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رَبَّنَا آتِنَا دَاوُدَ وَسُلَيْمَانَ عِلْمًا

وَقَالَ الْحَمْدُ لِلَّهِ الَّذِي

فَضَّلَنَا عَلَى كَثِيرٍ مِّنْ عِبَادِهِ الْمُؤْمِنِينَ

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Editorial

“To ensure the strength and enduringness of a rope, examine it at its weakest point!”

An old Axiom

Dear SMLJ readers;

More than a year has elapsed, before our anticipated issue of SMLJ could see light! We are *sorry!* But this argument does not suffice for an apology that counts. There must be a convincing explanation!

The defacto *Status Quo* was perplexing, namely after the instructions, issued by the academic authorities. Accordingly, the aspirants for higher degrees as well as baccalaureates are to, mandatorily, publish (at least) part of their work as a prerequisite for receipt of the degree claimed for. For us in the journal that bespoke the heavy duty that pends upon our shoulders. We schemed for the arduous brooding effort, and planned June 2018 as the term of our first issue. All this hope crumpled to dust! From this we learned that success cannot be earned through compulsion, rather attraction.

Yet, attraction comes through advertising and the only available forum could be the website. Some technical administrative obstacles stood and still stand on the way, but those could, hopefully, be resolved soon.

This issue, in your hands, bespeaks the potential hidden within our authors. Because suddenly at the deadline we received a formidable number of contributions, amongst which we discovered the high caliber of scientist work, beyond anticipations. I think this issue will exonerate our unforgivable delay. We are sure you will find it encouraging.

Sincerely Yours,

Dr. Ali Suleiman Elwakeel



Executive Editor

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For details turn to the [General Directives for Manuscripts Submitted to Publishing!](#)

Genial Welcome



Assistant Professor
Mohammed E. H. Ournasseir
SMLJ Editor-in-chief
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Dear Readers and Colleagues,

It gives me a great pleasure to bring before you the first issue, volume 6 of the SMLJ, assembled under our joint and articulate editorship.

The main objective of this journal is to publish peer-reviewed research articles, reviews or communications pertaining to the various aspects of medical laboratory sciences.

We are confident that our editorial board constituted by reputable staff members of in different specialties and diverse expertise. The ultimate goal behind the dissemination of knowledge is to improve the patient's management and to enhance health care delivery.

I would like to take this opportunity to thank prof. Ali S. Elwakeel for his effort in establishing the journal and special thanks are extended to the authors for their interest and encouragement.

We aspire to see the SMLJ to rank among the major resources for medical laboratory sciences and other related fields, yet this can only be achieved through your contribution and support.

All the Best of Wishes and Regards,

Mohammed E. H. Ournasseir

A handwritten signature in blue ink that reads "Mohammed E. H. Ournasseir". The signature is stylized and cursive.

Dean Faculty of Medical Laboratory Sciences

Antimicrobial Potential of *Acacia nilotica*, Subspecies, *tomentosa* against some Microorganisms that cause Urogenital Infections.

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ABSTRACT

Background: For centuries, aromatic plants with therapeutic properties were used to treat human illnesses

Objective: The aim of this study is to investigate and compare the antimicrobial susceptibility of microorganisms that cause urogenital infections to leaves and stem bark extracts of *Acacia nilotica* spp. *Tomentosa*, using standard strains and clinical isolates.

Result: The minimum inhibitory concentration (MIC) showed by leaves and bark methanol extracts on the standard bacteria ranged from 3.125 mg/ml to 12.50 mg/ml and the minimum bactericidal concentration (MBC) ranged from 6.25 mg/ml to 50 mg/ml. where the Mean Diameter of Inhibition Zone (MDIZ) ranged from 22.367.40 mm to 25.542.47 mm. On the other hand, isolates of *Candida albicans* showed MDIZ which was 23.203.44 mm for leaves methanol extract and 25.251.92 mm for bark methanol extract. The MIC exhibited by leaves and bark methanol extracts on bacterial isolates ranged from 3.125 mg/ml to 12.50 mg/ml and the MBC was ranged from 6.25 mg/ml to 25 mg/ml.

Conclusion: The study revealed that the extracts of *A. n. ssp. tomentosa* possess significant antimicrobial compounds, which showed formidable effectiveness against both standard strains and clinical isolates.

Keywords: Antimicrobial potential; *Acacia nilotica* subsp. *tomentosa*; leaves extracts; Bark extract; Antifungal activity.

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INTRODUCTION:

In developing and under-developed countries low-income people, such as farmers, workers, people of isolated villages and native communities use folkloric medicine for treatment of their common infections ^[1]. As well as, in the poor communities, synthetic drugs are expensive and sometimes inadequate for the treatment of some infectious diseases. On the other hand, indiscriminate use of broad-spectrum antimicrobial agents used in the treatment of urogenital infections represents one of those mechanisms that increase the development of multi-drug resistant microbial strains ^[2,3]. Moreover, synthetic chemotherapeutic agents may cause undesired side effects. Therefore, there is an urgent need to search new, safer and effective natural medicinal products to

fight and control the microbial infections ^[4,5,6]. Plants that used for traditional medicine contains a wide range of substances that can be used to treat chronic infections as well as acute infectious diseases. A perfect knowledge of how to use a plant against different illnesses may be expected to have been accumulated in areas where the use of the plant remains to be of great importance. [7] A wide range of phytochemical compounds contained in plants are known to inhibit microbial pathogens [8,9]. Successful determination of such biologically active compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. Organic solvents such as ethanol, acetone, and methanol are often used to extract bioactive compounds [10]. *Acacia nilotica*

(L.) Willd. Ex. Del. is a genus of shrubs and trees [11]. It is a moderate to large size tree that reaches a height of 10 to 20 meters. The barks are blackish grey [12]. The leaves are twice compound, [13]. This genus is spread around the tropical to warm-temperate regions including Europe, Africa, Southern Asia, and the Americas [14]. In Sudan, it grows in drained locations along water courses such as on the river banks of the White and Blue Nile [15]. Regarding traditional medicine uses, *Acacia nilotica* differs from ethnic group to another; the Zulu use barks for cough; the Chipi use roots for tuberculosis and root decoction, and is believed to impart courage [16]. The barks are used for diarrhea, dysentery, and leprosy. Decoction of pods is used as gargle in urogenital diseases [17].

The barks, gum, leaves and pods are strongly astringent due to tannin and are used medicinally in the East and West Africa. Some of rural communities in Sudan use different parts of *Acacia nilotica* spp. *tomentosa* to cure dysentery, leprosy, impotence, colds, congestion, coughs, diarrhea, dysentery, fever, gallbladder disease, hemorrhage, hemorrhoids, ophthalmic, diabetes and tuberculosis [16,18]. This study was conducted to investigate and compare the antimicrobial activity of methanolic and aqueous extracts of *A. n.* subsp. *tomentosa* on some pathogens recovered from urogenital infections.

MATERIALS AND METHODS

Sample Collection:

Leaves and fruits of *A. n.* spp. *tomentosa* were collected in

September 2012 from SHAMBAT Area. It is located in Khartoum North, Khartoum State, Sudan, and its geographical coordinates are latitude 15° 40' 5" North and longitude 32° 32' 1" East. The plant parts were spread, air-dried in the shade for twenty days and their botanical identification was kindly made by Professor Hatil Hashim El-Kamali, Department of Botany – Faculty of Science and Technology, Omdurman Islamic University (OIU), Omdurman, Sudan. Voucher specimens of the plant (HHK 383) have been deposited at Faculty of Science and Technology. Botany Department Herbarium, OIU. The microbiological works has been carried out in the microbiology laboratory, Medicinal and Aromatic Plants Research Institute (MAPRI), Khartoum, Sudan.

Preparation of Plant Extract and Standard Antimicrobial Agents;

Preparation of Aqueous Extract:

Aqueous extracts were prepared according to the method described by EL-Kamali and Awad EL-Karim, [19]. Exactly 100 g of the plant powder poured in 1 liter (1000 Milliliters) of hot sterile distilled water and left for 24 hours at room temperature. The mother liquor was filtered through Whatman No.2 filter paper (Sigma-Aldrich, Inc. USA). Extracts were kept in deep freezer for 48 hours, then introduced in freeze dryer till completely dried. The dried plant extracts were crushed from the flask using a spatula. Each residue was weighed and the yield percentage was determined. The crude extracts were stored in dark dry sterile containers in the microbiology laboratory,

MAPRI, Khartoum, Sudan, until the use for antimicrobial screening. At the time of testing, the aqueous residue (2 g) was dissolved in sterile distilled water 20 ml (con. 100 milligram/milliliter), and kept in refrigerator until used.

Preparation of Methanol

Extract: The plant parts were washed with distilled water, then dried at 60°C over night, cut into small pieces and crushed in a mechanical mortar to a coarse powder. Powder sample (100 g) were soaked in 200 ml of 98 % methanol (Chem. Lab. Ltd. Belgium) for 3 days at room temperature and then filtered through What-Man No.2 filter paper. The methanol extract was filtered and evaporated under reduced pressure again using Rotary Evaporator to complete dryness. Each residue was weighed and the yield percentage

was determined. Then, the residue was stored dry in sterile containers in the microbiology laboratory, (MAPRI), until use for antimicrobial testing [19].

Preparation of Standard Antibiotics and Antifungal

Drugs: entamicin (Lunik Pharma Pvt. Ltd., India), ampicillin (Therapeutic Pharmaceutical, India) and candizole (The United Pharmaceutical. Ltd. Jordan) were prepared immediately before use by diluting them in sterile de-ionized water. A series of concentrations were prepared by double fold dilution method from the original concentration to get 40 µg /ml, 20 µg /ml, 10 µg /ml and 5 µg /ml for each reference antibiotic, whereas the antifungal drug (Candizole) was prepared at concentration of 5 mg/ml, 10 mg/ml, 20 mg/ml and 40 mg/ml.

Preparation of Culture Media

and inoculum: Bacteria were grown in enriched and selective culture media. Mueller-Hinton agar (Oxoid, Ltd, England) was used as base medium for screening of antibacterial activity. Sabouraud's dextrose agar (Oxoid, Ltd, England) was used as a medium for identification and is used for screening of antifungal activity [20].

Preparation of McFarland

Standard: 0.5 McFarland standard was prepared by mixing 9.95 ml of 1% Sulphuric acid (Shree Pushkar Chemicals & Fertilizers Ltd. India) with 0.05 ml of 1% anhydrous barium chloride in distilled water in order to estimate the approximate bacterial density [21]. The tube was used for comparison of bacterial suspension with the

standard inoculum whenever required.

Preparation of Bacterial

Inoculum: One ml aliquots of a 24 hours broth culture of the test organisms were aseptically distributed onto Mueller-Hinton agar slopes and incubated at 37°C for 24 hours. The bacterial growth was harvested and washed off with 100 ml sterile normal saline, to produce a suspension containing approximately 10^7 Colony forming Units, (CFU), per ml. The suspension was stored in the refrigerator at 4°C until used. The average number of viable organisms per ml of the stock suspension was determined by means of the surface viable counting technique [22]. Serial dilutions of the stock suspension were made in sterile normal saline solution and 0.02 ml volumes of the appropriate

dilution were transferred by micropipette onto the surface of dried Mueller-Hinton agar plates. The plates were allowed to stand for two hours at room temperature for the drops to dry and then incubated at 37°C for 24 hours. After incubation, the number of developed colonies in each drop was counted. The average number of colonies per drop (0.02 ml) was multiplied by 50 and by the dilution factor to give the viable count of the stock suspension, expressed as the number of colony forming units per ml suspension. Each time a fresh stock suspension was prepared; all the above experimental conditions were maintained constant so that suspensions with very close viable counts would be obtained [23].

Preparation of Fungal Inoculum: The inoculum of *C.*

albicans was prepared as previously described by Cormican and Pfaller, [24]. Briefly, *C. albicans* was grown on Sabouraud's Dextrose Agar (SDA) plates for 24 hr. For each isolate five colonies were grown until their diameters were at least 1 mm. Then, the colonies were picked off and suspended in 0.85% saline solution. The suspension was adjusted to the turbidity of a 0.5 McFarland standard at a wavelength of 530 nm. Quantitative colony plate counts were determined on SDA to verify the inoculum size. The final inoculum concentration was standardized to approximately 1×10^6 CFU/ml. The suspension was stored in the refrigerator at 4°C until used [25].

Purification of Cultures by Streaking Plate Method: The bacteriological and fungal techniques followed in the

present work were described by Mackie and McCartney, [20], Koneman *et al.*, [21], Kavanagh, [23]; Cruickshank *et al.*, [26]; Arvidson *et al.*, [27]; Cheesbrough, [28]. Once the primary inoculum from the clinical specimens was made, a wire loop was used to spread the material into the four quadrants of the plate, as described by Cheesbrough, [28] and Koneman *et al.* [21]. The wire loop was sterilized between each successive quadrant streak. The inoculated plates were incubated at 37°C for 24 hours. The purpose of this technique is to dilute the inoculum on the surface of the Agar medium, so that single isolated colonies of bacteria and fungi, known as colony forming units, can be isolated. We used this method for sub-culturing the standard strains and obtained clinical isolates on

Nutrient agar, MacConkey's Agar, Mannitol salt agar, Blood Agar, Chocolate Agar, Nutrient Agar and Sabouraud's dextrose agar (Oxoid limited, England).

Sources and Maintenance of tested Microorganisms Sources of Standard Microorganisms:

Five standard strains of bacteria and one fungus were obtained from American Type Culture Collection (ATCC) Rockville, Maryland, USA. Those reference strains include *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumoniae* (ATCC 53657), *Proteus vulgaris* (ATCC 6380), *Staphylococcus aureus* (ATCC 25923) and *Candida albicans* (ATCC 7596). The strains were activated and sub-cultured three successive times and stored at 4°C.

Sources of the Clinical Isolates:

The clinical isolates were obtained from urogenital infections specimens of Sudanese patients (urines, urethral discharges and high-vaginal swabs). These isolates were collected from different General and Private Hospitals of Khartoum State, Sudan. After explaining the purpose of the study, an ethical verbal approval from the directors of these Hospitals has been taken. Clinical isolates were maintained frozen in Trypton Soya Agar TSA, (Oxoid Ltd. England).

Identification of Clinical

Isolates: Each clinical isolate was inoculated on a suitable culture media and incubated aerobically and the other an aerobically. The obtained isolates were then purified by streaking on plates containing the appropriate selective and

differential culture media that mentioned above. The purified isolates were then identified by microscopic examination, cultural characters and biochemical tests and stored in a refrigerator until they were used.

Activity of standard antibiotics and antifungal against standard microorganisms:

The agar well diffusion method was followed to determine the antimicrobial activity of standard antibiotics and antifungal drug against standard microorganisms. 20 ml of two culture media; Mueller-Hinton agar and Sabouraud's dextrose agar were poured on a glass Petri-dish of same size and allowed to solidify. Agar surface of each plate was streaked by a sterile cotton swab with the standard strain. Agar plate was punched with a sterile cork borer (No.4) and agar discs were removed. Alternate cups

were filled with 0.1 ml sample of each antibiotic using automatic micro-pipette, and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37°C for 18 hours. After incubation the diameters of the resultant growth inhibition zones were measured [20,26].

Antibacterial Activity of Extracts' Determination:

The agar well diffusion method [20] was adopted with some minor modifications to assess the antibacterial activity of the prepared extracts. One ml of the standardized bacterial stock suspension 10^7 CFU/ ml were thoroughly mixed with 100ml of cooled molten sterile Mueller-Hinton agar which was maintained at 45°C. 20 ml aliquots of the inoculated Mueller-Hinton agar were distributed into sterile Petri-dishes. The agar was left to

set and in each of these plates 4 cups (10 mm in diameter) was cut using a sterile cork borer (No. 4) and agar discs were removed. Alternate cups were filled with 0.1 ml sample of each extracts using automatic micropipette, and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37°C for 18 hours. Two replicates were carried out for each extract against each of the test organisms. After incubation, the diameters of the resultant growth inhibition zones were measured averaged and the mean values were tabulated.

Antifungal Activity of Extracts' Determination:

Testing of antifungal activity of extracts was performed by the agar well diffusion method with minor modifications. Two ml of the standardized *Candida albicans* stock suspension 10^6

CFU/ ml were thoroughly mixed with 100 ml of cooled molten sterile Sabouraud's Dextrose agar which was maintained at 45°C. 20 ml aliquots of the inoculated Sabouraud's Dextrose agar were distributed into sterile Petri-dishes. The agar was left to solidify and in each of these plates 4 cups was cut using a sterile cork borer (No. 4) and agar discs were removed. Alternate cups were filled with 0.1 ml sample of each extracts using automatic micropipette, and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37°C for 24 - 48 hours. Two replicates were carried out for each extract against each of the test fungus. After incubation, the diameters of the resultant growth inhibition zones were measured averaged

and the mean values were tabulated [29].

Determination (MIC) for standard strains and clinical isolates:

The plant extract that was found active, as antimicrobial agent, and was later tested to determine the MIC values for each strain. Mueller-Hinton broth medium was used to test the inhibitory effect of tested extracts on standard bacteria and clinical isolates, while Sabouraud's dextrose broth was used to determine the Minimum Fungicidal Concentrations, (MFC), of extracts on the standard strains and clinical isolates of *Candida albicans*. MIC was determined using broth dilution method as described by Mackie and McCartney [20]. Tubes were prepared in series of increasing concentrations of the plant extracts. The extracts were

double-fold diluted to give the final concentrations of 3.125, 6.25, 12.50, 25, and 50 mg/ml. The organisms tested were growing in broth over night to contain 10^7 CFU/ml. A loop-full of diluted culture was spots with a standard wire loop that delivers 0.001 ml of inoculum and inoculated in tubes with equal volume of Mueller Hinton broth, Sabouraud's dextrose broth and the plant extracts. The tubes were incubated aerobically at 35°C for 24-48 hours. Three control tubes were maintained for each strain (media control, organism control and extract control). The lowest concentration (highest dilution) of the extract that produced no visible growth (no turbidity) in the first 24 hours when compared with the control tubes that were considered as the initial MIC.

