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SMLJ

هَرَقَدْ آتَيْنَا دَاوُودَ وَسُلَيْمَانَ عِلْمًا

وَقَالَا الْحَمْدُ لِلَّهِ الَّذِي

فَضَّلَنَا عَلَى كَثِيرٍ مِّنْ عِبَادِهِ الْمُؤْمِنِينَ

العدد 15

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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.

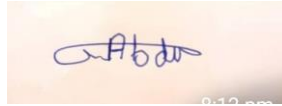
Aims and scopes

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 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.

A handwritten signature in blue ink, appearing to read 'Abdelsamee', is centered within a light orange rectangular box. The signature is fluid and cursive.

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

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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.

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

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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*).

Iron profile and some hematological parameters among Sudanese patients with Rheumatoid Arthritis at Khartoum state

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Abstract:

Background Rheumatoid arthritis (RA) is a chronic inflammatory disease characterized by the involvement of numerous joints and by systemic symptoms, which leads to disability and premature death, Anemia is the most often hematological manifestation issue that occurs in RA.

Objective: This study aimed to evaluate CBC and to assess iron deficiency in RA Sudanese patients in Khartoum state.

Material and methods: This retrospective observational study was conducted on 63 RA patients and 63 healthy individuals as control between 2018 and 2021. The CBC and iron profile (serum ferritin, serum iron and total iron binding capacity) were carried out, the Transferrin saturation (TS) was calculated using the formula: $TS (\%) = [\text{serum iron (ug/dl)} / \text{serum transferrin (mg/dl)}]$ (Theodoros Eleftheriadis, et al. 2010)

Result: In anemic RA patients the MCV, MCH levels, serum iron, serum Ferritin, and TS% were significant decrease while there was a significant increase in platelets count compared to control.

Conclusion: Iron deficiency is common (77%) of anemic RA patients while there was (22%) of ACD. RA patients had lower serum iron, lower ferritin and lower TS%, while there was increase in platelets count, Decreased hemoglobin was the strongest determinants of iron deficiency.

Keywords: IDA anemia ,ACD, Rheumatoid arthritis;RA, Serum iron; ferritin; total iron binding capacity; TIBC, Transferrin saturation; TS%.

Corresponding author: Rouaa Babikir Ahmed, rorybabiker@gmail.com

Introduction:

Rheumatoid arthritis is a chronic autoimmune disease that affect hand and feet small joints causing swelling and bone erosion and joint deformity (2). Rheumatoid arthritis may occur at any age but the prevalence increases until age of 70. The disease Infects 1% to 2%of the adult

population and its incidence is greater in women than in men (3:1). Anaemia is the most common and serious blood abnormality seen in rheumatoid arthritis either anaemia of chronic disorder or iron deficiency anemia (3). Anemia of chronic disease. is a clinical entity, which is commonly observed in patients with various underlying diseases that

include anemia of chronic infection, anemia of cancer, anemia associated with rheumatoid arthritis, and anemia in chronic kidney diseases (4). Anemia associated with rheumatoid arthritis, known as rheumatoid anemia, is a typical example of anemia of chronic disease (5, 6). In the absence of effective treatment, anemia is highly prevalent among rheumatoid arthritis patients. The mechanisms involved in rheumatoid anemia include shortening of the erythrocyte lifespan, inadequate bone marrow erythropoiesis in response to the anemia, and iron metabolism abnormalities (5-7).

Materials and methods:

This a retrospective observation study was conducted in Khartoum State, between 2018 and 2021 sample collected from Military Hospital, Khartoum, Sudan and Modern Medical Center, Khartoum, Sudan. A total of 126 volunteers were enrolled in this study; 63 were Sudanese patients diagnosed with anemic RA as a patient's group; this study was approved by Omdurman Islamic University ethical committee. The consent was also taken from every participant before the samples were gathering. Five ml of venous blood were collected from all participants, and then measure CBC using sysmex Kx-21. The iron profile (serum ferritin, serum iron and total iron binding capacity) was carried out using automated Chemical analyzer Roche/Hitachi cobas c311 and

e411 (Roche Diagnostics GmbH, Mannheim, Germany). Transferrin saturation (TS) was calculated with the formula: $TS (\%) = [\text{serum iron (ug/dl)} / \text{serum transferrin (mg/dl)}]$. Data were collected by direct questioner and analyzed by using statistical package for social sciences (SPSS) version 23. T. test and ANOVA test were used. While Correlations between variables were evaluated by Spearman's correlation coefficient. The results were expressed as means + standard deviation. Significance was set at $P < 0.05$.

Results:

162 individuals were included in this study, 63 were patients with anemic rheumatoid arthritis and 63 were healthy as control group, the mean age of patients and controls were (46.04 ± 10.07) and 43.76 ± 3.91 years old, respectively).

The mean of Hb, RBCs, and HCT was significant decrease in case $(10.67 \pm 1.00, 4.38 \pm 0.540, \text{ and } 32.96 \pm 2.60, \text{ respectively})$ with $p.\text{value} = 0.00$

The mean of MCV and MCH was significant decrease in case $(76.07 \pm 7.52 \text{ and } 24.69 \pm 3.04, \text{ respectively})$ with $p.\text{value} = 0.00$, while there was insignificant increase in MCHC in case (32.35 ± 1.24) with $p.\text{value} = 0.07$. The mean of TWBC count was insignificant increase in RA (10.67 ± 1.00) , with $p.\text{value} = 0.1$ while there was significant increase in platelet count in RA patients (326.66 ± 108.62) , with $p.\text{value} = 0.04$

Table 1: Mean of age, CBC results among patients with rheumatoid arthritis compared to control

	Study population	Mean±STD	p.value
Age	Case	46.04±10.07	0.09
	Control	43.76±3.91	
Hb	Case	10.67±1.00	0.00
	Control	12.96±1.62	
WBC	Case	6.49±2.58	0.1
	Control	5.84±1.82	
RBC	Case	4.38±0.540	0.00
	Control	4.95±0.42	
HCT	Case	32.96±2.60	0.00
	Control	41.08±2.49	
MCV	Case	76.07±7.52	0.00
	Control	83.52±5.81	
MCH	Case	24.69±3.04	0.00
	Control	26.71±1.99	
MCHC	Case	32.35±1.24	0.07
	Control	31.98±1.08	
PLT	Case	326.66±108.62	0.04
	Control	291.73±79.20	

The mean $\pm$ STD of Serum Iron, Serum Ferritin, and TS% was significant decrease in case (5.66 $\pm$ 3.13, 14.45 $\pm$ 19.78, and 12.98 $\pm$ 11.29, respectively) with P.value = 0.00, while there was insignificant different in TIBC between case (50.68 $\pm$ 12.43) and control with P.value= 0.9.

Table 2: Mean of iron status among patients with rheumatoid arthritis correlated to control

	Study population	Mean $\pm$ STD	p.value
S. iron	Case	5.66 $\pm$ 3.13	0.00
	Control	16.09 $\pm$ 5.79	
S. Ferritin	Case	14.45 $\pm$ 19.78	0.00
	Control	28.24 $\pm$ 19.39	
TIBC	Case	50.68 $\pm$ 12.43	0.9
	Control	50.84 $\pm$ 5.43	
TS%	Case	12.98 $\pm$ 11.29	0.00
	Control	31.83 $\pm$ 10.58	

The correlation of different age group with CBC parameters were insignificant in the two age group of RA patients.

Table 3: Mean of CBC results among patients with rheumatoid arthritis correlated to age group

	Age (years)		p.value
	<40	>40	
Hb	10.37 $\pm$ 1.05	10.79 $\pm$ 0.97	0.1
WBC	5.77 $\pm$ 2.13	6.78 $\pm$ 2.71	0.1
RBC	4.27 $\pm$ 0.35	4.43 $\pm$ 0.59	0.2
HCT	32.00 $\pm$ 2.44	33.35 $\pm$ 2.58	0.06
MCV	75.89 $\pm$ 7.18	76.14 $\pm$ 7.73	0.9
MCH	24.55 $\pm$ 3.00	24.75 $\pm$ 3.09	0.9
MCHC	32.35 $\pm$ 1.19	32.36 $\pm$ 1.28	0.8
PLT	321.88 $\pm$ 103.39	328.57 $\pm$ 111.72	0.8

The correlation of different age group with Serum iron, serum ferritin, TIBC, and TS% were insignificant in the two age group of RA patients.

Table 4: Mean of serum iron, ferritin, TIBC and TS% among patients with RA correlated to age group

	Age (years)		p.value
	<40	>40	
Serum iron	5.75±3.86	5.62±2.84	0.8
Ferritin	12.26±18.58	15.33±20.38	0.5
TIBC	52.65±12.96	49.89±12.27	0.4
TS%	11.16±6.80	13.70±12.65	0.4

The Hb, WBCs, RBCs, MCV, MCH, MCHC, and PLT correlated to duration of disease were insignificant different in RA patients, while HCT was significant decrease with duration >10years compared to other group (p.value=0.04).

Table 5: Mean of CBC results among patients with RA correlated to duration of disease

	Duration (years)			p.value
	<5	5-10	>10	
Hb	10.84±.81	10.48±1.24	10.00±1.16	0.05
WBC	6.53±2.18	6.35±3.35	6.82±2.48	0.9
RBC	4.46±.49	4.28±.56	4.17±.87	0.1
HCT	33.42±2.30	32.41±2.83	31.25±3.64	0.04
MCV	75.6±7.4	76.95±7.79	76.3±9.08	0.6
MCH	24.63±3.03	24.88±3.12	24.37±3.49	0.9
MCHC	32.47±1.25	32.20±1.30	31.97±.970	0.3
PLT	336.46±120.82	315.95±92.56	284.75±17.03	0.3

The Serum iron, serum ferritin, TIBC, and TS% different compared to other group (p.value=0.3, correlated to duration of disease were insignificant 0.8, 0.2, 0.6 respectively).

Table 6: Mean of serum iron, ferritin, TIBC and TS% among patients with rheumatoid arthritis correlated to duration of disease

	Duration (years)			p.value
	<5	5-10	>10	
Serum iron	5.44±3.05	5.75±3.54	7.27±1.02	0.3
Ferritin	14.20±21.47	14.64±18.51	16.02±8.50	0.8
TIBC	49.18±12.36	52.66±12.82	55.32±11.55	0.2
TS%	13.77±13.57	11.34±6.55	13.45±2.42	0.6

Discussion:

This study was done to evaluate the CBC and iron profile in anemic Sudanese patients with Rheumatoid Arthritis. It was shown that Hb, RBCs, and HCT were significantly decreased in those cases, these outcomes match with the studies done by W.TAŃSKI 2021 (8), Tahani Mursal 2016 (9), Eman Tariq 2019 (10) and Abbas Sabbar Dkhil 2017 (11).

The Iron deficiency was common with 77% of anemic RA patients in current study, while it was 64% in the study done by W. TAŃSKI 2021(8), 60% by Wojciech Tański 2021 (12) and it was only 23% with the study done by Tahani Mursal 2016 (9).

The MCV and MCH were found to be significant decreased and the serum iron, serum ferritin and TS % were significantly decreased in RA Sudanese patients ,This agree with the studies done by Eman Tariq 2019 (10),W.TAŃSKI 2021 (8), Tahani Mursal 2016 (9) and Noha Mohamed Saeed 2016 (13).

Basak TB 2013 refer the decrease in the indices as

reflect of the iron deficiency anemia and explain the association between RA and iron deficiency (IDA) caused by gastrointestinal bleeding associated with drug treatment (14). Iron deficiency anaemia (IDA) is characterised by impaired haemosynthesis and the production of erythrocytes, which are smaller than normal and contain less haemoglobin as a result of iron deficiency, also reduced ferritin levels and transferrin saturation (TS) of less than 20% ,according to Wojciech Tański 2021, Babitt J.L 2010 and Franchini M.2010 all if these are due to excessive inflammatory activation and the resulting increased production of proinflammatory cytokines (mainly interleukin-6) and hepcidin (12) (15) (16)

There was a significant increase in platelet count in RA patients in this study which agree with the studies done by W. TAŃSKI 2021(8), and Li Xue 2022 (17).

ErtenliI explain that an increase in platelets count may be due to the action of particular interleukins, namely IL- 6 and IL-1, with pro-inflammatory

mediator activities which have been shown to be involved in the regulation of megakaryocytopoiesis (18) (19). Gasparyan AY 2010 showed the reactive megakaryocytopoiesis increases circulating platelets count, proinflammatory pleiotropic cytokines of RA also have megakaryocytopoietic / thrombopoietic properties (20). Abbas Sabbar Dkhil 2016 showed that platelets count, interleukin-6 IL-6 plays a major role in the pathogenesis of RA including thrombocytosis (21).

Conclusion:

Iron deficiency is common (77%) of anemic RA patients while there was (22%) of ACD.

RA patients had lower serum iron, lower ferritin and lower TS%, while there was increase in platelets count, Decreased hemoglobin was the strongest determinants of iron deficiency.

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Identification of COX1 gene in urinary Schistosomiasis among School Children at Al-Fasher Town Northern Darfur- Sudan

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Abstract

Background: Schistosomiasis is one of the most important human diseases in terms of morbidity and mortality particularly in tropical area. The disease is endemic in many developing countries that adds primarily on water bodies. The disease remains highly prevalent in Sudan, especially amongst rural communities.

Objective: The study was conducted to detect the frequency of schistosomiasis and to investigate possible genetic variations in *S. haematobium*, in AlFasher Town.

Methods: Urine samples were collected from 195 school children enrolled in this study and examined using microscopic and molecular analysis involved PCR amplifications and visualization (on agarose gel) detection for of possible genetic variants in the mitochondrial genes (COX1) .

Results : The study showed the frequency rate of *Schistosoma haematobium* in urine samples was 12/196 (6.1%). All the cases identified by microscopy got amplified, some were found to vary in their ability to express the mitochondrial genes COX1.

Conclusions: Out of 12, positive microscopically urine samples eight was positive by COX1 gene PCR (8/12(66.6%), This finding will therefore add to knowledge on genetic variations in *S. haematobium*.

Keywords: Urinary schistosomiasis, PCR, COX1gene.

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Introduction:

Urogenital schistosomiasis is caused by infection with *Schistosoma haematobium*, a dioecious blood fluke that utilizes specific freshwater snails of the genus *Bulinus* as obligates intermediate hosts (1). Adult female flukes lay terminal-spine eggs, which cross the bladder wall, leading to macro-hematuria or red urine. Through poor environmental sanitation control, eggs that reach freshwater subsequently hatch to release a miracidia that actively searches and penetrates a

freshwater snail where they undergo sexual reproduction, emerges. The cercaria is able to cross unbroken skin directly (2).

Water contact activities such as washing and bathing in water where cercariae are present enables the *Schistosoma* to gain entry into the human host. Indeed, any demographical groups, irrespective of age or gender with unsafe water contact are at risk of infection (2, 3).

Like all neglected tropical diseases, schistosomiasis is a disease of poverty.

The broader social environment in which people live and work is key to understand vulnerability to the disease (4, 5, 6). Although there are some studies on *S.haematobium* infections in some parts of the country there are no published reports in Darfur (7), fact that justifies the importance of conducting the study in Al Fasher Town. This study reveals *S. haematobium* infections and COX1 gene at Al Fasher Town. COX1 is a protein, also known as mitochondrially encoded cytochrome c oxidase I (MT-CO1) that in humans is encoded by the MT-CO1 gene (8).

The *cox1* gene is universally carried by the mitochondrial genome and has been acknowledged as a global standard DNA barcoding approach for identifying organisms at the species level (9, 10).

It is a highly conserved region in the mitochondrial genome (10). The *cox1* gene is very sufficient for rapid discrimination between closely related species and to evaluate intraspecific diversity among species (10, 11).

Material and Methods:

This is a cross sectional, descriptive community-based study conducted in the period from May 2017 to August 2021 in Al Fasher, Sudan. The study involved 195 school aged children (mean age 12+); all of them were males. A few of these children showed hematuria, abdominal pain and weakness symptoms. The study area was specified as: city center (Tombase ,Makraka ,Awlad Elreef), city peripheries and IDP (Internally displaced person) Zamzam and Nifasha camps.

The samples were collected between 10 am and 2 pm as this is the maximum eggs excretion period that was reported by Gray *et al.*, (2011). The containers were place into plastic bags before being in suitable cool boxes with a temperature of 4–6 °C for subsequent examination. The samples were further subjected to microscopic examination to identify the presence and intensity of *Schistosoma* eggs and the sociodemographic and associated risk factors were obtained via a standardized questionnaire.

10% formalin was added to the positive samples before transformed to Tropical Medicine Research Institute, National Center for Research.

Molecular techniques

For the preparation of DNA extraction, G-DEX Genomic DNA Extraction Kit was used. Each fixed urine samples were washed three times in 1XPBS buffered, then samples were centrifuged for 5 min at 2000 rpm to remove formalin. For obtaining the genetic material of *Schistosoma* eggs, 300µl cell lysis solution was added to the positive urine samples to lyse the cells wall of *Schistosoma* ova, samples were chilled to room temperature and 100µl PPT was added to the cell lysate and vortexed vigorously at high speed for 20 seconds. Afterward samples were put to the ice for 5 min in order to remove the protein contamination then centrifuged at 13,000 rpm for 5 minutes. After centrifugation 300µl of supernatant containing the DNA was transferred (leaving behind the precipitate protein pellet) into 1.5ml tube. Next 300 µl 100% isopropanol was added and mixed with the sample by inverting

gently several times then centrifuged at 13,000 rpm for 1 minute; the DNA was visible as a small pellet. Equal volume of 70% ethanol was added and centrifuged at 13,000 rpm for 1 minute (to remove isopropanol) and the supernatant was discarded carefully without discard the DNA pellet. Finally, the tubes were inverted on clean paper to dry from alcohol and 150µl DNA rehydration solution was added and incubated at 65°C for 30 min. Then DNA purity was measured. To detect *Schistosoma* DNA in urine sample, PCR was performed using the extracted DNA. The *Schistosoma* partial cox1 mitochondrial DNA (mt DNA) region was amplified using a universal forward primer ShbmF (Macrogen, Korea) (5'-TTTTTTGGTCATCCTGAGGTGTAT-3') with species-specific reverse primers, ShR (5'TGATAATCAATGACCCTGCAATAA-3') for *S. haematobium*, (12). PCR amplification was performed in total volume of 25 µL containing 5µl master mix ready to load (SOLIS BIODYNE FIREPoi, Estonia), 1 µl from each

primer and 5 µl DNA (13). PCR cycling conditions were subjected to an initial denaturing step of 95 °C for 3 min, followed by 30 cycles of 94 °C for 30 s, 58 °C for 1 min 30 s and 72 °C for 1 min 30 s, with a final extension of 7 min at 72 °C. Amplicons were visualized and sized on a 1.5 %.

Statistical Analysis:

Data obtained from this study were entered into Statistical Package for Social Sciences (SPSS) version 25 and analyzed using Chi square to determine the **association** between variables and risk factors. P. value less than 0.05 was considered significant.

Results:

Macroscopic examination

Among 14 samples with hematuria twelve had *S. haematobium* infection and two did not harbor *S. haematobium* eggs. 182 samples without hematuria which also found negative for the parasite. There was significant association Between hematuria and eggs. Table 1

Table (1) Occurrence of hematuria in urine samples

Haematuria	Total	Result of urine (Conc.Tech)	
		Positive	Negative
Positive	14	12	2
		85.7%	14.3%
Negative	182	0	182
		0.0%	100.0%
Total	196	12	184
		6.1%	93.9%

(P. value< 0.05(0.001))

Overall frequency of schistosomiasis

Microscopic examination of 196 urine samples obtained from school children revealed that eggs

were found in 12 (6.1%) of the urine samples and that the remaining 184 (93%) samples were clear (Table 2).

Table (2) Overall frequency. of *S. haematobium* in urine samples of school children

Negative	Positive	Total specimens examined
184 (93.8%)	12 (6.1%)	196

Frequency of urinary Schistosomiasis according to age group

The most common urinary schistosomiasis was found among the age group ranging from 10 to 15 years (66.7%) followed by the age group ranging

from 16 to 19 years and 4 to 9 years 16.7 %. There is significant association between age group and the presence of *S. haematobium* as represented in Table (3)

Table (3): Frequency of urinary Schistosomiasis According to Age Group

Age Group	Total	Result of urine (Conc.Tech)	
		Positive	Negative
4-9 Years	55	2	53
	28.1%	16.7%	28.8%
10-15 years	120	8	112
	61.2%	66.7%	60.9%
16-19 Years	21	2	19
	10.7%	16.7%	10.3%
Total	196	12	184
	100.0%	100.0%	100.0%

P =0.020

Identification of COX 1 Gene in samples positive for *Schistosoma* eggs

Among the twelve positive urine samples that were

examined by concentration technique it was found that only eight were positive by PCR COX1 gene. This result is showed in table 4.

