and the 2015 American College of Rheumatology guidelines (12), Methotrexate is the most important and useful DMARD and is usually the first treatment. In this study, one patient using methotrexate was on remission and three had a high CDAI score. Paradoxically, three of the four patients on methotrexate alone had a mild disease CDAI, and three of the four patients using Methotrxate in combination with HCQ had a high CDAI index. This opposes the results obtained by Schapink et al in 2019, where Methotrxate–HCQ combination proved to be more effective after 6 months than MTX monotherapy in early RA patients, however, there was no significant difference between the two groups after one year (13). The previous study have the upper hand when compared with our own study, as there were more than thirty folds the sample size for Methotrxate and HCQ (325 patients), and the samples were re-taken twice.

In our study, 67% of the patients using Lefulonomide alone were on remission, and 33%had a high CDAI. While in the patients using Lefulonomide in combination with steroids, one patient (6%) was on remission while eight (50%) had a high CDAI. There were five (31%) having a moderate CDAI, and two (13%) with a low CDAI. The result, however, lacks the presence of an adequate sample size, as the former group contained only 3 patients and the later contained 19 patients.

Although the differences in mean serum UA levels between the groups using different drug regimens was insignificant, some attention should be paid to the Lefulonomide group that recorded the lowest mean UA, as the result agrees with previous studies(4, 14)

Study limitations:

1. The small sample size with inadequate distribution ofmedication, as most of the patients-two thirds-are using steroids.
2. The study design, which is a cross sectional while in this case, a prospective randomized controlled trial is more suitable.
3. In addition, the patients’ serologies were not determined.

Conclusion.

This study was executed to measure two variables in association with the common drug described for RA in Sudan: the uric acid levels, and the clinical disease activity index. The drug regimens were classified into five major groups: Steroids, Hydroxychloroquine, Lefulonomide, and Methotrexate. The overall result was Lefulono-dmide gave the highest rate of remission and the lowest serum UA mean, while HCQ gave the least percent of severe CDAI. There must be further studies for drug effects in these variables.

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Original Article Sudan Medical Laboratory Journal

Assessment of bone profile and Liver Enzymes among Triple negative versus Hormone Receptor Positive Sudanese Breast Cancer Patients

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Abstract:

Background: Some serum biochemical parameters may be used as prognostic markers of breast cancer; these are studied broadly, but not studied yet in Sudan.

Objective: In this study we aimed to assess calcium, phosphate, Alkaline phosphatase (ALP), Gamma glutamyle trans peptidase (GGT), and Lactate dehydrogenase (LDH) among hormone breast cancer subtypes.

Methods: In a comparative cross‑sectional 178 BC patients, ages ranged from 28 to 95 were randomly selected. Bone minerals and liver enzymes were measured using Mindray-BS-480 Clinical Chemistry Analyzer.

Results: In total, 30.3% were triple negative, were 69.7 % hormone receptor positive, and52.8 % out of total was late stage. T. test analyses show significant difference in GGT between triple negative and hormone receptor positive also between late stage and early stage (P = 0.017 and 0.016, and 0.018), respectively.

Conclusion: Hormone receptor positive and late stage BC patients have increased GGT activity.

Key words: Liver enzymes, Bone, Triple negative, Hormone, Receptor

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Introduction

Breast cancer (BC) is the most prevalent malignancy and a significant factor in both morbidity and mortality among women. More than 90% of mortalities are due to metastasis(1). Since Sudan lacks a population-based national cancer registry, hospital case series are typically used to estimate cancer rates in Sudan (2). According to data from the Khartoum State Malignancy Registry for the years 2009 to 2010, BC was the most prevalent cancer among Sudanese women. According to data from the National Cancer Institute—University of Gezira (NCI-UG), 34% of female cancer patients in 2017 had BC (2). In spite of 20% to 30% of people experience its recurrence in distant locations, researchers observed that, the death rate was decreased during the past ten years, which attributed to the early detection and intervention (3).

BC molecular subtypes include: (luminal A, luminal B, Human epidermal growth factor receptor 2 [HER2]-enriched and basal-like) based on the status of estrogen receptor (ER), progesterone receptor (PR), and HER2 (3, 4). Besides their different ability to metastasize to distant organs, these subtypes possess different pathways to the preferred metastatic sites, as well as differing survival responses after relapse (5). Several factors can increase the likelihood of developing a metastatic breast cancer MBC, including age, race, ethnicity, endogenous hormones, menopause, smoking, first-degree relatives, number of metastatic sites, length of breastfeeding, mutations, and the grade and size of the initial tumor (6).

Bone is the most frequent location for both metastasis and the first distant recurrence (3,7,8). The three most typical signs of bone metastases are severe osteopenia, pathologic fractures, and nerve compression. However patients continue to present with no clinical symptoms and miss detection at the diagnosis, therefore the bone metastasis associated with worse outcomes and lower quality of life (9, 10).

Furthermore, the rate-limiting factor for patient survival is hepatic metastases, which involve in about half of all cases (11).The early detection of liver metastases is essential for prompt treatment to improves outcomes. Fine-needle aspiration cytology (FNAC) of hepatic lesions has become a common diagnostic tool (11). Serological examination is used to monitor metastatic disease during treatment with low sensitivity and specificity. Liver function test that includes estimation of enzymes activity, showed poor results in 92% most cases, the glutamyl transferase (GGT) and alkaline phosphatase (ALP) are exhibit higher levels (11). The alteration in the levels of serum bone minerals is also noticed in BC patients. The hypercalcemia in BC has been attributed in part to osteolytic bone metastases (12). This study hypothesis that, bone minerals (Calcium & Phosphate) and liver enzymes {alanine aminotransferase ALT), aspartate aminotransferase (AST), gamma-glutamyl transpeptidase (GGT), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH)} are useful for prognosis and screening of bone and liver metastasis in breast cancer patients.

Study populations

Eighty-ninediagnosed adult female patients with breast cancer who were presented to Radiation and Isotope centre at Khartoum (RICK) a period of consecutive 24 months from December 2018 till December 2020 were recruited into this cross-sectional study after informed consent. The clinco-pathological data (stage, number of treatment doses and hormone receptor status) and demographic data (age, BMI and marital status) were recorded from the pathology reports of the patients. Patients diagnosed with bone disease, asthma, autoimmune disease and other types of cancer were excluded, all participants were candidates for chemotherapy with or without adjuvant. The study populations were classified in to groups according to hormone receptor status (Triple negative, hormone receptor positive group) and stage (Early, Late group). These groups were age and BMI matched.

Ethics Approval: The study protocol was approved by the Scientific Research Committee and Institutional Review Board (AL-Neelain University) IRB serial No: NU-IRB-17-10-10-106 and National Health Research Ethics Committee – Sudan. All participants were informed by the aim of the study.

Methods

Under aseptic condition 2.5 ml blood was withdrawn on lithium heparin container, plasma was obtained after centrifugation at 3000 rpm and kept at -20°.

Measurement of serum parameters: Parameters were measured using Mindray-BS-480 Clinical Chemistry Analyzer-Shenzhen Mindray Bio-Medical Electronics Co., Ltd.

Statistical analysis: Data analysed using Statistical Package for Social Sciences (SPSS Inc, ver. 23, IL-Chicago- USA). The descriptive results were presented as percentages mean ± SD. Independent t.test was employed to compare between two groups. *P value* of ≤0.05 was considered significant.

Results

One hundred and seventy-eight breast cancer female's patients; mean age 48 (range; 28 - 95) years, were included in this study. The descriptive results showed that the percentage of triple negative is30.3% in Sudanese BC while the hormone receptors positive is 69.7%; and the early stage was 47.2 % while 52.8 % was late stage(**Table 1**).The serum level in tables 2, 3 and 4 were analyzed using independent sample *t*-test. GGT was significant different between hormone receptor groups and Stage groups (*P value* 0.017, 0.016, and 0.018) (table 2, 3 and 4). While there was no significant difference in Ca, Pi, ALP, and LDH (*P value* 0.518, 0.347, 0.077, and 0.300) between hormone receptor groups (**Table 2**); also Ca, Pi, ALP, and LDH showed no significant difference between stage groups (*P value* 0.518, 0.347, 0.077, 0.300) and (0.459, 0.500, 0.129, 0.810) triple negative and hormone receptor positive respectively (**Table 3**& 4).

Table (5) showed significant positive correlation between cancer stage, Ca level and GGT activity while the other biochemical's showed no correlation. Also there is no correlation between age and all investigated parameters.

Table (1): Characteristics of Breast Cancer Patients

Percentage (%)	Frequency	Class
30.3%	54	Triple Negative
69.7%	124	Hormone. R Positive
47.2 %	84	**Early stage**
52.8 %	94	**Late stage**

Table (2): Differnces in bone profile and liver Enzymes between hormone receptor positive and triple negative BC patients

PARAMETER	Number	Mean ± SD	*P value*
CaTriple negative	54	8.5 ± 1.11	0.518
Hormone.R positive	124	8.6 ± 1.7	
PiTriple negative	54	3.9 ± 0.67	0.347
Hormone.Rpositive	124	3.8 ± 0.73	
ALP Triple negative	54	41 ± 42	0.077
Hormone.R positive	124	55 ± 46	
GGTTriple negative	54	25 ± 13	0.017
Hormone.Rpositive	124	46 ± 30	
LDH Triple negative	54	230 ± 114	0.300
Hormone.R positive	124	201 ± 181	

Ca = calcium, Pi = phosphate, ALP = alkaline phosphatase, GGT = gama glutamayl transpeptidase, LDH = lactate dehydrogenase.

Table (3): Comparison of Bone profile and liver Enzymes between early and late stage Triple negative BC patients

PARAMETER		Number	Mean ± SD	P value
Ca	Early	24	8.3 ± 1.4	0.459
	Late	30	8.6 ± 0.66	
Pi	Early	24	3.8 ± 0.83	0.500
	Late	30	3.9 ± 0.50	
ALP	Early	24	33 ± 20	0.129
	Late	30	51 ± 12	
GGT	Early	24	21 ± 16	0.016
	Late	30	30 ± 9.6	
LDH	Early	24	226 ± 97	0.810
	Late	30	233 ± 126	

Ca = calcium, Pi = phosphate, ALP = alkaline phosphatase, GGT = gama glutamayl transpeptidase, LDH = lactate dehydrogenase.

Table (4): Comparison of Bone profile and liver Enzymes between early and late stage Hormone receptor positive BC patients

PARAMETER		Number	Mean ± SD	P value
Ca	Early	56	8.8 ± 0.7	0.541
	Late	60	8.5 ± 2.2	
Pi	Early	56	3.7 ± 0.73	0.803
	Late	60	3.8 ± 0.74	
ALP	Early	56	57 ± 24	0.570
	Late	60	52 ± 37	
GGT	Early	56	31 ± 20	0.018
	Late	60	57 ± 25	
LDH	Early	56	168 ± 82	0.047
	Late	60	234 ± 117	

Ca = calcium, Pi = phosphate, ALP = alkaline phosphatase, GGT = gama glutamayl transpeptidase, LDH = lactate dehydrogenase.

Table (5): Correlation between Bone profile and liver Enzymes and cancer stage among BC patients

		Stage	Age
Ca	Pearson's correlation (r)	0.311**	0.075
	Sig (*P.value*)	0.000	0.166
	N	178	178
Pi	Pearson's correlation (r)	0.050	-0.065
	Sig (*P.value*)	0.270	0.199
	N	178	178
ALP	Pearson's correlation (r)	0.069	0.005
	Sig (*P.value*)	0.199	0.475
	N		178
GGT	Pearson's correlation (r)	0.263**	-0.055
	Sig (*P.value*)	0.000	0.239
	N	178	178
LDH	Pearson's correlation (r)	0.033	-0.114
	Sig (*P.value*)	0.342	0.060
	N		178

Ca = calcium, Pi = phosphate, ALP = alkaline phosphatase, GGT = gama glutamayl transpeptidase, LDH = lactate dehydrogenase. *P.value* less than 0.05 is considerd significant

Discussion:

In clinical practice, ALT, AST, GGT, ALP, and LDH are five serum biochemical markers that have been examined. All concern liver function (13,14), however, the latter three have also being looked into potential tumor invasion markers (15).

The present study detected that GGT was significantly higher in hormone receptor positive when compared to triple negative BC.This might be owing to Long-term estrogen inhibition can cause fatty liver disease (non-alcoholic fatty liver disease; NAFLD) (16); and Serum GGT activity is a sensitive marker of NAFLD (17)

Also this study observed significant increase in GGT in late stage compared to early stage. This is explicable by the function of GGT in the re-synthesis of GSH, which forms the cornerstone of cellular resistance to a number of electrophilic chemicals and is assumed to be implicated in the cellular defense mechanism (15). Chemotherapeutic resistance and a worse linked to increasing GGT may be explained by increased GSH, which metabolizes toxic compounds, including chemotherapy drugs. (18, 19). Additionally, it has been noted that GGT-mediated metabolism of GSH may result in a low but persistent amount of oxidants, which activate defense mechanisms. In fact, numerous studies showed that cells that overexpress GGT are less sensitive to a number of cytotoxic medicines (18).

Also this studty showed that LDH in Late stage hormone receptor positive patients was significant higher than in early stage hormone reeptor positiveBC patients. This can be explained by how LDH regulates the conversion of glucose to lactic acid, which explains why higher levels of LDH in cancer cells were linked to worse prognosis and higher rates of cell proliferation (20, 21). Similar to earlier research, which showed that elevated serum LDH levels were frequently observed in cancer patients and were associated with a poor prognosis and therapy resistance (20).

In this study the correlation results showed significant positive correlation between BC stage, calcium level and GGT activity; the reason for high GGT activity was explained before. While the reason of hypercalciumcemia with stage is related to the pathological bone resoption caused by cytokines that stimulate osteoclast activity; one of these cytokines is parathyroid hormone related protein (PTHrP) (22). In other hand the remaining parameters (Pi, ALP, LDH) showed no correlation with stage.

One of the limitation of this study it was a cross sectional hospital‐based study. Second, we did not measure the bone density, ALP and LDH isoenzymes which might help in the explanation of the results.

Conclusion

The data of present study suggests that, Hormone positive and late stage BC patients have increased GGT activity. Therefore, GGT activity might use as metastatic and prognostic markerfor BC**.**

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Original Article Sudan Medical Laboratory Journal

Serum Zinc and Copper Levels among Patients with Prostatic Cancer Attending National Cancer Institute, Gezira University, Sudan

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Abstract

Backgrounds: Prostate cancer is the most common cancer among Sudanese men and is equally distributed among its different tribes. Zinc (Zn) and copper (cu) are one of the most important microelements and ubiquitously present in the human body, and it is critical for maintaining prostate health and inhibiting prostate cancer development.

Objective: To assess serum zinc and copper levels among Sudanese prostatic cancer patients.

Materials and Methods: Case control study carried out in National Cancer Institute from August 2020 to December 2020. The study included 60 Sudanese subjects, 30 of them were patients with prostate cancer and 30 were as controls. The age of patients ranged between58-88. (43%) of patients were >70 Years and (57%) were <70 Years serum zinc and copper were measured by using atomic absorption spectrophotometer. Data was analysed using SPSS version (22).

Results: Highly significant decrease in zinc level in case group (0.08±0.06) when compared with control group (0.7±0.2) with p. value (p=0.000) and highly significant increase in copper level in case group (1.6±0.3) when compared with control group (0.9±0.2) with p.value (p=0.000). There were no significant differences on Zn and copper levels according to age, BMI and duration with p. value (0.10, 0.70, 0.30), (0.28, 0.06, 0.52) respectively. Regarding gleason stages patients with advanced stage > score 7 (43%) (0.06±0.05) showed more decrease in zinc than patients with non-advanced stage ≤ score 7 (57%) (0.09±0.06) without significant differences (P=0.3) andshowed more increase in copper than patients with non-advanced stage ≤ score 7 (57%) (1.47±0.50) without significant differences (P=0.37).There was weak negative correlation between age, grade, stage and PSA with serum zinc with a coefficient r (-0.3, -0.03, -0.003, -0.2) respectively. While there was weak positive correlation between BMI and duration with serum zinc with a coefficient r (0.07, 0.07) respectively.There was moderate negative correlation between age, and duration with serum copper with a coefficient r (-0.20, -0.14).While serum copper had moderate positive correlation with BMI, Stage, grade and PSA levels with a coefficient r (0.34, 0.28, 0.27, 0.27) respectively.

Conclusion: Serum zinc level in cases was lower than in controls; whileserum copper level in cases was higher than in controls. The study recommended regular monitoring of serum zinc and copper in prostatic cancer patients.

Keywords: Prostate cancer, Zinc, Copper, Risk factors, Grade, Stage, Sudanese.

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Introduction

It is recognized now that prostate cancer is one of the most serious medical problems facing men with a number of additional factors leading to increased risk of the condition, such as age, race and family history (1), Western industrialized countries face a significant challenge with prostate cancer; as it is the most common type of cancer among men, and with an aging male population, the number of cases being diagnosed is increasing (2). It is estimated that 1.3 million new male cases of (PCa) occur annually, and it is also the fifth most prevalent male cancer in Saudi Arabia (3). However, its mortality rate and future incidence are predicted to be stronger in the Middle East (4). Among men in Sudan, prostate cancer is the most common cancer and affects all tribes equally. About 85.4% of these men present with stage III and IV at the mean age of 72.2 ± 9.25 years (5). Despite being a sporadic disease in Sudan among men under 50 years of age, prostate cancer rate increases exponentially later in life (6). Age, education level, and a family history of prostate cancer are the most common risk factors. Among the common risk factors associated with prostate cancer in Sudan are age, education level, and a positive family history. Other factors include smoking and alcohol consumption, body mass index (BMI), and occupation (7,8). Several studies have reported that PCa is greatly affected by lifestyle and diet, including the intake of zinc (Zn) and other trace elements (TE) (9). TE serve vital physiological functions, such as maintaining and regulating cell function, regulating gene expression, and modulating metabolic reactions and membrane function (10). Excessive accumulation or an imbalance of the TE may disrupt cellular functions and result in cellular degeneration or death, in fact, one of the main functions of the prostate gland is to produce prostatic fluid with high levels of zinc and other chemical elements (11). The presence of trace elements is known to play a significant role in a wide variety of biological functions through activating or inhibiting enzyme cascades, which can lead to metabolic disorders and cellular growth impairment, including cancer (12). An essential element deficiency is most commonly suggested to cause cancer as it may lead to oxidative stress and failure of antioxidant defense, among them, Copper, Zinc, Selenium, Iron, and Manganese play a critical role in intra- and extracellular antioxidant defense (13). There are several studies showing that Cu is an important component of many enzymes that significantly affect many biological processes, including the growth of cancer cells by creating free radicals (14). Monitoring and periodic measurement of serum zinc levels is important in prostatic cancer patients to prevent zinc deficiency (15). Zinc is essential to keeping the prostate healthy and inhibiting prostate cancer, and its level in serum is easier to calculate than that in the prostate tissue (16). This research was done to evaluate the serum zinc and copper levels among patients with prostatic cancer attending national cancer institute, Wad medani city, Gezira state, Sudan.

Materials and Methods

This is a case control hospital based study was done in National Cancer Institute, Gezira State, Sudan from August to December 2020. Total of 60 individual were include by randomized sampling technique, 30 patients with prostate cancer and 30 control healthy subjects. Any known diagnosed patients with prostate cancer who agree to participate in the study was included in this study, whereas any patients with other disease that may disrupt zinc and copper normal distribution as diabetes and gastrointestinal diseases or patients under treatment that contains zinc and copper was excluded. Data was collected by using a questionnaire which includes all necessary information like (age, grade, stage, duration, serum PSA, wright, length and calculated BMI). Three ml of venous blood were collected into plain tube to measured serum zinc (normal value 0.5-1.2 mg/L) and copper (normal value 0.7-1.4) mg/L) levels by using Atomic absorption spectrophotometer (ASC-7000-Shimadzu) instrument in Mohamed Obaid Mubarak Laboratory, Gezira University. Written consent from each patient had been taken, and overall study was approved by the ethical committee at Gezira University, faculty of Medical Laboratory Sciences and Ministry of Health, Gezira State, Sudan. Data was analyzed using statistical package for social science (SPSS) computer program Version (22) when used t-test presented as (mean and SD) and correlation test (R value). A *P*-value of less than 0.05 was considered statistically significant.

Results

A total of 30 patients and 30 normal men were included in this study, the distribution of demographic data showed that 13 (43%) of patients age >70 years and 17 (57%) of patients age <70 years. According to the BMI distribution 8(27%) of patients was overweight and 22 (73%)of patients was normal weight. According to grade 8(27%) of patient were in grade I, 3 (10%) in grade II, 6 (20%) in grade III, 9 (30%) in grade and 4 (13%) in grade V. The copper level in case group was highly significant increase (1.6±0.3) when compared with control group (0.9±0.2) with p. value (p=0.000), while zinc level in case group was highly significant decrease (0.08±0.06) when compared with control group (0.7±0.2) with p. value (p=0.000). There were no significant differences on Zn and cupper levels according to age, BMI and duration with p. value (0.10, 0.70, 0.30),(0.28, 0.06, 0.52)respectively. Regarding gleason stages patients with advanced stage > score 7 (43%) (0.06±0.05) showed more decrease in zinc than patients with non-advanced stage ≤ score 7 (57%) (0.09±0.06) without significant differences (P=0.3) andshowed more increase in copper than patients with non-advanced stage ≤ score 7 (57%) (1.47±0.50) without significant differences (P=0.37).There was weak negative correlation between age, grade, stage and PSA with serum zinc with a coefficient r (-0.3, -0.03, -0.003, -0.2) respectively. While there was weak positive correlation between BMI and duration with serum zinc with a coefficient r (0.07, 0.07) respectively.There was moderate negative correlation between age, and duration with serum copper with a coefficient r (-0.20, -0.14) respectively. There was moderate positive correlation between BMI, Stage, grade, PSA and serum copper with a coefficient r (0.34, 0.28, 0.27, 0.27) respectively.