Determination (MIC&MFC) for standard strains and

clinical isolates: The Minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) determine the lowest concentrations that completely kill bacteria and fungi after 48 hours of incubation at 35°C. MBC and MFC were determined for each standard organism and clinical isolate after determination of MICs of microorganisms has completed. The streaks were taken from the two lowest concentrations of the plant extract plates that exhibiting invisible growth (from inhibition zone of MIC plates) and subcultures onto the Mueller-Hinton agar plates (for bacteria) and Sabouraud's dextrose agar plates (for fungi). The plates were incubated at 35°C for 24 hours then examined for bacterial and fungal growth in corresponding to plant extract concentration. MBC and MFC

were taken as the concentration of the plant extract that did not exhibiting any bacterial or fungal growth on the freshly inoculated agar plates [21].

Statistical analysis: The data analysis was carried out using SPSS version 20 (IBM, Corporation, New York, USA). The mean diameter of inhibition zone between different categories was compared using one-way analysis of variance and Tukey's *post hoc* test. The mean value between extract and antibiotics was compared using independent sample t-test. The statistical significance was fixed at 0.05.

Ethical Consideration:

Approval was obtained from Medicinal and Aromatic Plants Research Institute (MAPRI), National Centre for Research

(NCR), Khartoum, Sudan to conduct the laboratory work.

Results and Discussion:

In this study the antimicrobial potential of methanol and aqueous extracts of the leaves and stem barks of *A. n. spp. tomentosa* at concentration of 100 mg/ml were assessed against standard strains and 133 clinical isolates obtained from urogenital infections. The results of identification tests, showed that the 133 clinical isolates were distributed as follows 29 *Escherichia coli*, 18 *Pseudomonas aeruginosa*, 16 *Proteus mirabilis*, 14 *Staphylococcus saprophyticus*, 13 *Neisseria gonorrhoeae*, 12 *Klebsiella pneumoniae*, 11 *Enterococcus spp.* and 20 *Candida albicans* (Table 1).

Table (1): Biochemical properties of examined clinical isolates

Character	Tested clinical isolates							
	E.c.	P.a.	P.m.	S.sa.	N.g.	K.p.	E.sp.	C.a.
Gram stain	G-ve	G-ve	G-ve	G+ve	G-ve	G-ve	G+ve	ND
Aerobic growth	+	+	+	+	+	+	+	+
Motility Test	+	+	+	-	-	-	-	ND
Catalase Test	+	+	+	+	+	+	-	ND
Coagulase Test	ND	ND	ND	-	ND	ND	ND	ND
Indole Test	+	-	-	-	-	-	-	ND
Methyl red Test	+	-	-	+	-	-	-	ND
Voges Proskauer	-	-	+/-	-	ND	+	+	ND
Oxidase Test	-	+	-	-	+	-	-	-
Urease Test	-	-	+	+	-	+	-	-
Citrate Test	-	+	+/-	-	ND	+	-	-
DNase Test	-	-	-	-	-	-	-	-
Nitrate Test	+	+	+	-	-	+	+	-
Germ tube Test	ND	ND	ND	ND	ND	ND	ND	+
Novobiocin(5µg)	S	ND	ND	R	ND	S	S	ND
Bacitracin	ND	ND	ND	ND	ND	R	ND	ND
Mannitol	+	+	-	+	-	+	-	-
Sucrose	+/-	-	-	+	-	+	-	+
Lactose	+	-	-	+	-	+	+	-
Acid from glucose	-	+	+	+	+	+	+	+
Gas from glucose	+	-	+	-	+	+	-	-

Key: E.c.: *Escherichia coli*; P.a.: *Pseudomonas aeruginosa*; P.m.: *Proteus mirabilis*; S.sa.: *Staphylococcus saprophyticus*; N.g.: *Neisseria gonorrhoeae* K.p.: *Klebsiella pneumoniae*; E.sp.: *Enterococcus* spp.; C.a.: *Candida albicans*; +: more than 80% of isolates had positive reaction. ; - : more than 80% of isolates had negative reaction. +/-: variable character. ; ND: not determined; R: Resistant S: Sensitive; G-ve: Gram negative reaction. ; G+ve: Gram positive reaction.

Activity of *A. n. spp. tomentosa* extracts on the standard strains

The yield percentages of leaves and barks methanol extracts of *A. n. spp. tomentosa* was 9.8% and 8.4% for each which was higher compared to the yield percentages of aqueous extracts of the same parts which produced 4% and 3.6%. The results of susceptibility were interpreted as active ($> 18\text{mm}$), moderate active ($14\text{-}18\text{ mm}$), and inactive ($< 14\text{ mm}$) [21, 30]. The leaves and barks methanol extracts showed high activity against standard strains where the diameters of inhibition zones ranged from 20 mm to 39 mm, while the leaves and barks aqueous extract

showed lower varied activity which was ranged from 20 mm to 23 mm (Table 2). The antimicrobial activity of *A. n. spp. tomentosa* extracts could be due to presence of bioactive components such as alkaloids, flavonoids, terpenoids, glycosides tannis, and phenolic compounds [31]. It is obvious that leaves and barks extracts of *A. n. spp. tomentosa* inhibited all the standard strains examined in this study. These results are pararell to those findings of El-Kamali and Awad El-Karim, [19], Saba *et al.* [18].

Table (2): Antimicrobial activity of *A. n. spp. tomentosa* against standard strains

Part Used	Solvent system	Conc./ mg/ml	Yield %	*Test standard strains MDIZ/mm** of 100 mg /ml extract.					
				E.c	S.a	P.a	K.P	P.v	C.a
Leaves	Methanol	100	9.8	27	33	39	36	30	21
	Aqueous	100	4	20	23	22	23	20	22
Stem Barks	Methanol	100	8.4	28	30	38	30	28	20
	Aqueous	100	3.6	22	21	23	22	21	21

Key: MDIZ: Mean Diameter of Inhibition Zone. *E.c. = *Escherichia coli* (ATCC 25922), Ps.a. = *Pseudomonas aeruginosa* (ATCC 27853), K.p.=*Klebsiella pneumoniae* (ATCC 53657), P.v. = *Proteus vulgaris* (ATCC 6380), S.a. = *Staphylococcus aureus* (ATCC 25923) and C. a. = *Candida albicans* (ATCC 7596).

**Standard antibiotics vs tested
extracts on the standard
strains:**

Statistical analysis revealed that there were highly statistical significant differences between activity of 100 mg/ml from *A. n. spp. tomentosa* extracts compared to the effect of gentamicin 40 µg/ml on the standard bacteria ($P = 0.000$). There was also statistical significant difference between effects of gentamicin 20 µg/ml and effect of *A. n. spp. tomentosa* extracts on the standard bacteria ($P = 0.000$). Extracts *A. n. spp. tomentosa* exhibited antibacterial activity which was higher than activity of ampicillin 40 µg/ml on the standard bacteria, this difference in the activity was highly

statistically significant ($P = 0.000$). The activity of leaves and barks methanol extracts were found to be higher compared to the activity of leaves and barks aqueous extracts, where these differences were statistically significant ($P = 0.000$). There was no statistical significant difference between effects of leaves aqueous extract and effect of barks aqueous extract on the standard bacteria ($P = 1.000$) (Table 3, fig 1). Regarding antifungal activity, *A. n. spp. tomentosa* leaves and barks extracts showed activity towards standard strain of *Candida albicans* which falls between 5 mg/ml and 10 mg /ml of Candizole (Table 4, fig 2).

Table (3): Antibacterial activity of standard antibiotics against the standard bacterial strains

Antibiotics	Concentration Used µg /ml	*Tested standard strains /**DIZ mm				
		E.c.	S.au.	Ps.a.	K.p.	P.v.
Gentamicin	40	28	28	21	23	24
	20	26	29	19	21	22
	10	16	24	18	20	20
	5	—	20	17	18	18
Ampicillin	40	14	23	15	20	-
	20	-	21	-	18	-
	10	-	18	-	16	-
	5	-	14	-	14	-

Key: E. c. = *Escherichia coli* (ATCC 25922), S. a. = *Staphylococcus aureus* (ATCC 25923), Ps. a. = *Pseudomonas aeruginosa* (ATCC 27853), K. p. = *Klebsiella pneumoniae* (ATCC 53657), P. v. = *Proteus vulgaris* (ATCC 6380) and C.a. = *Candida albicans* (ATCC 7596). **DIZ/ mm: Diameter of Inhibition Zone in Millimeters. µg /ml= microgram/milliliter

Table (4): Antifungal activity against standard *Candida albicans*

Antifungal Drug	Concentration mg/ml	<i>Candida albicans</i> DIZ (mm)
Candizole	40	26
	20	25
	10	23
	5	20

Key: *Candida albicans*. **DIZ/ mm: Diameter of Inhibition Zone in Millimeters. mg.= milligram.

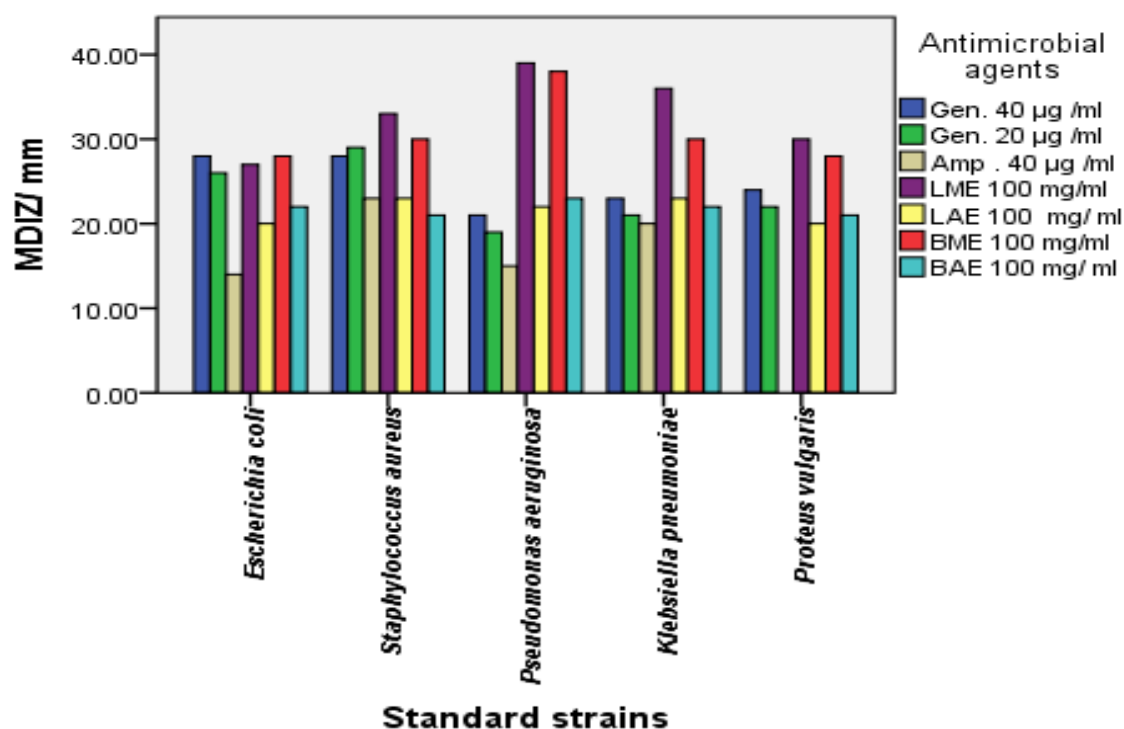


Fig. 1: Activity of standard antibiotics and tested extracts versus standard strains

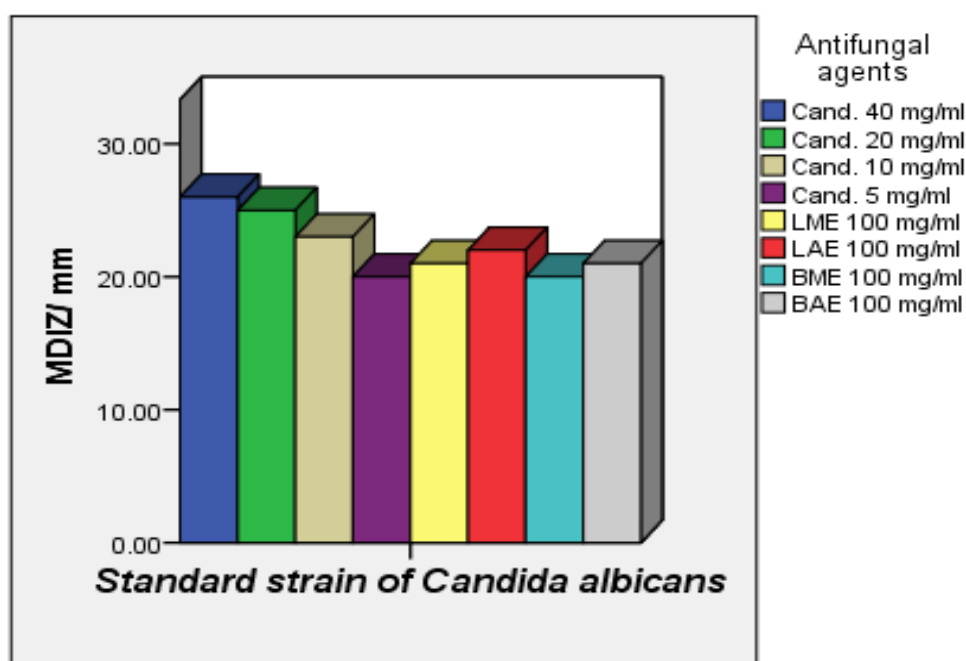


Fig. 2: Activity of standard antifungal agents and tested extracts versus standard fungus

Activity of *A. n. spp. tomentosa* extracts on the clinical isolates

The methanol extracts of leaves and barks of *A. n. spp. tomentosa* were only assessed against clinical isolates. The antimicrobial activity of extracts on the clinical isolates was expressed as MDIZ/ mm $\pm$ SD. The highest mean diameters of inhibition zones obtained by leaves methanol extract on the clinical isolates was 24.10 $\pm$ 2.94 mm for *Escherichia coli*, 23.92 $\pm$ 3.95 mm for *Neisseria gonorrhoeae*, 23.45 $\pm$ 8.31 mm for *Enterococcus* spp. and 23.00 $\pm$ 8.15 mm for *Klebsiella pneumoniae*, while the rest isolates showed semi-equal activity which was 22.94 $\pm$ 6.84 mm, 22.72 $\pm$ 4.97 mm and 22.36 $\pm$ 7.40 mm for *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Staphylococcus saprophyticus*, respectively. Clinical isolates of *Neisseria*

gonorrhoeae revealed higher susceptibility toward barks methanol extract which was 25.54 $\pm$ 2.47 mm, whereas the lower susceptibility was shown by *Enterococcus* spp. which was 23.36 $\pm$ 8.18 mm. Barks methanol extract showed equivalent MDIZs on the rest isolates which was 24.24 $\pm$ 3.27 mm, 24.17 $\pm$ 4.13 mm, 24.06 $\pm$ 6.73 mm and 24.00 $\pm$ 7.83 mm for *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Staphylococcus saprophyticus* and *Klebsiella pneumoniae* (Table 5). Antifungal activity of *A. n. spp. tomentosa* methanol extracts on the clinical isolates of *Candida albicans* was 23.20 $\pm$ 3.44 mm for the leaves and 25.25 $\pm$ 1.92 mm for the barks, where this activity was higher than 10 mg/ml of candizole (Table 6).

Table (5): Antibacterial activity of *A. n. spp. tomentosa* extracts and standard antibiotics on clinical isolates

No. of Clinical isolates	MDIZ/ mm $\pm$ SD of tested extracts and standard antimicrobial agents				
	LME 100 mg/ml	BME 100 mg/ml	Gent. 40 μ g/ml	Gent. 20 μ g/ml	Amp. 40 μ g/ml
<i>E. c.</i> (29)	24.10 $\pm$ 2.94	24.24 $\pm$ 3.27	28.0	25.0	14.0
<i>Ps. a.</i> (18)	22.72 $\pm$ 4.97	24.17 $\pm$ 4.13	21.0	19.0	15.0
<i>P. m.</i> (16)	22.94 $\pm$ 6.84	24.06 $\pm$ 6.73	24.0	22.0	16.0
<i>S. s.</i> (14)	22.36 $\pm$ 7.40	24.00 $\pm$ 7.31	28.0	24.0	18.0
<i>N. g.</i> 13	23.92 $\pm$ 3.95	25.54 $\pm$ 2.47	21.85	18.5	14.0
<i>K. p.</i> 12	23.00 $\pm$ 8.15	24.00 $\pm$ 7.83	23.0	21.0	15.0
<i>E. spp.</i> 11	23.45 $\pm$ 8.31	23.36 $\pm$ 8.18	21.36	19.5	17.0
Total: 113					

Key: E.c. = *Escherichia coli*, Ps.a. = *Pseudomonas aeruginosa*. P.m = *Proteus mirabilis*; S.s. = *Staphylococcus saprophyticus*; N.g.= *Neisseria gonorrhoeae*.; K.p.= *Klebsiella pneumoniae*, E.spp.= *Enterococcus sp.*; C. a. = *Candida albicans* MDIZ/ mm: Mean Diameter of Inhibition Zone SD: Standard Deviation; LME: Leaves Methanol extract; BME: Barks Methanol extract; Gent.: Gentamicin; Amp.: Ampicillin.