Table (4): Detection of COX1 gene by PCR

Result of urine (Conc.tech)	PCR cox1 gene	
12	PCR result Positive	PCR result Negative
	8	4
12/196 (6.1%)	8/12 (66.7%)	4/12(33.3%)

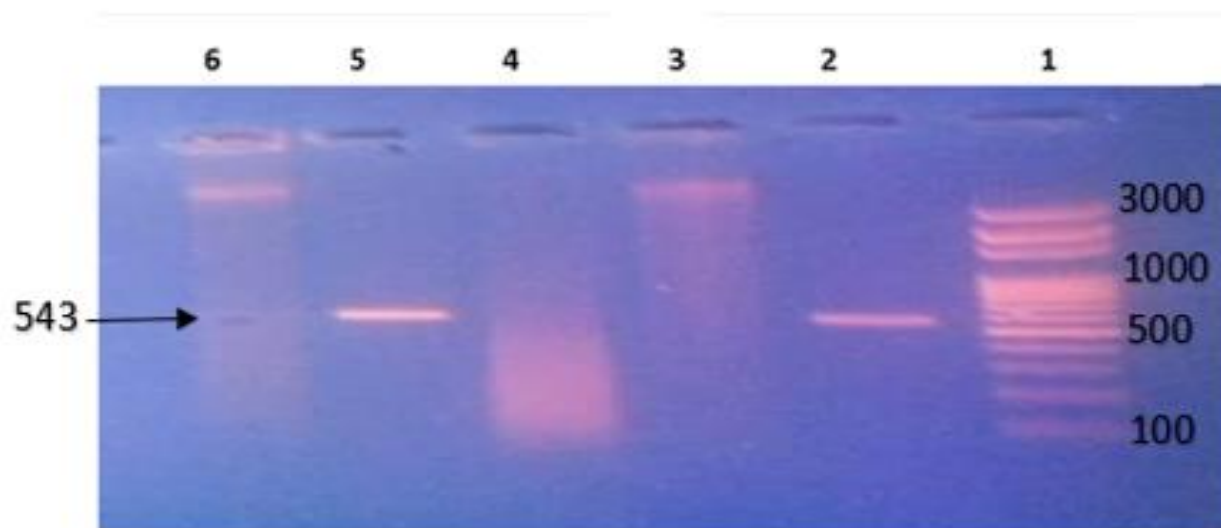


Figure 1: PCR result of *COX1* gene by gel electrophoreses. Lane 1: 100 bp DNA ladder9(1Kb+).

PCR result of *COX1* gene by gel electrophoreses. Lane 1: 100 bp DNA ladder. Lane 2 and 5: positive result (543 bp). Lane 3 and 6: non-specific bands. Lane 4: negative result. This result is showed in figure 1.

Discussion:

Human schistosomiasis is a neglected tropical disease of great importance that remains highly prevalent in Africa, especially among rural communities. (14). in this study the prevalence of *Schistosoma haematobium* was 6.1%. This finding was in agreement with study done in Egypt that was 7.8% (15), but sightless in agreement with study in Um-Asher area -Sudan (12.9%) (16), and in Southern Kordofan State 23.7% (17).

In contrast with study done in Alsafia and Abuselala South Darfur in Sudan, which found a prevalence rate of 56% (7) , and other study in White Nile River basin (Sudan) which found 45.0% (18).

In this study, macroscopic examination of hematuria showed the prevalence of 85.7% among infected patients. Statistically there was significant correlation between hematuria and the presence of *S.haematobium* eggs ($P.value= 0.001$) which it similar to in Al-Lamab Bahar Abiad Area, Khartoum State, that showed 77.8% among infected patients (19).

It was found there was insignificant association between the infection and age group which it in agreement with other study that found there was no significant relationship between the infection and the age group although other study in Al-Lamab Bahar Abiad Area, Khartoum State showed there was significant of infection with age group (19). This difference may be due to difference in sample size and diagnostic method used.

The present study showed that the children who live in Tambasae and Makraka area were the most infected (14.3% and 13.6% respectively)

compared with those who live in Nifasha and Zamzam camps (0%) this finding may be due to that the camps were designed considering the hygienic status.

Out of 12 (8/12(66.6%)), positive microscopically urine samples eight was positive by COX1 gene due to diversity to the gene other than COX1 in the current study we agreed with studies done by Angora, E., and his colleague (20) on school-aged children in different parts of Côte d'Ivoire which found 42.0% of 2164.

We disagreed with study done by Sady, H., et al (12) in Al Yamen which found 13%.

Other study by Webster, et al 2010 found that COX 1 gene is highly sensitive to discriminate *S.haematobium* and *S.bovis* which showed 100% (22).

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Sodium Voltage-gated Channel Alpha Subunit 1 gene (SCN1A) among Epileptic Sudanese Patients

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Abstract

Background: Epilepsy is a pathological condition characterized by recurrent, unprovoked, epileptic seizures the SCN1A gene represents one of the most commonly mutated human epilepsy genes.

Aim: The study aimed to investigate mutated SCN1A gene in patients with idiopathic epilepsy. It also determined the serum level of sodium, potassium.

Methods: the current study is a cross-sectional study that had been performed at Sheikh Mohamed Khair Centre, Banat, Omdurman, and National Centre for Neurological Sciences (NCNS) Khartoum state, during the period from November 2016 to February 2019. A total of 100 patients were enrolled in this study. Sodium and Potassium concentrations were obtained by the full automated Easylyte instrument (Na/K analyzer) and the SCN1A gene which located on chromosome 2 of humans, using conventional PCR. The data were analyzed using SPSS version (25) and bioinformatics tools were used to analyze sequencing results.

Results: One hundred subjects were recruited in this study, (47% males and 53% females) diagnosed with epilepsy. Patients aged between 18-40 years old were the highest category with a 55%. Onset of seizure was in age group of less than 5 yrs were 50% of the patients, in age group 5-10 yrs 28% and more than 10 yrs were 22%. Ninety percent of the patients had a Family history with epilepsy while the rest of them (10%) had not. Generalize epilepsy presented in 68% focal to bilateral in 26% and focal in 6 patients two of them (2%) developed impairment. Serum sodium and potassium analysis showed normal results, mean 134.82 mg/dl, 3.65 mmol/l, respectively. All patients were tested positive for SCN1A gene.

Conclusion: Genetic mutations have an effective role in developing epilepsy. AT deletion in SCN1A gene, indirectly affects gamma amino butyric acid function.

Key words: SCN1A gene, epilepsy, neurological disorders, Sudanese, serum electrolytes

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Introduction

Epilepsy is a pathological condition characterized by recurrent, unprovoked, and epileptic seizures (1). It affects approximately 42 million people worldwide and it is the most common heterogeneous neurological disorder with distinct symptoms, etiology, prognosis and

treatments (2). Nearly 70% of patients with epilepsy lack an obvious pathogenetic cause. Genetic is believed to play an important role in the cause of epilepsy (3). Among the genes known to be involved in epilepsy, the SCN1A gene represents one of the most commonly mutated human epilepsy genes, referred to be as

super culprit gene (4). The SCN1A gene codes for the alpha subunit of the neuronal voltage-gated sodium ion channel, type 1 (NaV 1.1) (5). It is located on chromosome 2 of humans, and is made up of 26 exons spanning a total length of 6030 nucleotide base pairs (bp) (6). It is expressed in the central and peripheral nervous systems and in cardiomyocytes (6). 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 (7). Action potential is the electric signal that travels along the axon and sends information to other neurons (8). It seems logical that mutations in voltage-gated sodium channels would cause epilepsy, because these channels are in part responsible for controlling electrical excitability (9). This study investigated SCN1A mutation in patients with idiopathic epilepsy in addition to sodium and potassium level measurement.

Materials and Methods

Study type and population: The current study is a cross-sectional study that had been performed at Sheikh Mohamed Khair Centre, Banat, Omdurman, and National Centre for Neurological Sciences (NCNS) Khartoum state, during the period from November 2016 to February 2019. Ninety nine patients were enrolled in this study. Only the patients who were diagnosed having idiopathic epilepsy were included in this study, the rest of them were excluded.

Sample collection and preparation: Blood samples were drained from each patient in two containers, (EDTA) and LI-heparin, the EDTA samples were processed for DNA extraction, and the heparinized samples were used for Sodium and potassium estimations.

Data collection: Clinical and demographic data were collected in a predesigned questionnaire. The demographic and clinical data relating to each patient were registered (age, gender, family history, class of seizure, onset of seizure).

Biochemical analysis

Serum was separated by centrifugation (Hettich Zentrifuge EBA200, Kirchleingern, Germany) at 3,000 RPM for 5 minutes and then stored at -20°C. Sodium and Potassium concentrations were obtained by the full automated Easylyte instrument (Na/K analyzer).

Molecular Genetic Analysis

Deoxyribonucleic acid (DNA) was extracted from whole blood using QIAGEN® DNA extraction kits (vacuum protocol). For polymerase chain reaction, 20 µl of ready-made MM (4 µL of 5× Firepol® Master Mix (Solis Bio-Dyne, Tartu, Estonia), 1 µL forward primer, 1 µL reverse primer, and 14 µL distilled water), and 2 µl of DNA were added into each PCR tube. Primers for SCN1A gene were 5'-TACCCTGTTCCGAGTGATCC-3' forward primer and 5'-GCTGTTGCCAAAGGTCTCAA-3' reverse primer were used for amplification, then the amplified PCR products (22 µL) were separated using 2% gel electrophoresis and then

the separated DNA was visualized using UV light.

DNA sequencing

Polymerase chain reaction products were transferred to china for sequencing at BGI solutions co.LTD.

Statistical Analysis

Analyses was done using Microsoft Office Excel and Statistical Package for Social Science Program 2010 SPSS version 25) for sequencing analysis, Bioinformatics tools were used to analyze sequencing results.

Results:

100 subjects were recruited in this study, (47% males and 53% females) diagnosed with epilepsy. Patients aged between 18-40 years old were the highest group with a 55%, followed by less than 18 years (32%), 41-65 years (11%) and more than 65 years were only 2%. Onset of

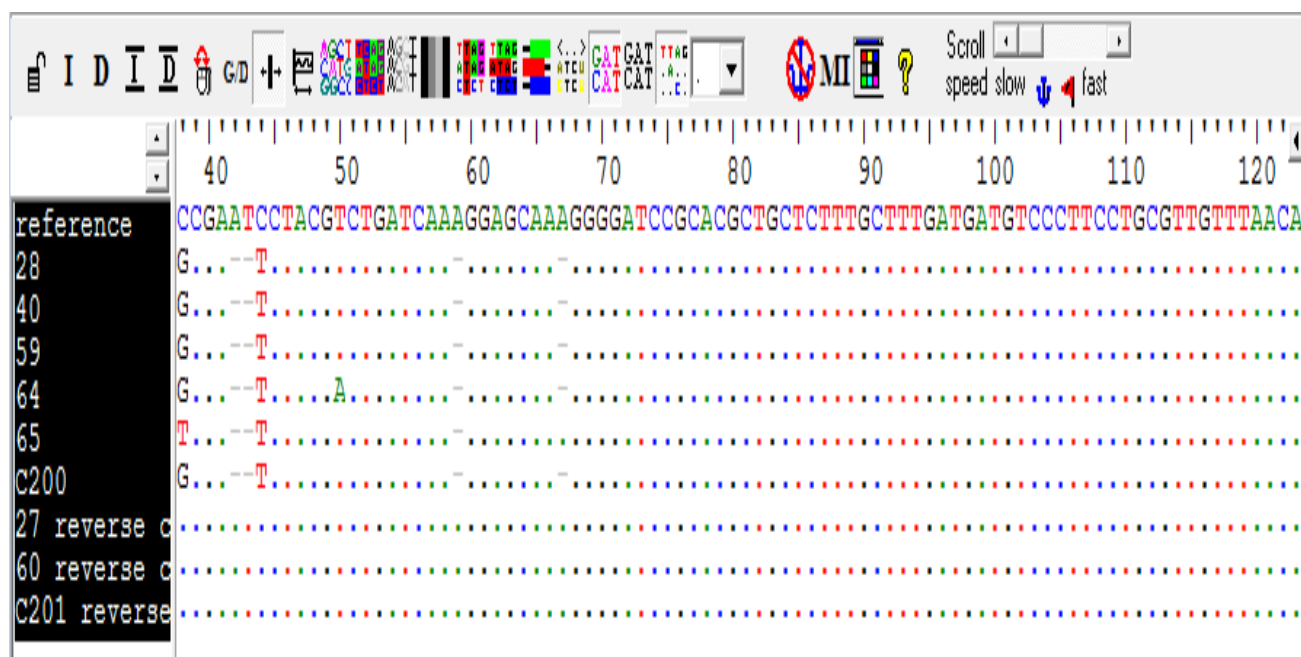
seizure was in age group less than 5 years were 50% of the patients. Ages between 5-10 years old was 28% and more than 10 years were 22%. Ninety percent of the patients had a Family history with epilepsy while the rest of them (10%) had not. Generalize epilepsy presented in 68%, focal to bilateral in 26% and focal in 6 % of the patients two of them (2%) developed impairment. Serum sodium and potassium analysis showed normal results, mean 134.82 mg/dl, and 3.65 mmol/l, respectively. The sequencing findings of SCN1A gene showed that, C > G (chr2:166848853) was detected in 57% of the samples and C > T in 14%, with splice site effect. Also A > T deletion with frame shift mutation (chr2:166848848) was appeared in 71% of the patients with splice site effect.

Table (1): The demographic data

		Count	Column N %
Age	More than 65	2	2.0%
	Less than 18	32	32.0%
	18-40	55	55.0%
	41-65	11	11.0%
Gender	Female	53	53.0%
	Male	47	47.0%
Onset of seizure	Less than 5 yr	50	50.0%
	More than 10 yr	22	22.0%
	5-10 yr	28	28.0%
Class of seizure	Focal	4	4.0%
	Focal to bilateral	26	26.0%
	Focal with impairment	2	2.0%
	Generalize	68	68.0%
Family history	No	90	90.0%
	Yes	10	10.0%

Table (2): Descriptive statistics (Mean±S) of chemicals

	Mean	Standard Deviation	Reference values
Na	134.82	7.95	135 - 145
K	3.65	.40	3.5 - 5

**Figure (1):** Sequence alignment of SCN1A gene of sample against reference sequence

Discussion

Epilepsy is a most common serious neurological disorder and is one of the world's most prevalent non-communicable diseases. Idiopathic (genetic) generalized epilepsies (IGEs) are common epilepsy syndromes. Regarding onset of epilepsy, this study showed that 50% of the patients had their onset of the disease at an age less than 5 years old. This was in favor to Ali A. Asadi's study only 15.2% were four years and under at the time of disease onset (10) though there is increasing evidence for the existence of IGE beginning beyond the third decade (11). In this study, females outnumbered males (53% females, 47% males). Similar findings were in Ali A.

Asadi study (females (57%), (43%) were males. Brain, among many other human tissues and organs, may be influenced by electrolyte disturbances. Seizures are often seen in patients with sodium disorders, especially, hypocalcemia, hyponatremia, and hypomagnesemia (12). numerous reports suggested that the body electrolytes (sodium (Na⁺), potassium (K⁺) and calcium (Ca²⁺) play a vital role in seizure condition to develop (13, 14). patient in the present study had sodium and potassium levels in the normal ranges .this might be because samples were collected when patients were in stable status as most of them were already under treatment. SCN1A is the most clinically relevant

gene for a large spectrum of epilepsy phenotypes and the search for a mutation in the SCN1A gene is the first widely accepted step in DNA diagnosis of patients assumed to have idiopathic epilepsy. Sequencing results showed that 71% of the sequenced samples had AT deletion chr2:166848848_166848849 resulting in amino acid substitution Isoleucine 1646 Proline. At the same exon (exon 26) analysis in patients with GEFS+2 identified a nucleotide substitution, G4943A, that results in the amino acid substitution Arg1648His (15). A substantial body of clinical data supports that high CNS L-proline may cause neuronal dysfunction by interfering with native neurotransmitter systems (16). In this regard, it was noted that the chemical structure of L-proline closely resembles that of GABA, suggesting pathological levels of L-proline might specifically disrupt normal GABA-ergic function(17)Lproline is a GABA-analogue, it's accumulation in the cytosol of GABA-ergic neurons causes competitive inhibition of glutamic acid decarboxylase (GAD) leading to deficient γ -Aminobutyric acid (GABA) production (17). GABA is recognized as the main inhibitory neurotransmitter in the cerebral cortex (18).It maintains the inhibitory tone that counterbalances neuronal excitation. When this balance is perturbed, seizures may ensue.

Conclusion:

Genetic mutations have an effective role in developing epilepsy, AT deletion in SCN1A gene, indirectly, affects Gamma aminobutyric

acid function which is inhibition of neuronal activity.

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Assessment of selective auto antibodies in pregnant women with spontaneous recurrent miscarriage and non-miscarriage in al-gezira state-sudan

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Abstract:

Recurrent miscarriage is a critical problem in the health sector. Antiphospholipid, anticardiolipin, antinuclear and antidouble strand DNA are group of autoantibodies that bind to negatively charged phospholipids which has relation with recurrent miscarriage during the first 10 weeks of pregnancy or the birth of a dead child (still birth). The aim of this study was to detect the presence of these antibodies among pregnant women in Al-Gezira state. A case-controls study was conducted at Wad Madani Teaching Hospital Al- Gezira, Department of Obstetrics gynecological in Sudan. The cases were (90 women) 45 women with miscarriage while 45 non-miscarriage women chosen as control. Antiphospholipid, Anticardiolipin, Antinuclear and Antidouble strand DNA for IgG antibodies were analyzed in the maternal sera of all of the participants by using ELISA. Furthermore all positive and borderline results were confirmed by using immunoblot test. Out of 90 women, 45 women with miscarriage showed 2(4.4%) were positive, 2(4.4%) were negative for IgG APA 1(2.2%), 2(4.4%) were positive and borderline ACL IgG, 3(6.7%), 1(2.2%) were positive and borderline ANA IgG and negative in dsDNA while 45 women with non-miscarriage 1(2.2%) was borderline APL IgG, 1(2.2%)was borderline ANA IgG and negative in ACL and dsDNA . There were significant difference in the age, biomass index, MCV, MCHC, MPV, RDWCV, RDWSD between the two groups ($P < 0.05$) .The analysis of In binary logistic regression women with variable, univariate and multivariate analysis showed that preeclampsia, vaginal diseases, vaginal bleeding, menstruation cycle, RDWC, RDWSD and biomass index were significantly associated with miscarriage in both ($P < 0.05$). While age were significant associated with miscarriage in univariate analysis (OR= 5, 95%CI=2-13, P value 0.001), family history (OR= 2.94, 95%CI=2.946-948, P value.000) and antiphospholipid antibodies associated with miscarriage in multivariate miscarriage (OR= 4.4, 95%CI=45-43.8, P value0.02). In the current study antiphospholipid, anticardiolipin and antinuclear IgG sero-positivity are associated with miscarriage.

Keywords: Recurrent miscarriage, Antiphospholipid, Anticardiolipin, Antidouble strand DNA, Antinuclear

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Introduction:

Recurrent miscarriage is defined by two or more failed clinical pregnancies and about 50% of reason of recurrent abortion is not clear, and there

is increasing evidence that immunological factors play an important role in the failure of these pregnancies [1-3]. These factors include various humoral abnormalities such as anti-nuclear

antibodies (ANA), anti-double stranded DNA antibodies (anti-dsDNA), lupus anti-coagulant (LACAb), anti-phospholipid (APA), anti-cardiolipin (ACA), anti-thyroglobulin (TgAb), anti-thyroperoxidase (TPOAb) and anti-thrombin III antibodies (ATIIIAb). Therefore, in the case of autoimmune-induced miscarriages the woman's body attacks the growing fetus or prevents normal pregnancy progression [4]. Further research also has suggested that autoimmune disease may cause genetic abnormalities in embryos, which in turn may lead to miscarriage [5]. Since its original description almost twenty years ago, the antiphospholipid syndrome (APS) has emerged as the most important treatable cause of recurrent miscarriage. The APS is also an important cause of early onset preeclampsia and of IUGR (6). Approximately 15% of women with recurrent miscarriage have persistently positive tests for either LA or ACA, compared to 2% of those with an uncomplicated obstetric history and hence it is important to screen for both LA and for IgG and IgM ACA [7]. Antiphospholipid antibodies are a family of closely related immunoglobulin's that react with anionic phospholipids. Recent studies would suggest these antibodies recognize complexes of anionic phospholipids and a variety of plasma proteins, including β_2 -glycoprotein I (β_2 -GPI), prothrombin, and proteins C and S [7], Anticardiolipin antibodies and lupus anticoagulants are members of the APA family. These antibodies have been associated with thrombotic events, which could lead to pregnancy loss [8]. Clinical features such as venous or

arterial thrombosis, unexplained fetal deaths after 10 weeks, delivery at 34 weeks as a result of severe pregnancy-induced hypertension (PIH) or recurrent pregnancy loss (RPL) before 10 weeks in combination with a positive laboratory results of antibodies to cardiolipin (CL) or a positive lupus anticoagulant (LAC) on two occasions at least 6 weeks apart are parameters for diagnosis of the antiphospholipid antibody syndrome (APS) [9]. However, the significance of APA in a woman without of a prior pregnancy or in the absence of prior thromboembolic phenomena is unclear [10]. The presence of an antiphospholipid antibody such as the lupus anticoagulant and anticardiolipin antibodies in an individual is associated with a predisposition for blood clots. Blood clots can form anywhere in the body and can lead to stroke, gangrene, heart attack, and other serious complications. Antiphospholipid and anticardiolipin antibodies are frequently found in sera of patients with systemic lupus erythematosus (SLE) and related diseases or in patients without any evidence of autoimmune disorders [11, 12]. These antibodies have been shown to be associated with thrombosis, thrombocytopenia, and recurrent fetal loss, which together comprise the antiphospholipid syndrome APS) [13, 14]. However, a few studies have been conducted in the Sudan about causative agents of miscarriage as case control studies to determine the association between the autoantibodies and recurrent fetal loss.