Table 1: Socio-demographic and clinical characteristics of study population

	Parameters	Frequency	Percent
Age Group	< 70 Years	17	56.7
	>70 Years	13	43.3
	Total	30	100
BMI Category	Normal weight	22	73.3
	Over weight	8	26.7
	Total	30	100
Grade	1	8	26.7
	2	3	10
	3	6	20
	4	9	30
	5	4	13.3
	Total	30	100
Stage	6	8	26.7
	7	9	30
	8	9	30
	9	2	6.7
	10	2	6.7
	Total	30	100
Status	Non advance	17	56.7
	Advance	13	43.3
	Total	30	100
Serum Cu Level	Normal	13	43.3
	Abnormal	17	56.7
	Total	30	100
Serum Zn Level	Normal	0	0
	Abnormal	30	100
	Total	30	100

Table 2: Compared serum Cu and Zn between case and control group

	Cases Control	N	Mean	SD	P. Value
Copper	Cases	30	1.53	0.45	0.000
	Control	30	0.90	0.18	
Zinc	Cases	30	0.08	0.06	0.000
	Control	30	0.72	0.22	

Table 3: Correlation between serum zinc and copper levels with age, PSA level, BMI and gleason stage of prostatic cancer

		Age	BMI	Stage	PSA	Cu	Zinc
Age	Pearson Correlation	1	-0.05	0.209	0.051	-0.202	-0.302
	Sig. (2-tailed)		0.803	0.269	0.787	0.284	0.105
	N	30	30	30	30	30	30
BMI	Pearson Correlation	-0.047	1	0.221	0.235	0.346	0.076
	Sig. (2-tailed)	0.803		0.241	0.211	0.061	0.688
	N	30	30	30	30	30	30
Stage	Pearson Correlation	0.209	0.221	1	0.239	0.284	-0.033
	Sig. (2-tailed)	0.269	0.241		0.203	0.128	0.863
	N	30	30	30	30	30	30
PSA	Pearson Correlation	0.051	0.235	0.239	1	0.273	-0.248
	Sig. (2-tailed)	0.787	0.211	0.203		0.145	0.187
	N	30	30	30	30	30	30
Cu	Pearson Correlation	-0.202	0.346	0.284	0.273	1	-0.096
	Sig. (2-tailed)	0.284	0.061	0.128	0.145		0.616
	N	30	30	30	30	30	30
Zinc	Pearson Correlation	-0.302	0.076	-0.033	-0.248	-0.096	1
	Sig. (2-tailed)	0.105	0.688	0.863	0.187	0.616	
	N	30	30	30	30	30	30

Discussion

Prostate cancer (PCa) is the second prevalent male cancer worldwide, with approximately 1.3 million annual new cases. Additionally, many dietary constituents may play significant roles in initiation and progression of the tumor such as trace element (17). Trace elements play an important role in the structure of proteins, enzymes and complex carbohydrates to participate in biochemical reactions. Copper (Cu) and Zinc(Zn)are essential trace element they involved in many biochemical processes as energy metabolism, iron homeostasis and antioxidant protection. Despite copper being an essential element for humans, high Cu concentrations may lead to cancer via toxic free radicals and producing DNA damage thus increasing serum Cu levels in several malignancies. Moreover, Cu levels could potentially cause progression of prostate cancer by improving blood supply to the tumor, which explains elevated Cu levels in involved tissues (18).While, Zinc level is significantly higher in prostate gland when compared to all tissues in the human body. Different studies indicated that Zn content increased in benign prostatic hyperplasia when compared to normal prostate tissue and that there was a decrease in prostate cancer, thus zinc levels are an important factor in the malignant prostate cells progression, which agrees with our study showing that serum zinc concentrations were significantly lower in prostate cancer patients than control subjects (19). Our study was case-control study conducted at National cancer Institute, Wad Medani, Gezira State, from October to December 2020, to assess the serum copper level and zinc level in prostatic cancer patients. A total of 30 patients and 30 normal men were included in this study, the distribution of demographic data showed that43% of patient's age >70 years and 57% of patient's age <70 years. According to the BMI distribution 27% of patients were overweight and 73% of patients were normal weight. According to grade 27% of patient were in grade I, 10% in grade II, 20% in grade III , 30% in grade and13% in grade V, and according to serum Cu levels among study subjects high level was 57%and 43% was normal level. In this study comparison means of serum copper level between case and control showed highly significant increase in copper level in case group when compared with control group, this finding agree with other studies (12, 14, 20) ,there were mild increase in copper level in patients <70 than patients >70 without significant differences and there were moderate negative correlation. Comparison means of serum zinc levels between cases and controls showed highly significant decrease in zinc level in case group when compared with control group and this agreed with study done by Li, D. *et al.*., While disagreed with study done with Białkowska, K. *et al.*.; which this disagreement may be due to genetic alterations among proteins related to zinc metabolism (16, 21). There was mild decrease in zinc in patients >70 years than those <70 years without significant differences, this agreed with Li, D. *et al.* Regarding to BMI there were mild increased in copper level in overweight group when compared with normal weight group without significant differences. Furthermore there were moderate positive correlation, and there was mild decrease in zinc level in normal weight group without significant differences. This agreed with research authored by Mahmoud, A.M.*et al*. (22).Regarding Gleason stages>7 was accepted as high (advanced) stage was (73%) and ≤7 as low grade tumors (non-advanced) was (27%), there were increase in copper level in high (advanced) stage when compared with low (non-advanced) stage without significant differences and there were moderate positive correlation, and showed more decrease in zinc than patients with non-advanced stage≤ score 7 without significant differences, this agreed with (23) .27% was grade I ,10% was grade II ,20% was grade Ⅲ,30% was grade Ⅳand 13% was grade V ,there were increase in copper level in advanced when compared with low advanced. Regarding to duration of disease there were increase in serum copper level in less than one year’s group when compared with group of more than one year without significant differences and there were moderate negative correlation, and mild decrease in serum zinc level than patients >1 year without significant differences. This study find moderate positive correlation between serum copper and serum PSA without significant differences, this finding disagree with study done by Saleh who find negative correlation (4).This may be due to sample size, environmental and Nutritional factors. There was weak negative correlation between age, grade, stage and PSA with serum zinc. While there was weak positive correlation between BMI and duration with serum zinc with, this agreed with study done by Mahmoud, A.M.*et al*. (22).

Conclusion

In conclusion, our study showed that there is an association between prostate cancer and trace elements. There is a significant Increased of serum copper among patients with prostate cancer and Serum zinc level significantly decreased compared to controls. In this study age, grade, stage and PSA showed negative correlations when correlated with copper and zinc levels, while BMI and duration showed weak positive correlation.

Limitations

Sample size lacked due to research time zone, as it was conducted during the pandemic peak and covid 19 spread due to high cost of reagents.

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OriginalArticle Sudan Medical Laboratory Journal

The association of hepatitis B virus genotypes with liver cirrhosis in HBV-infected Sudanese patients

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ABSTRACT

Background: The genome of hepatitis B virus (HBV) includes genetic variations arranged into ten genotypes, eight well-known genotypes labeled from A to H, plus two new genotypes called I and J. Several studies suggest that the variations between HBV genotypes are closely associated with disease progression, treatment, and clinical outcome. This study aimed to associate HBV genotypes with liver cirrhosis among HBV-infected patients in Sudan.

Methods: Ninety sera were collected from HBV-infected patients; 30(33.3%) of the participants were suffering from liver cirrhosis, and the rest were non-cirrhotic HBV patients as the control group. HBV genotypes were determined by PCR, and HBV viral load was estimated by real-time PCR. The concentration of AST and ALT was measured by a fully automated chemistry analyzer.

Results: Genotype D is the most frequent genotype **(94.4%)** in this study population, followed by genotypes B (52.2%), A (20%), and E (1.1%). Genotypes C, F, G, H, and I were not detected. Mixed genotypes were detected in most (60%) participants. Genotype D was the most frequent genotype **(93.3%)** among the cirrhotic group, as well as showing the highest level of viral loads, AST, and ALT.

Conclusion: Genotype D is more associated with severe manifestations because it showed the highest levels of viral load, AST, and ALT. Genotypes B and B+D mixed infections are more probably associated with the development of liver cirrhosis in HBV infected patients, and genotypes B and D are more frequent among patients with liver cirrhosis regardless of whether they are a single genotype or mixed with other genotypes.

Key words: HBV, genotypes, Liver cirrhosis

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Introduction

Hepatitis B virus is a *hepadnavirus* that belongs to the genus *Orthohepadnavirus* of the *Hepadnaviridae* family (1). The virus is an enveloped icosahedral nucleocapsid virion containing the length of 3200 base pairs (bp) of a circular, partially double-stranded deoxyribonucleic acid (DNA) genome. The two unequal strands of the DNA genome consist of the short strand (S strand), which is the positive sense, and the long strand (L strand), which is a negative sense or non-coding and complementary to viral messenger ribonucleic acid (mRNA) (2, 3). The viral genome contains four genes (C, P, S, and X) or four open reading frames (ORFs) that encode seven proteins that include structural proteins of the virion surface and core, a small transcriptional trans-activator (X), and a large polymerase (2). The C gene encodes HBcAg and hepatitis B e antigen (HBeAg), the P gene encodes DNA polymerase, which also acts as reverse transcriptase, and the S gene encodes HBsAg. This gene is divided into three sections; pre-S1, pre-S2, and S sequences, and the size of the final polypeptides of these sequences is varied according to the number of build-up sequences into large (pre-S1+pre-S2+S), middle (pre-S2+S), and small (S) (2, 3, 4). The last X gene encodes for hepatitis B virus X protein (HBx), which may be involved in the oncogenesis prosperities of the virus because it can inactivate the p53 tumor suppressor protein besides its role as an activator of viral RNA transcription (2).

Genotypes

The genetic variations of HBV were arranged into ten genotypes, eight well-known genotypes labeled from A to H, plus two new genotypes called I and J (5, 6, 7). Genotypes A, D, and G have global distributions, whereas genotypes B and C are commonly found in East and Southeast Asia, genotype E is found in West Africa, while genotypes F and H are in Central and South America (5). Usually, there is a 10–13%, or at least an 8–9% difference in nucleotide sequences between each genotype, except for the genome of genotype F, which diverges by 14% (5, 8, 9). There are at least 26 subtypes with a difference of 4-8% in the genome sequences (8).Type A has three subtypes (A1, A2, and A3), Aa (A1), which is predominant in Africa and Asia, and subtype Ae (A2), which is found in Europe and the United States. Genotype B is found in Asia and is divided into Ba and Bj. Ba (B1) is further sub-typed into (B2, B3, B4, and B5) whereas Bj is found in Japan. Type C was divided into six subgroups (C1-C6): type Cs (C1) is found in Southeast Asia, Ce (C2) in East Asia, C3 in New Caledonia and Polynesia, C4 in Australia, C5 and C6 in the Philippines. Genotype D is globally distributed and subdivided into nine sub-genotypes (D1-D9) (7). Genotype F was sub-grouped into four subtypes (F1–F4) with further sub-typing of F1 into F1a and F1b (9, 10, 11). The variations between HBV genotypes are closely associated with disease progression, treatment, and clinical outcome (12).

Materials and Methods

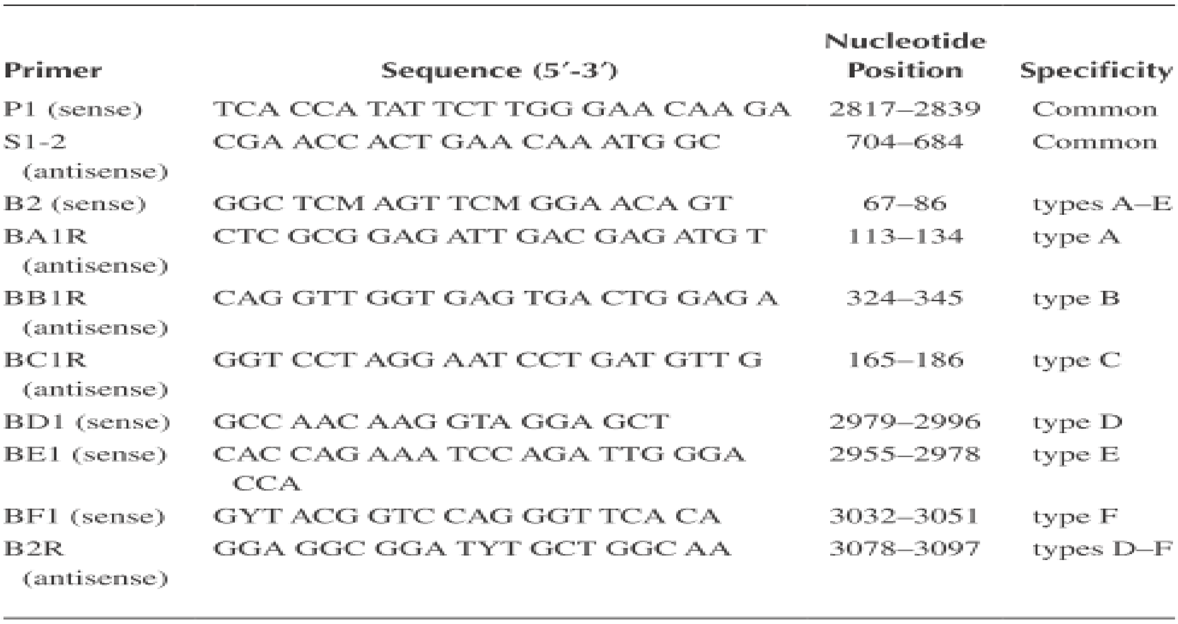
This study was conducted at *Ibn Sina* specialized hospital and Alzaiem Alazhari University in Khartoum State during the period from June 2016 to June 2017. Ninety blood samples were collected from HBV-infected patients, and their infections were confirmed by being positive for HBsAg using ELISA (*Fortress Diagnostics).* The population was divided into two categories: Category A includes HBV-positive patients with liver cirrhosis, and they were set as a case group in the study population. Category B contains HBV carriers, acutely infected patients, and recently infected individuals. All participants in category B had no liver cirrhosis, and they were used as the control group.

Ethical considerations: The ethical considerations and conformity of individuals in this study were considered with the approval of the ethical committees of Alzaiem Alazhari University and Ibn Sina Specialized Hospital, in addition to the documented agreements included in the questionnaire and signed by the participants.

DNA extraction: HBV DNA was extracted from all specimens using the G-spinTM Total DNA Extraction Kit from Invitrogen. HBV viral load was estimated by real-time *PCR (iNtRON Biotechnology Incorporation*). The concentration of AST and ALT was measured by a fully automated chemistry analyzer (*Mindray Bs120*) and *BioSystem* reagent kits.

Determination of HBV genotypes: HBV genotypes were determined by the PCR-Based Genotyping method, which is a rapid and specific genotyping system for HBV that corresponds to six major genotypes (A–F) by PCR using type-specific primers.In the first PCR, primer set P1/S1-2 was used to generate a 1103 bp product; in the second PCR, primer mix A contains B2/BA1R (68 bp) for genotype A, B2/BB1R (281 bp) for genotype B, B2/BC1R (122 bp) for genotype C; primer mix B contains BD1/B2R (119 bp) for genotype D, BE1/B2R (167 bp) for genotype E, and BF1/B2R (97 bp) for genotype F (13).

Table (1): Specific primers sequences use for HBV genotypes



Primer	Sequence (5′-3′)	Nucleotide Position	Specificity
P1 (sense)	TCA CCA TAT TCT TGG GAA CAA GA	2817–2839	Common
S1-2 (antisense)	CGA ACC ACT GAA CAA ATG GC	704–684	Common
B2 (sense)	GGC TCM AGT TCM GGA ACA GT	67–86	types A–E
BA1R (antisense)	CTC GCG GAG ATT GAC GAG ATG T	113–134	type A
BB1R (antisense)	CAG GTT GGT GAG TGA CTG GAG A	324–345	type B
BC1R (antisense)	GGT CCT AGG AAT CCT GAT GTT G	165–186	type C
BD1 (sense)	GCC AAC AAG GTA GGA GCT	2979–2996	type D
BE1 (sense)	CAC CAG AAA TCC AGA TTG GGA CCA	2955–2978	type E
BF1 (sense)	GYT ACG GTC CAG GGT TCA CA	3032–3051	type F
B2R (antisense)	GGA GGC GGA TYT GCT GGC AA	3078–3097	types D–F

Method

Forty µl of the first PCR mixture were prepared, containing 50 ng of each outer primer (P1/S1-2), 200 micromolar (µ*M*) of each of the dNTPs, 1 U of Takara Ex Taq DNA polymerase, and 1 1 × PCR buffer containing 2 millimolar (m*M*) MgCl2. The samples were incubated in a thermocycler at 95°C for 2 min; 40 cycles of 94°C for 20 sec, 55°C for 20 sec and 72°C for 30 sec. The second PCR mixture was prepared as the first PCR (two second-round PCRs are performed for each sample), with the common universal sense primer (B2) and mix A for types A–C, and the common universal antisense primer (B2R) and mix B for types D–F, respectively, together with a 2 μl aliquot of the first PCR product. The second PCR mixture was preheated at 95°C for 2 min, amplified for 20 cycles of 94 94°C for 20 sec, 58°C for 20 sec, 58 °C for 20 sec, and 72°C for 30 sec, and additional 20 cycles of 94°C for 20 sec, 60°C for 20 sec, and 72°C for 30 sec. Each of the two different second-PCR products from one sample was separated by electrophoresis on a 3% agarose gel, stained with ethidium bromide, and evaluated under UV light. Determination of the genotypes of HBV for each sample was done by identifying the genotype-specific DNA bands (13). The size of amplicons was estimated according to the migration pattern of a 50-bp DNA ladder (Pharmacia Biotech).

Results

The participants were divided into two main groups. One group included 30 participants with liver cirrhosis. The second group was composed of 60 HBV positive participants without liver cirrhosis. The final group included 30 HBV carriers, 19 acute hepatitis patients, and 11 newly diagnosed HBV patients.

Out of all ninety patients with HBV infection, genotype D was the most frequently detected genotype and identified in 85 (94.4%) of the specimens, whereas the other genotypes of B, A, and E were detected in 47(52.2%), 18(20%) and 1(1.1%) patients respectively.

As a singular genotype, D was found in 32(35.6%), B in 3(3.3%) and A in 1(1.1%). Mixed genotypes either two or three genotype was detected. The most frequent mixture was B+D, which occurred in 37(41.1%) followed by A+D in 10(11.1%) then A+B+D in 6(6.7%) and finally A+B+E in 1(1.1%) out of the 90 blood samples (Table 1).