Table (6): Antifungal activity of *A. n. spp. tomentosa* extracts and standard antifungal drug on clinical isolates

No. of Clinical isolates	MDIZ/ mm $\pm$ SD of tested extracts and standard antimicrobial agents				
	LME 100 mg/ml	BME 100 mg/ml	Cand. 40 mg/ml	Cand. 20 mg/ml	Cand. 10 mg/ml
<i>Candida albicans</i> (20)	23.20 $\pm$ 3.44	25.25 $\pm$ 1.92	26	25	23

Key: LME: Leaves Methanol extract; BME: Barks Methanol extract; Cand. : Candizole.

Effects of standard antibiotics compared to the tested extracts on the clinical isolates

The outcomes of statistical analysis illustrated that there are highly significant differences between the effects of (100 mg/ml) methanol extracts of *A. n. spp. tomentosa* and the effect of standard antibiotics on the clinical isolates ($P = 0.000$). The effect of gentamicin 40 µg/ml was stronger compared to the effect of leaves and barks methanol extracts on the clinical isolates where the differences were statistically significant ($P = 0.964$ for leaves and $P = 0.998$ for barks). The effect of leaves and barks methanol extracts on the clinical isolates was stronger compared to effect of ampicillin 40 µg/ml and the difference was statistically significant ($P = 0.000$). The

effect of barks methanol extract on the clinical isolates was also stronger compared to the effect of leaves methanol extract where the difference was statistically significant ($P = 0.871$) (Fig. 3).

Results of MIC, MBC and MFC of *A. n. spp. tomentosa* extracts on standard strains

The MIC showed by the methanol leaves extract toward standard bacteria was ranged from 6.25 mg/ml to 12.50 mg/ml, while the MIC of barks methanol was ranged from 3.125 mg/ml to 6.25 mg/ml. On the other hand, the MBC of leaves methanol extract on the standard bacteria was ranged from 12.50 mg/ml to 25 mg/ml, whereas MBC of methanol barks extract was 6.25 mg/ml to 12.50 mg/ml. The MIC showed by leaves and barks extracts on standard fungi was 6.25 mg/ml for while the MFC of

the same extracts 12.50 mg/ml for leaves and barks (Table 7).

Results of MIC, MBC and MFC of *A. n. spp. tomentosa* extracts on the clinical isolates

The MIC exhibited by leaves methanol extract on the bacterial isolates was ranged from 3.125 mg/ml to 12.50 mg/ml, while the MIC of the barks extract was ranged from 3.125 mg/ml to 6.25 mg/ml. On the other hand, the

MBC of the leaves was ranged from 6.25 mg/ml to 25 mg/ml, whereas the MBC of the bark was ranged from 6.25 mg/ml to 12.50 mg/ml (Table 8). The MIC of methanol extracts toward *Candida albicans* was 6.25 mg/ml for leaves and bark, while the MFC of methanol extracts on the standard fungi was 12.25 mg/ml (Table 9).

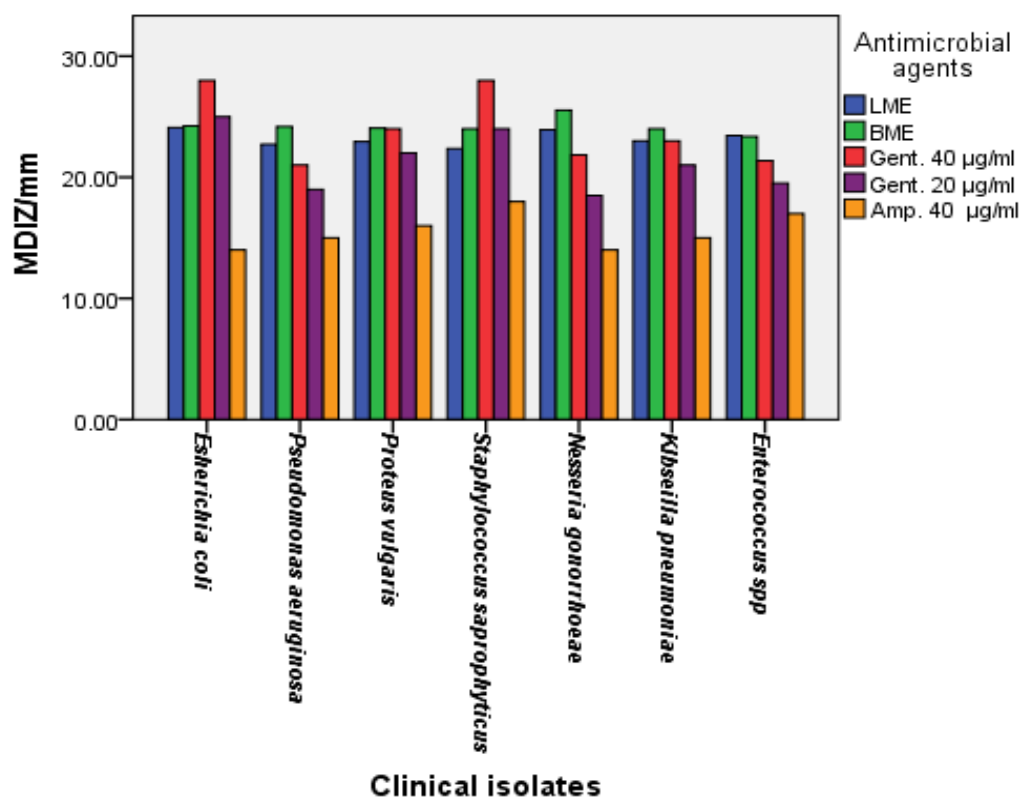


Fig. 3: Activity of tested extracts and standard antibiotics on the clinical isolates

Table (7): MIC, MBC and MFC readings of methanol extracts of *A. n. spp. tomentosa* on the standard strains.

	MICs, MBCs and MFC values per mg /ml											
Standard organisms	E.c.		Ps.a.		P.v.		S.au.		K.p.		C.a.	
Antimicrobial assay	MIC	M	MIC	M	MIC	M	MIC	M	MIC	M	MIC	M
Leaves extract	6.25	12.50	12.50	25	12.50	25	12.50	25	12.50	25	6.25	12.50
Barks extract	6.25	12.50	3.125	6.25	6.25	12.50	6.25	12.50	6.25	12.50	6.25	12.50

Key: MIC: Minimum inhibitory concentration; MBC: Minimum Bactericidal concentration; MFC: Minimum Fungicidal concentrations. E.c. = *Escherichia coli* (ATCC 25922), S. a. = *Staphylococcus aureus* (ATCC 25923), Ps. a. = *Pseudomonas aeruginosa* (ATCC 27853), K. p. = *Klebsiella pneumoniae* (ATCC 53657), P. v. = *Proteus vulgaris* (ATCC 6380) and C.a. = *Candida albicans* (ATCC 7596).

Table (8): MIC and MBC readings of methanol extracts of *A. n. spp. tomentosa* against clinical bacterial isolates.

Plant part	MICs and MBCs values of bacterial clinical isolates per mg /ml													
	E.c.		Ps.a.		P.m.		S.sa.		N.g.		K.p.		Ent. spp.	
	MIC	MB	MIC	MB	MIC	MB	MIC	MB	MIC	MB	MIC	MBC	MIC	MB
Leaves extract	3.125	6.25	3.125	6.25	12.50	25	3.125	6.25	3.125	6.25	12.50	25	3.125	6.25
Barks extract	6.25	12.50	3.125	6.25	3.125	6.25	3.125	6.25	3.125	6.25	3.125	6.25	6.25	12.50

Key: E.c. = *Escherichia coli*, Ps.a. = *Pseudomonas aeruginosa*, P.m. = *Proteus mirabilis*; S.sap. = *Staphylococcus saprophyticus*; N.g.= *Neisseria gonorrhoeae*.; K.p.= *Klebsiella pneumoniae*; E.spp.= *Enterococcus* spp.

Table (9): MIC and MFC of methanol extracts of *A. n. spp. tomentosa* on the clinical isolates of *Candida albicans*.

Plant part	MIC and MFC values of clinical isolates of <i>Candida albicans</i> per mg /ml	
	MIC	MFC
Leaves extract	6.25	12.50
Barks extract	6.25	12.50

MFC: Minimum Fungicidal Concentration

Conclusion

Leaves and barks methanol extracts of *A. n. spp. tomentosa* exhibited remarkable activity on the standard strains and clinical isolates, however, bark methanol extracts of was found to be more effective than leaves extract. This may elucidate the effectiveness of the plant part as a source of natural remedy. The antimicrobial efficacy of this plant may relies on the presence of certain chemical substances such as alkaloids, tannin, flavonoids and phenolic compounds that are responsible

for production of definite physiological changes on the microorganisms. Further phytochemical studies and clinical trials are needed to explain how the plant extracts kill the microorganisms.

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Pattern Susceptibility of Antibiotics against Clinical Isolates from Diabetic Foot Ulcer in Khartoum State

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Abstract

Diabetes constitutes a global emergency with case numbers increasing exponentially every year. Diabetic Foot Ulcers, DFUs, are one of the major complications of Diabetes Mellitus, DM, and are a significant risk factor for lower extremity amputation.

This study is a cross sectional study was performed in Khartoum state for isolation and characterization of aerobic bacteria and antimicrobial pattern among diabetic patients with foot ulcers. Wound swabs were collected from 50 patients and were cultured and bacterial isolates were identified using standard methods including Gram stain and biochemical test according to Feltham, Cowan and Steel methods. Then Chromogen agar was used as a confirmation for identification. Antibiotic susceptibility Testing was conducted on bacterial isolates. The antimicrobial susceptibility Testing was performed using the agar diffusion method according to Kirby-Bauer.

A total of 70 bacteria were isolated from 50 clinical samples, which revealed 47, [94%] growth and 3 [6%] no growth, among all isolates, 8 [11.4%] were Gram-positive bacteria, including *Staphylococcus aureus* 6 [9%], *Staphylococcus saprophyticus* 1[1%], *Enterococcus fecalis* 1[1%]. While Gram-negative bacteria constituted 62 [88.6%] of all the isolates, including *Proteus spp* 25 [40.3%], *Pseudomonas spp* 15[24.2%], *Klebsiella spp* 8 [12.9%], *E. coli* 6 [9.7%], *Citrobacter spp* 6 [12.7 %] and *Serratia spp* 2 [3.2%]. The antimicrobial susceptibility test showed 100% resistance to Oxacillin, 56%. Ampicillin/Cloxacillin, 57%, Ampicillin, 54%, Cefixime, 54%, Cephalexin, 35.2 %, Gentamicin, 26%, Ampicillin, 18.3% Doxycycline hydrochloride, 18.3%, Carbenicillin, 14%, Doxycyclin, 12.7%, Piperacillin, 7%, Meropenem and 5.6% to Erythromycin.

Keywords: Oxacillin, Ampicillin/Cloxacillin, Cefixime, Cephalexin, Gentamicin, Meropenem.

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Introduction:

Diabetes Mellitus DM is a chronic disorder that affects a large number of people globally and is a major public health problem ^[1] Diabetics are more susceptible to infections due to increased glucose levels and suppressed immune response as well as the neuropathy and decreased blood flow to extremities that lead to slow-healing wounds ^[2]. The individuals with diabetes have at least a 10-fold greater risk of being hospitalized for soft tissue and bone infections of the foot than individuals without diabetes ^[3]. Wound is defined as any anatomical and physiological disruption in skin structure leading to skin cell damage. Thereby, wound healing is a complex and overlapping process of reaction and interaction among cells and mediators to return natural skin ability, beginning immediately after skin loss. Inflammation, proliferation and remodeling are three overlapping and continuous

wound healing phases. Diabetes wounds infection is major long term impact on the morbidity, mortality and quality of patients ^[4]. DFUs (Diabetic Foot Ulcers) are one of the major complications of DM and are a significant risk factor for lower extremity amputation. Fluctuating blood sugar and hypoxia from poor circulation may impair the ability of white blood cells to destroy pathogenic bacteria increasing infection risk ^[5]. The increasing association of MDR pathogens with diabetic wound further compounds the challenge faced by the Physician or the surgeon in treating diabetic ulcers without resorting to amputation. Infection with MDR, ([Multi-Drug Resistance), pathogens is also responsible for the increased duration of hospitalization and cost of management ^[6]. MDR profiles can be transmitted to human through the food chain adding serious burden to human health ^[7]. Previously the most commonly

bacteria isolated from DFUs for Gram-positive bacteria were *S. aureus*, followed by *Staphylococcus saprophyticus*, *Staphylococcus epidermidis*, *Streptococcus agalactiae*, and *Streptococcus pneumonia* and the most commonly isolated Gram negative bacteria were *Proteus spp* followed by *E.coli*, *Pseudomonas spp*, and *Citrobacter spp*. Today MDR has rendered most of the original antibiotics obsolete for many infections. The emergence of pathogenic bacteria resistant to most, if not all, currently available antimicrobial agents has become a critical problem in modern medicine, particularly because of the concomitant increase in immune-suppressed patients^[8].

Materials and methods:

Study area

Study was confined on diabetic patients who have wound infection in different hospital Khartoum state [Arabian specialized hospital, Gaber Abualez Center and Al eamtiaz Center].

Study design

Cross sectional study hospital based for isolation of an aerobic bacteria associated with diabetic foot ulcers wound infections.

Sample size and study population

Study population is all Diabetic patients which has wound infection in Al Khartoum State all group of age. Size of sample include 50 inclusion criteria patients who suffer wound infections and another group that had no diabetics foot ulcers.

Data collection

Data were collected by using designed in structured questionnaire to obtain data from the patients.

Ethical clearance: The research approved and received ethical clearance from the management of hospital. Permissions were taken from patients and the research ethic committee. Sample obtained were only diabetic wound infection patients.

Methods

Specimens Collection:

Clinical samples were collected in this study included 50 wound cotton swabs by using sterile cotton swabs then Deeping in amies' transport media. All steps were performed under aseptic Technique.

Culture media:

All Collected specimens were cultured directly on blood agar and MacConkey agar for primary isolation. Then plate were inoculated and incubated for 24hours [hrs] at 37 ° C. Gram stain and other biochemical tests were also performed according to conventional identification and then identified according to Feltham and Cowan Steel methods [9]. Then Chromogen agar was used as confirmation of identification Gram- positive and negative bacteria.

Antimicrobial susceptibility test:

All isolated bacteria were examined for their susceptibility to a number of antibiotics by disk diffusion

technique [Kirby-Bauer method]. The isolated bacteria were subcultured onto nutrient agar and incubated at 37 ° C for 24 hrs. Then were made bacterial suspension [by taken a different colony from nutrient agar and emulsified in normal saline] and matched with, 5 McFarland standard [BaSO₃ was formed by mixing BaCl and H₂SO₄] then sterile cotton swab was immersed and inoculated on a plate of Muller-Hinton agar. The inoculation was evenly distributed all over the plate. Commercially prepared antibiotic discs of HI-media., bioanalyses were placed on the surface of the media using sterile forceps. Discs were gently pressed to ensure full with the medium. Plates were incubated at 37 ° C for 24 hrs. The zone of growth inhibition around each disc was measured in millimeters and the result was reported sensitive, intermediate or resistant. Antibiotics was used in susceptibility test, Antibiotics used

for Gram positive bacteria include Ampicillin [10µg], Oxacillin[1µg], Amoxicillin[25µg], Gentamycin [10µg], Cefotaxime [30µg], Erythromycin [15µg]. Furthermore antibiotics were used for Enterobacteriaceae Ampicillin [10µg], Amoxicillin [25µg], Gentamycin [10µg], Cefotaxime [30µg], Cephalexin [30µg], Cefixime [5µg]. On other hands antibiotics were used for *Pseudomonas spp* Carbenicillin [100µg], Meropenem [10µg], Doxycycline [30µg], and Piperacillin [100µg].

Results

A total of 50 wound swab of diabetic patient with DFUs were Collected. The specimens were collected from different hospital in Khartoum state during a period of December to March. Out of the 50 patients there is 31 male and 19 female, 30 specimens from male yield growth and 17

specimen from female yield growth as showing in Table [1]

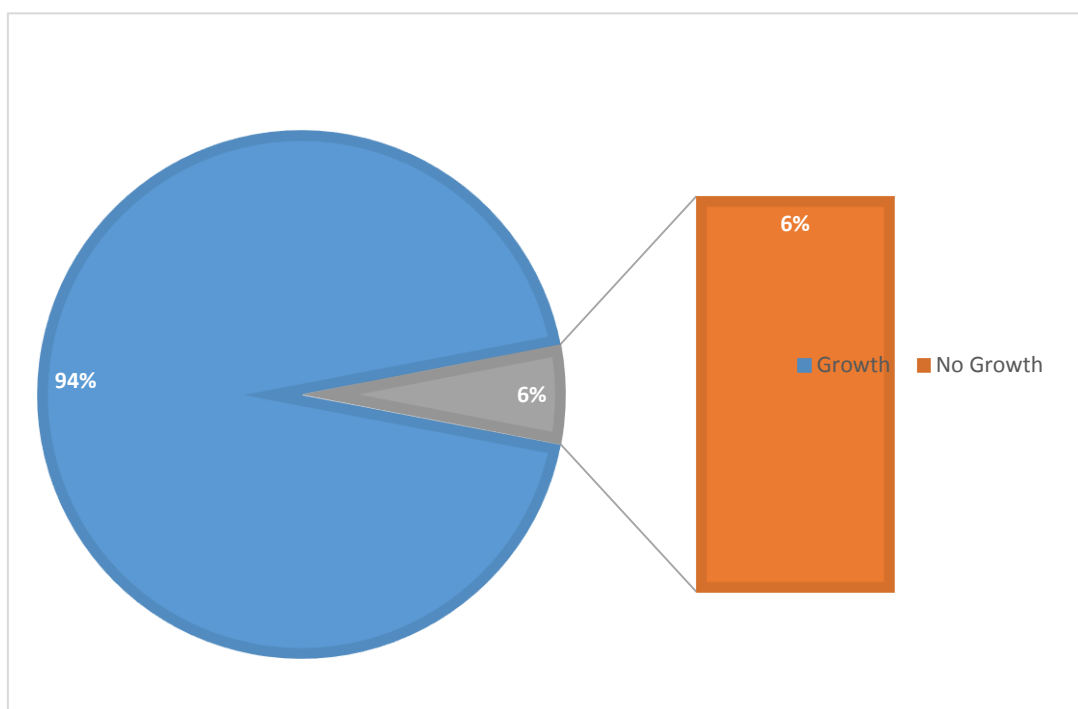
Out of the 50 patients specimens from 47 patients were positive bacterial growth 3 patients were sterile [no growth] as showing in fig1.