Methods

A case – control study was conducted at Wad Madani Teaching Hospital, Al-Gezira, Sudan during the period of August through November 2018. Including 90 (100%) participants women with and without history of miscarriage were enrolled, 45(50%) women were pregnant with history of miscarriage, while the others 45(50%) were pregnant without history of miscarriage, five ml of blood specimens were collected from each participated women and dispensed into sterile EDTA and plain blood containers, then serum samples were extracted from blood of women by centrifugation at 3000 for 5 minutes. Enzyme-linked immunosorbent assay (ELISA) was used for antiphospholipid, anticardiolipin, antinuclear and Antidouble strand DNA for (IgG) using commercial diagnostic kits (AESKU, Germany). Quantitative analysis for APL, ACL, ANA, dsDNA (IgG) were performed, and the assay result interpreted as positive more than ($1.2 \times \text{OD cut-off}$), borderline ($0.8 \times \text{OD cut-off} \leq \text{OD patient} \leq (1.2 \times \text{OD cut-off})$), negative less than ($0.8 \times \text{OD cut-off}$). Finally, all positive and borderline results were confirmed by using immunoblot test using commercial diagnostic kits (AESKU). Antibodies were detected using EUROIMMUN immunoblot strips coated with parallel lines of highly purified antigens. The EUROLINE test kit provides a qualitative *in vitro* assay for human autoantibodies of the IgG class to 14 different antigens (n-RNP, Sm, SS-A native and RO52, SS-B, Scl70, PM-Scl, JO-1, CENP B, PCNA, dsDNA, nucleosomes, histones, ribosomal-P-protein, and AMA M2). 1.5ml of the

diluted enzyme conjugate (alkaline phosphate labeled ant human IgG) were pipetted into each channel, and incubated for 30 minutes at RT on a rocking shaker, afterwards this liquid was aspirated off. A Second wash was carried out 5X with 5 minutes interval with 1.5ml working strength wash-buffer per a strip, on a rocking shaker, then 1.5 ml of a substrate solution were aspirated down into the channels of the incubation trays the trays were covered with clean papers and incubated for 10 minutes at RT on a rocking shaker. After the substrate solution was aspirated off, each strip was washed with distilled water as 3times 1minutes. The strips were left to air dry then were evaluated using the EUROIMMUN sheet.

Statistical analyses

Data was analyzed by statistical package for social sciences (SPSS) program version 21 for interpretation of the results and binary regression analyses, logistic regression analysis test was employed to assess the association between variables. Odds ratio (OR) with 95% confidence interval (CI) was calculated and statistical significance was set at $P < 0.05$. Frequencies, proportion were drawn for numerical data and recurrent response was done for quantitative data mean and standard deviation.

Results

A total of 90 participants women were enrolled to the study there were 45 cases and 45 controls, table (1) showed that the occurrence of APA ,ACL, ANA, Ads IgG between two groups, there are 2(4.4%) positive, 2(4.4%) borderline and

41(91.1%) negative for APL-IgG, 1(2.2%) positive, 2(4.4%) borderline and 42(93.3%) negative for ACL-IgG, 3(6.7%) positive, 1(2.2%) borderline and 41(91.1%) negative for ANA-IgG, while dsDNA-IgG was 0(0.0%) positive and borderline, 45(100%) negative cases of women

with recurrent miscarriage. And IgG for non-miscarriage women was 0(0.0%) positive, 1(2.2%) borderline and 44(97.8%) negative for APL and ANA antibodies, 0(0.0%) positive and borderline, 45(100%) negative for ACL and dsDNA antibodies.

Table 1: Distribution of women with recurrent miscarriage and non-miscarriage according to Antiphospholipid, Anticardiolipin, Antinuclear and Antidouble strand DNA antibodies for IgG:

Item	Women with recurrent miscarriage		Women with non-miscarriage	Total
Antiphospholipid Ab	Positive	2(4.4%)	0(0.0%)	2
	Borderline	2(4.4%)	1(2.2%)	3
	Negative	41(91.1%)	44(97.8%)	85
Total	45		45	90
Anticardiolipin Ab	Positive	1(2.2%)	0(0.0%)	1
	Borderline	2 (4.4%)	0(0.0%)	2
	Negative	42(93.3%)	45(100%)	87
Total	45		45	90
Antinuclear Ab	Positive	3(6.7%)	0(0.0%)	3
	Borderline	1(2.2%)	1(2.2%)	2
	Negative	41(91.1%)	44(97.8)	85
Total	45		45	90
Antidouble strand Ab	Positive	0(0.0%)	0(0.0%)	0
	Borderline	0(0.0%)	0(0.0%)	0
	Negative	45(100%)	45(100%)	90
Total	45		45	90

Table2: Confirm positive and borderline results by using immunoblot test:

SNO	Antibody	Diseases
86	CENP-B	Systemic sclerosis
55	SS-A/RO60 +SS-A/RO52	Sjorgen's syndrome
39	PCNA	Systemic lupus erythematosus
75	dsDNA + CENP-B +AMA M2	Systemic lupus erythematosus
77	dsDNA + PCNA+ SS-B	Systemic lupus erythematosus
61	AMA	Primary biliary cirrhosis

Socio-demographic and clinical characteristics with (p value < 0.05) include: age (P= 0.0003), Biomass index (P=0.0104), MCV (P=0.0001), MCHC (P=0.0125), MPV (P=0.0006), RDWCV (P=0.0044), RDWSD (P=0.0001). While there

was no significant difference between case and control include Hb, RBCs, TWBCs, Platelet, PCV, MCH, Antiphospholipid IgG, Anticardiolipin IgG with means as showed in Table (3).

Table 3: Socio-demographical and clinical characteristic of the cases and controls.

Variable	Control N=45 Mean $\pm$ SEM	Case N=45 Mean $\pm$ SEM	P-value
Age	26.02 $\pm$ 0.8531	30.89 $\pm$ 0.9504	0.0003 "-7.409 to -2.324"
Biomass index	25.66 $\pm$ 0.6089	27.85 $\pm$ 0.5751	0.0104 "-3.860 to -0.5250"
Hb	10.93 $\pm$ 0.2420	10.58 $\pm$ 0.3481	0.4187 "-0.4995 to 1.188"
RBCs	10.65 $\pm$ 6.849	3.843 $\pm$ 0.1349	0.3235 "-6.836 to 20.44"
TWBCs	9.109 $\pm$ 0.4661	7.907 $\pm$ 1.214	0.3577 "-1.386 to 3.790"
Platelates	251.7 $\pm$ 12.61	243.8 $\pm$ 14.61	0.6803 "-30.43 to 46.39"
PCV	33.94 $\pm$ 0.6871	31.84 $\pm$ 1.053	0.0984 "-0.4025 to 4.602"
MCV	90.72 $\pm$ 1.057	84.22 $\pm$ 1.010	0.0001 "3.590 to 9.410"
MCH	29.00 $\pm$ 0.5027	28.11 $\pm$ 0.5391	0.2311 "-0.5784 to 2.356"
MCHC	31.91 $\pm$ 0.3579	33.16 $\pm$ 0.3316	0.0125 "-2.216 to -0.2733"
MPV	8.687 $\pm$ 0.1015	9.593 $\pm$ 0.2327	0.0006 "-1.412 to -0.4012"
PCT	0.2115 $\pm$ 0.01113	0.2579 $\pm$ 0.03219	0.1762-0.1143 to 0.02136"
RDWCV	15.88 $\pm$ 0.2821	14.59 $\pm$ 0.3397	0.0044 "0.4121 to 2.170"
RDWSD	52.48 $\pm$ 0.8195	44.98 $\pm$ 0.8974	0.0001 "5.078 to 9.917"

RBCs (Red blood cells), Hb (Hemoglobin), PCV(Packed Cell Volume),MCV (Mean Cell Volume), MCH (Mean cell hemoglobin), MPV (Mean Platelet Volume), PCT (Plateletcrit), RDW-CV (Red Blood Cell Distribution Width), RDW-SD (Red Cell distribution width it measures the width of red cells size distribution).

In binary logistic regression women with variable, univariate and multivariate analysis showed that preeclampsia, vaginal bleeding, menstruation cycle, RDWC, RDWSD and biomass index were significantly associated with miscarriage in both univariate and multivariate. While age were significant associated with miscarriage in

univariate analysis (OR = 5, 95% CI = 2 -13, P value 0.001), family history (OR = 2.94, 95% CI = 2.946 - 948, P value.000) and antiphospholipid antibodies associated with miscarriage in multivariate (OR = 4.4, 95% CI = 45 - 43.8, P value0.02). Table (4).

Table 4: Binary regression analyses of the predictors for miscarriage and non-miscarriage:

Variable	Univariate			Multivariate		
	OR	95% CI	P value	OR	95% CI	P value
Age	5	2-13	0.001	.336	.090-1.250	.104
Biomass index	5	2-12	0.001	1.73	0.062-4.79	0.001
Rate of Miscarriage	.000	0.000-0.000	0.993	1.000E-013	0.000-1.000	0.9987
Family history	2.94	2.946-948	.000	1.000	0.000-1.000	1.000
Menstruation Cycle	3.775	1.2-11.5	0.02	2.59	0.078-8.61	0.028
Vaginal disease	0.230	0.211-1.453	0.230	0.689	0.239-1.987	.491
Vaginal Bleeding	6.353	2.1-19.2	.001	1.39	.043-4.47	0.001
Hypertension	1.08	0.065-17.8	0.96	0.972	0.057-15.741	0.951
Preeclampsia	16.1	1.9-131.1	0.01	2.9	1.3 -6.7	0.000
Blood group	0.000	.010-1.722	0.1	7.2	5.4-8.42	0.997
HB	1.3	0.56-3.1	0.4	0.574	0.126-2.615	.473
RBCs	2.3	0.85-6.2	0.1	0.494	0.156-1.564	0.230
TWBCS	0.7	0.254-1.97	0.5	1.928	0.635-5.855	.246
Platelets	0.7	0.21-2.2	0.52	1.208	0.343-4.251	.768
PCV	.432	0.162-1.157	.095	.594	0.180-1.959	.392
MCV	1.000	0.234-4.271	1.000	1.571	.327-7.549	.573
MCH	.577	0.248-1.343	.202	0.442	0.165-1.188	0.106
MCHC	1.545	0.616-3.878	.354	2.112	0.759-5.881	.152
MPV	.302	.058-1.587	.157	0.677	0.085-5.401	0.713
PCT	1.000	0.269-3.724	1.000	0.811	0.144-4.576	0.813
RDWCV	4.375	1.750-10.9	.002	3.531	1.190-10.472	.023
RDWSD	19.158	5.158-71.1	.000	17.019	4.187-69.179	.000
Antiphospholipid IgG	43	.46-40.01	.2	4.4	.45-43.8	.02
Anticardiolipid IgG	1	1-1	0.748	0.57	.007-.460	.017
Antinuclear IgG	0.9	0.56-15.9	0.9	1.170	.06-23.6	0.9
Antidoublestrand DNA IgG			1.000	0.063	0.01-0.5	.017

CI: Confidence interval , OR: Odds ratio RBCs(Red blood cells),Hb (Hemoglobin), RBCs (Red Blood cell), TWBCs(Total White blood cells),PCV(Packed Cell Volume),MCV(Mean Cell Volume),MCH (Mean cell hemoglobin),MPV (Mean Platelet Volume), PCT (Plateletcrit),RDW-CV (Red Blood Cell Distribution Width),RDW-SD(Red Cell distribution width it measures the width of red cells size distribution)

Discussion:

In this study women with fetal loss had relatively high incidence of raised antiphospholipid antibodies, anticardiolipin antibodies and antinuclear antibodies of the IgG class Table (1), and three of them were diagnosed with Systemic lupus erythematosus (SLE), Table (2) a finding which accordance with previous reports. Presence of clinical features previously associated with raised antiphospholipid, anticardiolipin, antinuclear antibodies levels [15].

Our study revealed there was no statistically significant difference in HB, platelets. While the age, (control) group were N = 45 Mean 26.02 and (case) group were N = 45 mean 30.89, this result showed statistical significant ($P < 0.0003$) between control and case group. The age of most positive cases in miscarriage women was belonged to more than 30, this result agrees with [16], who found that women of (31-35) years had a 28% miscarriage rate. Because the causative agents of miscarriage are many reasons, still need further studies. In the present study in all participants the biomass-index seropositivity of APL, ACL, and ANA-IgG were found higher in abnormal participant, the results that agreed with previous studies, who established there were strong associations between abdominal obesity and seropositivity in women participating. Interestingly, according to our study revealed many factors related to the biomass-index the (case) group was N=45 mean 25.66 and (control) group was N = 45 mean 27.85 this result showed statistical significant ($P = 0.0104$) and another

factors as remarkable signs of MCV ($P=0.0001$), MCHC ($P=0.0125$), MPV ($P=0.0006$), RDWCV ($P=0.0044$), RDWSD ($P=0.0001$) Table (3).

In binary logistic regression women with variable, univariate and multivariate analysis showed that preeclampsia, microcytic hypo- chromic anemia, vaginal bleeding, menstruation cycle, RDWC, RDWSD and biomass index were significantly associated with miscarriage in both univariate and multivariate. While age were significant associated with miscarriage in univariate analysis, antiphospholipid antibodies associated with miscarriage in multivariate (OR= 4.4, 95%CI=45-43.8, P value0.02). On the other hand, the results revealed that was statistical relation between the disease and the family history (OR= 2.94, 95%CI=2.946-948, P value.000), this result agreed with that of [17] who found about 60% of early pregnancy losses associated with sporadic chromosomal anomalies. Table (4).

The high level of antibodies is known to be harmful to both mother and child, so diagnosis and treatment can improve pregnancy outcome [18]. In these study, Presence of clinical features previously associated with raised anticardiolipin antibody levels: thrombosis (both venous and arterial), [15] fetal loss, [19] and thrombocytopenia [20] confirms the validity of our study. In our study presented that there are many risk factors associated with recurrent miscarriage.

Many studies agreed with our results that found significant association between repeated miscarriage and presence of high level of APA

ANA and ACL- Abs of clinical significance in identifying women at a risk of pregnancy loss then ACL-IgG antibodies. In different studies there was high a variation in rate of APA, ACL-Abs. which could be due to many causes of repeated abortion in women and the different methods that employed to determinate antibodies so, these results indicated the importance of systemic investigation of these antibodies in pregnant women at risk of miscarriage, and Identification of patients positive for antiphospholipid antibodies. it is importance to record. However, that several reports had described successful pregnancies in patients with antiphospholipid antibodies, treated during pregnancy [21, 22]. Whether antiphospholipid antibodies are causative, a consequence or coincidence remains unknown but there is general agreement that the pregnant woman is particularly vulnerable to the adverse effects associated with these antibodies [23]. Limitation of our study needs increasing of sample size and cohort study design to follow up pregnant women and further studies on molecular and cytokines levels.

Conclusion:

The current study revealed that seroprevalence rate in women with fetal loss there was a relatively high incidence of raised antiphospholipid antibodies, anticardiolipin antibodies and antinuclear antibodies of the IgG class and three of them were Systemic lupus erythematosus (SLE) patients. Also there was a significant association between menstruation cycle preeclampsia,

vaginal disease, vaginal bleeding and family history with recurrent miscarriage.

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Evaluation of Serum Uric Acid Level among Sudanese Obese Subjects

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Abstract

Background: Besides gout, elevated uric acid level is related to a variety of other conditions including increase alcohol and purine rich food consumption and obesity. Hyperuricemia is considered to be a common lifestyle disorder related with obesity.

Objectives: To evaluate serum uric acid level in Sudanese obese subjects.

Materials and Methods: Analytical case control study conducted at Khartoum state during the period from July to August 2021. Fifty obese subjects as a case group and 50 as a control groups. Uric acid level was assayed using spectrophotometer. The data analyzed using SPSS version (25).

Results: The serum uric acid and BMI in obese is a significantly higher than non obese with p value = 0.000. In obese group show mean uric acid concentration of males is significantly higher than females ($p < 0.05$). The risk of developing CVD is 8 time higher in obese subjects with high uric acid levels. Serum uric acid level showed strong significant positive correlation with BMI ($r=0.79$, $p= 0.000$), and no correlated with age ($r=0.03$, $p= 0.7$).

Conclusion: The obese had a significantly higher of serum uric acid. The elevation of uric acid level is sex dependent and is positively correlation with BMI.

Key words: uric acid, obese, cardio disease, hypertension, hyperuricemia

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Introduction

Obesity is becoming a more serious public health issue on a global scale, as global mortality and the incidence of chronic diseases continue to rise. Obesity is linked to a variety of disorders (such as hypertension, atherosclerosis, cardiopulmonary disease, metabolic syndrome, and cancer) and is also a risk factor for cardiovascular disease and all-cause mortality (1). Sudan has a population that is obese, ranking 174th in the world. Obese patients had a 2.1 fold increased risk of hyper-

uricemia (high uric acid [HUA]) compared to non-obese patients, according to research. Uric acid is also a byproduct of purine metabolism in the human body. Increased serum uric acid (SUA) is not only a risk factor for gout, but it is also an important risk factor for obesity, which can lead to an increased risk of cardiovascular disease. SUA has been shown to be associated with the risk of obesity in an independent and favorable manner (2). There are a number of mechanisms

that could explain the link between HUA and obesity. Obesity or extra body fat may be linked to increased uric acid synthesis and inadequate excretion as a result of insulin resistance (IR), leading in impaired uric acid metabolism and even HUA. Meanwhile, HUA can cause obesity by increasing the formation of liver and peripheral fat. Disorders of glycolipid and uric acid metabolism may encourage the coexistence of the two variables. HUA appears to be a risk factor for hypertension, cardiovascular disease, and cerebrovascular disease (3, 4). SUA levels are strongly linked to the occurrence of CVDs and its components. In Bangladeshi adults, a substantial positive association between SUA and obesity has been demonstrated (5). According to the most recent data, HUA has become a major health problem in southern China among urban people aged 35–79 years, with men receiving special attention. Further research on the comorbidities and causality of HUA is warranted (6). In study conducted by (7), Serum Uric Acid Levels in Overweight and Obese University Undergraduate Students In Relation To Anthropometric Indices Obese and overweight males had considerably higher serum uric acid levels than females and control subjects, according to the study. Also in study done by (8), the researchers wanted to see how serum uric acid levels differed between normal-weight people and severely obese people. When compared to the control group, laboratory markers, particularly blood uric acid levels, were shown to be greater in morbidly obese patients.

The objective of present study was to evaluate serum uric acid level in Sudanese obese subjects.

Materials & Methods

Analytical case control study conducted at Khartoum state during the period from July to August 2021. Fifty obese subjects as a case group and 50 as a control groups. BMI more than 30 and no history of any medical condition. Other 50 healthy individuals were used as a control group, whose age and sex matched healthy. Overweight subjects and obese subjects with history of medical condition were excluded from the Study. Informed consent was taken from the patients and subjects who participated in the present study. Ethical committee approval has also been obtained from Omdurman Islamic university. In both groups serum uric acid was estimated using uricase method and BMI was calculated by dividing by weight (kg) by height square (m^2).

Statistical Data: The result was expressed as mean $\pm$ SD. Student T test was used to compare the mean concentration of uric acid; chi-square test was used for risk estimate and person correlation coefficient to examine the relationship. P value less than or equal 0.05 was consider statistically significant.

Results

The study revealed that the average age of case and control was (40.5 ± 9.2) and (38.7 ± 7.9) respectively, with no significant difference ($P=0.311$). The mean BMI was significantly (0.000) higher in obese (34.7 ± 3.4) compared to control group (21.5 ± 1.9) (Table 1). Among the

50 obese individuals who participated, (50% men and 50% women), the mean serum concentrations was significantly higher in case (7.2 ± 0.27) compared to control group (5.3 ± 0.86) with p value (0.000) (Table 2). According to gender, Females and males mean of uric acid concentrations in the obese group is significantly higher than the mean that in the control group ($p < 0.05$) (Table 3). When considering obese group only, findings show mean uric acid concentration of males is significantly higher

than that of females ($p < 0.05$), while no significant difference was observed when compared according to age (Table 4).

This Study also showed that the risk of developing cardiovascular disease is 8 times higher in obese subjects with hyperuricemia than those with normal uric acid levels (table 5).

Serum uric acid level showed strong significant positive correlation with BMI ($r = 0.79$, $p = 0.000$), and no correlated with age ($r = 0.03$, $p = 0.7$). (Figure 1, 2).