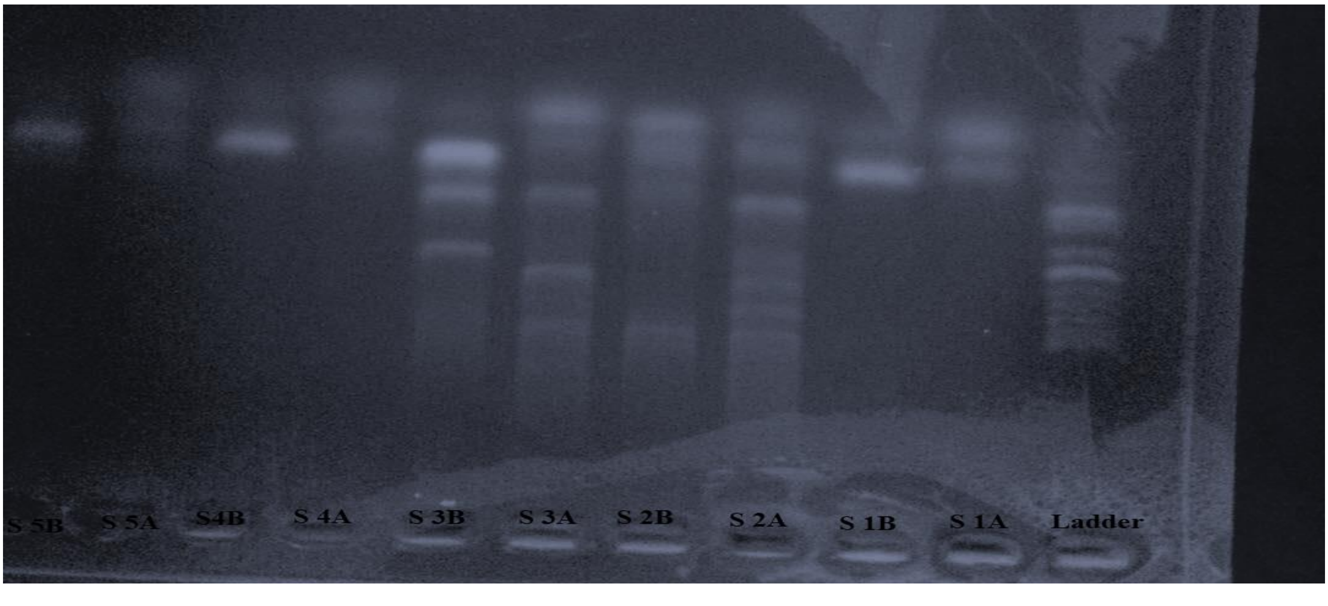
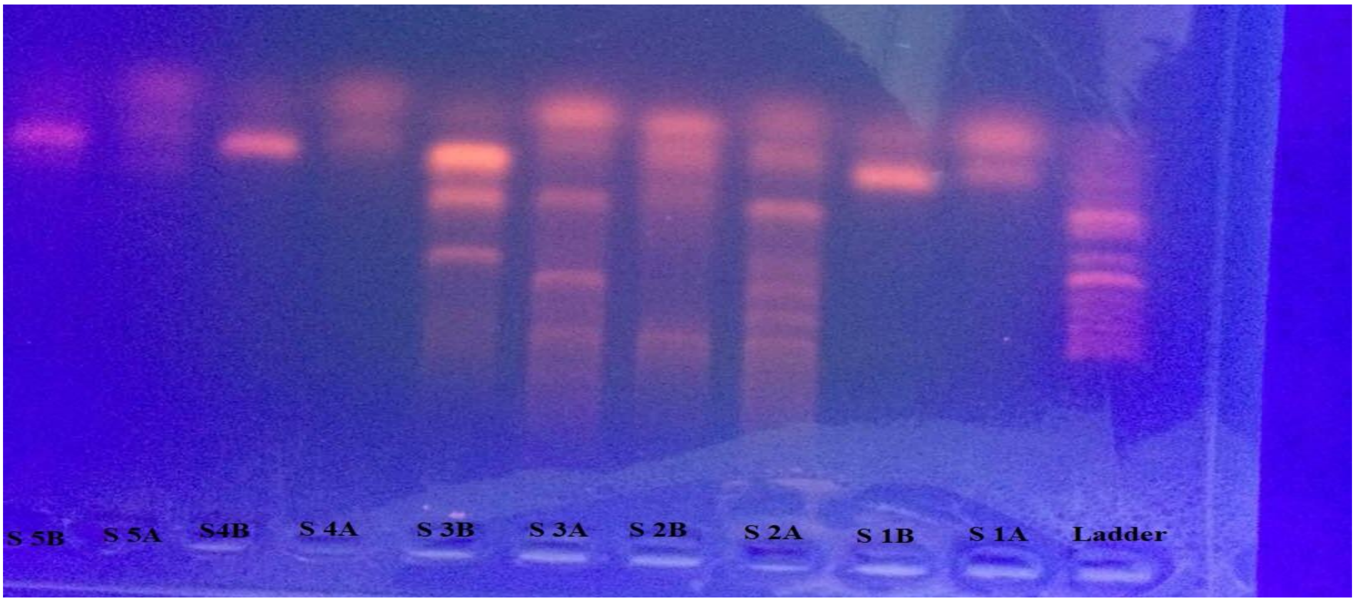


Figure 1: HBV DNA amplification from a plasma sample of liver cirrhosis among HBV infected patients



Figuren2: Characteristic bands of HBV genotypes

Table (2): The frequencies of HBV genotypes according to study groups

Genotypes	Study groups				Total
	HBV infection with liver cirrhosis	**HBV carriers**	**Acute HBV infection**	**Recent HBV infection**	
B+D	18(60.0)	6(20.0)	8(42.1)	5(45.5)	37(41.1)
D	9(30.0)	14(46.7)	4(21.1)	5(45.5)	32(35.6)
A+D	1(3.3)	5(16.7)	4(21.1)	0(0.0)	10(11.1)
A+B+D	0(0.0)	4(13.3)	1(5.3)	1(9.1)	6(6.7)
B	1(3.3)	1(3.3)	1(5.3)	0(0.0)	3(3.3)
A	0(0.0)	0(0.0)	1(5.3)	0(0.0)	1(1.1)
A+B+E	1(3.3)	0(0.0)	0(0.0)	0(0.0)	1(1.1)
Total	30(100.0)	30(100.0)	19(100.0)	11(100.0)	90(100.0)

Genotype D was the most frequent in patients with liver cirrhosis (93.3%) (Table 2), being present alone in 9(30%) patients, in contribution with genotype B in 18(60%) patients, and with genotype A in only one (3.33) patient. (Table 8)

Table (3): The prevalence of HBV genotypes among HBV infection groups with liver cirrhosis

Genotypes	Out of all 90 patients	Out of all 30 cirrhosis patients	Cirrhosis occurrence ratio from all genotypes
D	85(94.4)	28(93.3)	28(32.9)
B	47(52.2)	20(66.7)	20(42.5)
A	18(20)	2(6.7)	2(11.1)
E	1(1.1)	1(3.3)	1(100)

Regarding the patients with acute hepatitis, genotype D was the most frequently identified; it was detected in 17 out of the 19 patients (89.5%). It was found alone in 4(21.1%) patients, together with genotype A in 4 (21.1%) patients, and with A and B in one additional (5.3%) patient. The second most frequent genotype detected was B in 10 (58.8%) patients (Table 1).

Genotype D was the most frequent genotype detected in patients with recent HBV infection (100%), followed by genotype B in 6 out of 11 (54.5%) patients (Table 1). The least detected HBV genotype was type E in only one patient (Table 1).

The most common genotype among HBV carriers was D, which was found in 29 out of 30 (96.7%) patients, singular in 14 (46.7%) patients, mixed with B in 20% of patients, A in 16.7% of patients, and A and B in 13.3% of patients. Genotype B alone was detected in only one (3.3%) patient.

A mixture of B and D genotypes is the most common mixture, occurring in 18 (60%) of HBV infections with liver cirrhosis, 8 (42.1%) of the acute hepatitis B group, 6 (20%) of the HBV carriers, and 5 (45.5%) of the recently infected HBV group of the study population.

A triple mix of genotypes A, B, and D is found as four (13.3%) carrier groups, one (9.1%) recent HBV infection group, and one (5.3%) acute HBV infection group. A, B, and E mixtures are found only in HBV-infected people with liver cirrhosis group as one (3.3%).

There was an insignificant association between genotype D and the development of liver cirrhosis among HBV infected individuals (P value 0.543), and the genotype is less probably associated with liver cirrhosis (odds ratio 0.737/likelihood ratio 0.103) (Table 3).

Table (3) shows that HBV genotype B has an insignificant association with (P value 0.052), despite being statistically more likely to be associated with liver cirrhosis (odd ratio 2.444/likelihood ratio 3.821).

The same conclusion was obtained from the association of both genotypes B and D with the development of liver cirrhosis in HBV infected patients: the two genotypes are more associated with liver cirrhosis (odd ratio 2.10/likelihood ratio 2.705) with an insignificant association (P value 0.078) (Table 3).

Table (4): Associations of HBV genotypes B and D with liver cirrhosis.

Genotypes		Study groups		P value	Odd ratio	Likelihood ratio
		HBV infection with liver cirrhosis (n=30)	**HBV infection without liver cirrhosis (n=60)**			
Genotypes D	Genotypes D	28(93.3)	57(95)	0.543	0.737	0.103
	Other genotypes	2(6.7)	3(5)			
Genotypes B	Genotypes B	20(66.7)	27(45)	0.052	2.444	3.821
	Other genotypes	10(33.3)	33(55)			
Mixed	Mixed Genotypes D+B	18(60)	25(41.7)	0.078	2.10	2.705
	Other genotypes	12(40)	35(58.3)			

Table (4) shows the frequencies of viral load among different genotypes, the highest mean was found in pure genotype B (29781698.00), followed by genotype D (29185647.34), B+D (20148544.59), A+D (54487.50), A+B+D (34908.33), (253875282522.34), A (31400.00), and the least value recognized in the mixture of A+B+E genotypes (0.0).

This indicates that the genotypes B and D showed the highest viral load regardless of whether they were pure genotypes or mixed with other genotypes, followed by A and E.

Table (5): Statistics of viral load copy/ml among different HBV genotypes

Genotypes	Mean	Std. Deviation	Minimum	Maximum
B	**29781698.00**	51284843.851	94	89000000
D	29185647.34	111960880.726	0	618000000
B+D	20148544.59	74561612.069	0	412000000
A+D	54487.50	55254.528	100	150000
A+B+D	34908.33	67171.854	87	169000
A	31400.00	.	31400	31400
A+B+E	.00	.	0	0

Tables (5) show the distribution of the liver enzymes AST and ALT among different genotypes. AST was high with genotype D (80.953), followed by genotypes B+D (66.703), A+B+E (63.000), A+B+D (50.167), A+D (49.400), A (48.000), and B (37.667). ALT was elevated in genotype D (58.869), followed by genotypes B+D (42.081), A+B+D (38.167), A+D (37.900), A (31.000), A+B+E (28.000), and B (20.667).

Table (6): Statistics of AST IU/L and ALT IU/L among different HBV genotypes.

Variable	Genotypes	Mean	Std. Deviation	Minimum	Maximum
AST IU/L of serum	D	**80.953**	88.233	16.00	345.00
	B+D	66.703	59.904	16.00	300.00
	A+B+E	63.000	.	63.00	63.00
	A+B+D	50.167	28.499	20.00	90.00
	A+D	49.400	24.070	25.00	100.00
	A	48.000	.	48.00	48.00
	B	37.667	21.197	15.00	57.00
ALT IU/L of serum	D	**58.869**	94.171	12.00	520.00
	B+D	42.081	25.902	15.00	105.00
	A+B+D	38.167	14.825	17.00	59.00
	A+D	37.900	11.939	16.00	60.00
	A	31.000	.	31.00	31.00
	A+B+E	28.000	.	28.00	28.00
	B	20.667	11.060	9.00	31.00

These findings illustrate that the liver enzymes AST and ALT were markedly elevated in genotype D, either pure or mixed with other genotypes.

Among the recent HBV-infected subgroup of the study population, genotype D showed the highest viral load, AST, and ALT (Tables 6).

Table (7): Statistics of viral load copy/mL, AST IU/L, and ALT IU/L within the recent HBV-infected group

Variable	Genotypes	Mean	Std. Deviation	Minimum	Maximum
Viral Load copy/ml of serum	B+D	183437.00	197641.529	395	467000
	D	**49028520.00**	67093275.121	26000	125000000
	A+B+D	1470.00	.	1470	1470
AST IU/L of serum	B+D	62.000	26.833	20.00	90.00
	D	**196.000**	133.869	53.00	345.00
	A+B+D	30.000	.	30.00	30.00
ALT IU/L of serum	B+D	42.600	18.636	17.00	68.00
	D	**173.800**	204.287	31.00	520.00
	A+B+D	30.000	.	30.00	30.00

Table (7) shows the statistics of study variables among the acute subgroup of the study population. Genotype D showed the highest viral load and AST, while genotypes B+D showed the highest level of ALT.

Table (8): Statistics on viral load copy/mL, AST, IU, and ALT, IU, and L HBV genotypes in acute HBV-infected patients

Variable	Genotypes	Mean	Std. Deviation	Minimum	Maximum
Viral Load copy/ml of serum	B+D	66328891.50	145084690.466	812	412000000
	D	**154582500.00**	308945000.184	101000	618000000
	A+D	103200.00	39355.135	53800	150000
	A+B+D	36100.00	.	36100	36100
	B	345000.00	.	345000	345000
	A	31400.00	.	31400	31400
AST IU/L of serum	B+D	116.375	95.403	36.00	300.00
	D	**138.250**	95.451	56.00	270.00
	A+D	68.000	26.445	37.00	100.00
	A+B+D	40.000	.	40.00	40.00
	B	41.000	.	41.00	41.00
	A	48.000	.	48.00	48.00
ALT IU/L of serum	B+D	**68.375**	33.619	20.00	105.00
	D	61.250	41.307	20.00	115.00
	A+D	45.000	10.000	40.00	60.00
	A+B+D	38.000	.	38.00	38.00
	B	22.000	.	22.00	22.00
	A	31.000	.	31.00	31.00

Tables (8) show the statistics of study variables among the carriers subgroup of the study population: genotype D showed the highest viral load, and genotypes A+B+D showed the highest concentrations of AST and ALT.

Table (9): Statistics of viral load copy/ml, AST IU/Land ALT IU/L among HBV genotypes within HBV carriers groups

Variable	Genotypes	Mean	Std. Deviation	Minimum	Maximum
Viral Load copy/ml of serum	B+D	5322.17	12531.552	69	30900
	D	**595805.36**	2226055.097	38	8330000
	A+D	7055.00	8658.764	100	20800
	A+B+D	42970.00	84025.364	87	169000
	B	94.00	.	94	94
AST IU/L of serum	B+D	29.667	8.335	21.00	40.00
	D	34.821	15.970	16.00	80.00
	A+D	39.400	12.621	25.00	55.00
	A+B+D	**57.750**	33.270	20.00	90.00
	B	15.000	.	15.00	15.00
ALT IU/L of serum	B+D	25.333	7.941	18.00	40.00
	D	24.200	9.083	12.00	42.00
	A+D	31.600	11.971	16.00	45.00
	A+B+D	**40.250**	18.392	17.00	59.00
	B	9.000	.	9.00	9.00

Tables (9) show the statistics of study variables among the cirrhosis group of the study population: genotype D showed the highest viral load, genotype D showed the highest concentration of AST, and genotype D showed the highest level of ALT.

Table (10): Statistics of viral load copy/ml, ALT IU/L among HBV genotypes within group of HBV infection with liver cirrhosis

Variable	Genotypes	Mean	Std. Deviation	Minimum	Maximum
Viral Load copy/ml of serum	B+D	11884216.67	42060594.409	0	178000000
	D	6902982.22	20623931.328	0	61900000
	A+D	96800.00	.	96800	96800
	B	**89000000.00**	.	89000000	89000000
	A+B+E	.00	.	0	0
AST IU/L of serum	B+D	58.278	43.985	16.00	155.00
	D	**63.333**	58.211	18.00	210.00
	A+D	25.000	.	25.00	25.00
	B	57.000	.	57.00	57.00
	A+B+E	63.000	.	63.00	63.00
ALT IU/L of serum	B+D	35.833	19.731	15.00	90.00
	D	**47.889**	47.179	17.00	170.00
	A+D	41.000	.	41.00	41.00
	B	31.000	.	31.00	31.00
	A+B+E	28.000	.	28.00	28.00

Discussion

The present study shows that 54 (60%) of the ninety tested specimens were mixed-infected with two or three genotypes, detailed as B+D in 37(41.1%), A+D in 10(11.1%), A+B+D in 6(6.7%) and A+B+E in 1(1.1%). These percentages are higher than the findings of other studies done in Sudan, which reported a 13.5% combination of genotype D+E (14), and much higher than the 4.3% D+E of *Shaza* and her group (15) and the 2% D+E mixed genotypes reported by another Sudanese worker (16).These percentages are higher than the findings of another study done in Sudan, which reported a 13.5% combination of genotype D+E (14), and much higher than the 4.3% D+E of *Shaza* and her group of *Shaza et al* (15) and the 2% D+E mixed genotypes reported by another Sudanese worker. Outside Sudan, our findings were higher than the 15.7% prevalence of mixed infections, especially mixed A/D genotype infections, found in Egypt (17) and the reported 10% of mixed infections in Saudi Arabia (18). A single genotype was detected in 36(40%) out of all ninety participants, the most frequent genotypes were D 23(35.6%), B 3(3.3%) and A 1(1.1%). This finding is not far from the publications of other Sudanese workers; they reported D 46.0%, E 21.6%, and A 18.9% (16); in another study, E 57.5%, D 40.5%, and A22% (15); and the third study reported D 48.%, E 24%, and A 7%(16). Another study reported E 57.5%, D 40.5%, and A2 2% (15), while a third study reported D 48%, E 24%, and A 7% (16). Various findings from various countries have been published, including Egypt, where the prevalence of HBV genotypes was 37.1% for genotype D, 25.7% for genotype B, 10% for genotype A, and 8.6% for genotype C (16). In Côte d'Ivoire, E (87%) and A (13%); in Cameroon, E (67%) and A (33%); in Ghana, E (100%); and in Uganda, A (100%) (19).Our findings agreed with a study conducted in Saudi Arabia and reported that genotype D is the predominant genotype (81.4%), followed by E (5.7%), A (1.4%), and C (1.4%) (18). Other studies in Turkey found that all the HBV detected belonged to genotype D (20, 21). In India, genotype A accounts for 46% of the population, while genotype D accounts for 48%.Whereas the prevalence of HBV genotypes in China was 53% and 41% for genotypes C and B, respectively (23).In France and the United States, HBV genotype A was found in 54% of the specimens, while genotypes B, C, D, and E were found in 4% (12%), 14% (19%), and 1% (24%), respectively.In Poland, genotype A (56%), and genotype D (4%) were found to be (25%).This study shows that genotype D was the most frequent genotype among all the 30 HBV isolates, with liver cirrhosis individuals being detected in 28 (93.3%), genotype B in 20(66.7%), genotype A in 2(6.7%) and finally genotype E in 1(3.3%).Our study reveals that genotypes B and B+D mixed infections are more probably associated with the development of liver cirrhosis in HBV-infected patients, although the associations were insignificant. And genotypes B and D are more frequent among patients with liver cirrhosis, regardless of whether they are pure genotypes or mixed with other genotypes. These differ from the findings of several studies done in China, which reported that the genotype C of HBV is more associated with aggressive disease and the development of liver cirrhosis (26, 27, 28, 29). Other studies found that genotype A of HBV is more associated with liver cirrhosis (30, 31); in addition to that, Kobayashi and his colleagues found genotype A to be associated with milder disease than genotypes B and C (32). These findings differ due to variation in the predominant genotypes' distribution among their study populations. The highest serum concentration of HBV DNA copies in this study was found in pure genotype D, followed by genotypes B+D, B, A+D (54487.50), A+B+D, A, and the mixture of A+B+E genotypes. Genotype D also showed the highest level of viral load in different subgroups of the study population. This was agreed by Feld and his colleagues, who reported that genotype-D infection has been associated with more advanced disease (33). The present study considers the concentration of liver enzymes among different genotypes. AST was high with genotype D (80.9531) and then genotypes B+D (66.7027), A+B+E (63.0000), A+B+D (50.1667), A+D (49.4000), A (48.0000), and B (37.6667). ALT was elevated in genotype D (58.8688), followed by genotypes B+D (42.0811), A+B+D (38.1667), A+D (37.9000), A (31.0000), A+B+E (28.0000), and B (20.6667). These findings illustrate that the liver enzymes AST and ALT were markedly elevated in genotype D, either pure or mixed with other genotypes.

Conclusion

Genotype D is more associated with severe manifestations because it showed the highest levels of viral load, AST, and ALT. Genotypes B and B+D mixed infections are more probably associated with the development of liver cirrhosis in HBV-infected patients, and genotypes B and D are more frequent among patients with liver cirrhosis regardless of whether they are a single genotype or mixed with other genotypes.

Recommendations

Based on the findings of this study, we recommend additional research to determine the role of different HBV genotypes in the development of liver cirrhosis.

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Original Article Sudan Medical Laboratory Journal

Correlation between Serum C-reactive protein Level with Severity of Covid19 Disease

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Abstract

Background: New coronavirus pneumonia (COVID-19) is a health emergency due to its high infectiousness and high case fatality in critically ill patients. C-reactive protein is one of the acute phase proteins and nonspecific markers of inflammation and has been found to correlate with disease severity and treatment of many infectious and non-infectious conditions.

Objective: To correlate the serum level of C-reactive protein among COVID-19 Patients with disease severity.

Materials and Methods: this analytical cross-sectional study, was conducted at Aliaa specialized hospital, Omdurman city, Khartoum state, during the period from January to March 2021. Fifty patients diagnosed with covid-19 using RT-PCR, were enrolled in the study and then classified into severe patients and non-severe patients The CRP was measured by Particle enhanced immunoturbidimetric assay in Integra 400 fully automated instruments. The data were analyzed using SPSS version (25).

Results: Out of fifty patients, 42 patients were the non-severe group, and the remaining eight patients were the severe group. The average age was 66.45 years (ranging between 30 and 120 years), and 54% of patients were males and 36% were females. The most common coexisting conditions were Diabetes Mellitus and hypertension; 60% of patients were having a Diabetes Mellitus, and 52% of patients were having hypertension. The study's findings indicated that the mean CRP level of severe group was considerably higher than that of the non-severe group (365.75± 209.378), with a p-value of (0.002) and the results of the correlation analysis revealing a strong positive link between the severity of the disease and CRP (r=0.419**, p=0.002).

Conclusion: serum C-reactive protein has been found to correlate with disease severity.

Keywords: Covid19, C- reactive protein, Coronavirus.

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Introduction:

The 2019 novel coronavirus (2019-CoV) or the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) as it is now called, is spreading rapidly around the world from its origin in Wuhan City in Hubei Province of China [1].

In total, 494,215,930 cases of Coronavirus disease 2019 (COVID-19) were reported worldwide and 2.35 million deaths were reported as of April 6, 2022, in Sudan 29851 cases were reported. In critically ill patients, COVID-19 is a health emergency due to its high infectiousness and high mortality rates. The pathological and physiological processes and diagnostic methods of COVID-19 are challenged specially for new variants of the virus. In order to improve case fatality, clinical monitoring and appropriate treatment strategies were essential [2].

On February 28, 2020, the World Health Organization raised the global assessment of the risk of COVID-19 spread and impact to very high [3,4].

Reducing the virus's spread and mortality has become a worldwide issue. Because of the rapid increase in the number of COVID-19 patients, hospitals in many regions are facing significant challenges. Severe patients are typically treated in the intensive care unit, while mild patients are admitted to the isolation ward [5]. However, a small percentage of non-severe patients will progress to severe cases. As a result, identifying this group of patients early and actively monitoring and treating them is critical to reducing mortality and improving outcomes in COVID-19 patients [6]. C-reactive protein (CRP) is a non-specific acute phase reactant produced by hepatocytes during infection or inflammation [7]. C-reactive protein (CRP) can be used to help differentiate between viral and bacterial infections. Higher levels indicate more severe infection and have been used as an indicator of COVID-19 disease severity in several studies [8,9]. It can also be used to make an early diagnosis of pneumonia, and patients with severe pneumonia had elevated CRP levels. Because of the high mortality rate of Covid-19 and the lack of effective treatments, it is critical to control covid-19 infections to reduce outcomes and prevent disease aggravation [9,10].