Overall number of clinical isolated from 47 [95.7] specimens was 70 bacteria were isolated. Number of Gram positive was 8 [11.4] where remaining was Gram negative 62 [88.6] Table [3].

Gram positive bacteria including *Methicillin* resistance *staphylococcus aureus* 6 [75%], *S. saprophyticus* 1 [12.5%] and *Enterococcus faecalis* 1 [12.5%] Table [2]. Gram negative bacteria include *proteus spp* 25 [40.3%], *pseudomonas spp* 15[24.2%], *klebsiella spp* 8 [12.9%], *E. coli* 6 [9.7%], *citrobacter spp* 6 [12.7 %] and *serratia spp* 2 [3.2%] Table [5]. The results of antimicrobial susceptibility test are shown in Tables 6, 7 and 8.

Table [1]: Cross tabulation between Gender and Growth

Gender	Frequency	Number of Growth plate	Percentage %
Male	31[62%]	30	96%
Female	19[38%]	17	89%
Total	50[100%]	47	

**Figure [1]: Percentage of yields growth of cultured swabs from diabetic food ulcer**

$P = 0.54$ [Insignificant more than 0.05 Level]

Table [2]: Cross tabulation between Age and cultivation

Age	cultivation		Total [percentage]
	Growth	NO Growth	
from 20 to 30	2	0	2[4.0%]
from 30 to 40	3	0	3[6.0%]
from 40 to 50	15	1	16[32.0%]
from 50 to 60	10	2	12[24.0%]
from 60 to 70	17	0	17[34.0%]
Total	47[94.0%]	3[6.0%]	50[100.0%]

$P = 0.35$ [Insignificant more than 0.05 Level]

Table [3]: frequency and percentage of Gram reaction for bacterial isolates from diabetic food ulcer

Types of bacteria	Frequency	Total %
Gram positive	8	11.4%
Gram negative	62	88.6%
Total	70	100%

Table [4]: Frequency and percentage of Gram positive bacteria isolated from diabetic food ulcer

Types of bacteria	Frequency	Gram-positive %	Total%
<i>MRSA</i>	6	75%	9%
<i>S.saprophyticus</i>	1	12.5%	1%
<i>E. faecalis</i>	1	12.5%	1%
Total	8	100%	11%

Table [5]: Frequency and percentage of Gram negative bacteria isolated from DFUs:

Types of bacteria	Frequency	%	Total %
<i>Proteus spp</i>	25	40.3%	36%
<i>Pseudomonas aeruginosa</i>	15	24.2%	21%
<i>Klebseilla spp</i>	8	12.9%	11%
<i>E. coli</i>	6	9.7%	9%
<i>Citrobacter spp</i>	6	9.7%	9%
<i>Serratia spp</i>	2	3.2%	3%
Total	62	100%	89%

Table [6]: Antimicrobial susceptibility pattern of Gram positive bacteria isolated from diabetic foot ulcers by using Kirby Bauer techniques:

Bacteria isolated Antibiotics		<i>S.aureus</i>	<i>S.saprophyticus</i>	<i>Enterococcus faecalis</i>	Total
AX	S	16.6%	0%	0%	12.5%
	I	0%	0%	0%	0%
	R	83.4%	100%	100%	87.5%
AMP	S	33.4%	0%	0%	25%
	I	0%	0%	0%	0%
	R	66.4%	0%	100%	75%
OX	S	0%	100%	0%	12.5%
	I	0%	0%	0%	0%
	R	100%	87.50%	100%	87.5
CTX	S	33.4%	0%	0%	25%
	I	0%	0%	0%	0%
	R	66.4%	100%	100%	75%
AX	S	16.6%	0%	0%	12.5%
	I	0%	0%	0%	0%
	R	83.4%	100%	100%	87.5%
GEN	S	33.4%	0%	0%	25%
	I	0%	0%	0%	0%
	R	66.4%	100%	100%	75%
E	S	16.6%	0%	0%	12.5%
	I	0%	0%	0%	0%
	R	83.4%	100%	100%	87.5%

AX= Amoxicillin

AMP= Ampicillin

OX = Oxocillin

CTX= Cefotaxime

GEN=Gentamycin

CFM= Cefoxime

E= Erythromycin

Table [7]: Antimicrobial susceptibility pattern of Gram negative bacteria isolated from DFUs by using Kirby Bauer techniques:

Antibiotic		<i>E.coli</i>	<i>Klebsiella Spp</i>	<i>Citrobacter SPP</i>	<i>proteus spp</i>	<i>Serratia spp</i>	Total
AX	S	0%	25%	16.4%	28%	0%	21.3%
	I	0%	0%	0%	8%	0%	4.3%
	R	100%	75%	83.6%	64%	100%	74.4%
CFM	S	0%	25%	0%	40%	50%	27.7%
	I	33%	25%	0%	16%	0%	17%
	R	67%	50%	100%	44%	50%	55.3%
CTX	S	17%	37.5%	33.4%	40%	50%	36.2%
	I	0%	0%	16.6%	8%	0%	6.4%
	R	83%	62.5%	50%	52%	50%	57.4%
E	Not effective		Not effective	Not effective	Not effective	Not effective	Not effective
GEN	S	67.8%	63%	50%	72%	100%	68.1%
	I	0%	0%	33%	4%	0%	6.4%
	R	32.2%	37%	17%	24%	0%	25.5%
CN	S	33%	37.5%	33.3%	48%	100%	44.7%
	I	0%	0%	0%	0%	0%	0%
	R	67%	62.5%	66.7%	52%	0%	55.3%

AX= Amoxicillin CFM= Cefoxime CTX= Cefotaxime E= Erythromycin
 GEN=Gentamycin CN= Cephalexin

Table [8]: Antimicrobial susceptibility pattern of *pseudomonas spp* isolated from DFUs by using Kirby Bauer techniques:

Antibiotic	Sensitive	Intermediate	Resistance
MEM	60%	0%	40%
DO	13.4%	0%	86.6%
PY	33.3%	0%	66.7%
PRL	33.3%	0%	66.7%
GEN	33.3%	0%	66.7%
CTX	33.3%	33.3%	33.4%
CN	*	*	*
CFM	*	*	*
E	*	*	*
AMP	*	*	*

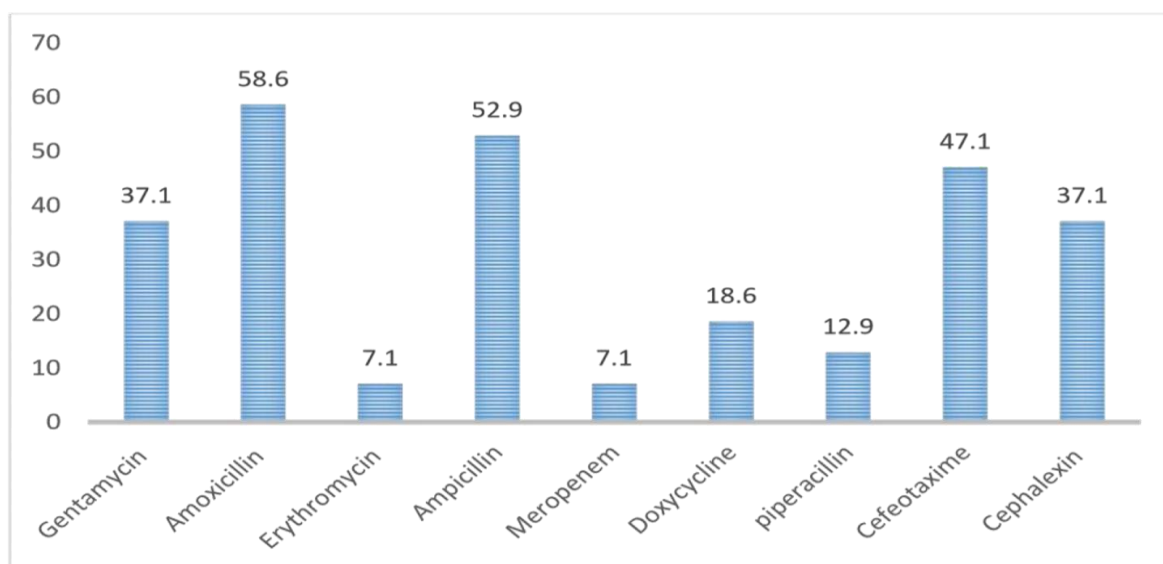


Fig 2 Resistance of bacterial isolates from diabetic foot ulcer

Discussion

DFUs are one of the major complications of DM and are a significant risk factor for lower extremity amputation, the increasing association of MDR pathogens with diabetic wound further compounds the challenge faced by the physician or the surgeon in treating diabetic ulcers without resorting to amputation [6, 10]. Today MDR has rendered most of the original antibiotics obsolete for many infections, the emergence of pathogenic bacteria resistant to most, if not all, currently available antimicrobial agents has become a critical problem in modern medicine, particularly because of the concomitant increase in immune-suppressed patients [11].

Although specimens from 94% of our diabetic patients had positive bacteria growth and 6% had no growth because of prior used of antibiotics. Out of the

total isolated bacteria Gram negative were the predominated isolates; it accounted [88.6%], while the remaining [11.4%] were Gram positive this result approximately in agreed with previous study was done in Sudan, Egypt and India by [12, 3, 13] respectively. Our findings results were disagreed with that reported in Pakistan that Gram positive bacteria were high prevalence [14], the variance may be due to selection of patients that exhibited sensory neuropathy and septic conditions during collection specimens. Interestingly out of total the most frequent bacterial strains in the present study was *Proteus spp* it is accounted 25 [35.7%] followed by *Pseudomonas aeruginosa* 15[21.4%]. Out of all Gram positive *S.aureus* is most common isolated 6 [75%] and they were MRSA, this results were agreed with studies was done in Brazil, USA, Iran [3,

^{15, 16]} respectively. Our present study showed that the most common Gram negative bacteria isolate is *Proteus spp* 25[40.3%] followed by *Pseudomonas spp* 15[24.2%], *klebsella spp* 8[12.9%], *E. coli* 6[9.7%], *Citrobacter spp* 6 [9.7 %] and *serratia* 2 [3.2%] as same observations with study was done in Malaysia^[17].

The Antimicrobial pattern of the current study for Gram positive showed that 87% resistance Oxacillin, Amoxicillin [75%], Erythromycin [75%], Cefotaxime [75%], Ampicillin [62.5%], Gentamycin [37.5%]. Five *Staphylococcus* were isolated and the antimicrobial pattern showed resistance to Oxacillin, Amoxicillin, Ampicillin, Cefotaxime, Erythromycin, and Gentamycin exhibited [100%], [80%], [60%], [60%], [60%] and [60%] respectively. These results were disagreed with

study done in Egypt ^[6] the different in result may be due to sample size and techniques used and manufactures company of antibiotics and type of strains used. Importantly the *Enterobacteriaceae* [*Proteus spp*, *Citrobacter spp*, *Klebsella spp* and *E.coli*] the resistant of antibiotics as following

Amoxicillin 78.7%, Cephalexin 57.4%, Cefotaxime 55.3%, Cefixime 55.3% and low resistance to Ampicillin 46.8%, Gentamycin 27.6%. This result is agree with Perim et al ^[1]. Antimicrobial pattern of *Pseudomonas aeruginosa* show resistant 100% resistant to Erythromycin, Ampicillin, Cefixime, Cefotaxime 66.6%, Gentamycin 73.3%, Meropenem 40%, and Doxycycline 66.6%. The result is disagree with Sivanmaliappan and Sevanan ^[18]. Their result of resistance is much lower than our results

because to excessive use of antibiotics today.

Conculsion:

According to finding in the study, we conclude thesis:

Different types of aerobic bacteria were isolated from DFUs. Including Gram- positive Cocci and Gram-negative rods. The *Proteus species* was the most frequent bacteria in DFUs followed by *Pseudomonas spp* and, *klebseilla spp*, Other less frequent bacteria included: *Staphylococcous aureus*, *Escherichia coli*, *citrobacter spp*, *serratia spp* and *enterococcus faecalis*. All of by *Staphylococcus aureus* isolates were Oxacillin resistant therefore are MRSA. Gentamycin was the most effective antimicrobial therapy against Gram- negative and Gram-positive microorganisms. Meropenium was the most effective

antimicrobial therapy against *Pseudomonas spp*.

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High Sensitive C-reactive Protein as Prognostic Marker for Cardiovascular Disease among Sudanese Hypertensive Patients.

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Abstract:

Background: Highly sensitive C-reactive protein, hs-CRP, is an acute phase protein which represents a sensitive systemic marker of inflammation and tissue damage especially in cardiovascular disease. Hypertension is a common health problem throughout the world and one of the major risk factors for Cardiovascular Disease, CVD, therefore this study aims at the evaluation of this highly sensitive C-reactive protein, hs-CRP, as a prognostic marker for CVD in hypertensive patients. **Materials and Methods:** A case control study was carried out in Abuda center during the period from January to February 2016. In this study 50 hypertensive patients were enrolled (13 males and 37 females) and 50 normotensive as a control group. Serum hs-CRP levels, TG and cholesterol were measured by Cobas, 311 automated analyzer. The data were, then, analyzed by using SPSS[®] software computer program. **Results:** The mean serum level of hs-CRP, cholesterol and triglyceride showed a significant increase in hypertensive patients compared to the control groups, assuming p-values of (0.002, 0.000 and 0.000) respectively. In case of the gender, age, BMI and duration of disease categories, the results indicated an insignificant increase in hs-CRP among females, overweight subjects and those of more than 5 years duration of HTN, with p-values of (0.382, 0.387 and 0.166), respectively. Females, overweight group, more than 60 years and more than 5 years duration of HTN showed insignificant increase in diastolic and systolic BP. The mean TG level showed insignificant increase in males, normal weight group and more than a 5 year duration of HTN with p-values of (0.862, 0.509 and 0.921), respectively, and insignificant decrease in patients more than 60 years with a p-value of (0.959). **Conclusion:** the study concluded that the hypertensive patients have increased hs-CRP, cholesterol and triglyceride compared to control HTN group with p-values of (0.862, 0.509 and 0.921), respectively, and an insignificant decrease in patients more than 60 years with a p-value of (0.959).

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Keywords: hs-CRP, HTN, BMI, CVD, triglyceride, cholesterol

Introduction:

Hypertension is defined as blood pressure, BP, equal or above 140/90 mmHg ^[1]. High blood pressure is the leading cause of cardiovascular disease and death globally. It is associated with at least 7.6 million deaths per year worldwide, (13.5% of all deaths), making it the major risk of CVD. The importance of BP as a modifiable risk factor for CVD is well recognized and many effective and inexpensive BP lowering treatments are available, therefore hypertension control and prevention of subsequent morbidity and mortality clearly should be achievable ^[2]. Sudan is considered one of leading countries in Africa for prevalence of HTN. However, a proper national registry on HTN is not available in Sudan. Nevertheless, a recent study showed an increasing incidence of HTN ^[3]. There is a compelling experimental and clinical evidence

suggesting crucial role for inflammation in initiation and progression of atherosclerosis. Among all blood biomarkers C-reactive protein, the classical acute phase reactant that can be measured with high sensitivity assays seems to be the most promising candidate ^[4]. CRP is a member of the pentraxin family of protein and is synthesized by liver and also produced by cells in vascular wall such as endothelial cells, smooth muscle cells and adipose tissue ^[5]. Its plasma half-life is 19 hours and it catabolized by hepatocytes ^[6]. The possible mechanistic role of CRP in plaque deposition is highly complex, exerting proatherogenic effects in atherosclerosis. CRP may facilitate monocyte adhesion and transmigration into the vessel wall, a critical early step in the atherosclerotic process ^[7]. CRP, an inducible protein secreted in response to inflammatory stimulus,

binds to pathogens and activates the complement to enhance opsonisation and clearance, even before the production of specific IgM or IgG. CRP bound to multivalent ligand initiates the assembly of C3 convertase through the classical pathway, which leads presentation of ligand with opsonic complement fragments ^[8]. Atherosclerosis is a chronic inflammatory disease process that occurs over a number of years and contributes to approximately 50% of all deaths in modern western societies ^[9]. A high level of plasma lipids, particularly low-density lipoproteins (LDL), is a major cause of vascular damage. Mechanistic studies suggest that they play a role in relevant feature for initiation and progression of lesions, such as endothelial dysfunction, intimal disorganization and thickening ^[10]. Triglyceride lipolysis by LPL on endothelial cell

surface elaborates high concentration of lipolysis products along the blood endothelial interface; contribute to atherosclerosis through mechanisms encompassing pro inflammatory, pro-coagulant, and pro-apoptotic gene activation. TG lipolysis releases neutral and oxidized free fatty acids that induce endothelial inflammation, vascular apoptosis, and reactive oxygen species production in endothelial cells and altered lipid raft physiology ^[11].