Table 1: Demographic and anthropometrics Characteristics of Study population

variables	case group (50)	control group (50)	p value
Age	40.5 ± 9.2	38.7 ± 7.9	0.311
BMI	34.7 ± 3.4	21.5 ± 1.9	0.000

Table 2: Compare mean uric acid concentration in study population

parameter	case group (50)	control group (50)	p. value
uric acid	7.2 ± 0.27	5.3 ± 0.86	0.000

Table 3: Serum Uric Acid level in the male and the female of case and control group

parameter	male		p value	female		p value
	obese	normal		obese	normal	
uric acid	7.6 ± 0.29	5.4 ± 0.87	0.04	7.2 ± 0.27	5 ± 0.85	0.00

Table 4: Compare mean uric acid concentration in case group according to variables risks (age & gender)

variables	uric acid	p value
age	above than 40 years 7.1 ± 0.26 less than or equal to 40 years 7.2 ± 0.29	0.31
gender:	male (25) 7.21 ± 0.28 female (25) 6.3 ± 0.22	0.04

Table 5: Show the Association of hyperuricemia with CVD in obese subjects

parameter	value	obese	OR	p value
uric acid	high	46	7.9	0.000
	normal	4		

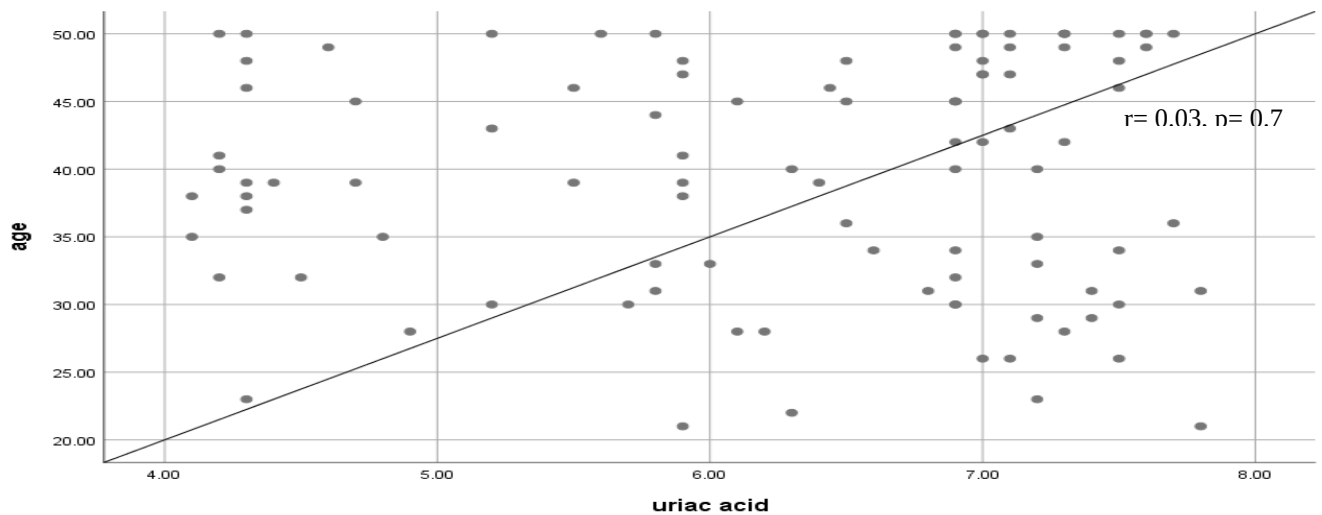


Figure 1: Correlation of serum uric acid with age

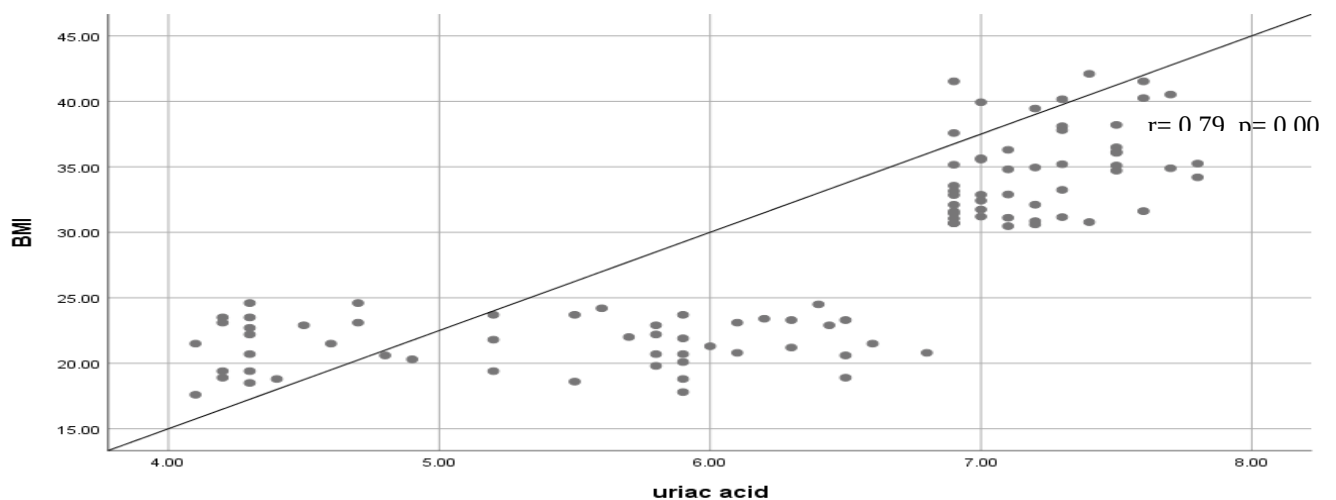


Figure 2: Correlation of serum uric acid with BMI

Discussion

Obesity in the last decade become a global problem and has been recognized as a risk factor with a variety of clinical conditions and adverse health consequences; hyperuricemia is one of these conditions. Nowadays, obesity and hyperuricemia and its complications, such as metabolic syndrome and cardiovascular diseases, have raised serious concern for public health in the international community because of their high prevalence, health consequences and substantial

economic burden 6). Several epidemiological studies have assessed the relationship between SUA and obesity in different population; however, to our knowledge, this is the first study that focused on SUA and obesity for the Sudanese population. The present study was carried out to measure the serum uric acid level among obese Sudanese subjects attending sabreen market of Khartoum during the period of July to august 2021. One hundred (100) heparin blood sample collected from the cases (obese)

and from the control (Healthy Subjects); so fifty (50) were Sudanese subjects with obesity whereas fifty (50) were apparently health Subjects. The results contribute a clearer understanding of effect obesity on fibrinogen levels. Compared with subjects with normal weight, obese subjects have significantly higher levels of SUA, which is consistence with study of (Mehmet İnanir, 2020) (8) who report that uric acid was found to be higher in morbidly obese patients compared to the control group. Increased serum uric acid in these participants may be due to reduced renal clearance and excessive secretion and production of uric acid from adipose tissues through xanthine oxidoreductase. On the other hand, this study revealed that obese males and females have significantly higher serum uric acid when compared with their control. These finding were consistence with report of (Simiao *et al.*, 2019) (9) who found that variation on serum uric acid is sex dependent. Also the study found that the mean uric acid concentration is significantly higher in obese male than female which in consistence with study of (EMMANUEL *et al.*, 2018) (7) who observed significantly higher serum uric acid in obese and overweight males than females. These finding maybe due to sexual dimorphism in female serum uric acid as result of higher renal clearance of urate, possibly due to their higher plasma oestrogen levels. In addition, This Study also showed that the risk of developing cardiovascular disease is 8 times higher in obese subjects with hyperuricemia than

those with normal uric acid levels. Ours finding in consistence with study of (Hefei *et al.*, 2019) (10) who says that hyperuricemia is an independent risk factor for the development of cardiovascular disease and its presence is associated with increased mortality. High uric acid level increases the activity of renin angiotensin aldosterone system causing more vasodilation and finally hypertension. Furthermore, the increased activity of RAAS is associated with an increased activation of activated mitogen protein kinase leading to an increase of apoptosis of cardiac muscles fiber. Thus hyperuricemia through these mechanisms may contribute to the pathophysiological process of heart failure and arterial fibrillation. As shown in Figure (2), along with increased BMI, SUA in obese patients increased gradually. This is similar to previous research of (Liu F *et al.*, 2020) (11) who concludes that SUA in in overweight/obese patients was significantly higher than in normal-weight subjects. The specific mechanism of the interaction between obesity and HUA be related to an imbalance of inflammatory factors and fat factors caused by chronic inflammation and oxidative stress, as well as IR. Finally, The BMI is a commonly recognized index to evaluate obesity. In this study, it was discovered that increased SUA was correlated with BMI in obese patients.

Conclusions

This study concluded that serum uric acid is significantly higher in obese compared to control.

The elevation of uric acid level is sex dependent and is risk factor for development of CVD.

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Determination of possible association between *Helicobacter pylori* and the infection of *Entamoeba histolytica*, *Giardia lamblia* and *Entamoeba coli* in Omdurman-Sudan

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Abstract

Background: There are multi factor aid the infection by parasite in Sudan, in addition to tropical area it may be due to contaminated food and water by parasite, hygiene and agriculture increase prevalence rate, the objective of this study is to Determinate the possible association between some intestinal protozoa and helicobacter pylori in ombada in Khartoum state.

Methods: This study was conducted to detect the infection rate of the *Entamoeba histolytica*, *Giardia lamblia* and *Entamoeba coli* parasite and to determine the relationship between them and *Helicobacter pylori* bacteria among patients attending ombada clinic center during the period from January 2019 to March 2020. The number of examined samples were 600, and the smear examined by direct microscopy.

Results the total infection rate was 77 (12.8%) positive samples and 523(87.1%) negative samples the *G.lamblia* was dominant in 28 (36%) patients, followed by *E.histolytica* with rate 25 (32.4%), and *E.coli* 24 (31.1%) the infection rates for males were higher than for females. 55(71.4%) and 22(28.5%) of females, respectively. The infection rates varied between age groups, as the highest rate of infection was in the age 5-15 years, 45(58.4%), and the lowest rate of infection was in the age group 30-45 years, with a rate of 5(6.4%). All positive samples were examined by rapid antigen test to detect *H.pylori* bacteria, and the results showed that 19(24.6%) samples were positive and 58(75.3%) were negative samples, and the co-infection of *H.pylori* with intestinal protozoa showed *E.histolytica* 10 (52.6%) were higher than that of *G.lamblia* 5(26.3%) and *E.coli* 4(21%).

Keywords- *Helicobacter pylori*, *Giardia lamblia*, *Entamoeba coli*, *Entamoeba histolytica*, association

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Introduction:

H.pylori, Gram-negative bacilli, is one of common bacteria cause gastrointestinal diseases (4). This bacterium infects at least 50% of the world's population. The infection is more common in early childhood and leads to chronic gastritis in children and adults (5). The faecal-oral route is the main route for transmission, although much is unknown in this regard (6). Studies have shown that *H.pylori* cause diarrhea (5). The urease

produced by *Helicobacter* bacteria helps intestinal protozoa and bacteria to cross the acidic environment of the stomach easily. In addition to *H.pylori* infection, intestinal protozoa have been reported to stimulate symptoms of gastrointestinal disorders (7). In different parts of the world a possible association between intestinal protozoa and *H.pylori* infection has been documented with a prevalence of 75% of *H.pylori* infection among patients with gastrointestinal parasites (8, 9, 10).

Diarrhea is a common health problem (1) it is caused by microorganisms such as parasites, bacteria, viruses and other unknown causes (3). The objective of this study is to Determine the possible association between some intestinal protozoa and *H.pylori* in ombada locality, Khartoum state-Sudan.

Methods:

Study Design: community-based descriptive cross-sectional study.

Study area: The study was conducted in ombada clinic center in Omdurman, Khartoum state-Sudan

Study time and population: From January 2019 to March 2020, the study included the examination of 600 patients of both sexes, aged 5-45, who complained of digestive disorders, abdominal pain, fever, vomiting and diarrhea,

Sample: a total of 600 Fresh stool samples were collected from patients using sterile, labeled, 30ml, wide-opening plastic containers with a tight seal to prevent the sample from drying out and to maintain its moisture. Stool samples were divided into two parts, one part used for direct microscopic examination for the purpose of detecting the phases of the intestinal protozoa, and the other part used for the detection of *H.pylori* bacteria

Stool examination: Stool samples were examined microscopically by direct wet smear using normal

saline solution, to detecting the stages of the intestinal protozoa, and Cassette is used in the rapid antigen test According to the manufacturer's instructions for detection of *H.Pylori* bacteria

Ethical Considerations: The verbal information and a written consent form were obtained from study patients prior to the commencement of the study and ombada clinic center management.

Results:

The results of this study are based on parasitological examination for intestinal parasites and serological tests for the diagnosis of *H.pylori*. The number of examined samples were 600, and they were examined by direct smear and the results of the study showed that the total infection rate was 77 (12.8%) positive samples and 523 (87.1%) negative samples the *G.lamblia* was dominant in 28 (36%) patients, *E.histolytica* 25 (32.4%), and *E.coli* 24 (31.1%), Where the infection rate of males was 55 (71.4%) and 22 (28.5%) females. The infection rates varied between age groups 5-15 years 45 (58.4%), 15-30 years 27(35%) and 30-45 years 5(6.4%%) the results showed that out of 77 positive sample 19 (24.6%) were *H.pylori* bacteria positive and 58 (75.3%) were negative samples, the co-infection infection rate was between the *E.histolytica* and *H.pylori* 10 (52.6%) *G.lamblia* 5 (26.3%) and *E.coli* 4(21%).

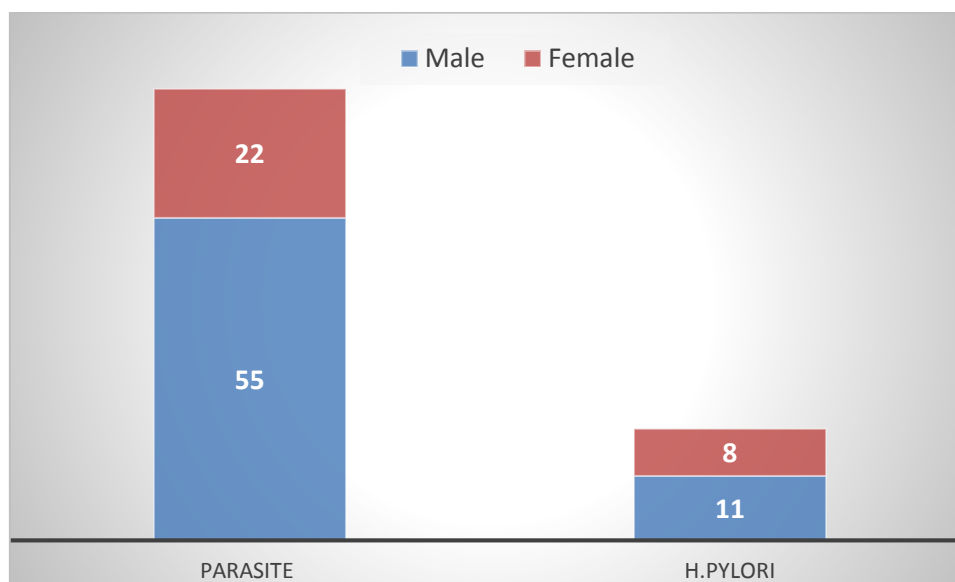


Figure1: The intestinal parasite and *H.pylori* among Gender

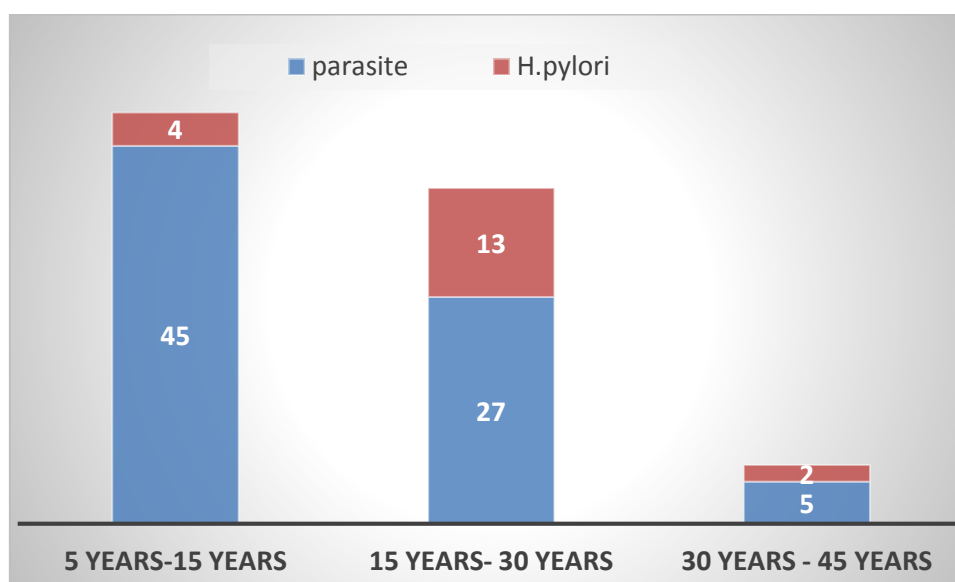


Figure2: The intestinal parasite and *H.pylori* among age groups

Table 1: The co-infection of the intestinal parasite and *H.pylori*

examine	Number of detected parasite	positive <i>H.pylori</i>
Total of examine 600	77(12.8%)	19(24.6%)
Giardia lamblia	28(36%)	5(26.3%)
Entamoeba histolytica	25 (32.4%)	10(52.6%)
Entamoeba coli	24(31.1%)	4(21%)

Discussion

The current study examined 600 stool samples from patients who complain of digestive disorders, as they were examined by direct wet smear method using a light microscope. The results showed that there were 77 (12.8%) samples containing intestinal parasite(*E.histolytica*, *G.lamblia* and *E.coli*) and 523 (87.1%) negative samples that may be there was another cause of diarrhea,

This result is almost in agreement with what he came up with Al-Kiz, and Fatima Sattar Jabbar when studied on primary and secondary school students in the city of Baiji in 2011, the infection rate is (7.52%) (11), the study by AL-Saeed, A.T. and Issa, S.H, the infection rate reached (7.1%) in the city of Dohuk by examining 1261 faecal samples (12). in the city of Tikrit, where it recorded a rate of infection (9.7%) by Kadir, M. A et al, 2018 (13). And recorded data by Abu Hussain, N.A 2005 in Saudi Arabia with a percentage of (9.2%) (14). The incidence of intestinal protozoa (*E.histolytica*, *G.lamblia* and *E.coli*) infections in males was higher than in females, as it reached 55 (71.4%) in males and 22 (28.5%) in female, the decreased incidence in females in the current study and previous studies depends on the habits of society.

The results of this study found that the highest rate of infection (58.4%) was in the age group between (5-15) years, While the lowest infection rate (6%) was in the age group (30-45 years), with one infected person, The reason for the high incidence of infection in the age groups (5-15) years is due

to several factors, including the fact that children are less resistant to infection compared to adults due to the incompleteness and maturity of their system, and that children are usually in a state of contact with the environment as well as mixing with each other. the low incidence of infection in the age groups (30-45) years, it is due to the increase in health awareness and the development of their immune system.

The results showed co-infection between *G.lamblia* ,*E.histolytica* and *E.coli* with *H.pylori* highest rate in *E.histolytica* 52.6% , lowest in *E.coli* 21% and 26.3% in *G.lamblia* This study agrees with the result of Abdurahaman Seid*, Zemenu Tamir in their study in Mekanesalem Hospital, northeast Ethiopia (7)

Conclusion

The results showed that co-infection of *H.pylori* among people who currently infected by *E.histolytica*, *G.lamblia* and *E.coli*

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Prevalence of Hepatitis B Virus and Hepatitis C Virus among Barbers at South Khartoum

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Abstract

Background: Barbers deal with sharp equipment all the time with a wide variety of clients leaving them at greater risk of exposure to hepatitis B virus (HBV), hepatitis C virus (HCV), and other harmful blood-borne pathogens.

Objective: This study aimed to determine the prevalence and knowledge of barbers on HBV and HCV among barbers at South Khartoum-Sudan.

Justification: Many hepatitis-infected individuals do not know their status and many barbers do not know that they could be transmitting or acquiring diseases during their work and how to protect themselves and their clients from contracting the diseases. Sharing contaminated sharp equipment increase the risk of transmission of HBV and HCV. Increasing the knowledge and awareness of HBV and HCV transmission routes among barbers will help in the prevention and control of new infections.

Methods: The current was a work-place based descriptive cross-sectional study conducted at South Khartoum. A structured and pre-tested questionnaire was used to collect demographic and clinical data. Enzyme-linked immunoassay (ELISA) was done to determine the presence of HBsAg and anti-HCV among 89 barbers.

The results: Among 89 barbers enrolled in this study, HBsAg was detected in 9 (10.1%), and anti-HCV was detected in 3 (3.4%). The HBsAg and anti-HCV positive rates were significantly higher in the age group of participants between 26 - 30 years. No participant has both positive results of HBsAg and HCV together.

Conclusion: High prevalence of HBV and HCV and low knowledge of viral hepatitis infections were found among barbers. Barbers in this area need to be educated on viral hepatitis and related diseases to reduce the acquisition of these infections.

Keywords: Barbers; HBV; HCV; Khartoum-Sudan; Prevalence

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Introduction

Hepatitis refers to an inflammation of the liver cells and damage to the liver. People with chronic HBV or HCV infection are at risk of developing a serious liver disease such as cirrhosis or hepatocellular cancer (HCC) (1). HBV and HCV are highly contagious and

transmission can occur via sexual or blood–blood contact (sharing of injection equipment and transfusion of unscreened blood and blood products) or vertically (from mother-to-child) (2).