Many studies were conducted to assess the serum level of C - reactive protein and correlate it with disease severity [11, 12]. Elevated levels of serum C-reactive protein (CRP) have been observed in patients with COVID-19 and used to assist with triage, diagnostics, and prognostication [13, 14]. The pathological, physiological, and diagnostic methods of COVID-19 are in the fact-finding stage. Upon examination, the clinical features can be interpreted more clearly through the use of biological markers like CRP. Therefore, investigation of the CRP level might have paramount importance for early diagnosis and appropriate management of COVID-19-related complications [15]. This article aims to explore C - reactive protein in the context of COVID-19 pathogenesis and assess how its level changes with the severity of the disease, and correlate it serum level of C - reactive protein with disease severity.

Materials and Methods

This was cross-sectional study was conducted in Khartoum state at Aliaa specialized hospital during the period from January to March 2021. Fifty subjects were enrolled in the study and then classified into severe patients and non-severe patients according to the clinical criteria, physical examination as po2, CT scan results.

Inclusion and exclusion criteria: patients diagnosed with covid-19 and accepted to participate in the study were included. Patient who refuse to participate in the study, in addition to patient with any type of infection and inflammation and patient with incomplete data were excluded.

Ethical consideration: The local committee of AL Neelain University, the Faculty of medical laboratory sciences, and the ministry of health ethically approved the study. All participants were informed verbally of the aim of the study whereas subjects who refused to participate were excluded from the study. A detailed history was taken from each individual using a pre-designed questionnaire.

Blood sampling: An expert lab technician under a septic condition collected venous blood sample (3ml) from Covid-19 patients in plain containers, blood samples were left to clot at room temperature then serum was separated by centrifugation at 3000 rpm for 5 min for measuring the CRP. The CRP was measured by Particle enhanced immunoturbidimetric assay in Integra 400 full automated instruments. Reference Range (Expected Values: <0.8 mg/dL).

Statistical analysis: The data were analyzed using SPSS version (25). Independent T-test was used to compare mean value C - reactive protein level between severe and non-severe groups, then person correlation coefficients was used to correlate C - reactive protein level with disease severity. Results were expressed as Mean± SD., with the level of significance set at P-value<0.05.

Results

Among the 50 included patients in the age range of 30 to 120 years, 42 patients belong to non-severe group and eight were severe. The average age was 66.45 years, and 54% of patients were male and 36% were female. 30 patients were have Diabetes Mellitus which represent 60%, and 26 patients have a Hypertension which represent 52% of patient were the most common coexisting conditions (Table 1, Figure 1).The study results showed that, the mean level of CRP was significantly high in severe group (365.75 ± 209.378) than in non-severe group (219.02 ± 95.349) with p value (0.002) (Table 2). The correlation analysis showed significant positive correlation between CRP ($r=0.284^*$, p= 0.045) with Disease severity (Figures 3).

Table 1: Demographic Characteristics of Study population

	Severe (8)	Non-severe (42)	P value
Age	72.75 ± 24.341	60.14 ± 13.968	0.04
Diabetes mellitus	8 (16.0%)	22 (44.0%)	
Hypertension	8 (16.0%)	18 (36.0%)	

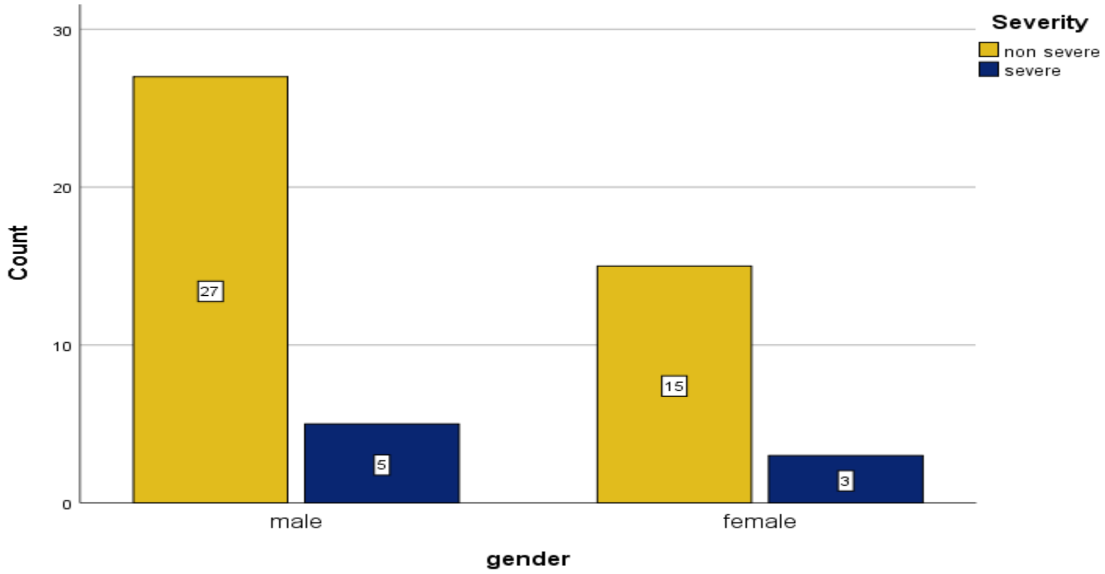

Figure 1: Gender distribution in study population

Table 2: Compares the mean level of CRP in study groups

	Severe (8)	Non-severe (42)	P value
Serum CRP	365.75 ± 209.378	219.02 ± 95.349	0.002

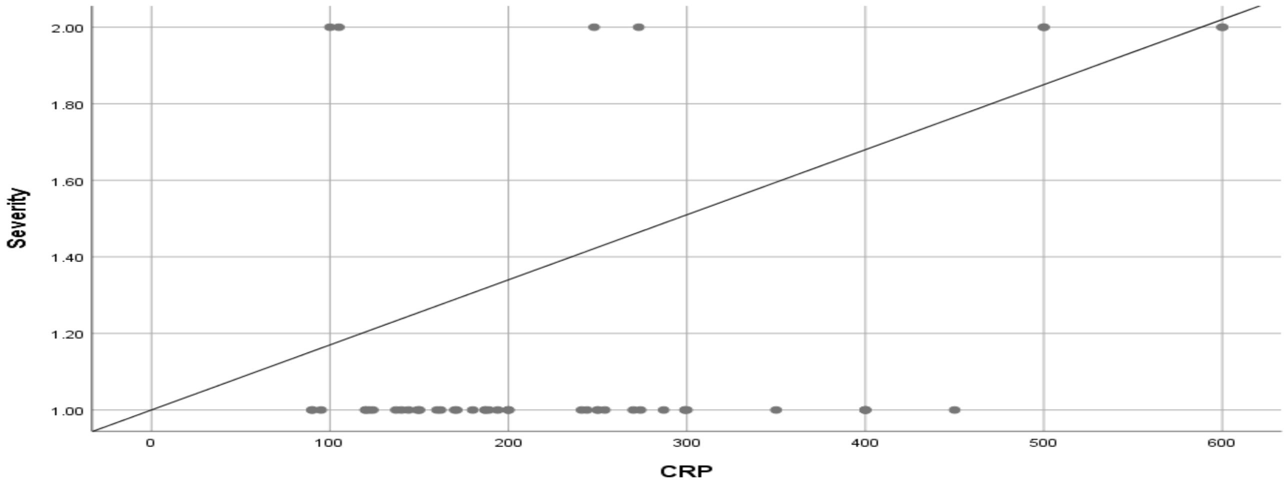

Figure 3: Show correlation of CRP with Disease severity

Discussion

C-reactive protein (CRP) is now considered as a biomarker of infection and can be used as a precise and fast indicator of the chronic inflammation in the human body, because CRP rises dramatically within hours of infection, which activate the complement system via the classical pathway and macrophages via Fc-receptors [16]. In the context of the SARS-CoV-2-induced disease COVID-19 it is remarkable that CRP plasma levels rise to an extent similar to bacterial infections .Further, CRP levels correlate with bad prognosis in COVID-19 and were emphasis to be a reliable marker for a huge number of deleterious processes [17, 18, 19]. In this study a 50 patients diagnosed with COVID-19 were recruited to correlate the level of CRP with disease severity, and the results showed a significant increase in the level of CRP within the COVID-19 which agrees with the previous study[3,20,21] and a more Elevated levels of CRP were observed up in severe COVID-19 patients than non-severe cases [3,12, 20,22,23,24,25] . This elevation indicates that covid19 disease is progressed and lung lesion of patients is large. The elevated levels of CRP might be linked to the overproduction of inflammatory cytokines (cytokines storm) in severe patients with COVID-19. Cytokines fight against the microbes but when the immune system becomes hyperactive, it can damage lung tissue. Thus, CRP production is induced by inflammatory cytokines and by tissue destruction in patients with COVID-19 [26]. The CRP levels were really interrelated with lung lesion and disease severity. This recommends that in the initial period of COVID-19, CRP levels may give the clinician first thought about severity of disease and size of lung lesions. It is clearly known that an appropriate treatment of infected individuals has a great implication in controlling the infectious diseases. Hence, there is an urge of early indicator biomarker of severity in the era of COVID-19 that may help to initiate timely and effective treatment strategies. Infection with COVID-19 is characterized by exuberant inflammatory reaction, particularly of the severe form of the disease resulting from excessive inflammation contributing to the loss of lives related to COVID-19. CRP is an indicator of systemic inflammation. Therefore, the level of CRP may clearly show not only the progression of mildly infected individuals but also dictate the recovery or adverse outcome of severe patients. CRP preferably binds to phosphocholine expressed highly on the surface of damaged cells. This binding makes active the classical complement pathway of the immune system and modulates the phagocytic activity to clear microbes and damaged cells from the organism. When the inflammation or tissue damage is resolved, CRP concentration falls, making it a useful marker for monitoring disease severity. In addition to being a biomarker of acute inflammation, it has recently been shown to be associated with chronic inflammation, such as cardiovascular diseases and Type II diabetes mellitus which may aggregated the severity of patient if infected with COVID- 19 [26, 27].

This study also showed that the number of male patients more than female in this study population with agree with previous study [28].

Conclusion

Finally, this study was concluded that elevation of CRP level increased in severe patients of covid 19 more than in mild cases, and the patients may be in a worse state of this disease.

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Original Article Sudan Medical Laboratory Journal

Cytomorphological Changes in Oral Mucosa as a Result of Cigarette Smoking in Wad Medani City, Gezira State, Sudan (2021)

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Abstract

Background: Smoking is a common practice and damages almost all organs and systems of the body. Oral cavity is rich of flora and contains many microorganisms that cause local and systemic diseases if microbiological flora is altered. Cigarette smoke renders oral mucosa epithelium to be susceptible for colonization of pathogens. These pathogens can cause or contribute formation of systemic diseases.

Objectives: This study aimed to assess the cytomorphological changes in oral mucosa Duo to cigarettes smoking using Pap stain and H&E stain, to find out the better staining results of the two stains, and to assess the effect of age, duration and frequency of smoking on the oral mucosa.

Materials and Methods: This study was cross-sectional study to assess the cytological changes in oral mucosa of cigarettes smokers using Pap stain in Wad Medani City, Gezira State, sudan (2021).The study included 100 cigarette smokers' samples. Samples tacked by plastic stick were spread on a slide and immediately fixed with fixative spray to avoid exposure to dry air. In the pathology laboratory, the samples were stained with Papanicolaou and Hematotoxlin and Eosin. Each specimen had two slides, which was reviewed by a cytopathologist according to criteria of benign and malignant.

Results: The mean age was 24 years. There was insignificant relation between the age of smokers and the cytological diagnosis of samples (P.Value 0.59), but most of normal cases in less than 24 years and most of a typical cases in more than 24years.There was insignificant relation between the duration of smoking and the cytological diagnosis of samples (P.Value 0.534), but most of a typical cases in more than 2years of smoking.

There was insignificant relation between the frequency of smoking/day and the cytological diagnosis of samples (P.Value 0.190).

Conclusion:The mean age was 24 years.Cytomorphological changes in oral mucosa associated with presence of a typical mucosal cell in the age more than 24years old, and in smoking duration more than two years.There was insignificant relation between the frequency of smoking/day and presence of cytological changes in the samples.PAP stain was more sensitive and specific than H&E stain in the diagnosis ofearly malignant oral lesions.

Key words: Cytomorphological Changes in Oral Mucosa; Cigarettes Smoking;PAP; H&E;Sudan

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Introduction:

In recent years cytology has played important role in the diagnosis of various diseases; specially those of neoplastic origin. Cytology is some time use to differentiate between a benign / reactive process and neoplastic or preneoplastic condition. Reagent and automation are now available, but the technical problems no longer present a major concern in this field (1). Oral cancer is a general designation of all malignant tumors appearing in the mouth. In 2018, the number of new oral cancer cases was approximately 354,000, accounting for 2% of the total number of 36 cancer cases. Oral squamous cell carcinoma (OSCC) cases constitute 90% of all oral carcinoma cases (2). In 2016, a total of 48330 oral cavity and oropharyngeal cancer incidents were reported, and an increase of 22.5% of human papillomavirus-related oropharynx cancer was recorded (3). The oral cavity and oropharynx considered the upper part of the digestive system. These two regions are differentiated from each other by their pathologic processes, prognosis, and histological grades (4). The oral cavity is the first part of the digestive system where the food is broken into small pieces by teeth, moistened and lubricated by saliva. The oral cavity consists of two parts, namely; the vestibule and the oral cavity proper. The oral cavity is lined by moist oral mucous membrane or oral mucosa which is continuous with the dry skin at the mucocutaneous junction of the lip (5). The oral **mucosa** has several functions. Its main purpose is to act as a barrier; It protects the deeper tissues such as fat, muscle, nerve and blood supplies from mechanical insults, such as trauma during chewing, and also prevents the entry of bacteria and some toxic substances into the body (6). The oral mucosa has an extensive innervation of nerves; which allows the mouth to be very receptive of hot and cold, as well as touch. Taste buds are also located in oral mucosa and are important for recognition of taste (7). The buccal mucosal membrane secretes moisturizing and lubricating fluids for the mouth and upper throat. These fluids are necessary to prevent drying effects, since this mucosa is part of the membrane system that lines the entire gastrointestinal tract, and this is open to exterior surfaces at both ends (8). Nicotine in cigar smoke (pH 8.5) is yields in small cigars averaged 1.24 and 3.49 mg/unit on ISO and CI regimens, respectively, compared with 0.73 and 2.35 mg/unit, respectively, for the research cigarettes. Nicotine yields per puff were similar between small cigars and cigarettes. We also found that FC (Filtered cigarettes) did not differ from LC (Little cigarettes) in nicotine yields. FC and LC differ from each other in many physical design features (unit weight, filter weight, and filter length), but are similar in others (unit length, diameter, and filter ventilation) (9).Oral mucosa is the first part that affected by smoking. The early significant response to cigarette smoke came from the basal and para basal layers of the oral epithelium, which appear as three-dimensional arrangement of the oral mucosa mimicking the inhalation/exhalation cycle during the exposure to cigarette smoke (10). Smoking can be associated with the decreasing gingival blood flow and epithelial changes. During the oral exfoliative cytology the presence of two or more features consistent with a typical change in cells which is a sign of malignancy. This study aimed to assess the cytomorphological changes in oral mucosa duo to cigarettes smoking using Pap stain and H&E, to find out the better staining results of the two stains, and toassess the effect of age, duration and frequency of smoking on the oral mucosa.

Materials & Methods:

The study design: This study was cross-sectional study to assess the cytological changes in oral mucosa of cigarettes smokers using Pap stain in Wad Medani City, Gezira State, sudan (2021).

The study area: This study was conducted in Wad Medani City and surround villages, Gezira state, Sudan. Wad Medani the capital of Gezira state which lies in the western bank of the Blue Nile, Central Sudan. The study included 100 cigarette smokers' samples.

The inclusion and exclusion criteria: The study included all patients smoke cigarette, but excluded patients with a history of radiotherapy or chemotherapy for oral or other malignancy, patients with history of alcohol consumption and tobacco users, patients with a history of systemic diseases, and patients with a history benign or malignant oral lesions.

The informed consent: The specimens and information were collected from the individuals under privacy and confidentiality and was not be used for any purposes rather than this study**.** Ethical committee approval has also been obtained from Medical Laboratory Sciences.

Collection and preparation: Participants were asked to rinse their mouths with normal saline before samples were taken to eliminate debris and excess saliva from the oral mucosa. Samples tacked by plastic stick were spread on a slide and immediately fixed with fixation spray to avoid exposure to dry air. In the pathology laboratory, the samples were stained with Papanicolaou and Hematotoxlin and Eosin. Each specimen had two slides, which was reviewed by a cytopathologist according to criteria of benign and malignant. Atypia was assessed cytologically by using the presence of two or more of the following features which were consistent with atypia: nuclear enlargement associated with increased nuclear: cytoplasmic ratio, hyperchromatism, chromatin clumping with moderately prominent nucleoli, irregular nuclear membranes and bi- or multi-nucleation, scant cytoplasm, and variation in size and/or shape of the cells and nuclei (11). The results for each stain compared with positive and negative control samples. Also assessment of other factors such as the most affected age group, duration and frequency of smoking.

Protocol of Papanicolaou stain: Ethyl alcohol fixed smear are hydrated in 95% alcohol for 2 min,through 70% alcohol for 2 min, rinse in water for 1 min, **s**tained in harries Hematoxylin for 5 min, rinsed in water for 2 min, differentiated in 0.5% aqueous hydrochloric acid for 10 seconds, rinsed in water for 2 min, blued in Scott's tap water substitute for 2 min, rinsed in water for 2 min, dehydrated in 70% alcohol for 2 min, dehydrated in 95% alcohol for 2 min, dehydrated in 95% alcohol for 2 min, stained in OG6 for 2 min, rinsed in 2 changed 95% alcohol for 2 min in each, stained in EA50 for 3 min, dehydrated in 95% alcohol for 1 min, through absolute alcohol, cleared in xylene and mounted in DPX (12)**.**

Protocol of Hematoxylin and Eosin: The smear was hydrated in 95% alcohol for 2 minutes and was hydrated in 70% alcohol for 2 minutes, then the smear was rinsed in water in for 1 minute and stained with Harris's Hematoxylin for 5 minutes and was rinsed in 1% acid alcohol for few second and blued by tap water for 5 minutes was stained with eosin for 3 minutes the smear was dehydrated through 70% alcohol for 2 minutes ,95%alcoho for

2 minutes then was dried and cleared by xylene and was mounted by DPX media (13).

Statistical analysis: The data was done by SPSS program. P value less than or equal 0.05 was consider statistically significant(*Chi test* was used for analysis).

Results:

The mean age was 24 years (table 1). The duration of smoking of most of the study population was more than 2 years in 52 sample (56.5%) and in duration less than 2 years was 40 samples (43.5%) (table 2). Most of study population frequency of smoking was only one time per day in 52 samples (57.1%), followed by two time per day 21 samples (23.1%), and finally three time per day in 17 samples (18.7%)%) (table 3). Most of samples were normal with H&E stain (91samples) and only one sample was Atypical, in PAP stain the normal samples were 78 , 3 inflammatory samples and 11 Atypical (P.Value 0.7)(table 4). The sensitivity of H&E samples was 100 % and specificity 1.4%. The sensitivity of PAP stain 100% and specificity 100% (Figure1). There was insignificant relation between the age of smokers and the cytological diagnosis of samples (P.Value 0.59), but most of normal cases in less than 24 years and most of a typical cases in more than 24years) (table 5).There was insignificant relation between the duration of smoking and the cytological diagnosis of samples (P.Value 0.53), but most of a typical cases in more than 2years of smoking(table 6). There was insignificant relation between the frequency of smoking/day and the cytological diagnosis of samples (P.Value 0.190) (table7). *Chi test* was used for analysis.

Table 1: age of study population

Age	Frequency	Percent
<24 years	50	54.3
>24 years	42	45.7
Total	92	100.0

Table 2: Duration of smoking of study population

Duration	Frequency	Percent
1-2	40	43.5
>2	52	56.5
Total	92	100.0

Table 3: The frequency of smoking of study population

No	Frequency	Percent
1	53	57.6
2	21	22.8
3	18	19.6
Total	92	100.0

Table 4: The cytological diagnosis of H&E stain and Pap stain

Stain	Normal	Inflammation	Cytological a typia	P.Value
H & E	91	-	1	0.7
Pap	78	3	11	

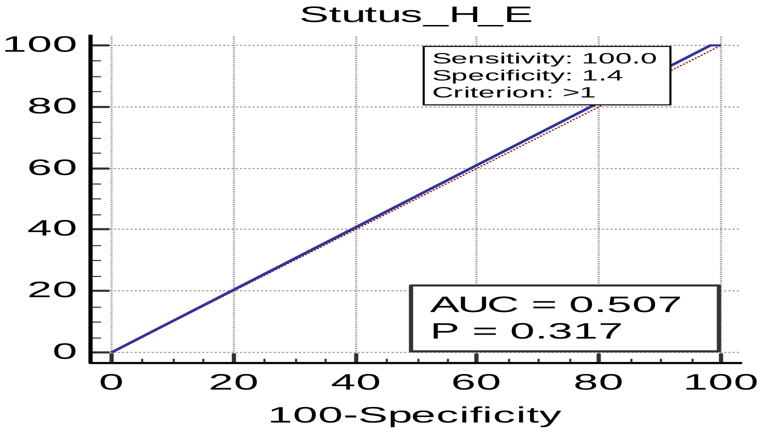

Figure 1: The sensitivity and specify of PAP stain and H&E stain

Table 5: comparison between the age of the smokers and cytological diagnosis of the study population

Age	<24 years	>24 years	Total	P.Value
normal	44	34	78	0.59
Inflammation	2	1	3	
Cytological a typia	4	7	11	
Total	50	42	92	

Table 6: comparison between duration of smoking and cytological diagnosis of the study population

Cytology diagnosis	1-2	>2	Total	P.Value
normal	34	44	78	0.249
Inflammation	2	1	3	
Cytological a typia	3	8	11	

Table 7: comparison between frequency of smoking/day and cytological diagnosis of the study population

Cytology diagnosis	1/day	2/day	3/day	Total	P.Value
normal	45	19	14	78	0.190
Inflammation	1	0	2	3	
Cytological a typia	7	2	2	11	
Total	53	21	18	92	

Discussion:

This study was cross-sectional study to assess the Cytological Changes in Oral Mucosa of Cigarettes Smokers using Pap stain in Wad Medani City, Gezira State, sudan (2021).