Materials and methods:

In this case control study, 100 subjects were included. Fifty were diagnosed with hypertension and 50 normotensive as control. Exclusion criteria involved patients with hepatitis and systemic illness.. Our sampling was performed from January to February 2016.

After overnight fasting, 5ml of venous blood was withdrawn. Blood samples were centrifuged at 1500 rpm for 10 min and stored until analyzed.

Ethical Considerations

This study protocol was formally approved by the Ethical Committee of Omdurman Islamic University. Permission of hospitals managers were taken before beginning. Informed consent was taken from every participant before enrolling.

Measurement of BMI:

Anthropometric data including weight and height were measured. Body mass index (BMI) was defined as weight (in kilograms) divided by the square of the height in meters.

Estimation of highly sensitive C-reactive protein:

According to the procedure provided, serum levels of hs-CRP were measured, using Cobas 311 automated analyzer. Particles enhanced immune-turbidimetric assay. Human CRP agglutinates with latex particles were coated with monoclonal anti-CRP antibodies. The precipitate is determined turbidimetrically.

Estimation of cholesterol:

Cholesterol esters were cleaved by the action of cholesterol esterase to free cholesterol and fatty acids. Cholesterol oxidase then catalyzed the oxidation of cholesterol to cholest-4-en-3-one and hydrogen peroxide. In the presence of peroxidase, the hydrogen peroxide triggered the coupling of phenol and 4-aminophenazone to form red quinone-imine dye. The absorption was

directly proportional to concentration of cholesterol and was measured, using Cobas 311 (autoanalyzer).

Triglyceride Estimation

Triglyceride was catalyzed by glycerol kinase peroxidase method through sequences of enzymatic catalysis step by lipases, triglyceride catalyzed to yield H_2O_2 , which on oxidation gives color dye quinonimine. The absorbance directly was proportional to concentration of triglyceride and was measured by cobas 311.

Statistical analysis:

Statistical analysis was performed using the statistical package for social science (SPSS). The data was displayed as (mean $\pm$ SD) for variables of analyzed concentration. The independent t-test was

employed to compare the difference between continuous variables. P-values less than 0.05 were considered statistically significant.

Results: In our study, 50 individuals with hypertension were (26% men and 74% woman) participated. The mean serum levels of cholesterol, triglyceride and hs-CRP were 195.1 ± 49.1 mg/dL, 171.5 ± 77.7 mg/dL, and 5.93 ± 9.96 mg/L respectively.

According to hypertension group, the mean level of hs-CRP and triglyceride show highly significant increase results and cholesterol level also show highly significant increase results compared with normal individuals as show in table (3-1).

Table (3-1) the mean of parameters level in study group classified as hypertensive patients and normal individuals.

Variable	Case	Control	P. Value
hs-CRP	5.93± 9.9	1.41± 1.2	0.002
Cholesterol	195.1± 49.1	151.6± 30.4	0.000
Triglyceride	171.5± 77.7	110.2± 50.2	0.000

Our study showed insignificant increase in mean triglyceride levels in males compared to females, while showed insignificant increase in mean levels of diastolic BP, systolic BP, hs-CRP and cholesterol in females compared to males as shown in table (3-2).

Table (3-2) the mean parameters level in study group classified as male and female.

Variable	Male	Female	P. Value
hs-CRP mg/L	3.3± 3.6	6.67± 11.3	0.382
Cholesterol mg/dL	187.3± 48.7	197.8± 49.4	0.511
Triglyceride mg/dL	174.8± 65.4	170.4± 82.4	0.286
Systolic BP mmHg	136.9± 15.5	141.9± 46.0	0.706
Diastolic BP mmHg	82.7± 8.8	84.5± 8.3	0.519

Our study revealed that there was insignificant increase in mean levels of cholesterol and triglyceride in normal weight

group in comparison to CRP level in overweight group in overweight and insignificant comparison to normal weight increase in mean levels of group as showed in table (3-3). systolic BP, diastolic BP and hs-

Table (3-3) the mean levels of parameters in study classified as normal weight and overweight.

Variables	Normal weight(BMI $\leq$ 26.5 Kg/m ²)	Over weight (BMI>26.5 Kg/m ²)	P-value
hs-CRP mg/L	4.59 $\pm$ 6.2	7.07 $\pm$ 12.31	0.387
Cholesterol mg/dL	204.5 $\pm$ 44.9	187.1 $\pm$ 51.8	0.213
Triglyceride mg/dL	179.5 $\pm$ 99.5	164.0 $\pm$ 53.9	0.367
Systolic BP mmHg	132.2 $\pm$ 16.3	147.8 $\pm$ 52.1	0.174
Diastolic BP mmHg	82.8 $\pm$ 9.0	85.0 $\pm$ 7.9	0.367

According to age of patients the results showed slightly insignificant increase in mean levels of diastolic BP, systolic BP and cholesterol and

insignificant decrease of hs-CRP and triglyceride in patients more than 60 years compared with less than 60 years as showed in table (3-4).

Table (3-4) the effect of age patients on hs-CRP, triglyceride, cholesterol, diastolic BP and systolic BP.

Variable	Less than 60 years	More than 60 years	P. value
hs-CRP mg/L	7.62± 13.68	4.71± 6.03	0.312
Cholesterol mg/dL	194.3± 45.8	195.7±52.1	0.922
Triglyceride mg/dL	172.2± 96.9	171.0± 62.2	0.959
Systolic BP mmHg	133.8± 14.8	145.6± 52.1	0.311
Diastolic BP mmHg	83.3± 7.1	84.5± 9.3	0.637

Also our study revealed that there was significant increase in the mean cholesterol level in patients have duration less than 5 year of hypertension compared with more than 5 years, we have observed that long history of disease (more than 5 years) have an insignificant increase in hs-CRP and triglyceride levels and insignificant decrease in systolic BP and diastolic BP compared to those (less than 5 years) group showed in table (3-5).

Table (3-5): the effect of duration of disease on hs-CRP, cholesterol, triglyceride, diastolic BP, systolic BP.

Variable	Less than 5 years	More than 5 years	P. Value
hs-CRP mg/L	3.81± 2.93	7.74± 13.13	0.166
Cholesterol mg/dL	209.9± 40.0	182.5± 53.2	0.048
Triglyceride mg/dL	170.3± 94.8	172.5± 61.4	0.921
Sytolic BP mmHg	144.6± 57.6	137.2± 14.9	0.521
DiastolicBP mmHg	84.1± 8.2	83.9± 8.7	0.920

Discussion:

Hypertension is most important public health problem in developing countries and one of major risk factors for cardiovascular disease.

In this study, 50 patients with hypertension were studied to evaluate serum hs-CRP, total cholesterol and triglyceride as predictor marker for CVD.

The results of independent t-test showed that, there was a highly significant increase in hs-CRP

level of the case in comparison with the control group of (p-value 0.002). This result agreed with the previous study that reported that HTN is a part of an inflammation, therefore observed elevated levels of hs-CRP in hypertensive individuals, which may be due to CRP, induce a decrease in endothelium-dependent relaxation and thus a potential risk factor for HTN, reverse causation might also be

implicated, whereby high blood pressure might induce inflammation and raise CRP levels. Our results indicate that high sensitive CRP could be a useful predictor marker for cardiovascular disease in hypertensive patients^[12].

The results of this study clearly showed that the total cholesterol and triglyceride were significantly higher in hypertensive patients in comparison to normotensive individuals with (p-value 0.000) for both parameters. These findings were completely in agreement with the result of previous studies^[13]. There is a close relationship between HTN and lipid abnormalities. This gives evidence that cholesterol induces endothelial dysfunction even at normal or high to normal by reducing the bioavailability of endothelium-derived nitric oxide. Endothelium vasodilation, in fact correlates inversely with

total cholesterol levels^[14]. These results indicate the routine investigation of lipid profile in hypertensive patients may help to prevent further aggravation and risk of CVD.

The results showed the mean serum hs-CRP has an insignificant difference in females compared to males with p-value of (0.387). These results agree with^[15].

In this study the mean TG and cholesterol levels showed insignificant difference when compared males with females with p-values of (0.86 and 0.511) respectively. These finding agree with^[16].

Our results revealed that there was an insignificant difference in mean hs-CRP in overweight hypertensive patients in comparison to normal weight with a p-value of (0.387). These finding agree with^[17].

In addition the results showed that there was an insignificant

difference in mean triglyceride levels between normal weight and overweight patients with p-value of (0.509). This agreed with previous study ^[12].

The results denoted that there was an insignificant increase in systolic and diastolic BP in overweight compared to normal weight with p-values of (0.174 and 0.367) respectively. These results are in accord with ^[18]. The possible justification that obesity characterized by the activation of the sympathetic nervous system, which leads to the activation of the renin angiotensin system and sodium retention might hold true ^[19].

As far as age is concerned the results found no significant increase in cholesterol in patients elder than 60 years compared to younger individuals with a (p-value 0.922), yet an insignificant decrease in hs-CRP and TG, with p-values (0.312

and 0.959) respectively, was observed.

The results also elucidated that there was no significant difference in systolic and diastolic BP between individuals below 60 years of age and those above with p-values (0.311 and 0.637) respectively. This was in controversy with ^[20].

On the other hand concerning the duration of disease there was no significant difference in TG, hs-CRP, diastolic BP and diastolic BP with p-values of (0.921, 0.166, 0.920 and 0.521) respectively. In contrast there was a significant increase in the mean cholesterol level in those, who spent less than 5 years duration of disease compared to those who suffered more than 5 years of disease duration with a p-value of (0.048). This finding agreed with the previous study that reported the deleterious effects of antihypertensive drugs, like thiazide on lipid

profile, observed mainly in the short term and almost disappear in long term studies that last for 5 or more years ^[21].

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Hypertension is most important public health problem in developing countries and one of

major risk factors for cardiovascular disease.

In this study, 50 patients with hypertension were studied to evaluate serum hs-CRP, total cholesterol and triglyceride as predictor marker for CVD.

The results of independent t-test showed that, there was a highly significant increase in hs-CRP level of the case in comparison with the control group of (p-value 0.002). This result agreed with the previous study that reported that HTN is a part of an inflammation, therefore observed elevated levels of hs-CRP in hypertensive individuals, which may be due to CRP, induce a decrease in endothelium-dependent relaxation and thus a potential risk factor for HTN, reverse causation might also be implicated, whereby high blood pressure might induce inflammation and raise CRP levels. Our results indicate that

high sensitive CRP could be a useful predictor marker for cardiovascular disease in hypertensive patients^[12].

The results of this study clearly showed that the total cholesterol and triglyceride were significantly higher in hypertensive patients in comparison to normotensive individuals with (p-value 0.000) for both parameters. These findings were completely in agreement with the result of previous studies^[13]. There is a close relationship between HTN and lipid abnormalities. This gives evidence that cholesterol induces endothelial dysfunction even at normal or high to normal by reducing the bioavailability of endothelium-derived nitric oxide. Endothelium vasodilation, in fact correlates inversely with total cholesterol levels^[14]. These results indicate the routine investigation of lipid profile in hypertensive patients may help

to prevent further aggravation and risk of CVD.

The results showed the mean serum hs-CRP has an insignificant difference in females compared to males with p-value of (0.387). These results agree with ^[15].

In this study the mean TG and cholesterol levels showed insignificant difference when compared males with females with p-values of (0.86 and 0.511) respectively. These finding agree with ^[16].

Our results revealed that there was an insignificant difference in mean hs-CRP in overweight hypertensive patients in comparison to normal weight with a p-value of (0.387). These finding agree with ^[17].

In addition the results showed that there was an insignificant difference in mean triglyceride levels between normal weight and overweight patients with p-

value of (0.509). This agreed with previous study ^[12].

The results denoted that there was an insignificant increase in systolic and diastolic BP in overweight compared to normal weight with p-values of (0.174 and 0.367) respectively. These results are in accord with ^[18]. The possible justification that obesity characterized by the activation of the sympathetic nervous system, which leads to the activation of the renin angiotensin system and sodium retention might hold true ^[19].

As far as age is concerned the results found no significant increase in cholesterol in patients elder than 60 years compared to younger individuals with a (p-value 0.922), yet an insignificant decrease in hs-CRP and TG, with p- values (0.312 and 0.959) respectively, was observed.

The results also elucidated that there was no significant

difference in systolic and diastolic BP between individuals below 60 years of age and those above with p-values (0.311 and 0.637) respectively. This was in controversy with ^[20].

On the other hand concerning the duration of disease there was no significant difference in TG, hs-CRP, diastolic BP and diastolic BP with p-values of (0.921, 0.166, 0.920 and 0.521) respectively. In contrast there was a significant increase in the mean cholesterol level in those, who spent less than 5 years duration of disease compared to those who suffered more than 5 years of disease duration with a p-value of (0.048). This finding agreed with the previous study that reported the deleterious effects of antihypertensive drugs, like thiazide on lipid profile, observed mainly in the short term and almost disappear in long term studies that last for 5 or more years ^[21].

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Elevation of Gonadotropin Hormones as Early Predictor Marker for Subfertility among Sudanese Patient with Chronic Kidney Disease Stage-5

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Abstract:

Background: Chronic kidney disease (CKD) is defined as the decrease of glomerular filtration rate (GFR) that leads to a progressive deterioration of fluid-electrolyte balance and metabolic or endocrinological functions. Furthermore, the relation of CKD with infertility and sexual dysfunction is well-known to affect medical health status as well as physiological status. Additionally, in men with CKD, gonadal dysfunction with elevation of serum gonadotropin concentration is a frequent finding, affecting 26% – 66 % of men with different stages of CKD. The objective of this study is to investigate the serum gonadotropins LH and FSH in patients with CKD.

Methods: This case-control study was conducted at military hospital, police hospital and Kidney transplantation association within six months from November 2017 to April 2018. More importantly, the study was carried out on a total of 80 samples including 40 CKD patients as case and 40 healthy individuals as control group. Finally, serum FSH and LH levels were estimated using ELISA technique and data was analyzed using SPSS software program version 21.

Results: This study has found significant elevation in FSH and LH levels with *p*-value (0.000, 0.001) respectively in CKD patients compared to control group. Moreover, the study found that FSH level is positively correlated with BMI and duration of disease, while LH level is positively correlated with the duration of disease only. **Conclusion:** Our study concluded that patients with CKD have elevated gonadotropins (FSH&LH) levels.

Key words: CKD, Obesity, Duration of Disease, FSH & LH.

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Introduction:

Chronic kidney disease (CKD) is defined as the decrease of glomerular filtration rate (GFR) that leads to a progressive deterioration of fluid-electrolyte balance and metabolic or endocrinological functions (KDIGO, 2013). The major risk factors of CKD include diabetes, hypertension, obesity, older age and smoking (Sarnak *et al.*, 2003; Gelber *et al.*, 2005). Furthermore, the relation of CKD with infertility and sexual dysfunction is well-known to affect medical health status as well as physiological status (Süleymanlar, 2011). Moreover, Hormone abnormalities and endocrine dysfunction are common in patients with CKD. Further, changes in gonadotropins levels are caused by altered function of the

hypothalamic-pituitary axis and uremic syndrome (Periklis *et al.*, 2014).

However, in men with CKD, gonadal dysfunction with elevation of serum gonadotropin concentration is a frequent finding, affecting 26% – 66 % of men with different stages of CKD (Iglesias, 2012). More importantly, changes of gonadotropins synthesis and metabolism develop early after the onset of renal insufficiency (Alice *et al.*, 2002). Multiple factors are responsible for the elevation in FSH and LH in CKD resulting in a reduced clearance of gonadotropin-releasing hormone (GnRH), LH and FSH leading to elevated serum levels of these hormones (Molina, 2013; Songul, *et al.*, 2016; Nannan, *et al.*, 2015). Moreover, the baseline levels of LH are high, due to

impaired negative feedback from low testosterone and because of to the reduced renal clearance as well. In addition to these secretory changes, the normal signaling of luteinizing hormone is inhibited in CKD (Matthew, 2017; Iglesias, 2012).

The plasma FSH concentration tends to be highest in those uremic patients with the most severe damage to seminiferous tubules and presumably the lowest levels of inhibin B (Mahboob and Shirin, 2008; Piotr *et al.*, 2015). In addition, Hormonal abnormalities might improve but rarely normalize with institution of maintenance dialysis (Rosas *et al.*, 2003; Diemont *et al.*, 2000). To elaborate, beside CKD, several other factors play a major part in the disturbance of Gonadotropins

hormones such as obesity and the duration of kidney disease.

Obesity, defined as BMI greater than 30 Kg/m², has been abundantly increasing among people in last decades. More importantly, it is an established factor of risk for numerous pathologic conditions including infertility (Mihalca *et al.*, 2014). Obesity is associated with adult male fertility as it affects the GnRH-FSH/LH pulses and impairs the function of Leydig and Sertoli cells (Sallmen *et al.*, 2006). Several studies showed strong relationship between obesity and gonadotropins hormones and different results were obtained. In fact, with the increase of BMI, the production of inhibin B decreases (Winters *et al.*, 2006; Miro *et al.*, 2016) the lack of negative feedback leads to

an increased level of production in FSH (Xin-Mei *et al.*, 2015).