Many people with hepatitis experience either mild or no symptoms, and symptoms appear

from 15 to 180 days after infection. The common signs and symptoms are diarrhea, fatigue, loss of appetite, mild fever, muscle or joint aches, nausea, abdominal pain, vomiting, weight loss, and jaundice (1). There were approximately 292 million HBV infections in 2016 globally. Of these infections, around 29 million were diagnosed, and only 4.8 million of the approximately 94 million individuals who were eligible for treatment actually received antiviral therapy (3). In fact, HBV infection is responsible for approximately 45% of HCC cases and 30% of liver cirrhosis cases (1). Approximately 80 million individuals are estimated to have chronic HCV infection, which corresponds to a global prevalence of 1.1%. Annually, an estimated 700,000 persons with chronic HCV infection die without receiving treatment (4).

The diagnosis of hepatitis can be by clinical examination, blood tests for liver enzymes, serological tests for the detection of antigens and or antibodies, and molecular techniques. Bio-marker surveys estimating the prevalence of HBsAg or antibodies to HCV are the reference methods more commonly used to measure the prevalence of HBV and HCV infections (5).

Sub-Saharan Africa is considered a highly endemic area for HBV. HBV seropositivity varies between different regions of Sudan, ranging from 5 to 8.2% among different geographical areas (6-10). The seroprevalence of the infection among health workers in Uganda has been reported to be between 8 to 11% (11). Regarding HCV, seroprevalence was

found to be ranging from 2.2 to 4.8% among the general Sudanese population (12), and up to 23.7% among hemodialysis patients (13). In a more recent study among the Sudanese population, a total of 2109 different patients were found to be infected by HBV during 2017; 1641 (77.81%) were males and 468 (22.19%) were females with the age group of 20-39 years predominating (64%). Besides, 16% of patients tested for HBeAg were found reactive, which highlights the acute phase as well as the high infectivity of the infection (14).

Treatment for viral hepatitis depends on the type and stage of the infection. Although treatment regimens exist for HBV and HCV, their efficacy is suboptimal, and problems with the cost and availability limit their use in low-resource settings (15). Globally, existing prevention and treatment interventions are estimated to have reduced the incidence of new HBV infections by 83%, thus preventing 310 million chronic infections that would otherwise have occurred between 1990 and 2020 (16).

Prevention of hepatitis can be by good hygiene and there is a highly efficient vaccine to prevent new infection by the HB viruses had been available since 1982 and is 95% safe and effective (17). Data on the prevalence, knowledge, and awareness of HBV and HCV among barbers are lacking in Sudan. This study, hence, sought to examine the prevalence and knowledge of HBV and HCV infections among barbers in the South Khartoum-Sudan.

Methods

Study design/study site: This workplace-based descriptive cross-sectional study was conducted at South Khartoum - Sudan

Study population: Eighty-nine (89) barbers working in the haircutting shops were conveniently recruited onto the study. Demographic and clinical data and knowledge on HBV and HCV infections of all barbers who were willing and consented to participate in the study were collected by face-to-face interview using a structured and pre-tested questionnaire before taking their blood samples. Participants with a history of HBV and HCV infections prior to the start of the profession were excluded.

Ethical consent: The study was approved by the Sudan International University ethical committee board and informed consent was obtained from each participant before collecting their data and samples.

Blood specimen collection: About 3 ml of venous blood was drawn from each participant, dispensed into a serum separator tube, and transported on ice to the laboratory. At the laboratory, the specimens were centrifuged at 1500 RPM for 5 minutes to obtain serum for serological testing. All sera were stored at – 20OC prior to testing.

Serological tests: The obtained sera were tested according to the manufacturer's protocol for the presence of HBsAg by enzyme-linked immunosorbent assay (ELISA) (ACON Laboratories, Inc-USA) and the presence of anti-HCV by ELISA (Fortress Diagnostics-UK). The presence of HBsAg was considered as evidence of prior exposure to HBV (Recent infection or chronic

carrier) and the presence of anti-HCV was considered as evidence of current infection or past exposure.

Statistical analysis: The data obtained from the questionnaire and sample results were entered into a database and analyzed using a statistical package for social sciences (SPSS). Descriptive statistics were reported as the mean $\pm$ SD for continuous variables and as the frequency (%) for dichotomous variables. To evaluate the relationship between different factors, we performed a chi-square analysis. Quantitative variables were compared using the independent t-test. *P. values* < 0.05 were considered statistically significant.

The Results: The age range was between 20 - 40 years with the majority between 26 - 30 years, and the mean age was 29 years. The level of education was 46% graduate, 47% secondary and only 7% primary. In expressing their knowledge of Hepatitis B and C, about 80% know about hepatitis B and C, but the majority of the group 72% do not know about the mode of transmission, and only about 3% of the respondents reported little knowledge of the signs and symptoms of hepatitis B and C. About 42% had a history of surgical and/or dental procedures; however, all participants had no history of blood transfusion. About 43% with repeated minor trauma and about 44% do not sterilize their equipment. Only about 6% of the participants were vaccinated against HBV. For HBsAg, the study revealed 9 (10.1%) positive cases and for HCV 3 (3.4%) positive cases.

The majority of the HBV and HCV positive participants were between the ages 26 - 30 years. Educational level, previous blood transfusion, previous surgical and/or dental procedures,

previous hepatitis B vaccination, minor trauma during the work, and sterilization of tools were not associated with HBV and HCV status.

Demographic characteristics, clinical and laboratory data

Characteristic	Frequency		HBV		HCV	
	No	%	No	%	No	%
Hepatitis status			9	10.1	3	3.4
Age						
20 – 25	19	21.35	0	0.0	0	0.0
26 – 30	42	47.19	6	6.8	3	3.4
31 – 35	16	17.98	1	1.1	0	0.0
36 – 40	12	13.48	2	2.2	0	0.0
Level of education						
Primary	06	06.74	1	1.1	0	0.0
Secondary	42	47.19	6	6.8	1	1.1
Graduate	41	46.07	2	2.2	2	2.3
Knowledge about hepatitis B and C						
Yes	71	79.78	8	9.0	3	3.4
No	18	20.22	1	1.1	0	0.0
Knowledge about hepatitis B and C modes of transmission						
Yes	25	28.09	1	1.1	1	1.1
No	64	71.91	8	9.0	2	2.3
Knowledge about hepatitis B and C signs and symptoms						
Yes	03	03.37	0	0	0	0
No	86	96.63	9	10.1	0	0
Previous blood transfusion						
Yes	0	0	0	0.00	0	0.0
No	89	100	9	10.1	3	3.4
Surgical and or dental procedures						
Yes	37	41.57	0	00.0	2	2.3
No	52	58.43	9	10.1	1	1.1
Vaccination against Hepatitis B						
Yes	05	05.62	0	0	0	0.0
No	84	94.38	9	10.1	3	3.4
Minor trauma during work						
Yes	38	42.70	4	4.5	3	3.4
No	51	57.30	5	5.6	0	0.0
Tools sterilization						
Yes	50	56.18	5	5.6	1	1.1
No	39	43.82	4	4.5	2	2.3
Total	89	100.00	9	10.1	3	3.4

Discussion

Many traditional and low-paid barbers in developing countries earn their livelihood by providing shaving services in unauthorized and non-conforming small marketplace and on the street side. Poor infection control during the use of sharp instruments may be a risk factor for serious blood-borne infections, potentially causing serious health problems for both the barber and the clients.

This cross-sectional study examined the prevalence of HBV infection and HCV infections among barbers in the South Khartoum-Sudan. The prevalence of HBV and HCV among the barbers were 10.1 % and 3.4 % respectively. Most of the participants had a low level of knowledge of HBV and HCV infections. The 10.1% prevalence of HBV among barbers was higher than the 4.1% prevalence among barbers in Gharbia-Egypt recorded in a cross-sectional study by Shalaby *et al.* (18), and also higher than the 5 - 8.2% prevalence among the general Sudanese population (6-10). However, it is lower than the 28% prevalence among barbers in Rabat-Morocco recorded in a cross-sectional study by Belbacha *et al.* (19), and also lower than the 14.5% prevalence among barbers in Obuasi-Ghana recorded in a cross-sectional study by Adoba *et al.* (20)

The 3.4 prevalence of HCV among barbers was higher than the 0.5% prevalence among barbers Obuasi-Ghana recorded in a cross-sectional study by Adoba *et al.* (20), and the 1.1% prevalence among barbers in Rabat-Morocco recorded in a cross-sectional study by Belbacha *et al.* (19), and fortunately within the 2.2 - 4.8%

prevalence among general Sudanese population (12). However, it is lower than the 12.5% prevalence among barbers in Gharbia-Egypt recorded in a cross-sectional study by Shalaby *et al.* (18).

The difference in prevalence rates could be due to the differences in sample sizes, geographical location, patient characteristics and sampling techniques used, lower prevalence of HCV found in this study compared to the findings of Shalaby *et al.* might be due to the differences in sample size, and the high endemicity of HCV in Egypt (18).

Adoba and colleagues (20) in a cross-sectional study to estimate the prevalence of HBV and HCV among barbers at the Obuasi-Ghana observed the highest prevalence of these viruses among barbers between the ages of 20 - 29 years. This supports the finding of the highest prevalence of HBV and HCV among barbers between the ages of 26 - 30 years in our study. The high prevalence of HBV and HCV infections among this group could be the result of risky lifestyles.

The finding of no significant association between the risk factors and HBV and HCV infections among the barbers indicates a probable high occupational risk of barbers to HBV and HCV infections.

The study revealed that the majority of barbers did not perceive themselves to be at risk of HBV and HCV infections and have little information about hepatitis B and C, but few know how the viruses are transmitted. This finding showed that the level of knowledge and awareness of barbers

about the concept of infectious risk associated with blood was generally very low. In the current study, all the participants knew that it was necessary to use a new blade for each customer and disinfect their tools, especially the sharp implements before using them on the next customer, but only 56.2% of them disinfect these tools.

The current study is limited by the small sample size, and the inability to use more sensitive and specific diagnostic methods like nucleic acid amplification techniques (NAATs) due to a lack of resources.

Conclusion

The study specifically sought to estimate the prevalence of HBV and HCV among barbers and suggested a high prevalence. Previous exposure to body fluid minor injuries with contaminated equipment, and lack of knowledge about the modes of transmission might be the source of infection in those barbers. Transmission of HBV and HCV among barbers is an underestimated route of infection due to the lack of knowledge, so more attention is needed than is currently offered and it should be mandatory to screen every barber for HBV and HCV annually.

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Molecular Detection of *oprD* resistant Gene among Carbapenem Resistant *Pseudomonas aeruginosa* Clinical Isolates, Khartoum State-Sudan

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Abstract

Background

Carbapenems are the most important therapeutic options that effect against serious infections caused by multidrug resistant *Pseudomonas aeruginosa* isolates; however recently carbapenems resistant isolates of *P. aeruginosa* are increasing worldwide. It is often due to the impermeability that arises via the loss of the OprD porin, the overproduction of an active efflux pump system, and the production of carbapenem-hydrolyzing β -lactamase

Objectives: This study aimed to detect *oprD* gene among carbapenem resistance *P. aeruginosa* clinical isolates using conventional PCR technique during the period from September 2018 to August 2021.

Methods: Hundred carbapenem resistance *Pseudomonas aeruginosa* clinical isolates were collected from different specimens from Soba Hospital, Fedail Hospital and Military Hospital for this study in Khartoum State, Sudan. All isolates were re-identified using conventional bacteriological techniques and their susceptibility to carbapenem was tested using Kirby-Bauer method for confirmation. All isolates were investigated for the presence of *oprD* gene using polymerase chain reaction (PCR).

Results: Of the 100 clinical isolates, the *oprD* gene expressed in 10 (10%) isolates and it was not detected among the remainder (90%), the higher percentage was found among the isolates from wound swabs 5 (50%), whereas it was not detected among the isolates from high vaginal swabs, nibble discharge and sputum samples (P value = 0.02).

Conclusions: The *oprD* gene was not detected among the vast majority of isolates such findings can supports the idea that the basic mechanism of carbapenem resistance could be via the loss of *oprD* porine gene.

Key words: Carbapenem resistance, *Pseudomonas aeruginosa*, OprD porin, Molecular technique, Portal of entry.

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Introduction

Pseudomonas aeruginosa is a motile, non-fermenting, gram negative opportunistic bacterium which it responsible for many different infections in patients with compromised host defenses (e.g., cancer, burn, HIV, and cystic fibrosis) [1]. Intensive care unit (ICU) hospitalized patients constitute one of the risk

groups that are more susceptible to acquire pseudomonas infections as they may develop ventilator-associated pneumonia (VAP) and sepsis [2–4]. Moreover, increasing emergence of multidrug-resistant *P. aeruginosa* (MDR PA) isolates during therapy is critical problem which is reported in 27%– 72% of patients with initially susceptible *P. aeruginosa*, leading to increased

health care costs and prolonged hospitalization [5]. Furthermore virulent *P.aeruginosa* is frequently life-threatening and often challenging to treat because it expresses a combination of bacteria-associated factors (intrinsic and acquired antimicrobial resistance, expression of different virulence factors [5].

It has become increasingly clear that resistance development in *P. aeruginosa* is multi- factorial, with mutations in genes encoding porins, efflux pumps, penicillin-binding proteins, and chromosomal β -lactamase, all contributing to resistance to β -lactam, carbapenems, aminoglycosides and fluoroquinolones [6].

Carbapenem antibiotics are recognized as one of the most potent agents with activity against *Pseudomonas aeruginosa* and remain important for the therapy of serious infections due to these pathogens. The mortality associated with carbapenem resistance *P.aeruginosa* infections is high (20% to 50%),making optimal and timely treatment is essential [7].

Recently, however, the strains with resistance to carbapenems have been increasing among clinical isolates of *P. aeruginosa* resistance to carbapenems in *Pseudomonas aeruginosa* is often due to impermeability that arises via the loss of OprD porin, the over production of an active efflux pump system, and the production of carbapenem-hydrolyzing β -lactamase [8].

The *oprD*-coding gene was mapped between 71 and 75 min on the *P. aeruginosa* PAO1 chromosome, and the OprD protein was shown to be coded by 1332-bp nucleotides [9,10]. Outer membrane protein OprD is considered the

preferred portal of entry for carbapenems and similar drugs such as imipenem and meropenem also enter the cell via OprD . Any loss of OprD expression from the outer membrane significantly decreases the susceptibility of *P. aeruginosa* to carbapenems and has been shown to play a major role in the acquired resistance to imipenem and, to a lesser extent, meropenem [11]. Terzi *et al.* (2015) suggested that loss of OprD might be the first antibiotic resistance mechanism induced by selective pressure of carbapenem [11]. So, this study aimed to detect the presence of *oprD* gene encoding carbapenem resistant *pseudomonas aeruginosa* by using molecular technique.

Materials and methods

Study Design and setting

A descriptive cross sectional study was conducted in different Khartoum State hospitals, (Omdurman Teaching Hospital, Fedail Hospital and Soba Teaching Hospital) during the period from November 2018 to November 2019. Hundred (100) Carbapenem (Meropenem and Imipenem) resistant *Pseudomonas aeruginosa* clinical isolates were obtained from different specimens including: wounds, urine, sputum, ear swabs, high vaginal swabs and nibble discharge were included in this study.

Phenotypic methods

The fresh cultured colonies of the clinical isolates were sub-cultured on nutrient agar and MacConkey agar media to ensure purity and optimum growth. Then they were re-identified using routine culture techniques and their susceptibility to carbapenems were tested using Kirby-Bauer method for confirmation.

DNA extraction:

Bacterial DNA was extracted by using boiling method. All bacterial strains were sub cultured on nutrient agar and the overnight pure bacterial growth were used. Three colonies were emulsified in Eppendorf tubes containing 200µl of distilled water and boiled at 100 °C for 30 minutes then centrifuged at 12000 rpm for 15 minutes. The supernatant containing DNA was transferred to new Eppendorf tubes and stored at - 20°C [12].

PCR amplification procedure

PCR was carried out for all isolates by using specific primer for *oprD* gene F5`GGGGTGTTTTTCGCAGAGTA3`, R5`ATCACAGTAAGAGGGGCC3`, 1780 bp, amplification was performed at final volume 25 µl (5µl Master mix, 5µl from DNA, 2µl primer and 13µl D.W) [13].

The PCR cycling conditions were as follows: 2 min of denaturation at 98 °C and 30 cycles of denaturation at 95°C for 30 sec, annealing at 55°C, for 30 sec, and extension at 72°C for 3 min. PCR Thermocycler machine (Promega, German) was used [13].

PCR products was separated on 1.5% agarose gel by electrophoresis to detect specific amplified product by comparing with standard molecular weight marker 100 base pair (DNA ladder). 5µl of PCR product was loaded into the well. Electrophoresis was carried out at 100 Volts, 60 AMP for 30 minutes. The amplified products of the study samples were visualized by Trans-illuminator Photographed by a digital camera

(German) and transferred to computer data for labeling and storage.

Statistical analysis

Data was analyzed using SPSS 21. Frequencies and percent were obtained for frequency tables, Chi-squared test was used for goodness of fit. The relationships between variables tested were obtained using cross tables and Chi-squared (Fisher exact) test for independence. P-value ≤0.05 was considered as significant.

Ethical consideration

The approval and informed consent were obtained from Al-Neelain University Ethical committee and informed consent was taken from the hospitals included in this study.

Results

Hundred (100) carbapenem resistant *P.aeruginosa* clinical isolates were collected from different hospitals in Khartoum State. They had been isolated from different clinical samples as represented in Table (1). Their identification was confirmed using conventional bacteriological techniques and their resistance to carbapenem (imipenem and meropenem) was confirmed using Kirby-Bauer method.

Based on conventional PCR results (Figure 1), 10 out of 100 (10.0%) carbapenem resistant *P. aeruginosa* clinical isolates were positive for *oprD* gene as in Figure (2)

The frequency of *oprD* gene among the clinical isolates according to type of specimens was represented in Table (2) (P value= 0.02).

Table (1): Distribution of *Pseudomonas aeruginosa* clinical isolates according to type of specimens

Specimens	Frequency (%)
Wound swab	56 (56.0)
Urine	23 (23.0)
High vaginal swab	11 (11.0)
Sputum	6 (6.0)
Ear swab	3 (3.0)
Nibble discharge	1 (1.0)
Total	100 (100.0%)

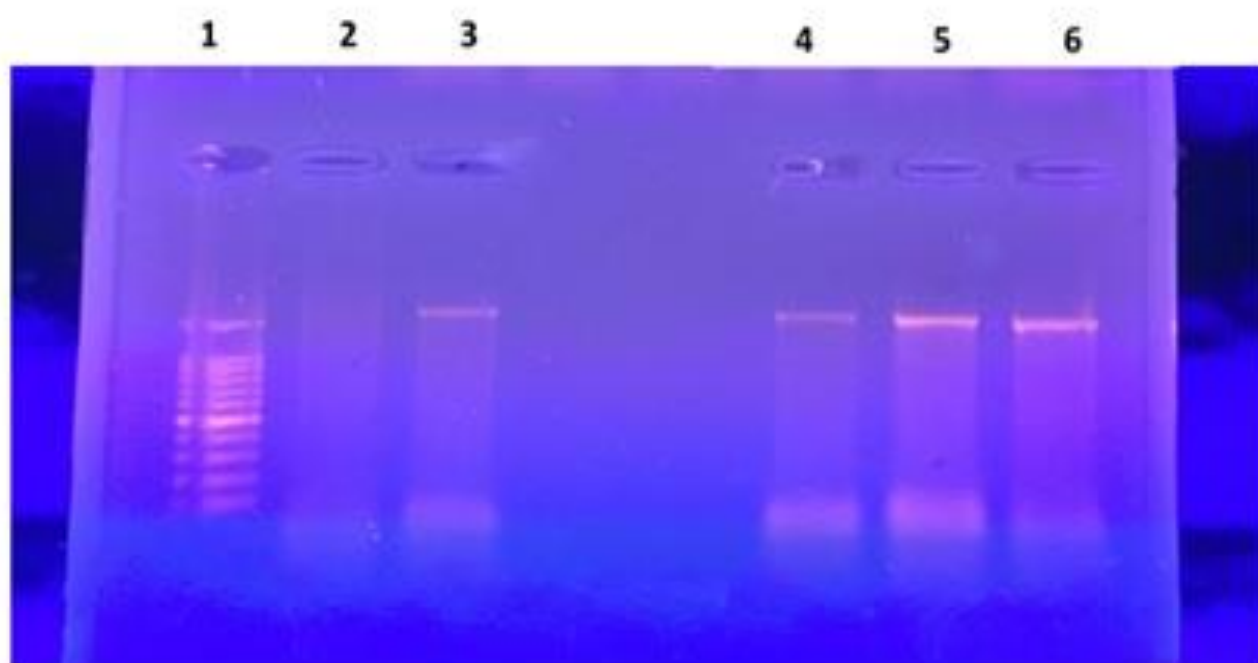


Figure 1 Agarose gel showing the results of amplified *oprD*. Lane: 1 ladder 100 bp, lanes (3, 4, 5, 6) positive *oprD* gene. Length 1780b