The mean age was 24 years (table 1). This result agree with Wen-Jiun Lin, *et al*,(2011), they investigated the association between smoking, alcoholic consumption, and betel quid chewing with oral cancer in a prospective manner. All male patients' age ≥18 years were included. They found that habitual cigarette smokers, alcohol consumers, and betel quid chewers have a higher risk of contracting oral cancer and should receive oral screening regularly to be detected as early as possible (14). The report from the Institute of Medicine (2007) published that tobacco kills more Americans yearly than other hazards**,** many studies detect the relation between the use of tobacco and the development of oral cancer, like a study done at the University of California and San Francisco, found that more than eight out of ten oral cancer patients were smokers (15). When we compare the age of smokers with the cytological diagnosis of samples, we found that there was insignificant relation between the age of smokers and the cytological diagnosis of samples (P.Value 0.59), but most of normal cases in less than 24 years and most of a typical cases in more than 24years) (table 5). Our results agree with Suhail,*et al*, (2014), they diagnosed the association between water-pipe smoking and the age of patients when diagnosed with oral cancer, they concluded that water-pipe smoking is a risk factor associated with the appearance of oral cancer at a younger age (16). Beth Israel Deaconess Center (2019), published According to the Mouth Cancer Foundation, approximately 90% of people with oral cancer are tobacco users, and smokers are six times more likely than non-smokers to develop oral cancer. Mouth cancer affects more men than women. A typical person at high risk for mouth cancer is male, more than the age 40, who uses alcohol and tobacco (17).

The duration of smoking of most of the study population was more than 2 years in 52 samples (56.5%) and in duration less than 2 years was 40 samples (43.5%) (table2). When we compared the duration of smoking with the cytological diagnosis of samplesThere was insignificant relation between the duration of smoking and the cytological diagnosis of samples (P.Value 0.534), but most of a typical cases in more than 2years of smoking (table 6). These findings agree with Ahmed HG and Babiker AA (2009), they established that, chemical carcinogenesis is a prolonged process and progressed with increasing of exposure (18).

Most of study population frequency of smoking was only one time per day in 52 samples (57.1%), followed by two time per day21 samples (23.1%), and finally three time per day in17 samples (18.7%) (table3). When we compare the frequency of smoking/day with the cytological diagnosis of samples, we found there was insignificant relation between the frequency of smoking/day and the cytological diagnosis of samples (P.Value 0.190) (table7). Our results agree with Brian L (2004), They demonstrated that approximately one third of patients with oral squamous cell carcinoma will report that they have never smoked. There was a strong association between a history of smoking and carcinoma involving the posterolateral tongue and floor of mouth (19). Our results agree with Julien Berthiller (2016), they suggest that low frequency of cigarette use leads to the development of head and neck cancer, also smoking duration play a major role in the development of head and neck cancer (20).

Most of samples were normal with H&E stain (91samples) and only one sample was Atypical, in PAP stain the normal samples were 78 , 3 inflammatory samples and 11 Atypical (P.Value 0.7)(table 4). The sensitivity of H&E samples was 100 % and specificity 1.4%. The The sensitivity of PAP stain 100% and specificity 100% (Figure1). When H&E and PAP Stain compare together we found there was no significant difference between the two stains but in spite of that PAP stain was accurate(Atypical cases 20) in the diagnosis than H&E stain (Atypical cases only one). PAP stain was more sensitive and specific than H&E stain. PAP Stain was better because it contain three stains (harries Hematoxylin, OG6 and eosin) .and it give us more details about the maturity of cell's nucleus and cytoplasm.This study agree with(Shukla, *et al*, 2015), they found increasing in the severity of the lesion (21), Beside that Papanicolaou (PAP) stain was found to be the most suitable stain . The study also agree with Rajput, *et al* (2010) they found that Sensitivity and specificity of PAP analysis in the oral smears for detection of oral cancer and normal cells was 91.176% and 100% (22). Gupta *et al* (2019) they concluded that PAP stain was the most suitable stain for screening of oral cancer and can be used as a prognostic indicator (23). Study not agree with Zafar, *etal,* (2020) they found that H&E stain showed sensitivity 44%, Pap 35%,. NPV–H&E 70%, Pap 66% (24).

Cigarettes, the most common form of tobacco used, causes about 90% of all lung cancers, according to the American Lung Association. Smokers are also at a 10 times higher risk for oral cancer compared to non-smokers. Smoking is linked to increased risk for more than 12 other types of cancer, too. In addition, cigarette smoking is linked to nearly 1 in 5 deaths in the U.S. Cigarettes contain more than 60 known cancer-causing agents (25)

Oral cancer ranks eighth among the most common causes of cancer-related deaths worldwide, and tobacco is one the most important carcinogenic factor (26).

Conclusions

The mean age was 24 years. Cytomorphological changes in oral mucosa associated with presence of a typical mucosal cell in the age more than 24years old, and in smoking duration more than two years. There was insignificant relation between the frequency of smoking/day and presence of cytological changes in the samples.PAP stain was more sensitive and specific than H&E stain in the diagnosis of early malignant oral lesions.

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Original Article **Sudan Medical Laboratory Journal**

Evaluation of Hematological Indices Changes in Response to Ramadan Fasting

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Abstract:

Introduction: Fasting in month of Ramadan is one of the five pillars of Islam. Fasting is obligatory for all adults and healthy Muslims during the day hours for the whole month every year. Effect of Ramadan on biochemical parameters is still a matter of debate. Several studies have reported the effect of Ramadan fasting on the values of certain hematological factors, and the impact of Ramadan fasting on various aspects of health, including lipid metabolism.

Objective: The study was aimed to assess the impact of fasting on hematological parameters among Sudanese healthy adults male.

Materials and Methods: An experimental study was conducted between 27 May and 26 June in 2017 (Ramadan of higri year 1438) in Khartoum state(Algerif East). The study was carried out in 20 healthy Sudanese volunteer subjects of aged between 18 to 45 years and within the same life style, food intake and work. After informed written consent; all volunteers were asked to complete a medical tests involving history taking, weight, height, and blood pressure were measured with standard techniques. Five ml of venous blood was collected by a standard procedure from each participant under complete aseptic conditionsand putted in a test tube containing an anticoagulant (Ethyl diamine tetra acetic acid/EDTA). Blood samples were taken in 2 separate sessions on 1st and 2nd day before Ramadan which represented the baseline and during the last 3days in Ramadan At 10 pm. Blood samples were assessed for haemoglobin, Hematocrit, red blood cells count and indices, white blood cells count, differential white blood cells and platelets count. The data were analyzed using SPSS version (24).

Results: Hemoglobin and white blood cells significantly increased at the end of Ramadan (respectively=0.002, P=0.05). There was direct correlation between hemoglobin and hematocrit significantly (P=0.000).

Conclusion: There was significant increase in hemoglobin during Ramadan fasting, because most of the individual eating high or rich source of iron (high biological value protein) and rich source of vitamin C during Ramadan in comparison to other months of the year.

Key words: Fasting, RBCs, WBCs, Indices

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Introduction

Ramadan is the holiest month in the Islamic calendar. Fasting in this month is one of the five pillars of Islam. Fasting is obligatory for all adults and healthy Muslims during the day hours for the whole month every year. The month occurs 11 days earlier every year due to the difference between the solar and lunar years, and may occur in any of the four seasons, making the length of fasting hours variable from 11-18 hours in different countries (1). Fasting of Ramadan is done by over 400 million of Muslims who spread across the globe; and live under various geographical, climatic, social, cultural and economic conditions It is the month during which Muslims refrain from food, liquids and tobacco smoking during daylight hours and eat a main meal after sunset. Drinking and eating are allowed from sunset to dawn. It teaches Muslims self – restraint and remind them of the feelings of the impoverished. Effect of Ramadan on biochemical parameters is still a matter of debate. Energy intake usually decreases during Ramadan (2). Several studies have reported the effect of Ramadan fasting on the values of certain hematological factors (3-7) and the impact of Ramadan fasting on various aspects of health, including lipid metabolism (8,9). In one study, which examined the effect of Ramadan fasting on basic hematological parameters, that is, hemoglobin (Hb), packed cell volume (PCV), RBC numbers, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) the results showed that Hb, PCV, and RBC decreased, MCV, MCH, and MCHC did not. In another study WBC count was found to be significantly lower after Ramadan fasting in both groups (Saleh et al,2004).and showed significant improvement in the mean levels of hemoglobin. Saleh et al found that the changes of Hct during study was not significant (P> .05) but Hb decreased during first 15 days of Ramadan markedly (P< .05), and again increased at second half of the month. The study was aimed to assess the impact of fasting on hematological parameters among Sudanese healthy adults male.

Materials and Methods

This experimental study was conducted in Khartoum state-sudan,algerif east in 2017 when Ramadan fasting occur between May 27 and June 26 [higriyear 1438], blood samples were taken in 2 separate sessions on 1st and 2nd day before Ramadan which represented the baseline and during the last 3days in Ramadan. All the samples were performed at night. All subjects had almost the same life style, food intake and type of work. The samples were taken from 20 healthy subjects volunteered to participate in this study. Their age groups vary between 18-45yrs, the first stage all volunteers were asked to complete a medical test involving history taking, blood pressure height and weight assessment. For examining the parameters of this study 5ml of venous blood was obtained at laboratory from anticubital vein and was collected in a test tube containing an anticoagulant (Ethyl diamine tetra acetic acid/EDTA), 2 days before ramadan and the same was repeated at the end of Ramadan. Blood samples were assessed for haemoglobin, red blood cells, white blood cells count, Hematocrit, MCV, MCH, MCHC, neutrophils, lymphocytes, Eosinophil, basophils and platelets. All data were expressed as Mean+ SEM .Paired t. test was used to compare hematological parameters changes before and during Ramadan fasting. The differences were considered significant when P values were less than 0.05 all analysis was performed using the SPSS.

Results

Hemoglobin and white blood cells significantly increased at the end of Ramadan (respectively P=0.002, P=0.05) other factors not significantly changed, also there was direct correlation between hemoglobin and hematocrit significantly (P=0.000). As shown in table 1.

Table (1): Hematological indices before and after Ramadan fasting (n=20)

	Before	After	P. value
Platelets count	268.65 ±27.58	298.80±33.77	0.07
Hb	13.26 ±0.47	14.03±0.51	0.002
WBC	6.90 ±0.67	7.75±0.82	0.05
HCT	45.20 ±1.20	47.41±1.93	0.06
MCV	80.85 ±2.06	80.13±2.02	0.602
MCH	23.50 ±0.44	23.82±1.27	0.628
MCHC	29.40 ±0.44	29.68±0.96	0.558

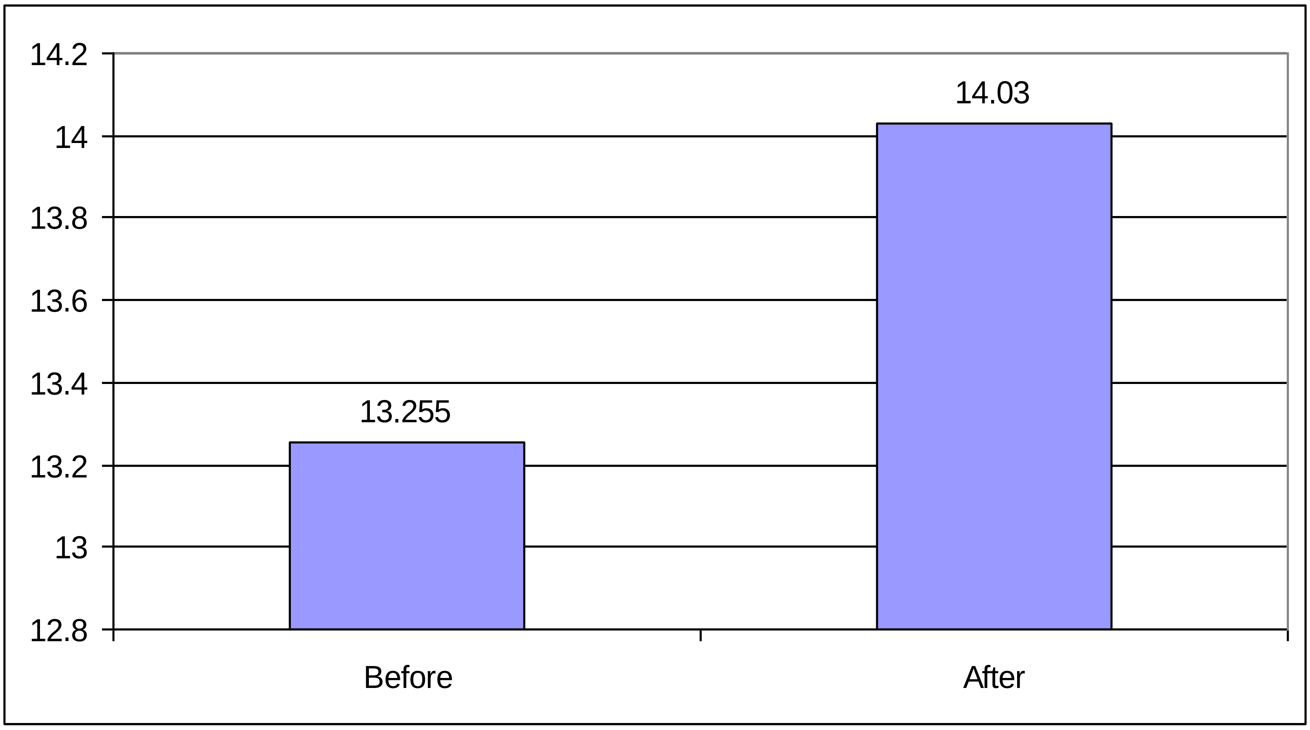

Figure (1) : Hb level before and after fasting Ramadan in adults male

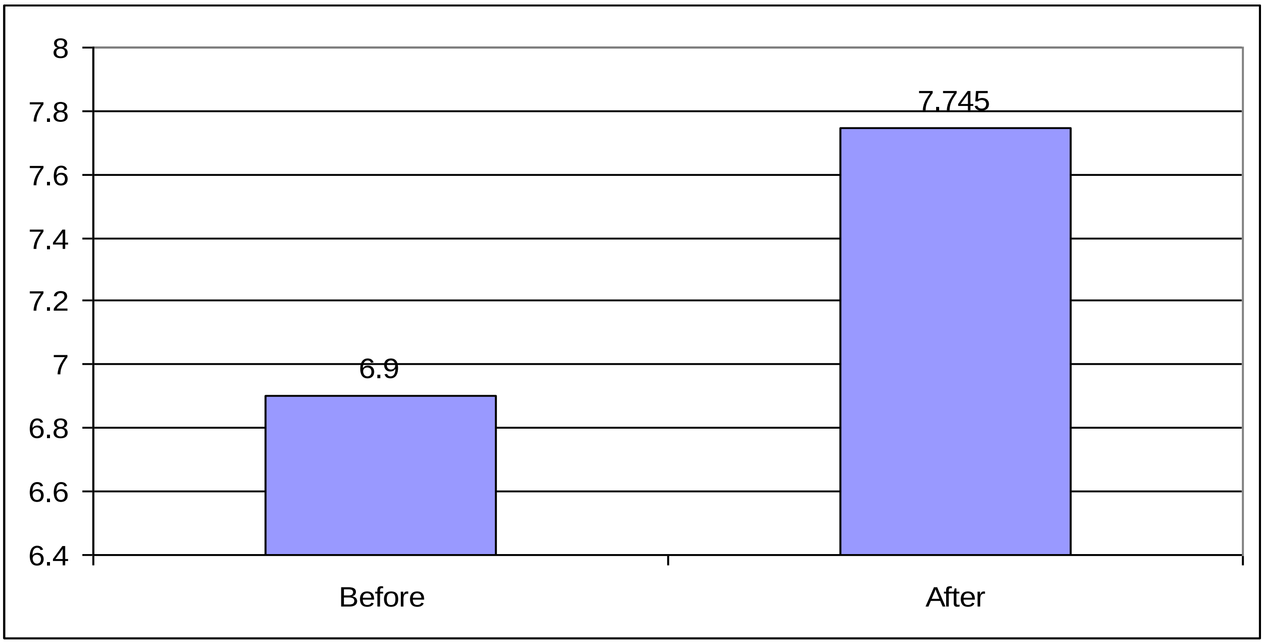

Figure (2): WBCs level before and after fasting Ramadan in adults male

Discussion

The hematological indices affected by many factor including fasting, dietary intake. The statistical association between the hematological indices and fasting was significantly changed, the result showed direct correlation between Hb and WBCS and Ramadan fasting (significantly increased). Also there was significantly direct correlation between Hb and HCT during Ramadan. Compared with other studies which showed: No significant changes were observed in haemoglobin, hematocrit, and red blood cell count values before and during Ramadan fasting. Platelets was decreased significantly during Ramadan compared to before Ramadan (P= 0.002).The WBC count was found to be significantly lower after Ramadan fasting in both groups, (Saleh et al.2004) noted significant improvement in the mean levels of hemoglobin. Indeed, Gharbi et al. (2003) found that eating behavior during Ramadan could lead to an increase in BMI among people at risk of nutritional deficiency.Because most of the individual in my study eat high or rich source of iron eg; red meat and legumes (high biological value protein) and rich source of vitamin C which enhance absorption of iron during Ramadan in comparison to before Ramadan .As a result of that nutritional behavior during Ramadan Hb level significantly increased.

Conclusion: Based on the results of our research there was significant increase in hemoglobin during Ramadan fasting, because most of the individual eat high or rich source of iron (high biological value protein) and rich source of vitamin C during Ramadan in comparison before Ramadan .

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Original Article Sudan Medical Laboratory Journal

A comparative study of the use of formal ether concentration, wet preparation, and zinc sulphate flotation as methods for diagnosing intestinal parasites. Omdurman, Sudan

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Abstract

Background: Fecal formal ether concentration technique has become routine methods as a part of complete parasite examination. It allows the detection of small organisms that may be missed by using zinc sulphate flotation and direct microscopy techniques.

Objective: To compare the reactivity of formal ether, zinc sulphate flotation, and direct microscopy techniques in detecting parasites ova, cysts, and larvae in stool samples.

Material and Methods: This is a descriptive cross sectional study conducted during the period from September to December 2021 in Ombada hospital. A total of 50 stool samples was collected and preserved in 10% formal saline. All samples were examined using wet preparation, Zinc sulphate flotation and formal ether concentration techniques.

Results: The Positive detection rate by Zinc sulfate flotation technique (28%) and types of parasites detected *E. histolytica* (29%), *Giardia. lamblia* (25%), and the detection rate by formal ether concentration technique detection rate (40%) and The most intestinal parasites type detected *E. histolytic a* (34%), *Giardia lamblia* (43.7%) *Ascaris.lumbericoide* (0%), *H. nana* (33%) and *Taenia SPP* (50%) and wet preparation detection rate (36%) the types of parasites detected *E. histolytic* (34%), *Giardia. lamblia* (31.3%),

Key words Comparison, reactivity detection, formal ether, Zinc sulphate ,flotation Techniques.

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Introduction:

Stool examination using conventional techniques or commercial kits which have low sensitivity (1). Are characterized by ease of performance and low cost for direct identification of infective agents (2). Fecal formal ether concentration technique has become a routine procedure as a part of the complete parasite examination. It allows the detection of small organisms that may be missed by using only direct wet smear (3). The zinc sulphate flotation technique is commonly used in laboratories to float parasite eggs and cysts in stool specimens (4). And remain the most performed, widespread, and appropriate methods to detect human intestinal parasites (5).However, *Microsporidium* Spp size 1-2.5 µm, *Cryptosporidium* Spp size 4-6 µm, *Cyclospora* spp size 8-10 µm, and *Cystoisospora* spp size 20-30 µm are difficult to detect by the conventional parasitological techniques. A major obstacle in the detection of these parasites is the need to perform specific permanent staining techniques (6). This technique also provides certain advantages including less alteration to organisms and increased recovery of helminthes eggs and protozoan cysts (7). That require specialized personnel or the time-consuming and labor intensive (8). In addition, errors in test interpretation can be occur because the parasitic structures (oocysts) are much smaller than larvae and helminth eggs. Therefore, clinicians may easily mistake real oocysts for yeasts or debris (9) Except for *Cysts isospora* spp. The technological development and combining alternative methods have improved the diagnostic accuracy of these tests. For instance, the parasitological examination unfortunately, extremely flammable, is highly volatile, produce anesthetic vapor, and forms explosive peroxides when exposed to light (10). Moreover, it can be a possible cause of mutagen, if inhaled or absorbed through skin with harmful long term health effect like neurotoxicity or cancer (11). The most commonly used parasitological diagnostic methods for detection of intestinal helminthic and protozoan infection in human are inexpensive and simple to perform, however they have important limitations, particularly regarding their sensitivity. Therefore, the use of more than one diagnostic methods is necessary to detect different parasitic evolving forms, such as eggs, larva, cysts, oocysts and trophozoites, due to the differences in size, morphology, density, and motility among them. More over the use of different parasitological methods is necessary to improve sensitivity for helminth and protozoa diagnosis in patients with low parasite burdens (12). This study aim to compare the reactivity of formal ether, zinc sulphate flotation, and direct microscopy techniques in detecting parasites ova, cysts, and larvae in stool samples.