In CKD patients, especially those with end stage renal disease, when kidney function is severely impaired, the renal clearance will probably be impaired as well. In fact, GFR decreases and, as a result, toxins build up in the body. More importantly, high levels of uremic metabolism in blood affect hypothalamic-pituitary function leading to elevated gonadotropin hormones levels (Kerstin *et al.*, 2017; Hylander and Lehtihet, 2015). These abnormalities are already seen in patients with moderate reduction in the glomerular filtration rate and often become more obvious as kidney failure progresses. Otherwise,

disturbances in the hypothalamic-pituitary-gonadal axis can be detected before the need for

dialysis but continues to worsen once dialytic therapy is initiated (Palmer, 2003).

Methods:

This is case-control study carried out in a total of 80 subjects including 40 patients admitted to three nephrology units for clinical assessment as case, and 40 healthy individuals as control matching in age. The inclusion & exclusion criteria cover patients who were older than twenty years old, Stage-5, and excludes smokers, patients suffering from pituitary tumor, patients with high dose or long-term steroid abuse and those who were older than sixty-five years old. The study was performed in accordance with the Declaration of Omdurman Islamic University regarding ethical principles for medical research involving

human subject and also permission was obtained from health authorities in military hospital, police hospital and Kidney transplantation association. All participants have been informed and consent is obtained prior to their inclusion in the study. Blood specimens were collected before dialysis. Levels of FSH and LH were estimated using direct ELISA kits.

Statistical analysis:

Data was analyzed using SPSS program version 21. Results were expressed as mean $\pm$ SD. Independent t-test and Pearson correlation test were used; *P-value* less than 0.05 regarded statistically significant.

Results:

The present study included 40 individuals with CKD whose ages are ranging between 20 - 62 years. Our results in table 1 reveal significant increase in gonadotropins (FSH, LH) in case group compared to control group, also show that is increase in gonadotropins with increase duration of disease, FSH also increase with increase BMI, figure 1 and 2. Further, FSH levels were positively correlated with BMI and duration of disease (figure 3A, $r = 0.651$, $p = 0.000$) (figure 3C, $r = 0.340$, $p = 0.034$). In addition, positive correlation is seen between LH and duration of disease (figure 3B, $r = 0.612$, $p = 0.000$).

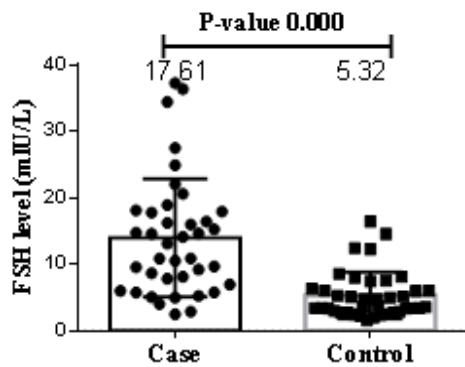


Figure1: FSH Level (P-value 0.000)

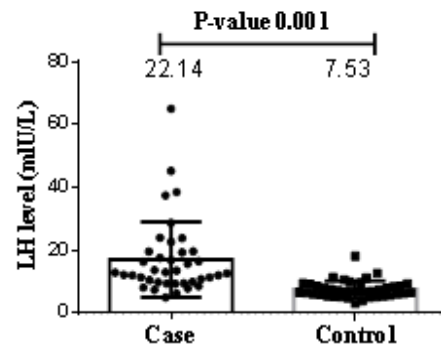
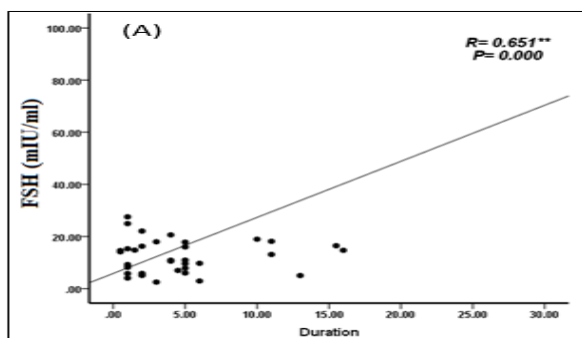
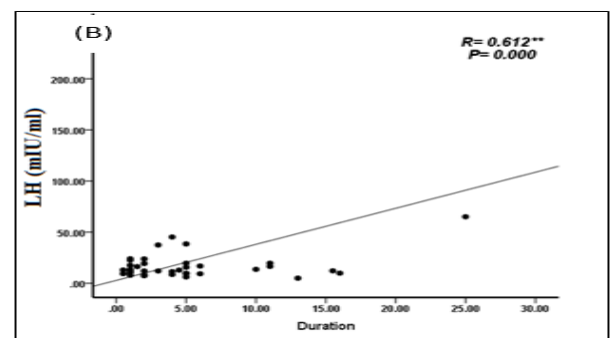


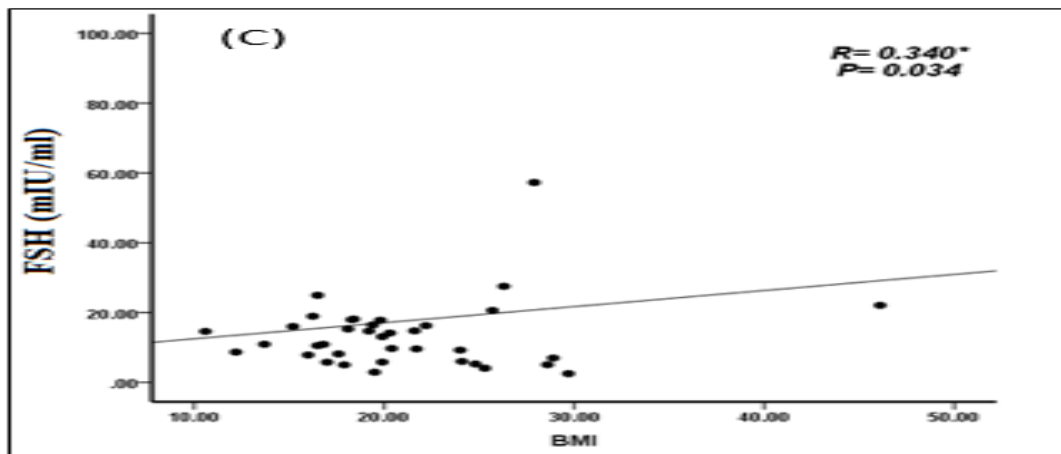
Figure2: LH Level (P-value 0.002)



Duration of disease



Duration of Disease



BMI

Figure 3: A: Duration is correlated significantly in a positive manner with FSH values ($r=0.651$, $p=0.000$). B: Duration is correlated significantly in a positive manner with LH values ($r=0.612$, $p=0.000$).

C: BMI is correlated significantly in a positive manner with FSH values ($r=0.340$, $p=0.034$).

Table (1): show mean levels of FSH and LH in case and control groups with duration of disease and BMI.

Parameters	Case (Mean $\pm$ SD)	Control (Mean $\pm$ SD)	P-value
FSH (mIU/ml)	17.61 $\pm$ 10.62	5.32 $\pm$ 3.43	0.000
LH (mIU/ml)	22.14 $\pm$ 12.94	7.53 $\pm$ 2.70	0.001
Variables	<5 Years (Mean $\pm$ SD)	$\geq$ 5 Years (Mean $\pm$ SD)	P-value
Duration of disease			
FSH (mIU/ml)	14.35 $\pm$ 11.39	22.80 $\pm$ 19.89	0.023
LH (mIU/ml)	15.72 $\pm$ 9.34	32.42 $\pm$ 16.37	0.013
BMI	Normal weight (Mean $\pm$ SD)	Overweight (Mean $\pm$ SD)	P-value
FSH (mIU/L)	14.92 $\pm$ 13.98	29.85 $\pm$ 24.05	0.005

Discussion:

Chronic kidney disease (CKD) is defined as the decrease of glomerular filtration rate (GFR) leading to a progressive deterioration of fluid-electrolyte balance and metabolic or endocrinological functions (KDIGO, 2013). Additionally, in men with CKD, gonadal dysfunction with elevation of serum gonadotropin concentration is a frequent finding, affecting 26% – 66 % of men with different stages of CKD (Britta and Mikael, 2015). The results of present study in table1, figure1 and 2, show that the FSH and LH are significantly increased in CKD patients compared to control group with *P*-values (0.000, 0.001) respectively. In agreement with the previous study (Songul, *et al.*, 2016) this presumably due to prolonged half-life, reduced renal clearance for both, and lack of normal feedback inhibition of LH by testosterone, the secretion of which from Leydig

cells is diminished. FSH concentration tend to be highest in uremic patient with the most severe damage to the seminiferous tubules and presumably the lowest level of inhibin (Molina, 2013). On other hand, table1 shows that there were significant increases in gonadotropin hormones (FSH and LH) in CKD patients with increase duration of disease *p-values* (0.023, 0.013) for both hormones respectively. The renal clearance may be impaired as GFR decrease and hence the observation of elevation in gonadotropin hormones, and uremic metabolism at advance stages of CKD affect hypothalamic-pituitary function lead to hormonal abnormalities (Kerstin *et al.*, 2017; Hylander and Lehtihet, 2015). Additionally there statistically significant increase in the level of FSH with increase BMI (*p-value* 0.034) in previous study found that there were decrease in production of inhibin B with increase obesity (Winters *et al.*,

2006) which act as negative feedback mechanism for FSH secretion. Further, in the present study in figure (3/A-B) we found FSH and LH levels have positive correlation with duration of CKD ($r = 0.651$, $p = 0.000$, $r = 0.612$, $p = 0.000$) respectively which is similar to the results found by (Britta and Mikael, 2015). On the other hand, FSH in figure (1-C) has positive correlation with BMI ($r = 0.014$, $p = 0.932$) and this matches with study of (Xin-Mei *et al.*, 2015).

Conclusion:

Gonadal dysfunction is very common in male with CKD. The pathophysiology of gonadal dysfunction in CKD is multifactorial. Hence, hypergonadotropic hypogonadism is a frequent finding in CKD patients. This study showed that gonadotropins levels are significantly increased in male patients with CKD compared to control group. Significant positive

correlation between FSH levels appears with BMI and duration of disease. In further, significant positive correlation appear between LH and duration of disease.

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Bacterial etiology of Community Acquired Pneumonia and their Antimicrobial Susceptibility in Patients Admitted to Alshaab Teaching Hospital

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Abstract

Background: Community Acquired Pneumonia (CAP) is a frequently encountered lower respiratory tract parenchymal lung infection which continues to be a major health problem leading to significant morbidity and mortality worldwide. This study was aimed to determine frequency and susceptibility pattern of bacterial etiology of community acquired pneumonia isolates from Sudanese patients.

Material and method: One hundred Sputum specimens were collected from patients with Community Acquired Pneumonia attended to chest unit in Alshaab Teaching Hospital during the period between Januarys to March 2017. The patients were grouped according their age as follows (year); 16 to 26, 27 to 37, 38 to 48, 49 to 60. Antibiotic susceptibility test of isolated organisms were carry out by the Kirby - Bauer disc diffusion method. **Result:** Out of 100 patient enrolled in the study the etiology was identified in 42/100 (42%) with most frequent isolates *Klebsiella pneumoniae* 18/42 (42.8%) followed by: *Pseudomonas aeruginosa* 13/42 (30.9%), *Staphylococcus aureus* 10/42 (23.9%) and *Escherichia coli* 1/42 (2.4%). Only 3 (16.7%) organisms among all isolated *klebsiella pneumoniae* were found resistant to all investigated antibiotic (third generation cephalosporin (3GC)) Six 6/10 (60%) from all isolated *Staphylococcus aureus* found resistant to methicillin (ME10µg) and Oxicillin (OX 1µg). all isolated *Pseudomonas aeruginosa* were sensitive to Meropenem. The isolated *Escherichia coli* found only resistant to Ampicillin. **Conclusion:** prevalence of community-acquired pneumonia in adult Sudanese patients is relatively high with major causative agents *Klebsiella pneumoniae*.

Keywords: Community-acquired pneumonia (CAP), *Klebsiella pneumoniae*, alcoholic abuse, Methicillin resistant *Staphylococcus aureus* (MRSA).

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Introduction

Community-acquired pneumonia (CAP) is a common lower respiratory tract infection and remains an important cause of morbidity and Mortality worldwide (1, 2) Detailed information on the etiology of CAP is required for the formulation of treatment recommendations and the introduction of preventive measures (3). Up to 36% of patients who have CAP require admission to the ICU (4). The range of microorganisms reported as being responsible for community acquired pneumonia varies between studies and depends on the patient population studied, the extent of laboratory investigations, and the microbiological techniques employed in some Studies have identified cause of pneumonia were *Streptococcus pneumoniae*, *Haemophilus influenzae*,

Staphylococcus aureus, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and other respiratory viruses as the main pathogens associated with CAP (5). The distribution of etiological pathogens of CAP may vary by country. Most of the data on pathogen distribution of CAP have been reported from western countries, whilst only a few data are available in the Asian region (6). The initial management of patients suspected of having community-acquired pneumonia is challenging because of the broad range of clinical presentations, potential life-threatening nature of the illness, and associated high costs of care. The initial testing strategies should accurately establish a diagnosis and prognosis in order to determine the optimal treatment strategy (7).

Method: Descriptive cross sectional Hospital base study

was performed from December 2016 to February 2017. A total of 100 patients attended into chest unit in Alshaab Teaching Hospital with sign and symptoms of lower respiratory tract infection were enrolled in this study.

All sputum samples were collected from enrolled patient in sterile container with screw cap and transported to research laboratory according to Clinical Laboratory Standard Institute (CLSI).

Then inoculated under aseptic technique in 5% Blood agar, MacConkey agar and Chocolate agar prepared according to manufacturer's instruction, the inoculated culture media were incubated aerobically at 37°C overnight and culture of chocolate agar incubated at 37°C in candle jar overnight.

Smears from detected growth were prepared for Gram stain which was used as basic

identification of possible causative agents, for Gram positive cocci catalase test was performed, for catalase positive organism DNase test, coagulase test and Novobiocin sensitivity test was done to identify *staphylococcus* spp. Gram negative identification was done by using motility test, oxidase test, kligler iron agar, citrate utilization test, urease hydrolysis test, indole production test and methyl red test. Antibiotic susceptibility test of isolated organisms The test was performed using Kirby-Bauer disc diffusion method as follows:

Preparation of inoculums:

The inoculums were prepared by transfer 2-5 well isolated colony of same appearance with sterile wire loop to 2ml of sterile physiological saline. The turbidity of suspension was adjusted to a 0.5 McFarland standard these suspensions were

used within 15 minutes of preparation.

Seeding of plates:

Sterile nontoxic cotton swab was dipped into the inoculum tube and then the swab was rotated against the side of the tube above the level of suspension to remove excess fluid.

The plate of Muller Hinton agar was inoculated by streaking the swab evenly over the surface of the medium in three directions the surface of agar was allowed 3-5 minutes to dry.

Antibiotic disc application: The selected antibiotics were applied on surface of agar by using sterile forceps which evenly distributed in the inoculated plate each disc was pressed down to ensure its contact with the agar.

Incubation: The inverted plates were incubated aerobically at 37°C for 18-24 hours.

Reading of zones of inhibition

Following overnight incubation by using a ruler on the underside

plates the diameter of each zone of inhibition was measured in millimeters.

Interpretation of result: The zone of each antibiotic was compared to their standard inhibition zone on the chart provided by manufacture the result were interpreted as sensitive (S) intermediate (I) or resistance (R).

Methicillin (10µg), Oxacillin (1µg) was used to detect community acquired methicillin resistance *staphylococcus aureus* (MRSA). Susceptibility test for *klebsiella pneumonia* was done by using standard disc diffusion (SDDF) as screening method for identifying potential extended spectrum B-lactamase (ESBLs) against a group of third generation cephalosporin (3GC) include Ceftazidime (CAZ, 30µg), Ceftriaxone (CRO, 30µg), Cefepime (FEP, 30µg), Cefotaxime (CTX, 30µg). Standard double disk synergy

test (DDST) was done to *klebsiella pneumonia* which found resistant to the group of cephalosporin (3GC) antibiotic. Eight antibiotic discs were tested for *Pseudomonas aeruginosa*, include Meropenem (MEM, 10µg), Cefepime (FEP, 30µg), Ceftazidime (CAZ, 30µg), Gentamicin (CN, 10µg), Colistin (CT, 10µg), piperacillin (PRL, 100µg), Aztreonam (ATM, 30µg), Imipenem (IPM, 10µg). Ten commercially available antibiotic discs were used for *Escherichia coli* antibiotics were Ampicillin (10µg), Amoxicillin (25µg), Gentamycin (10µg), Cefixime (5µg), Amikacin (30µg), Ceftazidime (30µg),

Ciprofloxacin (5µg), Imipenem (10µg), and Norfloxacin (10µg), Trimethoprim/Sulfamethoxazole (1.25µg/23.75µg).

Results: The results showed 42 (42%) were positive for bacterial growth. Out of investigated specimen CAP agents were identified as 18/42 (42.8) *Klebsiella pneumoniae* followed by 13/42 (30.9%) *Pseudomonas aeruginosa*, 10/42 (23.9%) *Staphylococcus aureus* and 1/24 (2.4%) *Escherichia coli*. CAP agents were more prevalent in males 23/42 (54.7%) than females 19/42 (45.3%) but this result was statistically insignificant(P-value=0.129) (Table1).