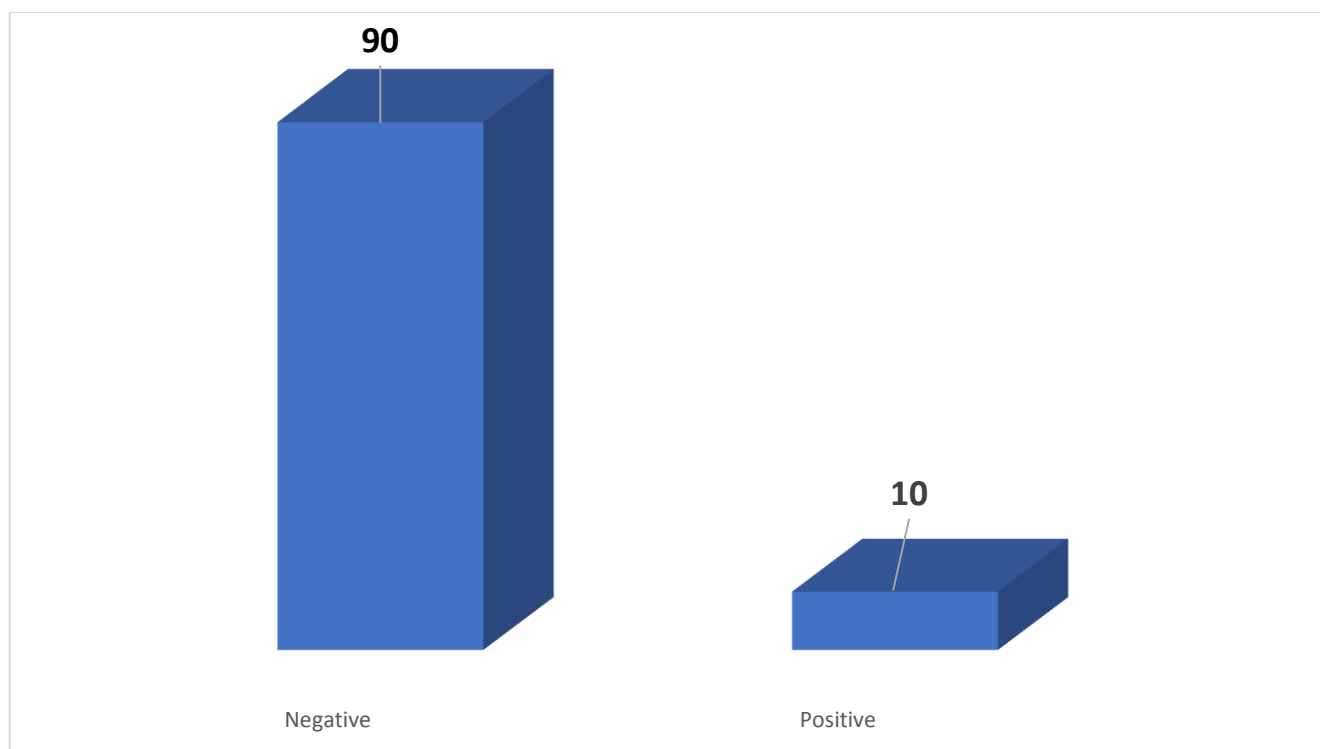


Figure 2: Percentage distribution of *oprD* gene among *P.aeruginosa* clinical isolates

Table 2: Frequency of *oprD* gene among *P. aeruginosa* clinical isolates according to type of specimens

Type of sample	Result	
	Positive (No %)	Negative (No %)
Wound swabs	5 (9.8)	51 (91.1)
Urine	3 (13.0)	20 (86.9)
High vaginal swabs	0 (0.0)	11 (100)
Sputum	0 (0.0)	6 (100)
Ear swabs	2 (66.7)	1 (33.0)
Nibble discharge	0 (0.0)	1 (100)
P value= 0.02		

Discussion

Due to the increase in multi-drug resistant *P. aeruginosa* infections, a greater emphasis has been placed on identifying genetic characteristics underlying bacterial resistance and the clinical implications of these mutations [14].

In the present study it was found that among the total (n= 100) clinical isolates, the most common isolates were from wound swab specimens this is may be due that *Pseudomonas aeruginosa* is most commonly associated with wound infections [15].

Outer membrane protein OprD is considered the preferred portal of entry for carbapenems and similar drugs such as imipenem and meropenem also enter the cell via OprD [16]. Any loss of OprD expression from the outer membrane significantly decreases the susceptibility of *P. aeruginosa* to carbapenems and has been shown to play a major role in the acquired resistance to imipenem and, to a lesser extent, meropenem [17]. Our study showed that *oprD* expression was negative in the majority of *P. aeruginosa* isolates (90%) and it may be played a significant role in their carbapenem resistance, similar study done by Fournier *et al.* (2013) in French public hospital, they detected loss of *OprD*, as a result of mutations or gene disruptions in 94 out of 109 (86.2%) imipenem-resistant *P. aeruginosa* isolates [18]. Also, other study in Bulgarian hospitals showed that OprD expression was decreased in the vast majority of 29 multi-drug resistant *P. aeruginosa* isolates (97%) and played a significant role in their carbapenem resistance

[19]. Other study done by Sanbongi *et al.*, (2009) in Japan that studied a total of 99 clinical isolates of metallo- β -lactamase-negative *Pseudomonas aeruginosa* for their susceptibilities to carbapenem agents and corresponding *oprD* gene mutations. The OprD sequence of each strain was grouped into two major classes, based on the pattern of alterations. Eighty strains (80.8%) were so-called 'full length type', whose OprD proteins were fully encoded. The remaining 19 strains (19.2%) were so-called 'defective type', which possessed deletions or major alterations that might cause conformational changes in the OprD porin protein. The changes in 'defective type' strains led to 15-, 17- and 23-fold increases in the geometric mean MIC for imipenem, meropenem and biapenem compared with 'full length type' strains, respectively [20].

The present study found that the frequency of *oprD* gene was significantly associated with the type of samples (P value=0.02), the *oprD* gene was not detected among 91.1% of isolates from wound specimens and completely did not express among high vaginal swabs (n=56), sputum (n=6) and nibble discharge (n=1). This indicates that these specimens may harbor isolates with OprD protein deficient which may conferred carbapenem resistance to them.

Conclusion

The present study concluded that the vast majority of isolates did not show the presence of *oprD* gene which it reflects the absence or deletion of OprD protein among these isolates. And this is may be

considered the main mechanism of carbapenem resistant among these clinical isolates.

Conflicts of interest

The authors declare no conflicts of interest.

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***Chlamydia trachomatis* IgM antibodies among women suffering infertility in Gezira State, Sudan**

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ABSTRACT

Background: Although the symptoms of genital chlamydia infection vary from symptomatic to asymptomatic, it has a profound impact on the female reproductive system causing ascending infections which may lead to infertility. We aimed to detect the genital chlamydia infection and common associated symptoms in both primary and secondary infertility cases.

Methods: One hundred and sixty two Sudanese women suffering infertility were recruited to this study. Blood samples were collected from each woman, and then the separated serum was tested for the presence of anti-*chlamydia trachomatis* IgM using ELISA technique. A designed questionnaire was used to collect demographic and clinical presentation.

Results: The percentage of chlamydia infection was 48.6% among women suffering primary infertility and 81.8% among those suffering secondary infertility with significant differences between the two groups (p value 0.00). Women with secondary infertility exhibited significantly more common chlamydia symptoms than those with primary infertility. Chlamydia infection was significantly associated with the age, burning in urination and urethritis (p value <0.05).

Conclusions: Secondary infertile women affected by chlamydia infection and symptoms more than women those have primary infertility. Screening for chlamydia infection is mandatory.

Keywords: *Chlamydia trachomatis*, Primary Infertility, Secondary Infertility, Symptoms

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Introduction

Genital chlamydia infection is a sexually transmitted disease (STD) of public health importance (1-3). Although asymptomatic *Chlamydia trachomatis* infection are quite common occurring in up to 70% of infected women, the symptomatic infection often cause less severe symptoms than other sexually

transmitted diseases. These may allow the infection to go unnoticed with minimal patient awareness until secondary or tertiary symptoms develop. The sequelae of undetected and untreated chlamydia infection such as urethritis, cervicitis, prostatitis or epididymitis may lead not only to significant morbidity but far more,

importantly ectopic pregnancy, tubal problems and then infertility(1, 4).

Infertility is a medical problem affects more than 80 million people worldwide. Large proportion of the developed countries has no access to medical diagnosis and treatment for infertility, and even in Sudan (5). The term primary infertility used for a couple who have never achieved pregnancy, the secondary infertility used for a couple who have previous successful pregnancy even if it ended in abortion. Reports represent primary infertility as a common than secondary infertility in Gezira state and the most common etiology was high prolactin level and tubal factor respectively(5, 6).

Infertility due to *chlamydia trachomatis* represents a preventable type of infertility, if detected early. However, *C.trachomatis* infection is not a notifiable disease in Sudan and data pertaining to infertility attributed to *chlamydia trachomatis* infection is very limited particularly in Wad Madani City, thus preventing the policies from being formulated regarding screening of patients.(2)

Diagnosis, treatment and prevention of sexually transmitted Chlamydia infection has become an important public health priority (7), especially when strong evidence linked this infection with clinical signs and symptoms . Few reports are available on the prevalence of current *C.trachomatis* infection in women with different clinical complications like urethritis and vaginal discharge; however, there is less reports in Sudan on its prevalence and association with infertility

or related clinical symptoms. Evaluation of present infection with the aforesaid types of manifestations could help in treatment.

The aim of this study was to detect the rate of chlamydia infection in both primary and secondary infertile women, also accordingly the most common clinical symptoms as an attempt for more clinical identification of the infection.

Methods

Study design and setting: From January to June 2021, 162 women suffering infertility attending Wad Medani Maternity Hospital were selected to detect anti-*chlamydia trachomatis* IgM in their serum samples using ELISA. Permission to carry out this study was granted from the Ministry of Health, Gezira State. The written consent was obtained from study participants after they had been informed about the study objectives.

Participants and selection criteria: The study included married women, suffering infertility selected during the routine clinic of the Hospital. The subjects were grouped according to infertility type into: primary infertile group (n= 74) those failed to get pregnant for at least one year of marriage, and the secondary infertile group (n= 88) those failed to get pregnant for at least five years after a history of abortion and/or successful pregnancy before. Pregnant women were excluded from this study.

Data collection and analysis: A designed questionnaire was used to collect data including demographic characteristics and relevant clinical symptoms. Collected data was analyzed using Statistical Package for the Social Science (SPSS)

software version 20.0 (IBM, USA). Descriptive statistics such as frequency tables, cross-tabulation, and chi test were used. The mean and standard deviation was also used for age. The level of confidence was at 95% and P value <0.05 was considered significant.

Clinical data was collected according to standard guidelines, from each participant using a sterile syringe five ml of venous blood were collected into a plain tube. Then the serum was separated in another tube after centrifugation of the blood sample. The serum samples were analyzed using ELISA technique to detect the presence of anti-*chlamydia trachomatis* IgM antibodies.

Enzyme Linked Immune-sorbent Assay: The ELISA kit from SERION ELISA classic *Chlamydia trachomatis* IgM Best-Nr: ESR 1372M company was used. When a participant's serum added to the microtitration plate, the fixed antibodies were bind to make an immune complex. After the incubation time the conjugate solution containing the enzyme and the secondary antibody was added. After that, the colourless substrate (p-nitrophenylphosphate) was added, and then the enzyme interacted with it to form a yellow colour compound. Finally, the signal intensity was measured photometrically using 405 nm filter. Steps and the cut off value were followed according to instructions by the kit sheet.

Results

The study included 162 participants, their age ranged between 17 and 46 years, with mean ages

of 33.9 ± 7.05 and 34 ± 7.08 for primary infertile women and secondary infertile women respectively. Almost half (54.1%) of the primary infertility women and quarter (27.3%) of the secondary infertility women were between 21-40 years of age. The majority of both are housewives residing in Wad Madani City with varying educational levels and socioeconomic status as shown in table 1.

Out of 162 participants 66.7% of them were suffering Chlamydia infection as the anti-*chlamydia trachomatis* IgM antibodies were detected in their blood

Using ELISA technology, the anti-chlamydia IgM antibodies were detected in 66.7% of study participants. According to study groups, 48.6% of women with primary infertility and 81.8% of women with secondary infertility with significant differences between the two groups (p value 0.00) as shown in table 2. The associated symptoms studied among these women were lower abdominal pain, urethritis, burning during urination, and vaginal discharge. Almost (90%) of the secondary infertile women suffer from these Chlamydia symptoms more than the primary infertile women. The risk factors associated with chlamydia infection were the age (p value 0.041), burning in urination (p value 0.036) and urethritis (p value 0.004) as shown in table3.

Statistical analysis showed significant differences between primary and secondary infertility groups.

Table 1. Demographic characteristics and common symptoms of study participants

Characteristics		Primary Infertility (n= 74) N (%)	Secondary Infertility (n= 88) N (%)
Age Group/ysr	0-20	4 (5.4)	0 (0)
	21-30	20 (27)	32 (36.4)
	31-40	40 (54.1)	24 (27.3)
	41-50	10 (13.5)	32 (35.4)
Occupation	Employees	36 (48.6)	32 (36.4)
	House Women	38 (51.4)	56 (63.6)
Residence	Wad Medani City	62 (83.8)	56 (63.6)
	Out Side Wad Medani City	12 (16.2)	32 (36.4)
Educational Level	High	26 (35.1)	16 (18.2)
	Medium	26 (35.1)	40 (45.5)
	Low	22 (29.7)	32 (36.4)
Socioeconomic Status	High	2 (2.7)	0 (0)
	Moderate	52 (70.3)	64 (72.7)
	Low	20 (27)	24 (27.3)
Common Symptoms	Lower abdominal pain	30 (31.9)	64 (68.1)
	Burning in urination	24 (27.3)	64 (72.7)
	Vaginal discharge	24 (27.3)	64 (68.1)
	Urethritis	26 (28.9)	64 (71.1)

Table 2. Frequencies and percentages of ELISA positive and negative samples case groups.

Test Cases		Primary infertility group N (%)	Secondary infertility Group N (%)	Total	P-value
ELISA (IgM)	Positive	36 (48.6)	72 (81.8)	108 (66.7)	0.00
	Negative	38 (51.4)	16 (18.2)	54 (33.3)	
Total		74 (100)	88 (100)	162 (100)	

Table 3. Risk factors associated with Chlamydia infection in study participants

Parameters		ELISA		OR (95% CI)	P-value
		Positive (n= 108)	Negative (n= 54)		
Age Group/ years	0-20	2 (1.9)	2 (3.7)	0.24 (0.03-1.90)	0.041
	21-30	42 (38.9)	10 (18.5)	3.27 (1.40-7.63)	
	31-40	36 (33.3)	28 (51.9)	0.65 (0.29-1.44)	
	41-50	28 (25.9)	14 (25.9)	0.64 (0.29-1.44)	
Lower Abdominal Pain	Yes	94 (87.0)	44 (81.5)	1.5 (0.63-3.70)	0.348
	No	14 (13.0)	10 (18.5)		
Burning in Urination	Yes	88 (81.5)	36 (66.7)	2.20 (1.04-4.64)	0.036
	No	20 (18.5)	18 (33.3)		
Vaginal Discharge	Yes	88 (81.5)	38 (70.4)	1.90 (0.87-3.96)	0.109
	No	20 (18.5)	16 (29.6)		
Urethritis	Yes	90 (83.3)	34 (63.0)	2.94 (1.39-6.22)	0.004
	No	18 (16.7)	20 (37.0)		

OR; odd ratio, CI; confidence interval

Discussion

Infertility is becoming an emerging health problem in many countries of the world (8), including Sudan. A WHO study reported the current chlamydia infection in infertile women 18-20 percent is high (2). When compared with other published studies (2-4, 9), the prevalence of *C.trachomatis* infection in our study is also high. This alarming finding should alert doctors and point out the importance to include the screening of chlamydia infections in the diagnostic workup for infertility. Screening for *C.trachomatis* in high risk populations is recommended by the Center for Disease Control and Prevention (CDC) (4). Early detection and treatment can prevent serious long-term sequelae of *C.trachomatis* infection including infertility.

Although the increase of primary infertility cases than secondary infertility cases in last previous report by Osman in Sudan (5), at this study, the *C.trachomatis* infection was more common in women with secondary infertility than primary infertility with significant statistical differences between them. Women with secondary infertility were more likely to be screened positive for chlamydia has been reported earlier by others in Nigeria, India, china, Turkey and Sweden (2, 3, 10-13). This higher rate could be due to the presence of tubal factor as the most common etiology among them and also their longer period of active sexual life thus enhancing their exposure and also susceptibility to chlamydia infection.

There was a statistical significant correlation between age and chlamydia infection in women

included in this study. The age distributions of study participants with *C.trachomatis* infection revealed a high proportion of women were in the group older than 20 years of age. With the exception of Japan (14), India (15) and New Zealand (16), where the rates were higher in women younger than 20 years old, other reports from Denmark, Australia, Nigeria and Sweden is similar and in harmony with this study showed that increasing chlamydia infection rates were associated with increasing age (3, 16). Our result supports other previous studies in Sudan, a study conducted by Mohammed at 2012 and another study conducted by Omer at 2020 whose documented highest rates of chlamydia infection in older age (8, 17). The reasons of why old adults are more susceptible for genital chlamydia infection than young adult in Sudan may be associated with certain aspects of social factors including the later sexual intercourse experience with optimum age of marriage usually above 20 years old. In addition, this infection was mostly asymptomatic; women only came to this hospital usually after they had married and/or developed complications such as symptoms of lower genital tract infection, repeated pregnancy loss, and had infertility at later age; thus, present observation revealed an increasing rate of infection with age. Ninety percentages of women those with secondary infertility suffer from chlamydia symptoms more than those with primary infertility as they exhibit high rate of infection. The classical symptoms of chlamydia infection are dysuria (burning in urination), urethritis,

abnormal vaginal discharge and lower abdominal pain (13). Urethritis is a condition in which the urethra, or the tube that carries urine from the bladder to outside the body, becomes inflamed and irritated and usually accompanied with the burning sensation during urination, it typically causes pain while urinating and increased urge to urinate. Urethritis is generally defined as > 4 PMNL per high power field (HPF) (13). In the current study, in the context of the routine urine general analysis at this hospital, smears with > 10 PMNL per HPF were considered as urethritis (15), however, urethritis was observed in 83.3% (90/108) of positive cases with chlamydia suggesting a significant association between urethritis and chlamydia infection. Also this was in line with Falk findings in Sweden, when he recorded that the chlamydia infection is strongly statistically associated with urethritis, when he observed 71% of infected women with chlamydia suffering urethritis versus 40% of women free of chlamydia infection (13).

The vaginal discharge has no significant association with chlamydia infection among study women. This finding was inconsistent with most of the *C.trachomatis* related studies (2, 4, 10) and this is explained by that the vaginal discharge among study participants may be associated commonly with other conditions than chlamydia infection, as it may be associated with normal physiologic occurrence or a pathological manifestation such as psychosocial factors or vaginal or cervical infections. It is often challenging to distinguish abnormal from normal

discharge, both for the patient's and the health care provider's perspective. (18). In the majority of settings here in Sudan, abnormal vaginal discharge is highly indicative of a vaginal infection, thus all women presenting with vaginal discharge receive treatment for *T.vaginalis*, Bacterial vaginosis and *Candida spp.*

While the majority of women are asymptomatic, the lower abdominal pain considered as one of the classical symptoms of genital chlamydia infection and the leading cause to pelvic inflammatory disease (19). The latter is not easy to be diagnose, and the minimum criteria for diagnosis includes but not limited to the presence of the lower abdominal pain, thus, those women need better advice on when to seek early medical attention to avoid risk of reproductive damage. For this study, the lower abdominal pain was observed in 87% and 81% of infected women and non-infected women for chlamydia respectively. Several previous studies reported different rates of chlamydia among those present with lower abdominal pain (8,20). Moreover, it may be associated with other infection such as *N.gonorrhoea* (20)

Prevalence of *C.trachomatis* varies considerable cross regions, subject population under study and the sensitivity of the laboratory method used. Our study suggests that all infertile women should be screened for *C.trachomatis* especially those suffering the secondary infertility. In the absence of requisite infrastructure and skills for culture, ELISA can play a significant role in screening for *C.trachomatis* high risk women. Studies with

larger sample size should further elucidate the extent of infertility caused by *C.trachomatis* in Sudan.

In conclusion, the chlamydia affects 66.7 % of infertile women, and the secondary infertile women suffer more than the primary infertile women in concerning the significant differences between them. Hence, we highly recommend active screening for *C.trachomatis* infection in women above 20 years of age irrespective of clinical presentation. Moreover, *C.trachomatis* infection should become one of the mandatory notifiable diseases in Wad Madani City.

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Effects of Hemodialysis on Serum Copper and Zinc Levels in Sudanese Patients with Chronic Kidney Disease

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Abstract

Background: Trace elements are essential nutrients with a gamut of functions in human being. Hemodialysis patients are at risk for deficiency of essential trace elements and excess of toxic trace elements, both of which can affect health. Alterations in trace element levels in patients receiving long-term renal replacement therapy have been implicated in immune dysfunction and increased oxidative stress.