Material and Methods:

This is a descriptive cross sectional study was conducted in Ombada hospitals in Omdurman-Sudan from September to December 2021. A total of 50 faecal samples was collected in containers contain 10% formal saline as preservative reagents. were examined by microscope, formal ether concentration and zinc sulphate flotation techniques as intestinal diagnostic methods, preserved faeces in formal saline were emulsified and homogenized on one drop of emulsified faecse suspensions, placed on a slide microscope, covered with cover class and examined microscopy by wet preparation technique, using the 10 x objective and 40x objective of microscope lens for detecting intestinal parasites in faeces. Using formal ether concentration method, 1 g of faeces emulsified in 4 ml of 10% forma water in test tubes, 3-4 ml of formal water added further in test tubes and mixed well, emulsified faeces sieved in beaker, 2-3 ml of diethyl ether added to the suspension and mixed in the test tubes, homogenate suspension was centrifuged at 750-1000 g for 1 minute and the sediment was examined microscopically by using the 10x objective and 40 x to identify intestinal parasites in faeces. Zinc sulphate solution concentration was 33%w\v Reagent, specific gravity 1.180-1.200, about one quarter of zinc sulphate solution added in to test tube mixed by 1gram of faeces (or2 ml if a fluid specimen), Using a rod or stick, emulsify the specimen in the solution, Fill the tube with the zinc sulphate solution and mix well, strain the faecal suspension to remove large faecal particles, Rectum the suspension to the tube and Stand the tube in completely vertical position in a rack. Using a plastic bulb pipette or Pasteur pipette add further solution to ensure the tube is filled to the brim. Carefully place a completely clean (grease –free) cover glass on top of the tube. avoid trapping any air bubbles, lave undisturbed for 30-45 minutes to give time for the cyst and egg to float, carefully lift the cover glass from the tube by a straight, pull up wards and examine microscopically using 10x and 40 x to identify intestinal parasites.

Results: A total of 50 faecal samples collected from Ombada hospitals Omdurman examined the detection rate by the three methods for intestinal parasites shown in (Table1) and Figure (1). The positive detection rate of the formal ether Concentration technique method showed the highest rate of positive detection among this three methods the positive detection counted 40 out of 50 samples, counted (80%) On the other hand the wet preparation technique comes in second which counted 36 out of 50 represented (72%) and Zinc Sulphate solution techniques comes the third one with positive detection rate counted 28 out of 50 represent (56%). The types of parasites detected by three methods, *Endameba*. histolytic a was the most frequent detected parasite, it was more detected by wet preparation method showed detection rate 17 out of 47 represent (36.2%) and formal ether concentration technique showed detection rate 16 out of 47 represent (34%), while zinc sulphate flotation technique showed lowered positive detection rate for *E. histolytica* by rate 14 out of 47 represent (29.8 %) as shown in the (Table 2) and figure (2). *Giardia*. l*amblia* was more detected by formal ether concentration technique showed detection rate 21 out of 48 represent (43.7%), while detection rate by Wet preparation reached (31.3%) from a total 15 out of 48 and zinc sulphate flotation technique showed lowered detection rate of *Giardia. lamblia* (25%) represent 12 out of 48 in the (Table 2) and figure (3). Other parasites like *Hymenolepis nana*, *Enterovirus. vermicular*, *Taenia spp* and *Ascaris .lumbericoid* showed lowered detection rate and was more detectable by the wet preparation method as shown in the (Table 2) and figure (2).

Table 1: Positive detection rate according to type of method used.

	Detection Method	Positive		Negative		Total	
		N	%	N	%	N	%
1	Wet preparation	36	72%	14	28%	50	100%
2	Concentration Technique	40	80%	10	20%	50	100%
3	Zinc Sulphate technique	28	56%	22	44%	50	100%

Table 2: Positive detection rate according to type of parasites and methods.

	Parasites	**Wet preparation**		**Concentration technique**		**Zin sulphate technique**		**Total**	
		N	**%**	**N**	**%**	**N**	**%**	**N**	**%**
1	E. histolitica/coli	17	36.2%	16	34%	14	29.8/%	47	100%
2	Giardia. Lamb Lia	15	31.3%	21	43.7%	12	25%	48	100%
3	Ascaris .lumbricoides	1	50%	1	50%	0	0	2	100%
4	Hymenolepis .nana	1	100%	0	0	0	0	1	100%
5	Enterobius vermicalaris	1	33.3%	1	33.3%	1	33.3%	3	100%
6	Taena Species	1	50%	1	50%	0	0	2	100%

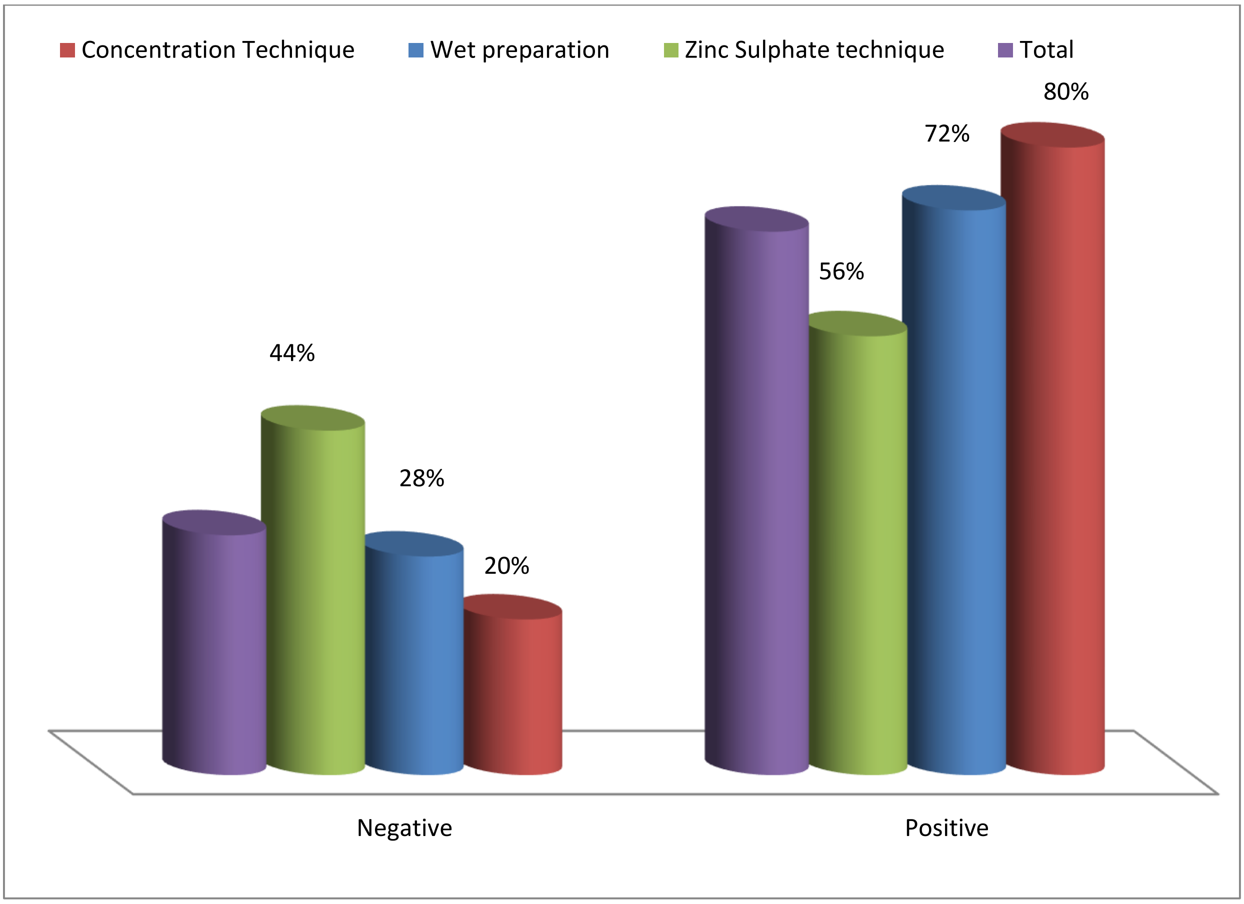

Figure 1: positive detection rate by type of methods

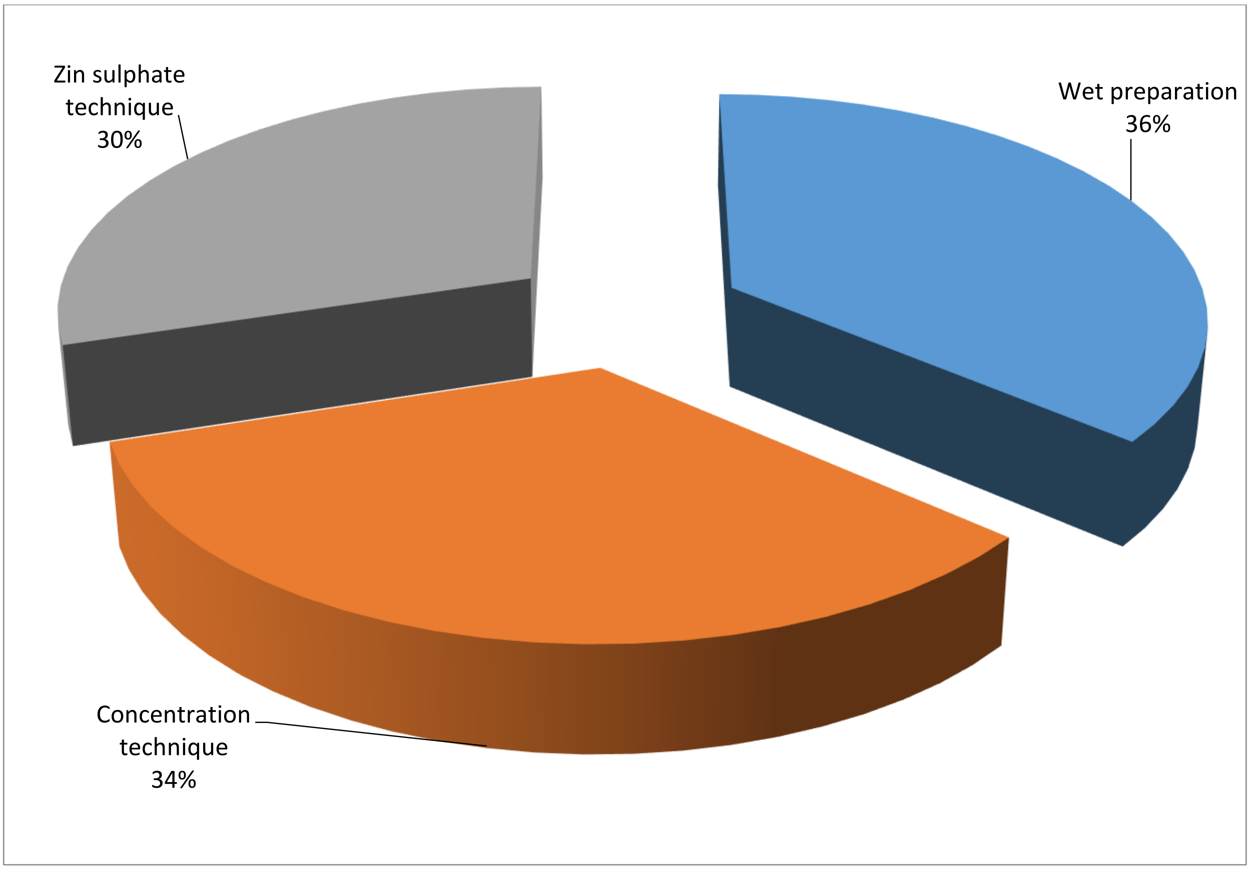

Figure 2: E. histolytic a detection rate by methods

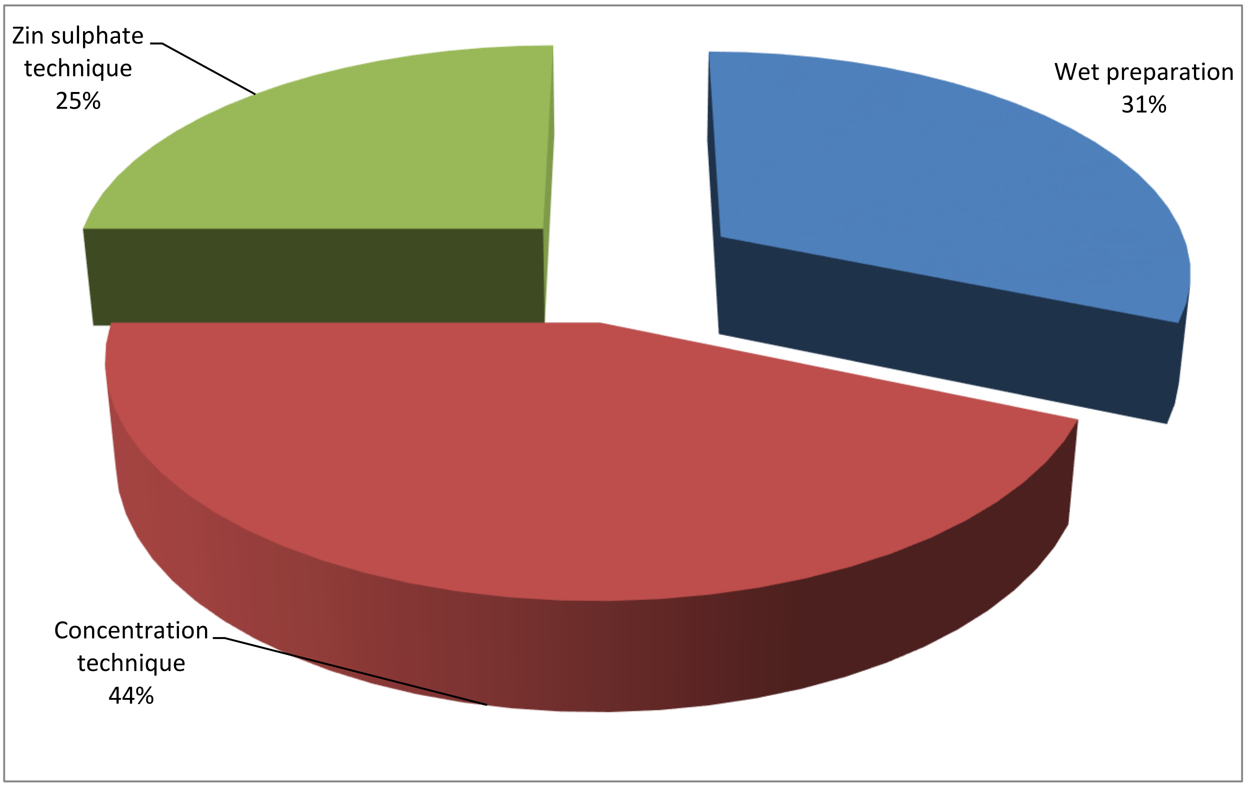

Figure 3: ***Giardia*.lamblia detection rate by three methods**

Discussion:

The goal in using the formal ether concentration method is to improve the detection of protozoan cysts and some helminths eggs that is missing in conventional techniques used in stools analysis by wet preparation and zinc sulphate flotation, some parasites their densities are higher than zinc sulphate and they cannot rise to the surface (13).Therefore the use of more than one parasitological methods is needed to detected different intestinal parasitic evolving forms such as eggs, larva, cyst and trophozoites due to their different in size, morphology, density and motility among them{ 14 }. In the present study the results showed that 50 stools samples examined ,six intestinal parasites detected by different methods employed the results reveal that the detection rate of protozoan parasites by formal ether concentration technique 40(80%) , wet preparation 36(72%) and zinc sulphate 28(56%) this detection rate showed significant differences in percentage between three methods used to identification of intestinal parasites when compared this results by pervious results reported by Katagiri G,etal . 2010(16) showed agreement with our results. Our results obtained in the present study showed that the frequency of protozoan parasites identified by three methods *Giardia. lamblia* counted 15/48 (31. %) by wet preparation ,21/48 (34%) formal ether concentration technique and 2/ 48 (25%) by zinc sulphate floatation technique this results showed that formal ether concentration technique and wet preparation technique were more effective techniques for identification and detection of *Giardia. lamblia* in stool samples than zinc sulphate technique which showed low accuracy in detection of *Giardia. lamblia* this can be explained that the hypertonic zinc solution may not be appropriate for the detection or the polymorphism of this protozoa with different micrometers when compared this results with pervious study results by Eymael D, *etal*.2010 (7) showed concordance. In The present study the frequency of *E. histolytica* detected by three methods employed showed higher frequency differences in diagnosis when detected by formal ether concentration method represent 16 out of47 (34%), wet preparation method counted 17out 47(36%) and zinc sulphate counted 14 out47 (29.8%) this results when compared by previous results reported by faust EC,*etal* .1939 (8) showed agreement. The results reveal that the heavy helminthes eggs such as those *Taenia spp , H.nana* and *E.vermicularis* were only detected by wet preparation method and formal ether concentration method due to their high density and subsequent tendency to settle along with various faecal debris , this results when compared with previous results reported by Scandrett WB, *etal***.**2004 (18) showed agreement. Other previous studies reported by Santos FLN, etal.2007 (17) agree with our present study results, the formal ether concentration method is a more effective technique for identification of *H.nana* and Giardia *lamblia* cyst and some eggs of helminthes in faecal samples. In our present study we observed that detection of *Taenia spp, E. vermicularis and*

H.nana diagnosed by different three methods showed significant differences detection rate in all methods because this heliminthes itis usually based on the recovery of typical eggs on perianal skin when compared this results with previous results reported by John DT, Petri WA, 2009 (20) showed agreement .

Conclusion: the prevalence of intestinal parasites diagnosed by these techniques showed that detection rate of intestinal protozoal parasites was higher significant detection rate than intestinal helminthic parasites infection, there were significant differences between these methods in the identification of intestinal parasites and formal ether concentration techniques consider to be the most accurate methods of chose in detection of intestinal parasites than wet preparation and zinc sulphate.

Conflict of interest: There are no conflict of interest.

Financial: This study has not received financial support.

Acknowledgment: We would like to appreciate laboratory staph of Ombada hospitals Omdurman.

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Original Article Sudan Medical Laboratory Journal

Analysis of environmental Infection Control practices among TB laboratories in Khartoum state-Sudan

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Abstract

The tuberculosis laboratory, patient care settings require specific strategies directed at the prevention of transmission of diseases among health care workers and their patients.

Aim: The aim of the present study was to assess the knowledge, attitude and practice of infection control among TB laboratories in Khartoum state- Sudan

Materials and Methods: A cross-sectional study using a structured distributed questionnaire was carried out included the key areas of infection control including Quality Control, personal protection, and environmental infection control.

Result: A total of sampling laboratory in Khartoum State in the study. Ten tuberculosis laboratories were visited. There was a collection area separate for TB samples collection and we found 50% only had a separated area for sputum sample collection.

Conclusion: Assessment of environmental Infection Control practices Among TB laboratories the indicated that the standards infection control was very low

Keywords: Barbers; Tuberculosis laboratory, TB-IC Infection Control, Khartoum-Sudan; analysis environment

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Introduction

Tuberculosis (TB), is a chronic highly infectious disease and from ancient times till today, it remains the captain of death worldwide. According to estimates, 10 million cases of TB will be reported in 2020, and approximately 1.5 million people will die from TB globally, including 214000 HIV-infected patients (1). The emergence of numerous drug-resistant strains of Mycobacterium tuberculosis, including mono, multi, pre-extensive, and extensive drug-resistant strains, is posing a threat to global efforts to control and eradicate the disease (2). It has been reported that a third of the estimated burden in 2020 was enrolled in MDR treatment regimens, which highlight the lack of early and accurate diagnosis of cases (2).

Sudan is the African country that suffered from mismanagement of resources for several decades and is still excruciating from poverty (3) and limited healthcare infra-structure and disease prevalence data which is a vital necessity to inform better introduce interceptive measures (4). In Sudan, it has been estimated 28,000 new TB cases in 2020, and that includes 160 confirmed cases of mono and multi-drug-resistant (5). All healthcare facilities, both public and private, and all other settings where TB patients or persons suspected of having TB congregate, should implement TB Infection Control (TB-IC) measures. The measures selected will depend on the infection control (IC) risk assessment, which in turn is based on the local epidemiological, climatic and socioeconomic conditions, as well as the burden of TB (6).

Methods

A cross-sectional study was conducted at the Khartoum State TB laboratories diagnosis Centre between April 2019 and January 2020. 10 tuberculosis diagnosis labs were visited. The purpose and research procedure were first explained to each subject. After a verbal consent to participate in the study, data were collected from each individual under study by interviewed questionnaire. Information was obtained on whether there is a allocated area separate for TB lab?, The facility design, patient flow and triage system comply with what is outlined in the infection control plan and/or national infection control policy. ? Waiting area is well ventilated (i.e. windows and doors open when feasible) and there is clear display of messages on cough hygiene in all areas frequented by patients? The data of the current study were statistically analyzed by SPSS version 26.0

Result

Total of 10 tuberculosis diagnosis labs were visited. Five of the visited laboratories had a separate collected area for TB (50%) and five of them had no separate collected area for TB (50%) (Figure 1), and there was no budget allocated for TB infection control activities for all labs (table 1).

Regarding the patient triage and patients flow for TB laboratories we found only one laboratory (10%) performing the patient triage and patients flow and comply with what is outlined in the infection control plan and or national infection control policy (Figure 2).

The waiting area is well ventilated in all the TB laboratories (100%) (ie. windows and doors open when feasible) and there is clear display of messages on cough hygiene in all areas frequented by patients. (Table 2).