Table.1 Frequency of isolated bacteria according to Gender

Isolated bacteria	Gender		Total
	Male	Female	
<i>Klebsiella pneumonia</i>	7 (16.6%)	11 (26.3%)	18 (42.8%)
<i>Pseudomonas aeruginosa</i>	8 (19%)	5 (11.9%)	13 (30.9%)
<i>Escherichia coli</i>	1 (2.4%)	0	1 (2.4%)
<i>Staphylococcus aureus</i>	7 (16.7%)	3 (7.2%)	10 (23.9%)
Total	23 (54.7%)	19 (45.3%)	42 (100%)

(P-value=0.129)

The result showed insignificant relationship between isolated bacteria and smoking (P-value=0.899).

CAP agents were found significantly more frequent among patients with alcohol abuse show in (Table.2).

Table.2 Relationships between isolated bacteria and alcoholism

Isolated bacteria	Alcoholism		Total
	Yes	No	
<i>Klebsiella pneumonia</i>	0	18	18
<i>Pseudomonas aeruginosa</i>	2	11	13
<i>Escherichia coli</i>	0	1	1
<i>Staphylococcus aureus</i>	3	7	10
Total	5	37	42

P-value =0.025

The result of standard disc diffusion (SDDF) which used as screaming method for identifying potential extended spectrum B-lactamase (ESBLs) producer was done against a group of third generation cephalosporin (3GC) include

Cefepime (CPM), Ceftriaxone (CL), Ceftazidime (CAZ) and Cefotaxime (CTX) the test result show that only 3 (16.7%) organism among all isolated *Klebsiella pneumoniae* were resistant to all investigated antibiotics while remain

15 (83.3%) were sensitive as show in (Table 3 below).

Table .3 Antimicrobial susceptibility of *Klebsiella pneumoniae* against third generation Cephalosporin.

Antibiotic discs	Inhibition zone		Total
	Sensitive	Resistant	
Ceftazidime	15	3	18
Cefepime	15	3	18
Ceftriaxone	15	3	18
Cefotaxime	15	3	18
Total	60	12	72

The double disc synergy test (DDST) was done to these 3 organism which showed resistant to the group of cephalosporin (3GC) antibiotics which indicated that these 3 organism could considered as ESBLs producers none of ESBLs harboring isolate was susceptible to combination of amoxicillin and clavulanic acid. Antibiotic susceptibility of *pseudomonas aeruginosa* that includes

Eight antibiotics detected that all isolates were 100% sensitive to Meropenem follow 92.3% sensitive to fifth antibiotics they were Cefepim, Ceftazidime, Gentamicin, Aztreonam, Imipenem, followed by 84.6% sensitive for Colistin, the less effective antibiotics is Piperacillin with 53.8% sensitivity as show in (Table 4).

Table.4 Antimicrobial susceptibility test of *Pseudomonas aeruginosa* against some antibiotic

Antibiotic Discs	Inhibition zone			Total
	Sensitive	Resistant	Intermediate	
Meropenem	13	0	0	13
	100.0%	0.0%	0.0%	100.0%
Cefepim	12	1	0	13
	92.3%	7.7%	0.0%	100.0%
Ceftazidime	12	1	0	13
	92.3%	7.7%	0.0%	100.0%
Gentamicin	12	1	0	13
	92.3%	7.7%	0.0%	100.0%
Colistin	11	2	0	13
	84.6%	15.4%	0.0%	100.0%
Piperacillin	7	6	0	13
	53.8%	46.2%	0.0%	100.0%
Aztreonam	12	1	0	13
	92.3%	7.7%	0.0%	100.0%
Imipenem	12	0	1	13
	92.3%	0.0%	7.7%	100.0%
	91	12	1	104
Total	87.5%	11.5%	1.0%	100.0%

Table.5 Antimicrobial susceptibility test for *Staphylococcus aureus*

Antibiotic Discs	Inhibition zone			Total
	Sensitive	Resistant	Intermediates	
Ciprofloxacin	8	2	0	10
	80.0%	20.0%	0.0%	100.0%
Ceftriaxone	9	1	0	10
	90.0%	10.0%	0.0%	100.0%
Clindamycin	8	2	0	10
	80.0%	20.0%	0.0%	100.0%
Vancomycin	8	2	0	10
	80.0%	20.0%	0.0%	100.0%
Penicillin	4	6	0	10
	40.0%	60.0%	0.0%	100.0%
Amikacin	9	1	0	10
	90.0%	10.0%	0.0%	100.0%
Cephalexin	8	2	0	10
	80.0%	20.0%	0.0%	100.0%
Cefuroxime	8	2	0	10
	80.0%	20.0%	0.0%	100.0%
Erythromycin	7	3	0	10
	70.0%	30.0%	0.0%	100.0%
Amoxicillin	6	3	1	10
	60.0%	30.0%	10.0%	100.0%
Rifampin	7	3	0	10
	70.0%	30.0%	0.0%	100.0%
Ceftazidime	7	2	1	10
	70.0%	20.0%	10.0%	100.0%
	89	29	2	120
Total	74.2%	24.2%	1.7%	100.0%

Six from all isolates *Staphylococcus aureus* which were penicillin resistant were tested to determine if they are methicillin resistant *Staphylococcus aureus* by using

methicillin (ME10µg) and Oxicillin (OX 1µg) the result showed that 100% resistant to methicillin (ME10µg) and Oxicillin (OX 1µg) as show in (table 6).

Table.6 Antimicrobial susceptibility test for *Staphylococcus aureus* to detect MRSA

Antibiotic Discs	inhibition zone	Total
	Resistant	
Penicillin	6	6
	100.0%	100.0%
Oxicillin	6	6
	100.0%	100.0%
Methicillin	6	6
	100.0%	100.0%
	18	18
Total	100.0%	100.0%

Antimicrobial Susceptibility test for *Escherichia coli*

One isolated *Escherichia coli* was tested using 10 antibiotics which showed 100%

Sensitive to Gentamycin, Cefotaxime,

Cefixime, Erythromycin, Ampicillin, Imipenem, Amikacin, Ciprofloxacin, Trimethoprim/Sulfolmethoxazole, Norfloxacin.(Table7).

Table .7 Antimicrobial Susceptibility test for *Escherichia coli*.

Antibiotic Discs	Inhibition zone		Total
	Sensitive	Resistant	
Gentamycin	1	0	1
	100.0%	0.0%	100.0%
Cefotaxime	1	0	1
	100.0%	0.0%	100.0%
Cefixime	1	0	1
	100.0%	0.0%	100.0%
Erythromycin	1	0	1
	100.0%	0.0%	100.0%
Ampicillin	0	1	1
	0.0%	100.0%	100.0%
Imipenem	1	0	1
	100.0%	0.0%	100.0%
Amikacin	1	0	1
	100.0%	0.0%	100.0%
Trimethoprim/Sulfamethoxazole	1	0	1
	100.0%	0.0%	100.0%
Ciprofloxacin	1	0	1
	100.0%	0.0%	100.0%
Norfloxacin	1	0	1
	100.0%	0.0%	100.0%
Total	9	1	10
	90.0%	10.0%	100.0%

Discussion

The present study showed that the most common etiologic agent isolated from patient with community acquired pneumonia was *Klebsiella pneumoniae* (42.8%) This result is consistent with other result by Liam (8) who showed that *Klebsiella pneumoniae* was the most frequently isolated pathogen and not compatible with Johansson *et al*(9) who showed that *Streptococcus pneumoniae* was the most common isolated pathogen, also not compatible with Goel *et al*(10) and Khawaja *et al*(11) whom showed that *Pseudomonas aeruginosa*, *Staphylococcus aureus* was the most frequently isolated pathogen respectively this

variation may be due to racial and geographical variations between each area.

The most affected age group in this study were those of the age 16 -26 (33.3%) and The present findings seem to differ from the results of Huijts *et al*(12) which showed that the most affected age group was those above the age of 60 years with percentage of 55.8%. Torres *et al*(13) reported that the community acquired pneumonia was statistically associated with alcoholic abuses, similar to our results which provided that significant relationship between occurring of community acquired pneumonia and alcoholic abuses. Previous studies done by Farr *et al*(14) and other one by Torres *et al*(13) showed increasing risk of CAP associated with smoking disagreement with the present result which show statistically insignificant relation between smoking and CAP (P-value =.899). As shown in present study all isolated ESBL producer

Klebsiella pneumoniae were 100% resistant to Cefepime (CPM), Ceftriaxone (CL), Ceftazidime (CAZ) and Cefotaxime (CTX), which is relatively high than that found by Romanus *et al*(15) found that all ESBL producer *Klebsiella pneumoniae* were resistant to Ceftazidime 69%, Cefotaxime 74% and Ceftriaxone 79.6%.

All isolated *Pseudomonas aeruginosa* was found to be 100% susceptible to Meropenem followed by 92.3% susceptible to Cefepim, Ceftazidime, Gentamicin, Aztreonam, and Imipenem and less susceptibility found to Piperacillin with percentage 53.8% with relatively similar to study done by Sadar *et al*(16) who reported that the most effective antibiotics against *Pseudomonas aeruginosa* were Amikacin, Meropenem, Piperacillin/tazobactam, Imipenem and Aztreonam with 68.7%, 67.1%, 67.1%, 65.5 %,

42.1% susceptibility respectively. In the present study 6/10 (60%) *Staphylococcus aureus* were methicillin resistant this result relatively high to that found by Francis (2005) (17) who report that 4 patients were identified with severe community acquired methicillin resistant *Staphylococcus aureus* (MRSA) pneumonia. All isolate MRSA were found to be resistant to Penicillin, Methicillin, Oxacillin and susceptible to Ceftriaxone, Amikacin and Rifampin with 83.3% susceptibility reduce to 66.7% for Vancomycin, 50.0% Erythromycin and 33% for Amoxicillin, which is slightly similar to study done by Hageman (2006)(18) who reported that antimicrobial susceptibility test results for the isolated MRSA showed that all isolate were resistant to Oxacillin but susceptible to Rifampin and Vancomycin.

Conclusion

The study concluded that *Klebsiella pneumoniae* was the major etiological agents of community-acquired pneumonia among enrolled patients. The frequency of ESBL producer *Klebsiella pneumoniae* in patients with community acquired pneumonia is relatively high. Most of the isolated *Staphylococcus aureus* were found resistant to Methicillin and Oxacillin consider MRSA and all of the isolated *Pseudomonas aeruginosa* were found susceptible to Meropenem. Further studies are recommended for the detection of the other bacterial species in sputum samples coupled with viral detection using polymerase chain reaction and also Studies addressing the detection of antibigrams resistance gene of the bacteria causing community acquired pneumonia are also needed.

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Molecular detection of *Chlamydia trachomatis* among children in Jarmai village - Gadarif State-Sudan

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Abstract

Background: Trachoma is the leading infectious cause of blindness and it caused by ocular infection with the bacterium *Chlamydia trachomatis* (Ct). While the majority of the global disease burden is found in sub-Saharan Africa, the Middle East, Central and South America, Asia, Australia, and the Pacific Islands bear a considerable burden. The World Health Organization and its partners are aiming to eliminate trachoma as a public health problem by 2020.

Objective: This study aimed to determine the prevalence of active trachoma and *Chlamydia trachomatis* among children in Jarmai village - Gadarif State, Sudan.

Methods: A population-based prevalence study was conducted in Jarmai village, Alrahad locality, Gadarif State, during the period from Nov 2016 to Nov 2017. A total of 509 children, whose ages ranged between 1 and 9 years old, were surveyed. Each eye was examined for trachoma follicles and trachoma inflammatory intense, (TF, and TI). Swab samples were collected from children, clinically diagnosed as suffering active trachoma, for the DNA analysis. Collections were done from the tarsal conjunctival surface with a Dacron polyester swab and with UTM media. DNA was, then, extracted and amplified by molecular technique with Touchdown protocol and primers for *C. trachomatis* outer membrane protein complex B, (omcB).

Result: The overall result of the 509 participants was as follows: 63 (12.4 %) were diagnosed for active trachoma, 7.0 (11.11 %) of them had follicles in the upper tarsals (TF), and showed 9 (14.29 %) had trachoma inflammatory intense (TI) and 47 (74.60 %) had the both signs of trachoma follicles and trachoma inflammatory intense (TF and TI). The prevalence of *Chlamydia trachomatis* in all the children in the village was 1.8%, and from the 63 children were, those who were clinically diagnosed as active trachoma patients i.e. 9, (14.9%).

Conclusions: This study revealed that the clinical signs of active trachoma can persist even when ocular *Chlamydia trachomatis*, were not detected. This may be due to long lasting clinical sign or there might be another causative agent other than *Clamydia Trachomatis*.

Keywords : Trachoma , *Chlamydia. trachomatis*, PCR, Prevalence, Jarmai village.

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Introduction

The World Health Organization (WHO) defines blindness as visual acuity in the better eye of less than 3/60 with available refractive correction, which predicts the inability to walk safely without assistance. The best published estimate suggests that 5.9 million people in the world fulfill this criterion because of trachoma, which makes it responsible for about 15% of all cases of blindness⁽¹⁾. In addition to those already blind, an estimated 600 million people live in areas of Africa, the Middle East, Central and South America, Asia, Australia, and the Pacific Islands, where trachoma is endemic^(1,2). The accuracy of these estimates is questionable, however⁽³⁾. There is a pressing need for further research on the global distribution and prevalence of disease⁽⁴⁾.

Trachoma, is a neglected tropical disease, caused by conjunctival infection with particular serovars

of *Chlamydia trachomatis*. The bacteria are spread by direct contact with ocular and nasal discharges from infected individuals, by contact with fomites, (inanimate objects that carry infectious agents, such as towels or washcloths), and by eye-seeking flies, particularly *Musca sorbens* that by repeated and/or prolonged episodes, may cause ocular infection by the bacterium *Chlamydia trachomatis*.⁽⁵⁾

The disease disproportionately affects individuals living in rural and resource-poor settings, and children are the primary carriers of ocular *C. trachomatis* infection and active disease⁽⁶⁾.

The presence of follicles and inflammation in the upper tarsal conjunctiva, known as active trachoma, is characteristic of childhood infection. Following years of repeated infection, the upper tarsal conjunctiva may become so severely scarred that the eyelashes turn inwards, rub on

the eyeball and cause corneal opacity and blindness⁽⁷⁾.

The year 2020 is the target date for the global elimination of trachoma as a public health problem. As the first step to achieving this goal, mapping is needed to assess the endemicity of trachoma and determine the need for interventions⁽⁸⁾.

The World Health Organization (WHO) advocates the implementation of the SAFE strategy (Surgery for trichiasis, Antibiotics for active infection, Facial cleanliness to prevent disease transmission and Environmental improvement to increase access to water and sanitation) for trachoma elimination. Mass Drug Administration (MDA) of azithromycin aims to clear infection from communities such that trachoma ceases to be a public health concern⁽⁹⁾.

In 2012, the World Health Organization (WHO) estimated that 21 million people had active

(inflammatory) trachoma (trachomatous inflammation – follicular, TF and/or trachomatous inflammation – intense, TI)⁽¹⁰⁾, and more than 7 million people had in-turned eyelashes (trachomatous trichiasis), TT, that could lead to corneal opacity and blindness⁽⁵⁾. Sub-Saharan Africa bears the largest burden of disease, with more than 80% of the cases of trachoma⁽⁵⁾.

In Sudan Trachoma mapping is complete in the northern states except for the Darfur States. TF prevalence was above 10% in three districts and between 5% and 9% in 11 districts. TT prevalence among adults was above 1% in 20 districts (which included the three districts with TF prevalence .10%). The overall number of people with TT in the population was estimated to be 31,072⁽¹¹⁾.

Trachoma grading

Grading systems are used in an effort to standardize diagnosis in

field surveys and research studies. The WHO simplified system was designed as a cut-down version of the FPC (Follicle, Papillae and Cornea opacity) system, with which it was intended to coexist. Thylefors *et al.* considered the simplified scheme suitable for use by “less experienced observers” in “population based surveys or for the simple assessment of the disease at the community level”. It provides considerably less information than the FPC scale ⁽³⁾. However, the simplified system has enjoyed broad acceptance and is now widely used in research, community assessment, and program monitoring by both medical assistant and ophthalmologists. The system requires the examiner to assess an individual for the presence or absence of each of five signs. The WHO simplified system uses the following criteria: TF, trachomatous inflammation, follicular, the presence of five or more follicles at least 0.5 mm in

diameter in the central part of the upper tarsal conjunctiva; TI, trachomatous inflammation, intense, pronounced inflammatory thickening of the upper tarsal conjunctiva obscuring more than half the normal deep tarsal vessels; TS, trachomatous conjunctival scarring, the presence of easily visible scars in the tarsal conjunctiva; TT, trachomatous trichiasis, and at least one eyelash rubs on the eyeball. ⁽³⁾.

Methods

Ethical approval

This study is in accordance with the declaration of NHREC Ethics Sudan Federal Ministry of Health, and Central Institutional review board, Al-Neelain University.

Oral informed consent was obtained from all study participants and their guardians. During survey, all infected children on the study village were treated with a single height-based dose of oral azithromycin in

accordance with WHO and national protocols, and Opticyclin ointment for the children had ocular infection other than trachoma.

Study area and Study design

A population-based prevalence study was conducted in Jarmai village –Alrahad locality, Gadarif State during the period from Nov 2016 to Nov 2017. According to trachoma map in 2010 surveys of trachoma prevalence in the northern states of Sudan TF prevalence was above 10% in three districts ,Gadarif state was one of these district ⁽¹⁰⁾.

Trachoma clinical diagnosis:

A total of 509 children in the age between 1 to 9 years old were clinically examined which are represent all eligible children residents on the day of the survey in Jarmai village. Examination for trachoma signs was conducted by ophthalmic medical assistants trained in using the WHO simplified grading system⁽¹⁰⁾. Potential examiners underwent

training to apply the simplified grading scheme led by an ophthalmologist experienced in trachoma grading. A reliability study was conducted using a set of standardized photographs.