Patients and Methods: This was a case control study conducted at Gezira Hospital for Renal Disease and Surgery, Wad Medani, Gezira State, from July 2020 to May 2021 was aimed to evaluate the effect of hemodialysis on serum copper and zinc levels in patients suffering from chronic kidney disease. A total of 90 individuals were included in this study which divided into two groups; 60 healthy person as control group and 30 patients with chronic kidney disease as case group. Blood samples were collected, one time from control group and two times from case group at pre and post dialysis. Serum copper and zinc were estimated with atomic absorption spectroscopy. Data were collected by questionnaire and analyzed by statistical package for social sciences version (21) and medical calculator.

Results: it showed that 66.7% of patients were males and 33.3% were females, 43.3% of patients age from 23-40 year, 40% from 41-57 year and 16.7% from 58-75year, 3.3% patient were underweight, 33.3% were with normal weight, 30% overweight and 33.3% obese. The family history showed that 13.3% had family history and 86.7% had no family history. Duration of disease showed that 60% were less than 5 years and 40% more than 5 years. The history of chronic disease found that 76.7% of patients were hypertensive and 26.7% were diabetic. There is no significant deference in serum zinc levels between pre hemodialysis and control group (p. value 0.656), while, a significantly decrease levels observed after hemodialysis when compared to control group and pre hemodialysis (p. value 0.014 and 0.000). Serum copper levels were significantly decreased in pre hemodialysis and post hemodialysis when compared to control group with P. value (0.000), also serum copper levels were significantly decreased in post hemodialysis when compared with pre hemodialysis with P. value (0.000). Serum copper and zinc levels had weak correlations with age, gender, BMI and duration of hemodialysis, whereas, the level of serum zinc after hemodialysis had strong positive correlation with their levels before dialysis (Person correlation: 0.727 and P. value 0.000). Homolateral, there is strong positive correlation in copper levels between post and pre dialysis (Person correlation: 0.716 and P. value 0.000).

Conclusion: Hemodialysis has an effect on serum copper and zinc levels by decreasing their levels in serum, thus, regular monitoring of serum copper and zinc levels in hemodialysis patients and copper - zinc supplements are recommended after hemodialysis.

Key words: Hemodialysis, Copper, Zinc, Trace Element, Sudanese, Renal disease.

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Introduction:

Kidney function declines slowly and steadily in chronic renal failure. It is typically a side effect of another serious medical illness, and it is currently one of the most significant health issues in the world (1). Early screening is essential to stop the onset of end-stage renal failure because the mortality rates in individuals with chronic renal failure are high both before and after the start of renal replacement treatment (2). Chronic renal failure (CRF) is a slowly progressing kidney illness that affects the kidneys over months and years (3). Low rates of glomerular filtration are its defining feature for CRF (4). CRF is a sign of a severe illness that requires specialized replacement therapy for the renal system, such as dialysis, to address (5). CRF has a 1 in 5000 incidence rate and primarily affects middle-aged and elderly individuals. It is largely irreversible and may eventually result in renal failure (6). Removal of waste materials and fluids from the bloodstream and disposal through the urine pathway are key components of the kidney's physiological function (7). Dialysis, which involves removing excessive toxic fluids and metabolic waste products from the body, is one form of treatment for patients who have unexpectedly lost their renal function or who have reached end-stage renal stage (8). To better understand how dialysis affects CRF patients, it is crucial to compare the mean values of serum renal biochemical indicators before and after dialysis. A trace element is an element in a sample with an average concentration of fewer

than 100 parts per million, or less than 100 micrograms per gram, as measured in atomic count (9). A trace element is a dietary mineral in biochemistry that is required in very small amounts for the appropriate physiology, growth, and development of the organism. In biology, a trace element, sometimes known as a micronutrient, is any chemical element needed by living things in very small amounts, typically as a component of an essential enzyme or a catalytic protein made by the cell (10). Despite being strong and relatively soft, copper (Cu) has outstanding electrical and heat transmitting qualities. Both in its elemental form and in compounds, copper are widely present in the natural world. Tin, nickel, and copper combine to make the alloy cupronickel, which is commonly used in coinage (11). Copper is a mineral that is necessary for several processes. Even though copper insufficiency is uncommon in the majority of people, people with end-stage renal disease (ESRD) are more likely to develop copper deficit than people without ESRD (12). First, those on HD have limited access to dietary copper. Second, antacid or alkali therapy may result in impaired copper absorption and/or copper insufficiency. The consumption of mineral supplements is a third factor that could have an impact on HD patients' copper status (13). The importance of zinc in human nutrition has been clearly shown by the identification of numerous clinical conditions that are related to zinc (14). It is the second-most prevalent trace element in the body after iron (15). Some

common uremic symptoms, such as immunological dysfunction, impairments of taste and smell, growth retardation in children, and male testicular shrinkage, have been linked to zinc deficiency (16). The objective of present study was to evaluate the effect of hemodialysis on serum copper and zinc levels in patients suffering from chronic kidney disease.

Patients and Methods:

The study was analytical case control hospital based study carried out in Gezira hospital for renal disease and surgery, Wad Madani city, Gezira State during the period from July 2020 to May 2021. Three ml of venous blood samples were collected in plain containers from healthy individuals as control and two times from patients with chronic kidney at pre and post dialysis. Ninety subjects; 30 patients with hemodialysis (sample pre-dialysis and second sample post-dialysis) and other 60 healthy subjects as control groups were enrolled in this study. Chronic kidney disease patients under indapamide and amlodipine treatment or with recently blood transfusion and patients receiving supplements containing zinc or copper were excluded from this study. The blood samples were collected randomly in the plain containers from study population after taking verbal consent, then the samples were mixed and centrifuged for 3 minutes. The plasma was transferred into new plain containers. Data were collected using structured interviewing questionnaire that include many variable such as age, gender, BMI, and duration of disease. Serum

zinc and copper were estimated with buck model 210 VGP atomic absorption spectroscopy. Serum copper normal value from 0.7 to 1.4 mg/L, and serum zinc normal value from 0.5 to 1.2 mg/L. Ethical consideration was include permission to conduct this study was obtained from Ministry of health and health insurance, research approval was obtained from research board faculty of medical laboratory sciences, University of Gezira, and written consent from each patient had been taken. The precision and accuracy of the methods used in this study were checked each time by using control material. The data of this study were analyzed using the statistical package for social study (SPSS) software (version21). T-test presented as (Mean $\pm$ SD) and correlation test (R value). A P-value of less than 0.05 was considered statistically significant.

Results:

A total of 90 individuals were included in this study when 60 healthy people as control group and 30 patients with chronic kidney disease as case group which two samples were collected at pre and post dialysis. Results showed that 66.7% of patients were males, 43.3% of patient's age from 23-40year, and 63.3% overweight and obese. The family history of renal disease showed that 13.3% had family history and 86.7% had no family history. Duration of the disease showed that 60% were less than 5 years and 40% more than 5 years. The history of chronic disease found that 76.7% of patients were hypertensive and 26.7% were diabetic.

Table 1: Distribution of chronic renal disease according to demographic data:

Risk Factors		Number	Percent %
Age	23 – 40 Year	13	43
	41 – 57 Year	12	40
	58 – 75 Year	5	17
Sex	Male	20	67
	Female	10	33
Obesity	Underweight	1	3.3
	Normal Weight	10	33.3
	Obese	10	33.3
	Overweight	9	30
Hypertension	Hypertensive	23	77
	Normotensive	7	23
Diabetes Mellitus	Diabetic	8	27
	Non-Diabetic	22	73
Disease Duration	Less than or equal 5 year	18	60
	More than 5 years	12	40
Family History of renal disease	Yes	4	13
	No	26	87
Total		30	100

Table 2: Means of serum copper and zinc levels in pre and post dialysis:

Trace elements		N	Mean	STD	P. Value
Copper	Pre	30	0.29	0.08	0.000
	Post	30	0.23	0.07	
Zinc	Pre	30	0.50	0.21	0.000
	Post	30	0.35	0.15	

Table 3: Comparison of serum copper and zinc levels between control group and cases (pre and post dialysis):

Trace elements		N	Mean	STD	P. Value
Zinc	Pre	30	0.50	0.21	0.656
	Control	60	0.47	0.37	
	Post	30	0.35	0.15	0.014
	Control	60	0.47	0.37	
Copper	Pre	30	0.29	0.08	0.000
	Control	60	1.13	0.45	
	Post	30	0.23	0.07	0.000
	Control	60	1.13	0.45	

Table 4: Correlation between serum zinc with age, gender, BMI and duration of dialysis:

Zinc	Correlation coefficient r	P. Value	95% Confidence interval for r
Age	-0.32	0.083	-0.61 to 0.043
BMI	0.03	0.884	-0.34 to 0.38
Duration	-0.01	0.952	-0.37 to 0.35

Table 5: Correlation between serum copper with age, gender, BMI and duration of dialysis:

Copper	Correlation coefficient r	P. Value	95% Confidence interval for r
Age	-0.16	0.403	-0.49 to 0.21
BMI	0.11	0.555	-0.26 to 0.45
Duration	-0.16	0.402	-0.49 to 0.21

Table 6: Correlations between serum zinc and serum copper levels in pre and post dialysis:

		ZnPre	ZnPost	CoPre	CoPost
ZnPre	Pearson Correlation	1	0.727	0.284	0.113
	P. value)	-	0.000	0.128	0.551
ZnPost	Pearson Correlation	0.727	1	0.101	-0.065
	P. value)	0.000	-	0.595	0.733
CoPre	Pearson Correlation	0.284	0.101	1	0.716
	P. value)	0.128	0.595	-	0.000
CoPost	Pearson Correlation	0.113	-0.065	0.716	1
	P. value)	0.551	0.733	0.000	-
	N	30	30	30	30

Discussion:

Slowly progressive disease of the kidney function progressing over a period of months and years was called chronic renal failure which manage usually by compensation of renal function especially the waste elimination by artificial purification systems as hemodialysis (17). This case control study conducted to evaluate the effect of hemodialysis on serum copper and zinc levels in patients suffering from chronic kidney disease at Gezira Hospital for Renal Disease and

Surgery, Wad Medani, Gezira State, Sudan. This research included 90 participants: 60 healthy individuals as control group and 30 patients with chronic renal failure. The trace elements (serum copper and zinc) levels were measured before and after hemodialysis. The CRF patients were age between 23 to 75 years and most of them male and above 40 years (67%, 57%). The most risk factors were found in study patients were 77% hypertensive, 63.3 obese and overweight, while decrease a probability of some risk factors

like diabetes and family history of CRF(73% non-diabetic and 87% without family history). Majority of patients were affected recently with disease duration less than 5 years about 60%.Serum zinc levels as same in pre hemodialysis and control group without significant deference but significantly decrease after hemodialysis when compeers with control group and pre hemodialysis, this finding agree with study done by Ashraf Naged Ismael *et al.* in Egypt (2022) (18) and other study done by Rana M. Hasanato in Sudia Arabia (2014) (19) but disagree with study done in Sudan by Shehab and Amar were concluded to increased serum zinc after dialysis (20). Serum copper level was significantly decreased in CRF patients when compeer with healthy individuals, Moreover, copper level was affected by hemodialysis which significantly decreased after dialysis less than before it, this result was near to study done in Rajasthan by Ritika Gupta *et al.* (2022) (21). Serum copper and zinc levels had weak correlations with age, gender, BMI and duration of hemodialysis, this finding was agree with study done in Sudan 2018 by Fayza A Rahamtalla *et al* (22). The serum copper level in post hemodialysis had strong positive correlation with copper level in pre hemodialysis and weak positive correlation with serum zinc levels in pre and post dialysis, on the other hand, serum zinc level in post hemodialysis had strong positive correlation with their level in pre hemodialysis. These results indicated to decreasing in serum

copper and zinc was affected by their concentration before hemodialysis.

Conclusions:

Serum copper and zinc levels were decreased after hemodialysis when compared to their levels before hemodialysis and control group. The levels of serum copper and zinc at post hemodialysis had positive strong correlation with serum copper and zinc at pre hemodialysis, respectively. Regular monitoring of serum copper and zinc levels in hemodialysis patients and minerals supplement (copper, zinc) are recommended after hemodialysis.

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Effect of ethanolic extraction of *croton zambesicus* fruit on some biochemical parameters

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ABSTRACT:

This current study aimed to evaluate the *C. zambesicus* extracts were harvested in Sudan, on biochemical parameters (Glucose, total protein, albumin, triglyceride, cholesterol, urea, and creatinine, ALT, AST and ALP) and toxic effects in albino male rats compared to representing control.

Thirty five male albino rats with initial weights ranging from 100-150 g were used in this study. Ethanol 70% for 72 hours at room temperature with occasional shaking and prepared extract was used for the various tests. Rats' plasma were tested for biochemical parameters. The data were subjected to one –way analysis of variance (ANOVA).

Different groups were treated with ethanolic extract.

Glucose, T. protein, AST and ALT they were increased in experimental group B 1 while the remainders they were decreased. In experimental group C 1 Glucose, T. protein, Albumin and AST they were increased, the remainders were decreased. In experimental group D 1 Glucose, T. protein, Albumin, AST, ALT, and ALP they were increased, while the remainders they were decreased.

In experimental group B2 all parameters they were decreased, except AST it was increased. Albumin, triglycerides, creatinine, ALT and ALP in the group C2 were decreased, while Glucose, T. protein, Urea and AST were increased. Cholesterol, AST, ALT and ALP in the group D2 were increased, while Glucose, T. protein, triglycerides, urea and creatinine were decreased.

Key words: *Croton zambesicus*, T. protein, triglycerides, urea, Sudanese, Ethanolic extract.

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Introduction

Croton zambesicus Muell. Arg. (syn. *C. amabilis* Muell. Arg.; *C. gratissimus* Burch) (Euphorbiaceae) and commonly known as “Um Glegla” in Sudan. Is a plant native in the Africa including Sudan and it is one of the plants that have wide application in African folkloric medicine. The plant is used by the Sudanese local people to treat fever associated with malaria, cough, and to relieve menstrual pain (1), the GC/MS chromatogram of the

C. zambesicus essential oil revealed the presence of 58.86% monoterpenes and 23.28% sesquiterpenes. Predominant mono terpenoids were m-cymene (21.56%), linalool (7.21%) and p-mentha -1-en-8-ol (5.28%) while the predominant sesquiterpenoids were alpha-guaiene (3.55%) and C15H24 (3.23%) (1). the lack of records of previous chemical and toxicological studies of the

fruit extracts stimulated us to initiate them through the present research work

Medicinal uses of *C.zambesicus* in Traditional medicine the leaf decoction is used in Benin as anti-hypertensive and anti-microbial and in parts of Nigeria as antidiabetic and malarial remedy (2). The roots are used as antimalarial, febrifuge and anti-diabetic in Niger Delta region of Nigeria (2). The root is also used in Sudan for menstrual pain (3). The plant fruit is used by the Sudanese local people to treat fever associated with malaria, cough and to relieve menstrual pain. (1)

Pharmacological activity and bioactive compounds of Croton: Numerous research works have proven its uses of *C. zambesicus* beyond the ethno medicinal ones in experimental animals. Studies have revealed its were as cytotoxic, vasorelaxant, antiulcer genic, anti-inflammatory, antipyretic, antimicrobial, antiulcer and, anti-convulsion, plasmodial and anti-diabetic activities.

Chemical constituents of *C.zambesicus* extracts the survey of literature revealed that *C.zambesicus* is enriched with many phytochemicals like (triterpenes, steroids, essential oils, fatty acids, etc.) The literature about the *C. zambesicus* indicates a high variability in the chemical composition of the essential oils of leaves, stem bark, flowering tops, fruits within the same plant species from different collections. Usman et al., reported that *C.zambesicus* leaves of the plants growing in north central Nigeria consists mainly of limonene, gamma-terpinene, beta-pinene, neral and geranial (4). Menut et al., have investigated the leaf oil of central African grown *C.zambesicus*

(5). The principal constituent of the oil were p-cymene, linalool and beta-caryophyllene. Caryophyllene oxide, beta-caryophyllene, alpha-copanene, linalool and beta-pinene have been identified as the major constituents of Benin grown *C.zambesicus* (6). Fekam et al., investigated the leaf and root and stem bark oils of Cameroonian grown *C. zambesicus*, their report showed that the oils from the leaves and stem bark oil consists mainly of monoterpenes, while the root bark oil contained sesquiterpenes (7). The root and stem bark oils contain spathulenol and linalool as the major components, respectively. It is worth mentioning that there was a report on the analysis of *C.zambesicus* flowering tops from Sudan by Mekkawi which reported the major constituents found in the flowering tops oil are pinene, limonene, menthol, carvone, thymol, alpha-humulene and ceisnerolidol (8). The essential oil composition of *C.zambesicus* fruits is described for the first time in the work of EL-Kamali et al., (1). The *C.zambesicus* fruit contain 2-Naphthalenemethanol, decahydro-alpha (6.45%), longifolene – (V4) (6.32%), n-hexadecanoid acid (5.57%) and benzo furan 2, 3-dihydro- (5.05%) as major components in ethanolic extract. A number of diterpenoids from the stem bark of *C.zambesicus* have been reported (Ngadjui et al., Ngadjui et al., Block et al., reported that ent-trachylobane diterpene isolated from dichloromethane extract of the leaves has cytotoxic activity on Hela cells (9, 10 ,11) . Antidiabetic activity of ethanolic leaf extract of *C.zambesicus* was evaluated using alloxan-induced hyperglycemic rats (2). The root extract

has been reported to possess antimalarial and anticonvulsant and ulcer activities (2). The aqueous leaf extract of *C.zambesicus* has been reported to possess anticonvulsant activity in mice and rats (12). The root extract and its fractions exhibit antispasmodial activity (2).

Animals and experimental set-up: Thirty five male albino rats with initial weights ranging from 100-150 g were used in this study .They brought from National Center for Research (NCR), Medical and Aromatic Research Unit, Khartoum, Sudan. All animal housed and reared at the premises of the (NCR) in metal cages (stain less steel wire mesh cage) they will be maintained for one week as an acclimatization period, and maintained under controlled conditions. The temperature of the room was thermostatically controlled at 22-24°C and a 12-hour light/dark cycle was maintained.

Protocol:

Thirty –five animals were randomly divided in to seven groups with each group five rats. The seven groups of rats were subjected to the following oral treatments once a day for **14 and 28days:**

Group (A): Served as normal control was received distilled water

Group (B1): rats were received 1000mg/kg of *C. zambesicus* ethanolic extract **14 days**.

Group (C1): rats were received 500mg/kg of *C. zambesicus* ethanolic extract **14 days**.

Group (D1): rats were received 250 mg/kg of *C.zambesicus* ethanolic extract. **14 days**

Group (B2): rats were received 1000mg/kg of *C. zambesicus* ethanolic extract **28 days**.