For waste management in the TB labs, all of them (100%) perform waste management (table 3).

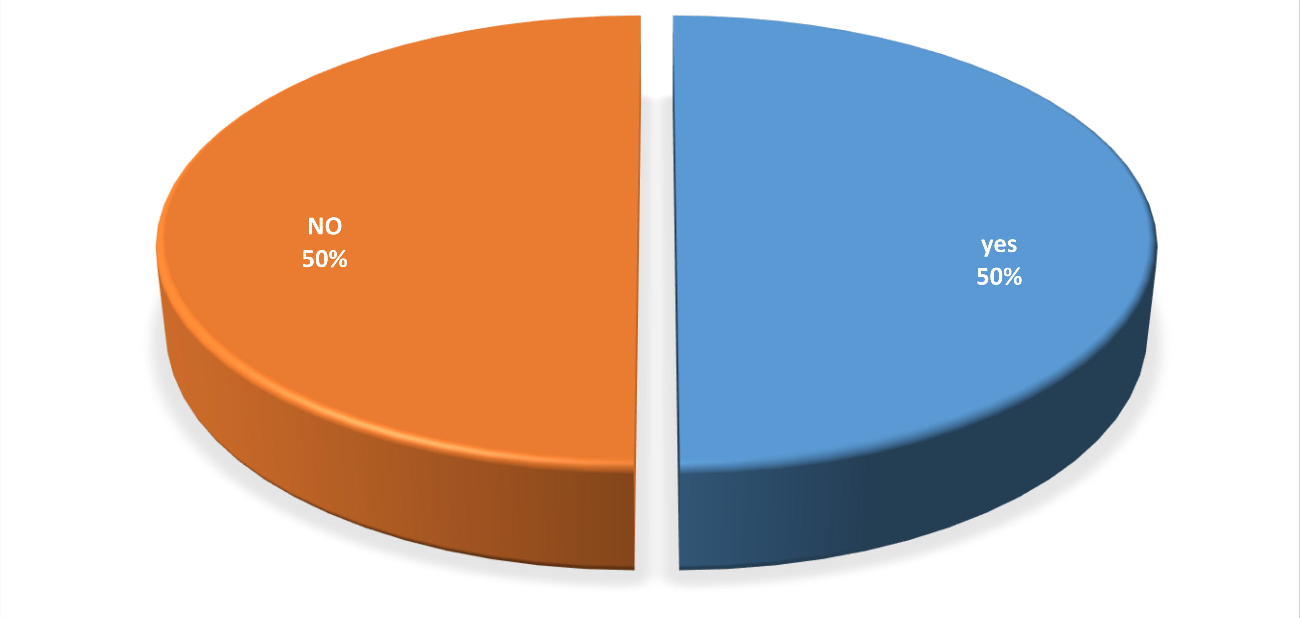

Figure 1: allocated area separate for TB lab

Table 1: The budget allocated for TB infection control activities*:*

budget allocated	Frequency	Percent
Yes	0	0.0%
No	10	100%
Total	10	100%

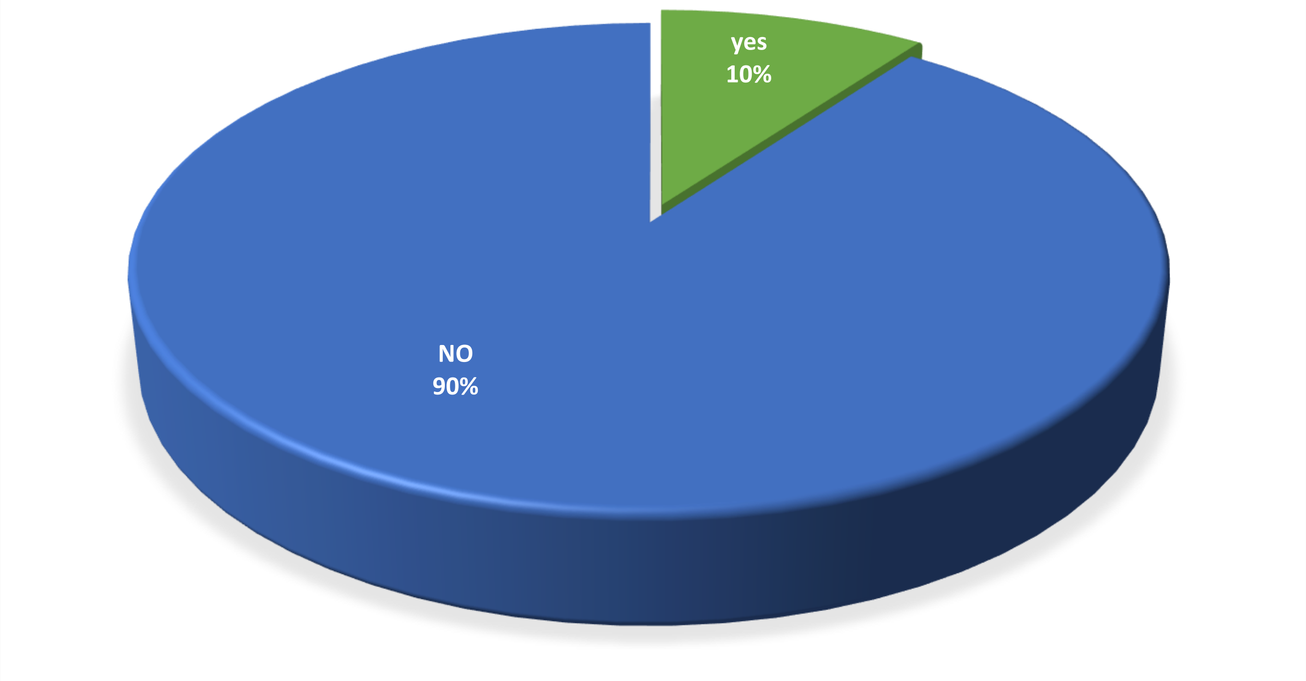

Figure 2: The facility design, patient flow and triage system

Table 2 : Waiting area is well ventilated

Waiting area ventilation	Frequency	Percent
Yes	10	100%
No	0	0%
Total	10	100%

Table 3: waste management for the TB labs

waste management	Frequency	Percent
Yes	10	100%
No	0	0%
Total	10	100%

Discussion

TB remains the leading cause of morbidity and mortality in adulthood globally, with approximately 10 million of TB cases reported annually [7]. High rate incidence of TB occurring in low income and below poverty line countries as TB considered a disease of poverty [8]. There was no written facility-specific infection control plan and also there was no Designated TBIC focal person has received documented TBIC training or refresher training. Similar were observed in the study, as Only 38 % of the study participants had proper overall TBIC practice in West Gojjam Zone. Measures of TBIC practices were variable from good in practicing natural ventilation (89.2 %) to low (23.5 %) use of N95 respirators by staff at the health centers Even though TBIC focal persons were assigned in each health centers the presence of TBIC policy/plan and national guideline for the management of TB was known by 72.9 and 75.8 % of participants respectively. This finding is in line with the study in South Africa[9.10].In which despite a TBIC officer appointed, there was no announced to all health workers. This may be due to the gap that health centers and woreda health offices didn't give emphasis to the program and it was left to those health workers working in TB rooms. In this study only 34.5 % of participants were ever trained on TBIC which is lower than the study done in Uganda (48.6 %) [11]

Conclusion

This study indicated that the standards infection control precautions adopted by the diagnostics laboratories in Khartoum state was very low. In addition to that, awareness of laboratory personnel towards infection control and biosafety principles implementation was very low too.

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OriginalArticle Sudan Medical Laboratory Journal

Frequency of urinary tract infection among cancer patients receiving the chemotherapeutic drug at radiation and isotopes center of Khartoum (RICK)

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Abstract

Background: Chemotherapy often decreases the number of white blood cells in the body, Neutropenic patients, usually have their course of treatment interrupted and stopped unless blood cells return to normal.

Aims: To isolate and identify the bacterial causative agent of UTI among cancer patients receiving a chemotherapeutic drug. Also, evaluation of antibiotics used for treatment and the incidence of infection in males, females and children

Methods: Fifty midstream urine (MSU) specimens were collected from patients who undergo chemotherapy treatment then specimens were analyzed to isolate and identify bacterial pathogens, then standard AST was performed to screen the antibiogram of isolates

Results: Out of 50 urine specimens investigated, 23 different isolates were recovered giving a percentage of (46%), and 27 urine specimens (54%) showed no growth. *S. aureus* (26.1%) followed by *E.coli* (21.7%), *S. epidermidis* (17.4%), *K. pneumoniae* (13%), *p. mirabilis* (13%), and *Ps. aeruginosa* (8.7%).

Conclusion: The most frequent isolate is *S. aureus* and *E.coli*. Females and adults were more infected than males and children respectively.

Keywords: UTI, a chemotherapeutic drug, and RICK

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Introduction

The therapies that are usually used to treat cancer (for example chemotherapy), often decrease the number of white blood cells in the body. White blood cells fight bacterial, fungal, and viral infections (1). Most patients with cancer who have few white blood cells have a very weak immune system and can't fight infection well. They get infections easily and as result can die, particularly if the infections are not recognized early and treated (2). The spectrum of bacterial and fungal infections undergo periodic change and is impacted by several factors including the use of antibacterial/antifungal prophylaxis, the use of foreign medical devices, and the nature and intensity of the antineoplastic used (3).

Fever is usually one of the earliest signs of infection. Thus, powerful antibiotics are used to treat an infection in a patient with cancer whose white blood cell count is low. Combinations of antibiotics that work against many different types of bacteria are successful.

Neutropenic patients, usually have their course of treatment interrupted and stopped unless blood cells return to normal (4).

Methods

Study area: The study was carried out at the Radiation and Isotope Center Khartoum (RICK).

Collection of specimens: Fifty midstream urine (MSU) specimens were collected from patients who undergo chemotherapy treatment, in sterile, dry, wide-mouth, leak-proof containers. These specimens were collected from the Radiation and Isotope Center of Khartoum (RICK).The specimens were immediately inoculated on blood agar and MacConkey agar (5).

Isolation and identification

Using several media and biochemical test for Isolation, identification and susceptibility of the antibiotics to the bacterial causative agent of UTI, urine sample cultivated on MacConkey agar medium and Blood agar (HiMedia Laboratories Pvt Ltd, India), to isolate the bacteria, according to colonial morphology and gram stain the isolated microorganism identified by set of biochemical test including), catalase test, Coagulase test, Deoxyribonuclease agar (DNase), Kligler iron agar (KIA), Simmon's citrate media, Christensen, urea agar media, Mannitol salt agar (HiMedia Laboratories Pvt Ltd, India).

Kirby-Bauer method used to detect the sensitivity of isolated bacteria to the antibiotic (table 1) using Muller Hinton agar (HiMedia Laboratories Pvt Ltd, India).

Table 1: Antimicrobial drugs

Antimicrobial agents	Symbol	Disc potency	Diameter of zone of inhibition (mm)		
			Susceptible	Intermediate	Resistant
Ampicillin	AMP	10 mcg	≥17	_	≤16
Chloramphenicol	C	30 mcg	≥18	13-17	≤12
Ciprofloxacin	CIP	5 mcg	≥21	16-20	≤15
Erythromycin	E	15 mcg	≥23	14-22	≤13
Gentamicin	GEN	10 mcg	≥15	13-14	≤12
Methicillin	MET	5mcg	≥14	10-13	≤9
Nitrofurantoin	NIT	300mcg	≥17	15-16	≤14
Novobiocin	NV	30mcg	≥17	18-21	≤22
Oxacillin	OX	1 mcg	≥20	11-12	≤10
Penicillin	P	10 units	≥29	-	≤28
Tetracycline	TE	30 mcg	≥15	12-14	≤11

Results

During the period between February and March 2022, a total of 50 samples was collected from cancer patients who receive chemotherapy treatment (29) were female and (21) were male, (78%) of female were infected and (22%) of male were infected, (Table 5).

The incidence of infection among the different ages was (26%) in children and (74%) in adults, (Table 6).

From the 50 urine specimens investigated, 23 different isolates were recovered giving a percentage of (46%), and 27 urine specimens (54%) were clear, (figure 1). The predominant isolate was *S.aureus* (26.1%) followed by *E.coli* (21.7%), *S.epidermidis* (17.4%), *K.pneumoniae* (13%), *P.mirabilis* (13%) and *Ps.aeruginosa* (8.7%), (Figure 2).

In-vitro sensitivity tests for four antimicrobial agents by the Kirby-Bauer method were carried out on (10) Gram`s positive organisms and (13) of Gram`s negative organisms.

S.aureus susceptibility, using four antibiotics, showed that Chloramphenicol is highly active (100%), followed by fusidic acid (83%), penicillin (67%), and methicillin (50%), (Figure 3).

S.epidermidis susceptibility, using four antibiotics, showed that gentamicin was highly active (100%), followed by Chloramphenicol (75%), penicillin (25%), and then methicillin (0%), (Figure 4).

E.coli susceptibility, using four antibiotics, showed that Chloramphenicol is highly active (80%), followed by ampicillin and nitrofurantoin (60%), and ciprofloxacin (40%), (Figure 5).

Proteus mirabilis susceptibility, using four antibiotics, showed that Chloramphenicol, ciprofloxacin, and tetracycline were highly active (100%), followed by ampicillin (67%), (Figure 6).

Klebsiella pneumoniae susceptibility, using four antibiotics, showed that ciprofloxacin was highly active (100%) followed by Chloramphenicol, tetracycline (67%), and nitrofurantoin (0%), (Figure 7).

Pseudomonas aeruginosa susceptibility, using four antibiotics, showed that Chloramphenicol and ciprofloxacin were highly active (100%), followed by ampicillin (50%), nitrofurantoin (0%), (Figure 8).

Table 2: Morphological characteristics on CLED agar and Gram`s reaction of isolates

Organisms	CLED	Gram stain
E. coli	Moderate, moist, yellow L.F Colonies	Gram- negative bacilli
S. aureus	Small, moist, yellow L.F Colonies	Gram- positive cocci
Ps. Aeruginosa	Moderate, moist, blue-green NLF colonies	Gram- negative bacilli
K. pneumonia	Large, mucoid, yellow L.F Colonies	Gram- negative bacilli
S. epidermidis	Small, moist, yellow LF Colonies	Gram- positive cocci
P. mirabilis	Moderate, moist, blue-green NLF colonies	Gram- negative bacilli

Table 3: Biochemical tests

species	Biochemical tests						
	Indole test	Urease test	Citrate test	KIA			
				slope	butt	Gas	H2S
E. coli	positive	Negative	Negative	Y	Y	+ve	-ve
K. pneumoniae	Negative	Positive	Positive	Y	Y	+ve	-ve
Ps. aeruginosa	Negative	Negative	Positive	R	R	-ve	-ve
P. mirabilis	Negative	positive	Positive	R	Y	+ve	+ve

Table 4: Biochemical test of isolated gram positive bacteria

Species	Biochemical tests		
	Mannitol fermentation	Catalase test	DNase test
S. aureus	positive	Positive	positive
S. epidermidis	negative	positive	negative

Table 5: Incidence of urinary tract infection among both males and females who receive chemotherapeutic drugs:

Gender	Urinary tract infection	
	Frequency	percentage
Female	18/23	78%
Male	5/23	22%

Table 6: Incidence of urinary tract infection among adults and children who receive chemotherapeutic drugs

Age	Urinary tract infection	
	Frequency	percentage
Adult	17/23	74%
Children	6/23	26%

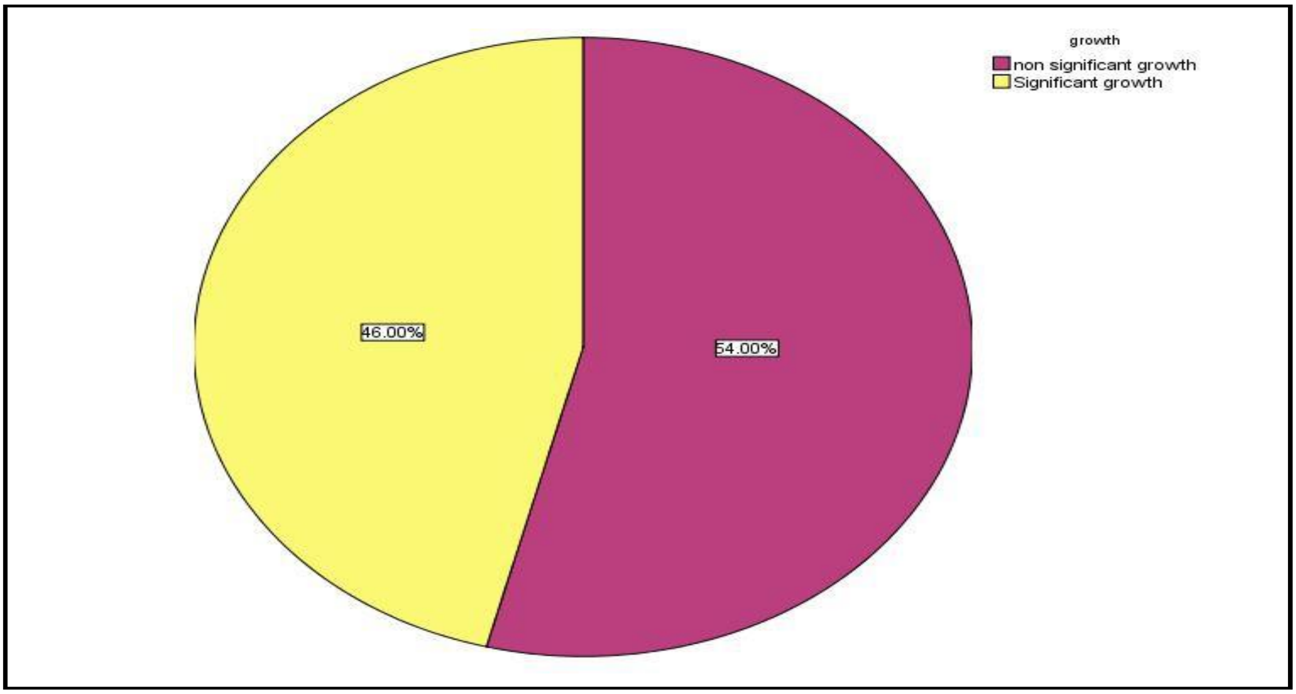

Figure 1: Bacterial growth on CLED media for primary isolation of causative agents.

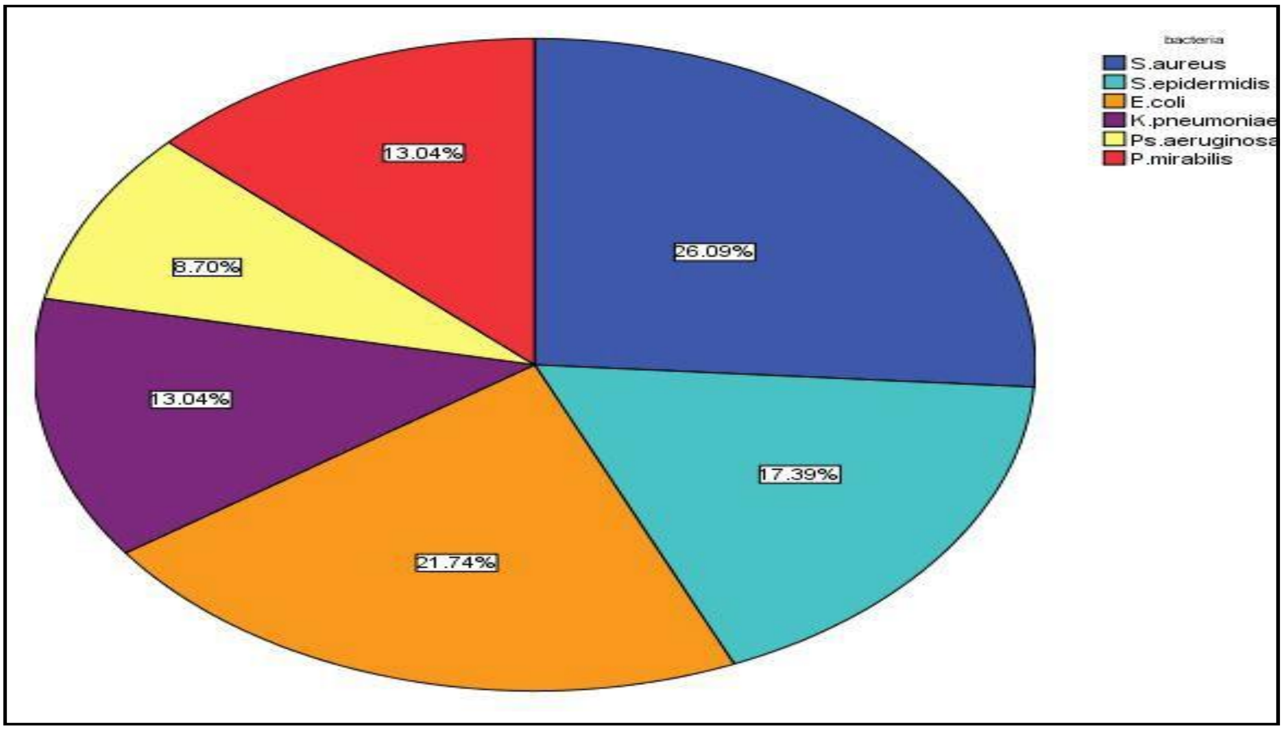

Figure 2: Percentage of the isolated bacteria that cause urinary tract infection.

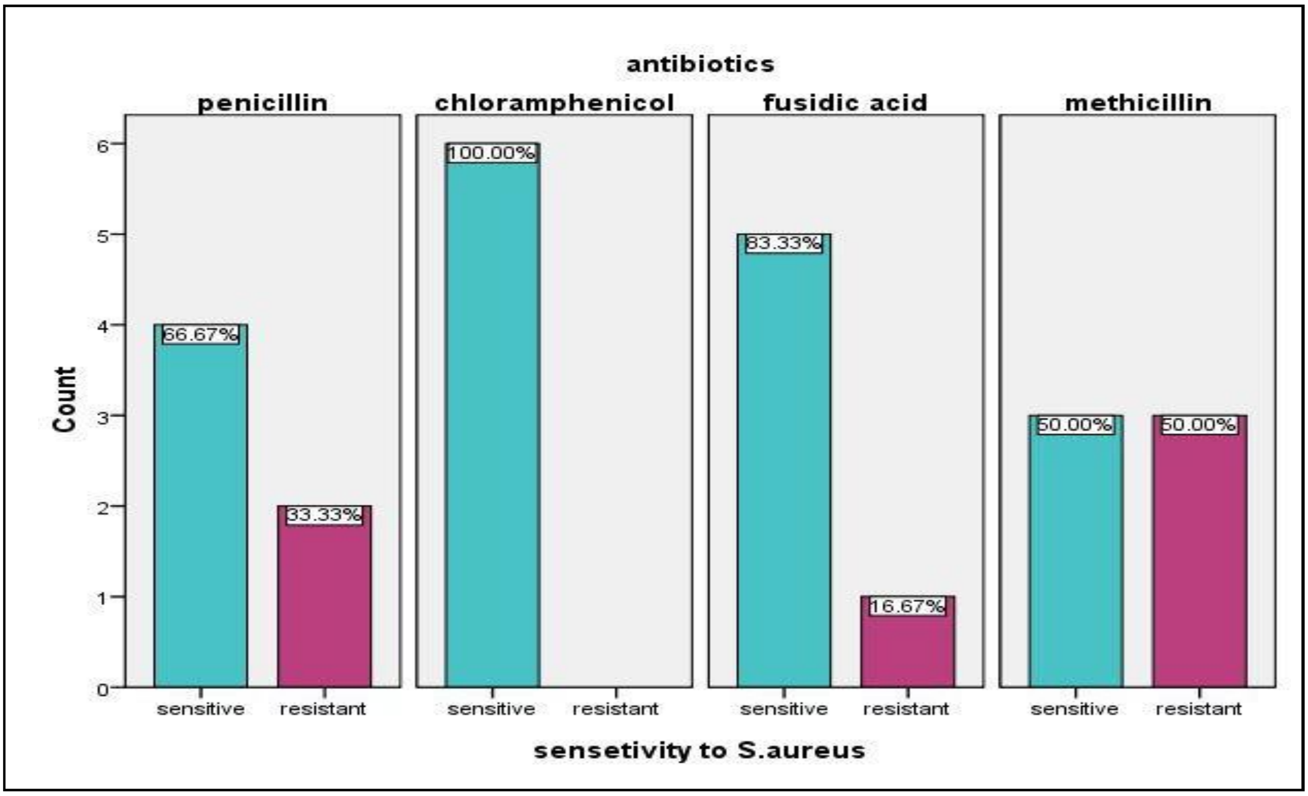

Figure 3: Antimicrobial susceptibility test for *S. aureus*

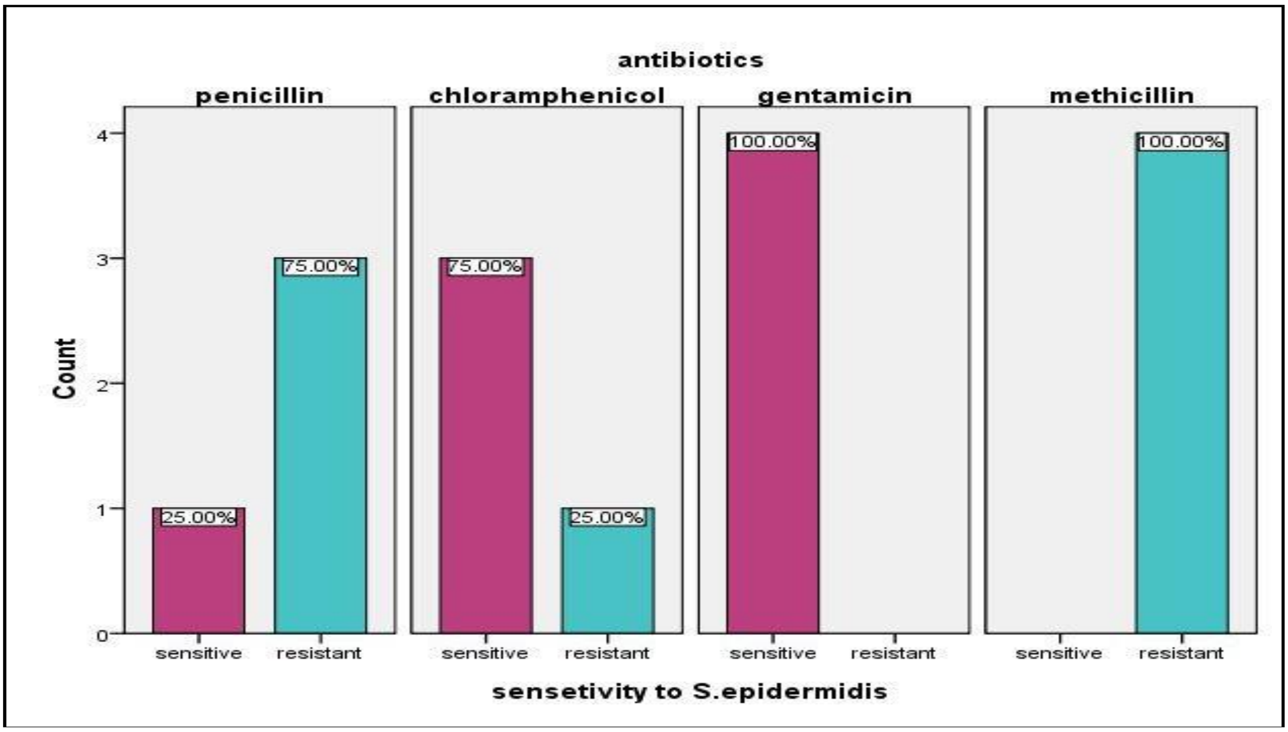

Figure 4: Antimicrobial susceptibility test for *S. epidermidis*

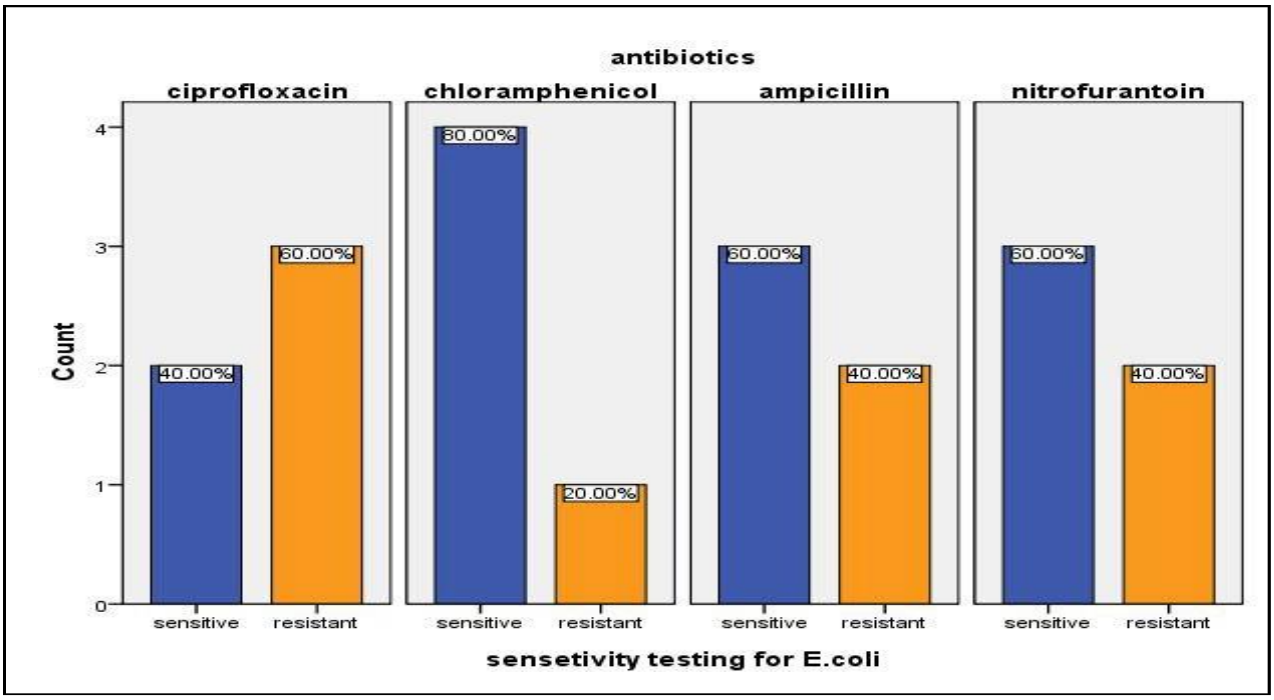

Figure 5: Antimicrobial susceptibility test for *E.coli*

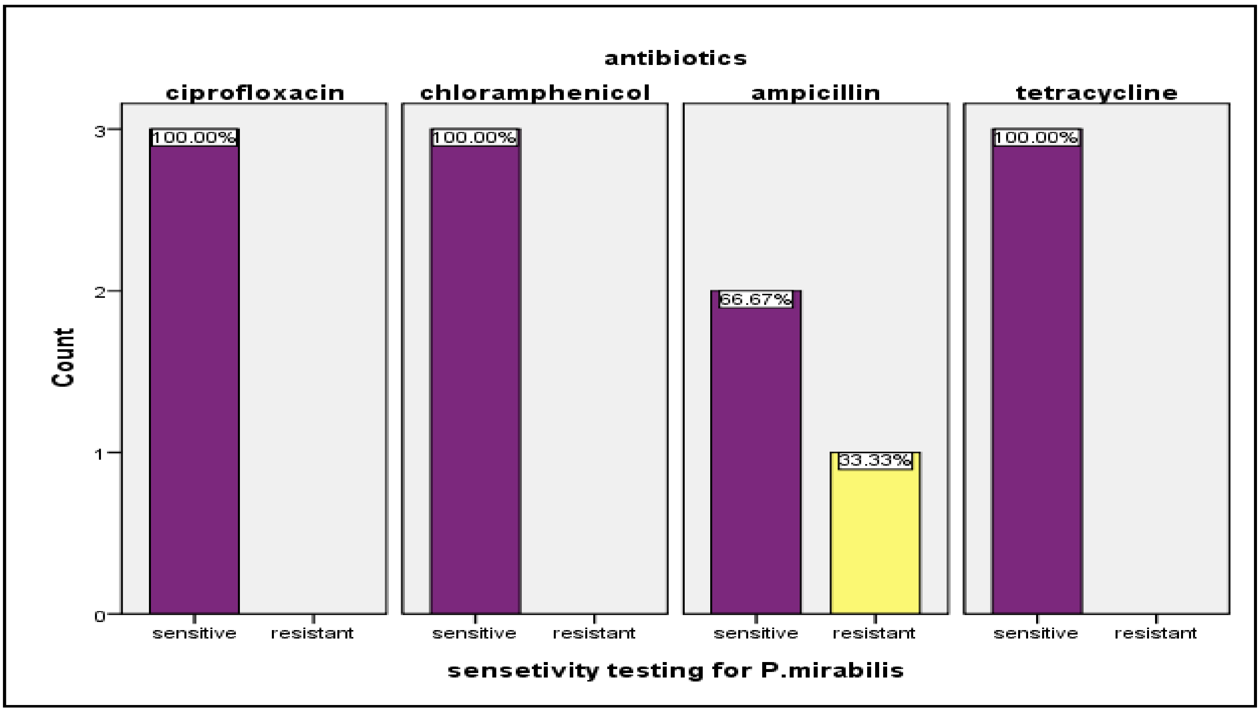

Figure3.6: Antimicrobial susceptibility test for *P. mirabilis*

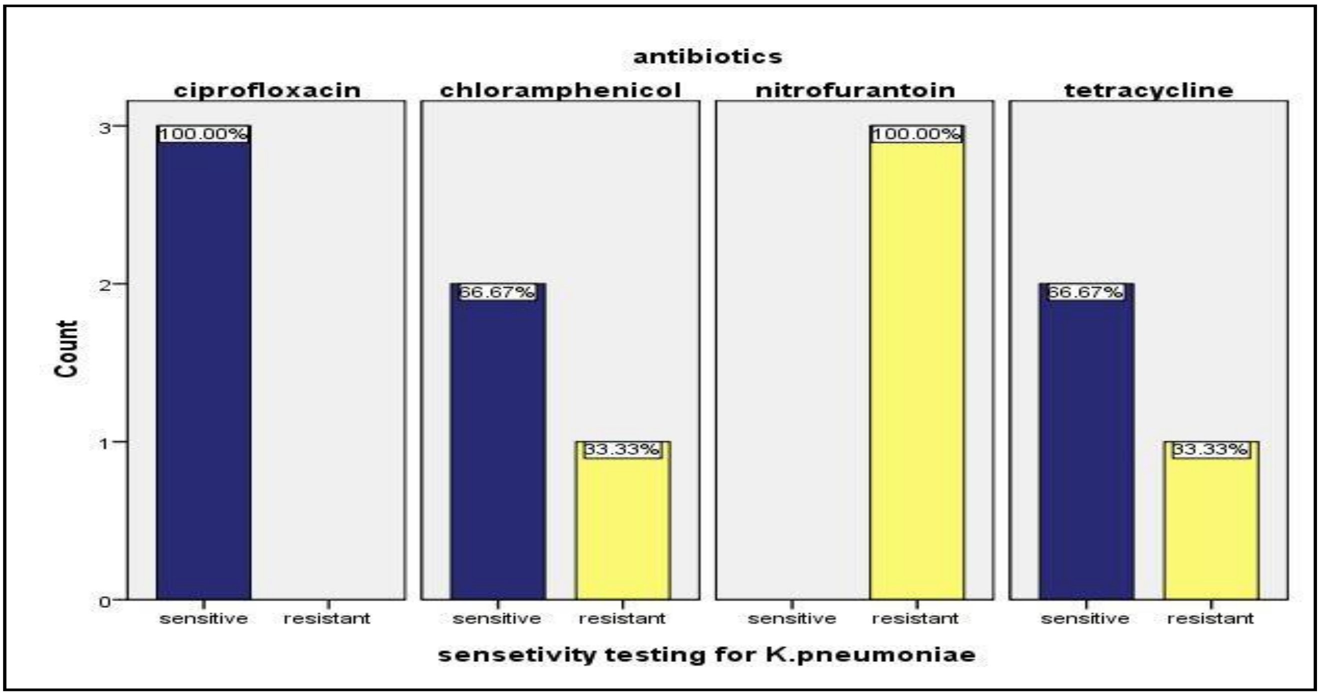

Figure 7: Antimicrobial susceptibility test for *K. pneumoniae*

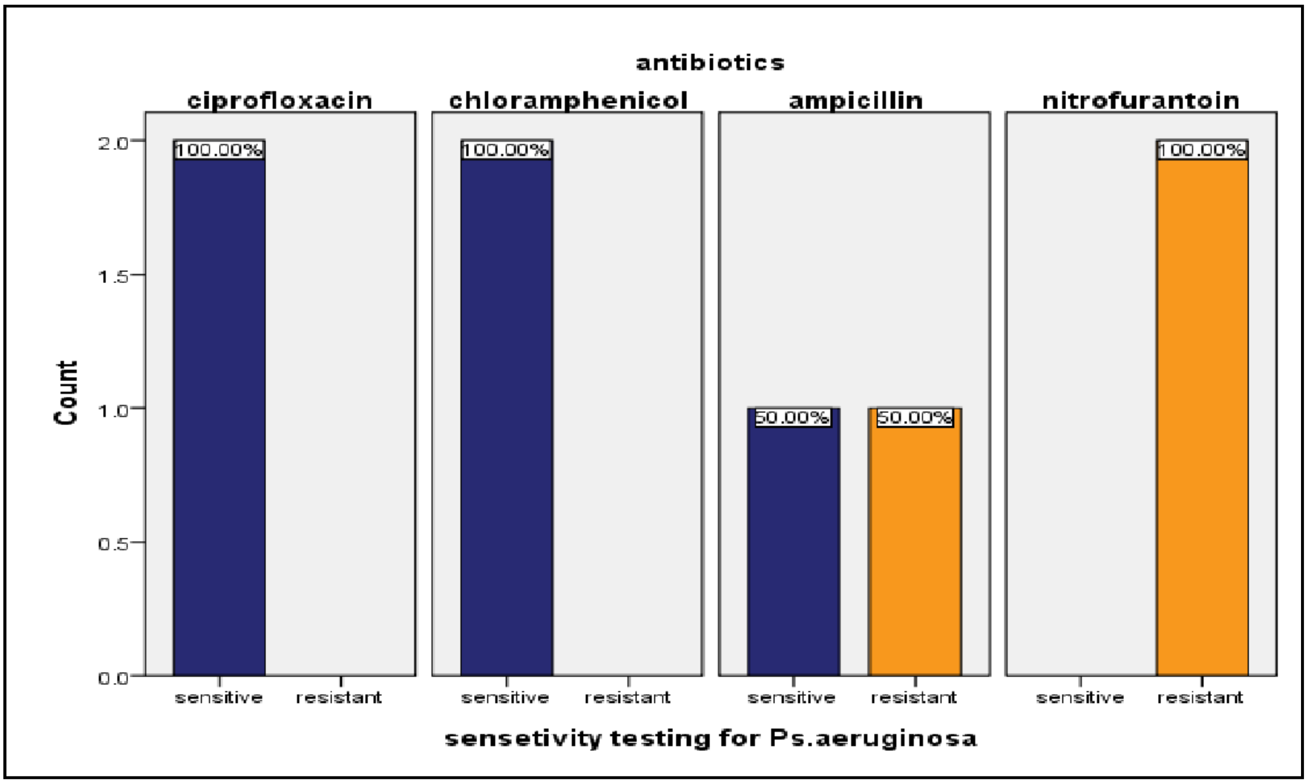

Figure 3.8: Antimicrobial susceptibility test for *Ps. aeruginosa*

Discussion

The main objective of the present study was to isolate the main common pathogens that cause urinary tract infections (UTI) among patients receiving a chemotherapeutic drug. Fifty specimens were collected for patients, prepared, and adopted different standardized tools and methods for the realization of the problem through isolation and identification of bacterial strains which cause UTI.

The study relived that gram`s positive pathogens are commonly isolated from the patients and *Staphylococcus aureus* was the predominant microorganism recovered (26.6%) disagrees with a study done by Purewal and his colleagues (6). Followed by *E.coli* (21.7%) agree with a study done by Purewal and his colleagues(11), while disagreeing with a study done by Tancheva S and his colleagues whom found that *E.coli* is the common pathogen isolated, then *S.epidermis* (17.4%), K.pneumonia (13%), *pr.mirabilis* (13%) and *Ps.aeruginosa* (8.7%) (7).

The percentage of urinary tract infections among patients whom receive chemotherapeutic drug is (46%) this result agrees with study done by Kenneth V. I. whom found that bout (45%) were positive (8), while disagree with a study done by Tancheva S and his colleagues their result show (68%) where positive (7). Our result showed that the distribution of disease among female (62%) higher than male (28%) which disagree with study done by Hayami and his colleagues which revealed that (88%) of infected were female (9).

Our results showed that the chloramphenicol was highly active against gram negative bacteria (84.6%), followed by ciprofloxacin (77%) agreeing with a study done by Theodore which showed that the Chloramphenicol (100%) was highly active against gram`s positive (10).

Susceptibility test for *E.coli* shows that Chloramphenicol is active (80%) which disagrees with a study done by Boris and his colleagues reported that the activity of Chloramphenicol (54%) followed by ampicillin and nitrofurantoin (60%) (11), while agreeing with a study done by Hayami and his colleagues which report the activity (65%), while the ciprofloxacin (40%) disagrees with the previous study which reports activity (85%) (9).

The susceptibility test for *Proteus mirabilis* shows that Chloramphenicol, tetracycline, and ciprofloxacin are highly active (100%) which disagrees with a study done by Sohail M and his colleagues, while our result shows ampicillin activity (67%) which agrees with the same study that reports ampicillin activity (61%) (12).

The susceptibility test for *klebsiella pneumoniae* shows that ciprofloxacin is highly active (100%) agrees with a study done by Archana and Harsh that report activity of ciprofloxacin (90%) followed by Chloramphenicol and tetracycline (67%) disagree with same study result which that report activity (40%) (13).

The susceptibility test for *staphylococcus aureus* shows that Chloramphenicol is highly active (100%) which disagrees with a study done by Adebola and Josiah, while the result of fusidic acid and penicillin, and methicillin agrees with the same study results (14).

Susceptibility test result for *Pseudomonas aeruginosa* shows that Chloramphenicol and ciprofloxacin were highly active (100%) and (100%) resistant to nitrofurantoin reported (96%) resistance which agrees with a study done by Jonathan and Jiyoung, while and show (50%) resistance to ampicillin which disagree with same a study result (15).

Susceptibility test for *S.epidermidis* shows that gentamicin is highly active (100%) disagrees with a study done by Hellmark and his colleagues that report activity of gentamicin is (21%), while Chloramphenicol, penicillin, and methicillin (100%) resistance which agrees with our study results (16).

Conclusion

Of the 23 positive samples, *S.aureus* and *E.coli* are the most frequent. Females are more infected than males. Adults are more infected than children. Chloramphenicol, gentamicin, and ciprofloxacin have good effects against causative agents and are recommended as the first line of treatment.

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