Group (C2): rats were received 500mg/kg of *C. zambesicus* ethanolic extract. **28 days**

Group (D2): rats were received 250 mg/kg of *C.zambesicus* ethanolic extract. **28 days**

The experiment was conducted for 28 days during the experiment, readings was taken in 14 days and after 28 days. After 14 days' blood samples were collected from all rats through retro orbital puncture. Rats were anaesthetized in chloroform-saturated chamber. Animal in test and control group were sacrificed on the 28 days. Five micro liters' blood was taken from animal eyes to determine biochemical parameters. The samples were collected into two different containers. The first container was a plain bottle containing no anticoagulant with the sample used for biochemical determination whiles the second container containing. Is an automatic multi-pair blood cell counter for in vitro diagnostic use in clinical laboratory? It performs speedy and accurate analysis of blood parameters and detects the abnormal samples

The effect of ethanolic extract of *C.zambesicus* fruit on biochemical parameters after treatment of rats for 14 days At 1000 mg /kg:

The rats of Group (B1) treated with 1000 mg /kg of fruit ethanolic extract after 14 days, showed no significant change at ($P<0.05$ and $P<0.01$) in Glucose, total protein, albumin, urea, Creatinine , AST and ALP. There was significant change at ($P<0.05$) in Triglyceride and cholesterol, whereas showed significant ($P<0.05$ and $P<0.01$) in ALT .
(Table 1)

At 500 mg/kg: The rats of Group (C1) treated with 500 mg /kg of fruit ethanoic extract after 14 days,

showed significant change at ($P<0.05$ and $P<0.01$) in urea, and showed no significant change at ($P<0.05$ and $P<0.01$) in other biochemical parameters tested except in glucose, triglyceride and ALT whereas showed significant change at ($P<0.05$) to comparison with control. **(Table 2)**

At 250 mg/kg: The rats of Group (D1) treated with 250 mg /kg of fruit ethanoic extract after 14 days, showed no significant change at ($P<0.05$ and $P<0.01$) in all biochemical parameters tested relative to their respective control. **(Table 3)**

The effect of ethanoic extract of *C.zambesicus* fruit on biochemical parameters after 28 days at 1000 mg/kg: Treatment of rats Group (B2) with 1000 mg/kg of fruit ethanoic extract after 28 days, did not produce any significant change at ($P<0.05$ and $P<0.01$) in glucose, total protein, urea, ALT, AST and ALP whereas was exhibit significant change at ($P<0.05$ and $P<0.01$) in albumin, and

were showed significant change at ($P<0.05$) in cholesterol, triglyceride and creatinine compared to control **(Table 4).**

At 500 mg/kg: Treatment of rats Group (C2) with 500 mg/kg of fruit ethanoic extract after 28 days, did not produce any significant difference in all biochemical parameters tested at ($P<0.05$ and $P<0.01$) except were showed significant difference at ($P<0.05$) in triglyceride and urea compared to control **(Table 5)**

At 250 mg/kg: -Treatment of rats Group (D2) with 250 mg/kg of fruit ethanolic extract after 28 days, did not produce any significant difference at ($P<0.05$ and $P<0.01$) in glucose, total protein, albumin, cholesterol, urea, AST, and ALP, and there was significant difference at ($P<0.05$) in triglycerides, creatinine and ALT relative to their respective control. **(Table 6)**

RESULTS

Table 1: Control 1000 mg / kg s of ethanolic extract of *C.zambesicu* after 14days for biochemical parameters in rats serum blood: -

Parameters	Control	Treatment	T. value	P-value	S at P<0.01	Sat p<0.05	Change	Change %
Glucose	47.4± 9.34	56.6 ±10.36	1.47469	0.178528	Ns	Ns	increment	19.41 %
T. protein	70.0 ±6.20	71.2 ± 2.86	0.39265	0.704831	Ns	Ns	increment	1.71 %
Albumin	20.2 ±2.77	9.8 ± 1.30	-0.29173	0.777915	Ns	Ns	decrement	1.98 %
Triglyceride	105.2±8.6	87.6 ± 9.53	-3.06377	0.015494	Ns	S	decrement	16.73 %
Cholesterol	73.2±8.56	54.4 ±14.15	-2.54193	0.03606	Ns	S	decrement	25.68 %
Urea	37.6±6.35	36.4 ± 4.34	-0.05745	0.95597	Ns	Ns	decrement	3.19 %
Createnine	0.64±0.15	0.56 ±0.15	-1.04257	0.327625	Ns	Ns	decrement	12.5 %
ALT	8.9±2.21	5.0 ±0.55	-3.45119	0.00868	S	S	increment	43.82 %
AST	4.26±0.94	4.52 ±0.41	0.44201	0.670185	Ns	Ns	increment	6.10 %
ALP	107.8±27.67	103.6 ±10.14	-0.2625	0.799569	Ns	Ns	decrement	3.90 %

Table 2: Control 500 mg / kg of ethanolic of *C.zambesicu* after 14 days for biochemical parameters in Rats' serum blood: -

Parameters	Control	Treatment	T. value	P-value	S at P<0.01	S at P<0.05	Change	Change %
Glucose	47.4 ±9.34	63.2 ±4.97	3.33836	0.010256	Ns	S	increment	33.33%
T. protein	70.0 ±6.20	76.0 ±5.87	1.57027	0.154993	Ns	Ns	increment	8.57%
Albumin	20.2 ±2.77	22.8 ±1.92	1.72189	0.123391	Ns	Ns	increment	12.87%
Cholesterol	73.2 ±8.56	65.6±6.66	-1.56776	0.155573	Ns	Ns	decrement	10.38%
Triglyceride	105.2 ±8.61	95.0±4.85	-2.30748	0.049885	Ns	S	decrement	9.70%
Urea	37.6 ±6.35	25.6 ±2.13	-3.62266	0.006757	S	S	decrement	31.92 %
Createnine	0.64 ±0.15	0.66 ±0.09	0	1	Ns	Ns	increment	3.13%
ALT	8.9 ±2.21	5.58 ±1.70	-2.66549	0.028562	Ns	S	decrement	37.30 %
AST	4.26 ±0.94	5.16 ±1.53	1.1222	0.294327	Ns	Ns	increment	21.13%
ALP	107.8±27.67	83.0 ±33.76	-1.27031	0.239672	Ns	Ns	decrement	23.01 %

Table 3: Control 250 mg / kg of ethanolic extract of *C.zambesicus* after 14 days for biochemical parameters in rats' serum blood:

Parameters	Control	Treatment	T.value	P-value	S at P<0.01	S at P<0.05	Change	Change %
Glucose	47.4 ±9.34	54.4 ±6.19	1.39665	0.200047	Ns	Ns	increment	14.77 %
T. protein	70.0 ±6.20	73.6 ±5.81	0.94671	0.371499	Ns	Ns	increment	5.14 %
Albumin	20.2 ±2.77	22.6 ± 1.52	1.69706	0.128133	Ns	Ns	increment	1.19 %
Cholesterol	73.2 ±8.56	66.2 ±7.12	-1.4062	0.197297	Ns	Ns	decrement	9.56 %
Triglyceride	105.2 ± 8.61	99.4±2.3	-1.45455	0.183876	Ns	Ns	decrement	5.51 %
Urea	37.6 ±6.35	31.8 ± 6.26	-1.19257	0.267207	Ns	Ns	decrement	15.43 %
Creatinine	0.64 ±0.15	0.64 ± 0.17	-0.19803	0.847961	Ns	Ns		0 %
ALT	8.9 ± 2.21	11.54 ± .70	1.69266	0.128978	Ns	Ns	increment	29.66 %
AST	4.26 ±0.94	4.5 ±0.49	0.37006	0.72094	Ns	Ns	Increment	5.63 %
ALP	107.8 ±27.67	123±14.16	1.09344	0.306029	Ns	Ns	Increment	14.1 %

Table 4: Control 1000 mg / kg fruit of ethanolic extract of *C.zambesicu* after 28 days for biochemical parameters in rats serum blood:

Parameters	Control	Treatment	T.value	P-value	S at P<0.01	S at P<0.05	Change	Change %
Glucose	53.2 ±6.41	44.6 ±5.11	-1.05699	0.321388	Ns	Ns	decrement	16.17 %
T. protein	67.8 ±2.29	64.8 ±1.77	-1.03633	0.330354	Ns	Ns	decrement	4.42 %
Albumin	37.6 ± 0.68	31.8±0.8	-3.80699	0.005185	S	S	decrement	15.43 %
Cholesterol	75.6 ±3.08	65.2 ±2.60	-2.5839	0.032419	Ns	S	decrement	13.76 %
Triglyceride	61.4 ±1.33	54.4 ±2.29	-2.64198	0.029622	Ns	S	decrement	11.40 %
Urea	36.8±1.50	34.8 ± 2.15	-0.76249	0.46765	Ns	Ns	decrement	5.43 %
Creatinine	1.36±0.07	1.04 ± 0.09	-2.59554	0.031838	Ns	S	decrement	23.53 %
ALT	9.6 ±1.29	7.8 ±1.14	-1.04605	0.326111	Ns	Ns	decrement	18.75 %
AST	3.36 ±0.22	6.04 ±0.47	1.29624	0.231035	Ns	Ns	increment	79.76 %
ALP	114.6 ± 12.61	101.6 ± 8.28	-0.86166	0.413952	Ns	Ns	decrement	11.34 %

Table 5: Control 500 mg / kg fruit of ethanolic extract of *C.zambesicus* after 28 days for biochemical parameters in rats serum blood:

Parameters	Control	Treatment	T. value	P-value	S at P<0.01	S at P<0.05	Change	Change %
Glucose	53.2 ± 6.41	59.8 ± 5.37	0.78896	0.452878	Ns	Ns	increment	12.41 %
T. protein	67.8 ±2.29	69.0 ± 0.89	0.48827	0.638458	Ns	Ns	increment	1.77 %
Albumin	37.6 ± 0.68	36.2 ± 0.49	-1.67332	0.132801	Ns	Ns	decrement	3.72 %
Cholesterol	75.6 ±3.08	74.4 ± 2.71	-0.2926	0.77276	Ns	Ns	decrement	1.59 %
Triglyceride	61.4 ±1.33	53.6 ± 2.25	-2.98677	0.017419	Ns	S	decrement	12.70 %
Urea	36.8±1.50	44.2 ± 2.65	2.42017	0.41256	Ns	S	increment	20.11 %
Creatinine	1.36±0.07	1.08 ± 0.11	-2.087	0.070346	Ns	Ns	decrement	20.59 %
ALT	9.6 ±1.29	7.6 ±1.01	-1.22375	0.255863	Ns	Ns	decrement	20.83 %
AST	3.36 ±0.22	5.48 ±0.32	0.30779	0.7661	Ns	Ns	increment	2.24 %
ALP	114.6 ±12.61	89.8 ±14.78	-1.27642	0.237613	Ns	Ns	decrement	21.64 %

Table 6): Control 250 mg / kg fruit of ethanolic extract of *C.zambesicus* after 28 days for biochemical parameters in rats serum blood:

Parameters	Control	Treatment	T. value	P-value	S at P<0.01	S at P<0.05	Change	Change %
Glucose	53.2 ±6.41	49.2 ±2.60*	-0.57807	0.579119	Ns	Ns	decrement	7.52 %
T. protein	67.8 ±2.29	66.0 ±1.90	-0.60541	0.561687	Ns	Ns	decrement	2.65 %
Albumin	37.6 ± 0.68	37.4 ±0.6	-0.22086	0.830732	Ns	Ns	decrement	0.53 %
Cholesterol	75.6 ±3.08	76.4 ± 3.36	0.17575	0.864858	Ns	Ns	increment	1.06 %
Triglyceride	61.4 ±1.33	54.4 ±1.69	-3.2567	0.011584	Ns	S	decrement	11.40 %
Urea	36.8±1.50	35.6 ± 2.48	-0.41404	0.689723	Ns	Ns	decrement	3.26 %
Creatinine	1.36±0.07	1.08 ± 0.04	-3.34664	0.010131	Ns	S	decrement	20.59 %
ALT	9.6 ±1.29	14.4 ±1.44	-2.49136	0.037443	Ns	S	increment	50 %
AST	3.36 ±0.22	5.36 ±0.58	0	1	Ns	Ns		0 %
ALP	114.6 ±12.61	12.28 ±7.59	0.65229	0.532503	Ns	Ns	increment	8.38 %

Discussion

In general, folk medicines uses plant extracts in tradition without taking into account the toxicity aspect. *C.zambesicus* is uses as medicinal plant in West and Central Sudan to treat malaria, cough, dysentery, fever , convulsions, tonic , antihypertensive and antimicrobial (urinary infections) (1). However, toxicity and phytochemical studies about the fruits/seeds of this plant are rare. Assessment of biochemical parameters can be used to determines the extent of deleterious effect of plant .In the rats that were received 1000mg/kg of *C. zambesicus* ethanolic extract with in 14 and 28 days (B1 and B2) , Triglycerides and cholesterol in this experiment treated with ethanolic extract were decreased to 16.73%, 25.68%, (after 14 days), and decreased to 13.76%, 11.40 % , ,(after 28 days) and they were showed significant result change at ($P<0.05$), respectively, while ALT in group B1 was increased to 43.82% , and was showed significant result change at ($P<0.01$ and $P<0.05$) but it was increased to 37.30 % and showed significant result change at ($P<0.05$). In group B1, Glucose, T. protein were increased to 19.41 % , 1.71%, and decreased to 16.17 % , 4.42 % , (after 28 days) and they were showed no significant result change at ($P<0.01$ and $P<0.05$). Albumin was decreased to 1.98 % with no significant result change at ($P<0.01$ and $P<0.05$) in group B1 , and it was decreased 15.43 % with significant change at ($P<0.01$ and $P<0.05$) (table 4). Urea and creatinine in the experimental treated with ethanolic extract were decreased in groups B1 and B2 to 3.19 % , 5.43 % , 3.19 % and 23.53%, respectively with

significant change. In group C1, Glucose, T. protein were increased to 33.33% , 8.57%, (with significant change at $P<0.05$ for glucose) and increased to 12.41 % , 1.77 % , (in group C2) and they were showed no significant result change at ($P<0.01$ and $P<0.05$). Albumin was increased to 12.87% with no significant result change at ($P<0.01$ and $P<0.05$) in group C1 , and it was decreased 3.72 % with no significant change . (table 5). Urea in the experimental treated with ethanolic extract was decreased in group C1 to 31.92 % with significant change, but was increased in C2 to 20.11 % with significant change at ($P<0.05$) , and creatinine was increased to 3.13% with no significant change,(Table 2) and it was decreased in C2 to 20.59 % with no significant change . In the last group D1 , glucose , T . protein , Albumin, ALT ,AST, and , ALP they were increased to 14.77 % , 5.41 % , 1.19 % , 29.66, 5.63 and 14.1%, respectively with no significant change, but in group D2 , cholesterol , ALT ,AST, and ALP they were increased to 1.06%, 50 % , 59.5 % , and 8.38%, respectively with no significant change for cholesterol , ALT ,AST, and ALP, except for ALT there was a significant change at($P<0.05$)

There are many enzymes such as phosphatases and transferases that are found in the serum which did not originate from the extracellular fluid. During tissue damage, some of these enzymes find their ways into the serum by leakage through disrupted cell membranes (13). This serum enzymes measurement provides a marker in toxicity studies as well as in clinical diagnosis, alkaline phosphatase (ALP) is a marker co-enzyme for the

plasma membrane and often used to assess the integrity of the plasma membrane . Liver damages/infections are characterized by elevated ALP concentration in serum as in experiment , and these may be associated with inflammation and / or injury to hepatic cell (14). Generally, enzymes such as ALT, AST and ALP are marker enzymes for function and integrity of organs such as liver. The enzymes found within organs and tissues are released into the blood stream following cellular necrosis and cell membrane permeability and are used as diagnostic measure of liver damage (15). Furthermore, serum ALT/AST has been used as an index to monitor liver pathology (16). Treatment of rats with 250 mg/kg of fruit ethanoic extract after 28 days, did not produce any significant difference in lipid profile that mean high dose of *C.zambesicus* fruit can cause lipid lower; there was no significant effect on glycemic, and kidney and there was no toxicity all liver enzyme were normal .The administration of *C.zambesicus* fruit caused an obvious alteration in the cholesterol compared to control. This reduction was found to be significant ($P<0.05$). Increase in HDL (high density lipoprotein) ratio is one of desirable of an ideal hypocholestromic agent since the higher the ratio that lowers the atherosclerotic risk. The decrease in cholesterol balance is indicate the total changes in body pools of cholesterol and may be due to the compensatory mechanism such as a decrease in resorption of endogenous cholesterol (17) or an increase in the rate of secretion into intestinal tract. The changes in the lipid profile with *C.zambesicus* fruit treatment may contribute

to decreased incidence of atherosclerosis. *C.zambesicus* fruit may activate the enzyme lipoprotein lipase which uptake cholesterol from blood vessels or enhance lecithin cholesterol acyl transferase in HDL, which has a role in scavenging of cholesterol (18).

Cholesterol is a biochemical parameter that is affected by many factors such as age, sex diet and genetic factors. One of such factors may provide an explanation to the pattern observed in this study, other factors could be attributed to the active components in *C.zambesicus* fruit under question. Coumarin has a role in antihyper lipidemic activity .Potent anti-hyper- lipidemic activity and was found to decrease the plasma Triacylglycerol (TAG) by 55% total cholesterol (TC) by 2% accompanied by increase in HDL. HDL /TG ratio 42% in hyper lipedemic rate to greater degree than some the references statin drug (19). Polyphenol inhibit fat cell formation and increase lipolysis by inhibition of digestion by lipase (in vitro) resulting in decreased lipolysis of Triacylglycerol which may be translated to reduced fat digestion in human and weight loss. Polyphenols inhibit pancreatic lipase and triacyl glycerol absorption (20) and inhibit apoprotein β synthesis in intestine mucosa and inhibit of cholesterol esterification and intestinal lipoprotein production .And inhibit protein in lipoprotein like apo protein then cholesterol level was decreased which prevent cholesterol absorption

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A case of conjunctivitis following mild illness COVID-19 infection

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Abstract

Background

Up to date, COVID-19 resulted in nearly 4 million deaths worldwide, making it the most serious global health disaster in hundred years [1]. Previously, there were multiple anecdotal and published reports of eye redness and irritation in COVID-19 patients, implicating conjunctivitis as an ocular presentation of the infection. COVID-19 has been linked to multiple ophthalmic illness, according to new research, but most of literature links the pandemic to Conjunctivitis as a common encounter escorting respiratory findings [2]. The authors here report a case of mild illness COVID-19 that developed conjunctivitis a week after being diagnosed with the illness.

Summary

In this case report, a 40 years male presented with mild symptoms of COVID-19 which proved to be positive. Six days later, he developed redness in his right eye, which was associated with mild pain and itching. The left eye acquired the same symptoms within 24 hours. Conservative management was given in the form of antihistamine eye drop which was prescribed two times daily. A diagnosis of COVID-19 associated conjunctivitis was issued, which is uncommon to be associated with mild COVID.

Key words: COVID-19, Conjunctivitis, mild illness

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Case presentation

A 40 years old male presented to the flu clinic in King Abdulaziz Medical city in Riyadh, Saudi Arabia with dry irritating cough, runny nose, and mild sore throat on 22 January 2022. He was using analgesics and antihistamine tabs for two days, afterwards the patient went to the COVID-19 screening clinic in 24 January. On the next day the results of COVID-19 PCR test (through oral and nasal swaps) was positive. Patient was advised to take home quarantine for 10 days, received oral steroid inhaler, Paracetamol tabs

1000mg B/D, antihistamine nasal drops, and vitamin C, and advised to be voluntarily on social isolation. Symptoms improved gradually within the next week, however on the sixth day, he developed redness in his right eye, which was associated with mild pain and itching, but there were no excessive lacrimation, intolerance to light (photophobia) or visual disturbances. On the next day the left eye was also affected; see figure 1. When seeing the GP, vitals were all normal. Eye examination revealed, by inspection: red congested sclera, no discharges or excessive

lacrimation. An antihistamine eye drop was prescribed two times daily. Within three days, symptoms on both eyes disappeared; see figure 2, and on day 12 of COVID-19 diagnosis, all the symptoms including the upper respiratory tract symptoms were recovered. He was diagnosed as a case of COVID-19 associated with viral conjunctivitis.

Investigations

Patient underwent COVID-19 PCR test via oral and nasal swaps. No general investigations were performed. No investigations were performed concerning the eyes, and no conjunctival swaps were to be taken.

Differential diagnosis

Given the patient's presentation and clinical probabilities, the most likely diagnosis was viral

conjunctivitis that has developed by some sort of contact with the nasal or oral secretions. Other differential diagnoses included other causes of viral conjunctivitis, bacterial conjunctivitis, allergic conjunctivitis, and chemical irritation.

Treatment

Patient received Paracetamol tabs 1000 mg B/D, steroid inhaler (Fluticasone 250µg + Salmeterol xinofoate 25µg), antihistamine nasal drops (Xylometazoline 0.1%), and vitamin C (Redoxon effervescent 100 mg OD). An antihistamine eye drop was prescribed for his eyes, and the patient did not use it.

Outcome and follow-up

Both the ocular and respiratory tract symptoms relieved in less than two weeks, and needed no further treatment. PCR test was not repeated.



Figure 1: Appearance of the patient's eye at presentation; 6 days after positive COVID 19 PCR.



Figure 2: Appearance of the patient's eyes one week after the previous photo.

Discussion

A mild illness COVID 19 is described as a disease with any of the following COVID-19 signs and symptoms: fever, cough, sore throat, malaise, headache, muscle pain, nausea, vomiting, diarrhea, loss of taste and smell, but no shortness of breath, dyspnea, or finding in the chest imaging [3]. Even in COVID-19 positive patients with lesser illness, moderate conjunctivitis appearing as conjunctival congestion is prevalent and one of the predominant ocular symptoms [4, 5].

The earliest reported cases of COVID-19 associated conjunctivitis were documented by Salducci and La Torre in a cruise's ship in May 2020 [6] followed by Navel, Chiambaretta, and Dutheil case report in September 2020 of an ICU-admitted patient [7].

Authors think this is an uncommon case because, According to Loffredo et al (2020), the chances of having conjunctivitis with severe COVID-19 is four times the chance of having it with milder

illness [8]. Güemes-Villahoz et al 2020 report, however, found no significant relationship between the COVID-19 severity score and the presence of conjunctivitis [9]. Authors weighs on Loffredo results as it's an in-depth meta-analysis. While the kind participation of Güemes-Villahoz et al was a cross-sectional study. Furthermore, the eye symptoms manifested while the upper respiratory symptoms were fading. Several studies mentioned that conjunctivitis can even precede the respiratory symptoms of COVID-19 [3, 10-13], also some inferred: it could be the only symptom and/ or sign of the disease [14-16]. As in this current case, the symptoms of conjunctivitis were frequently reported to be bilateral, but some studies reported an involvement of only one eye [17, 18]. Authors think it is attributed to the transmission through hands or adjacent structures, such as clothes or bed sheets, to the other eye. The tears and eye discharges are known to be contaminated with COVID-19 virus [19, 20]; hence, a conjunctival

swap could be used for diagnosing of COVID-19. In this current case, a swap was not taken because the patient is already diagnosed and because this procedure is not routinely performed in Saudi Arabia.

This study have the disadvantages of lacking routine laboratory testing and the close follow-up of the patient, also a post recovery testing was not performed, nonetheless, due to the current pandemic circumstances, only patients with serious illness or concomitant chronic diseases are closely monitored.

Learning points

- COVID-19 associated conjunctivitis can accompany a mild illness.
- Attention should be paid to hand contact to the eyes in COVID-19 patients.
- Wearing gloved is preferable particularly at nighttime.
- When the patient coughs, sneeze, or clean his nose, vigorous hand washing is necessary.

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