Each eye was examined for trachoma follicles and trachoma inflammatory intense (TF, and TI). Both eyes were examined and findings for the worst affected eye recorded. Signs had to be clearly visible in accordance with the simplified grading system in order to be considered present. Alcohol-soaked cotton was used to clean the examiner's fingers between examinations. Individuals with signs of active trachoma (TF and/or TI) were offered free treatment with antibiotics according to WHO and national guidelines.

Sample Collection:

Conjunctival swab samples collected from children clinically diagnosed as active trachoma (TF and TI) for DNA analysis

collection were done from tarsal conjunctival surface with Dacron polyester swab and with UTM media (Fisher Scientific, UK) sampling was standardized as four horizontal passes across the conjunctiva, with a one-quarter turn between each pass. After collection, swab will be returned to transporting media and stored until used. The sample divided into two parts; a group covered by mass drug administrations, (MDA), with azithromycin, and another group not included in the MDA program. The patients with conjunctivitis or any other eye infections were excluded from investigation.

DNA extraction

DNA was extracted from all samples using the G-spin Total DNA kit (iNtRON Biotechnology, Korea) according to the manufacturer's instructions.

PCR amplification with omcB gene primers:

Chlamydial DNA from clinical samples was amplified using a conventional PCR machine (SENSOQUEST, USA). Touchdown protocol was performed using Maxime PCR PreMix kit (iNtRON, Biotechnology, Korea), and primers for *C. trachomatis* outer membrane protein complex B (omcB) (F/ GAC ACC AAA GCG AAA GAC) (R/ACT CAT GAA CCG GAG CAA CCT)⁽¹²⁾

Amplification was performed in 20µl reaction volumes containing 17µl by add H₂O, 1 µl of primer and 2µl of DNA template. The 106 bp fragment indicates the presence of *C. trachomatis* outer membrane protein complex B (omcB).

PCR Products Detection

All PCR products were subjected to electrophoresis on agarose gel along with a 100 bp DNA marker.

An amounts of 5 -µL of each PCR mixture separated in 1.5%

agarose gel, then stained with 3 μ ethidium bromide and viewed under gel documentation system with a 100 bp DNA marker. A result was considered positive when a band of the size 106 bp was visible in the gel. Standard procedures for reducing contamination were strictly followed.

Results

In total, 509 eligible children were examined in this survey over the Jarmai village. The children participants, of age between 1 to 9 years old i.e. 63 (12.4 %) were clinically

diagnosed as active trachoma (figure 2); of whom 7.0 (11.0%) had follicles in the upper tarsal (TF), 9 (14.0%) had trachoma inflammatory intense (TI) and 47 (75.0%) had the two signs of active trachoma, the follicles and trachoma inflammatory intense (TF and TI) (figure 3). Among those examined 26 (41%) were females, and 37 (59%) of those examined were males (table 1).

The prevalence of *Chlamydia trachomatis* among all children in the village was 1.8% and out of 63 children 9 (14.9%) were clinically diagnosed as active trachoma patients, (figure 4).

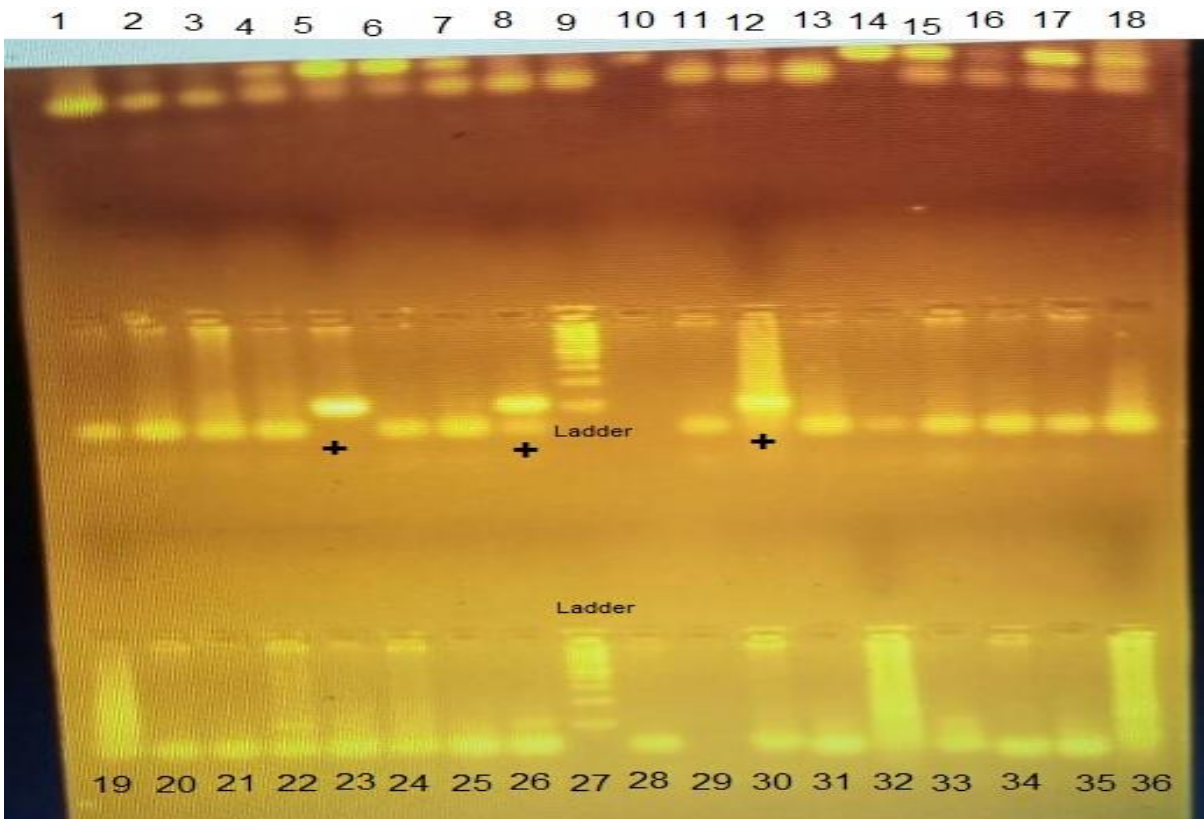


Figure 1 : PCR result of *Chlamydia trachomatis omcB* gene

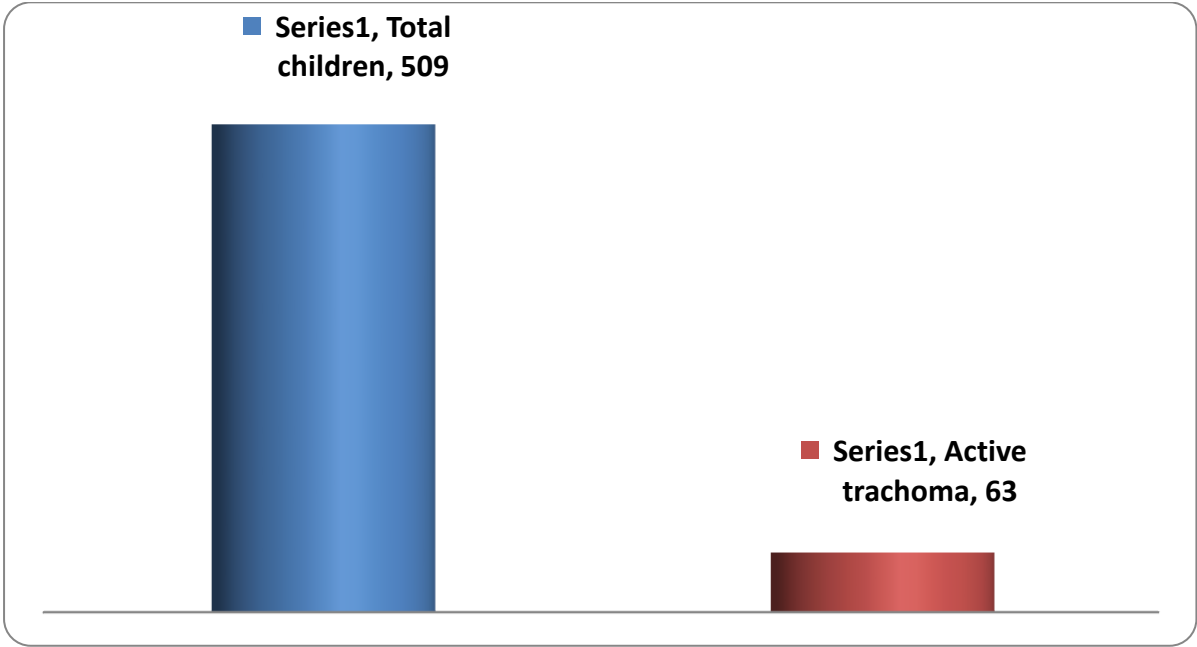


Figure 2 : The prevalence of active Trachoma among children of age 1–9 years old in Jarmai village

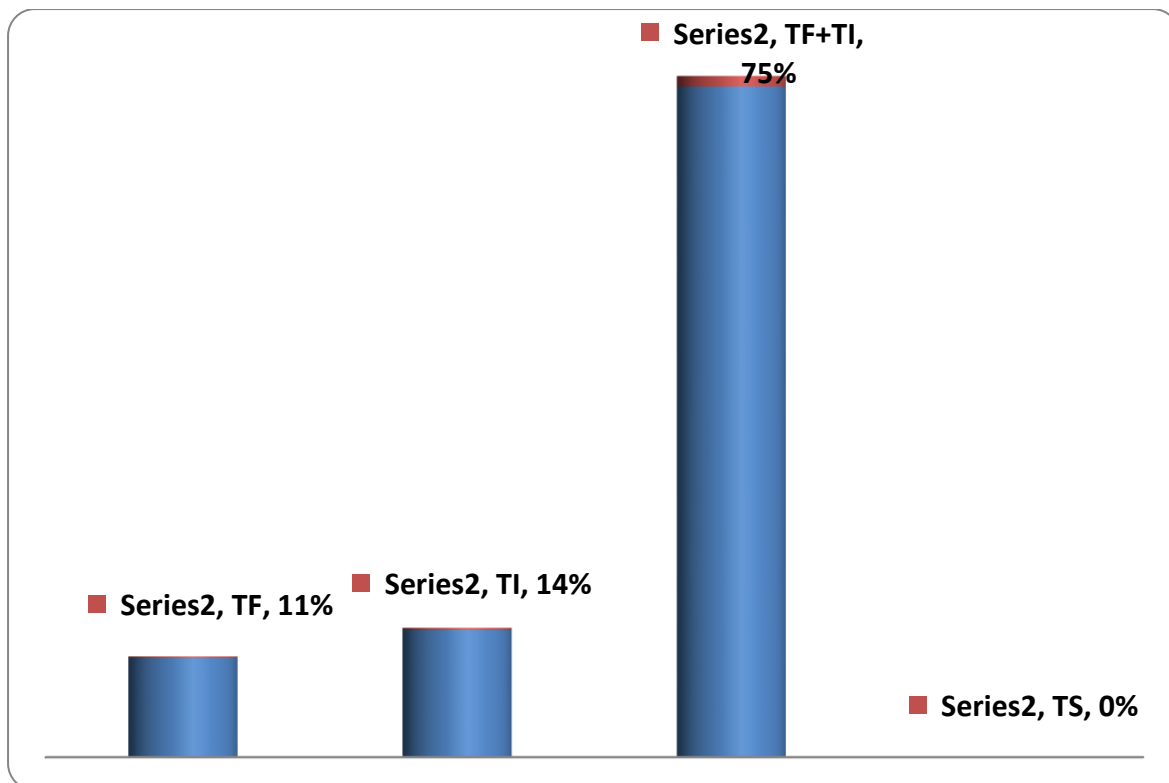


Figure 3 : The prevalence of Trachoma Follicular (TF), Trachoma Inflammatory Intense (TI) and TF+TI among children of age 1 – 9 years old in Jarmai village

Table 1 : The frequency of Trachoma Follicular (TF), Trachoma Inflammatory Intense (TI) and TF+TI in gender

		Trachoma grade			Total
		TF	TI	TF + TI	
Sex	Male	3	4	30	37
		5%	6%	48%	59%
	Female	4	5	17	26
		6%	8%	27%	41%
	Total	7	9	47	63
		11%	14%	75%	100%

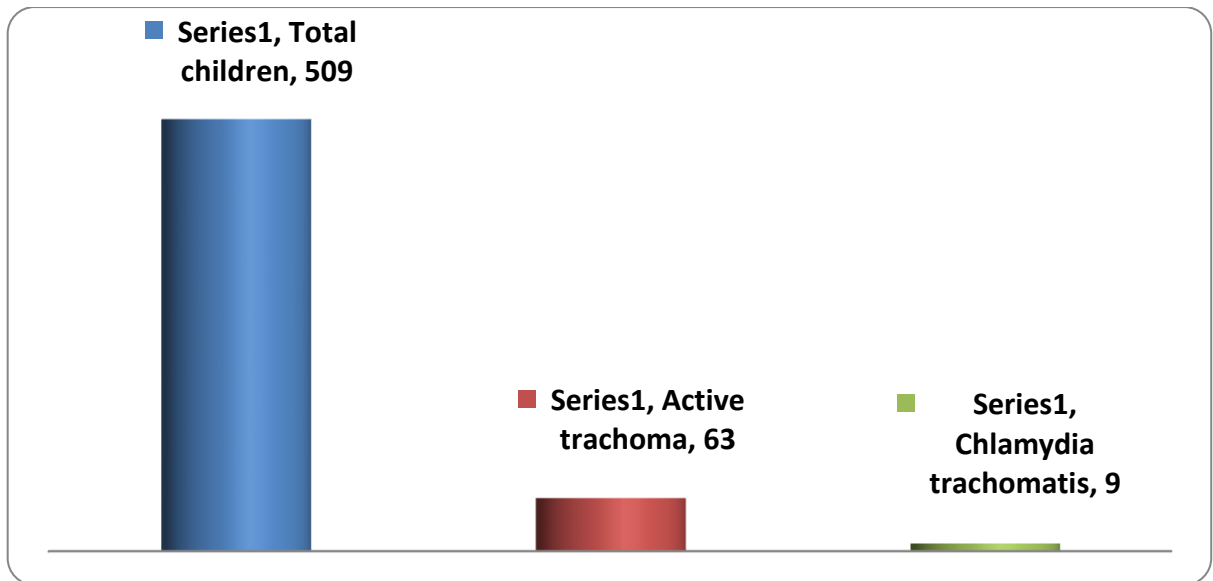


Figure 4 : The prevalence of Trachoma and *Chlamydia trachomatis* among children of age 1 – 9 years old in Jarmai village

Discussion:

The WHO led Global Alliance for the Elimination of Trachoma (GET2020) aims to elimination the disease as a public health problem in the world by 2020. The key trachoma control strategy is SAFE strategy, which is endorsed by WHO. The acronym stands for: “Surgery”; for patients with advanced disease, “Antibiotics”; namely azithromycin or tetracycline eye ointment for active trachoma, “Facial” cleanliness and “Environmental” improvement

on water supply and sanitation^(13,14).

Knowledge of trachoma prevalence at district level is particularly important in planning the national trachoma control program in order to achieve the goal of eliminating trachoma as a blinding disease by the year 2020. District level mapping of trachoma prevalence was carried out in the Northern States of Sudan 2006- 2010⁽¹¹⁾.

The total number of children in the village was 509 of ages between 1 – 9 years. All of them

were clinically examined for ocular trachoma by ophthalmic medical assistants trained in using the WHO simplified grading system. The prevalence of trachoma in Jarmai village was found to be 12.4%. A previous study of mapping trachoma made in Northern State of, Sudan, in Gadarif State where the village of our study is located, registered a prevalence of trachoma between 0.9 and 19.8⁽¹¹⁾.

The results of this population-based impact study demonstrated that the prevalence of active trachoma among children, of ages 1 to 9 years was greater than 10%; indicating that trachoma remains one of the major public health problems in that area of Sudan. and that coincides with the views reported from, South Sudan ⁽¹⁵⁾, countries in Pacific island ⁽¹⁶⁾. India ⁽¹⁷⁾ and Ethiopia ⁽¹⁸⁾ have classified trachoma as an epidemic. The rate of active

trachoma ranged from 25.2%–71.0% .

The prevalence of *Chlamydia trachomatis* children in entire village was 1.8% and out of 63 children, 9 (14.9%) were clinically diagnosed as afflicted by active trachoma , this may be due to a persisting clinical sign of trachoma or there may be due to hosting a causative agent other than *Clamydia. Trachomatis*.

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Screening of Hepatitis C Virus among Blood Donors in the Central Blood Bank, Wad Medani Teaching Hospital - Gezira State, Sudan

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Abstract

Hepatitis C virus (HCV) infection is a major worldwide public health problem. The World Health Organization (WHO) estimates that 3% of the world's populations are chronically infected with HCV, most of these cases occur in Africa. The main objective of this study was to determine the prevalence Hepatitis C Viruses among blood donors attending to the Central Blood Bank in Wad Medani Teaching Hospital. This study was conducted during the period from 2012-2015. One thousand blood samples were collected. The mean age of the subjects was 28.1 ± 6.2 with a range between 18 and 49 years of age. When the selected subjects were screened for HCV by ELISA, only 2/1000 (0.2%) tested positive. This study concludes that the prevalence rate of HCV among blood donors in Wad Medani Blood Bank is 0.2%. Vaccination of HCV for the negative blood donors in is thence recommended.

Keywords: Hepatitis C, Blood Bank, ELISA, blood donors, Vaccination

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Introduction:

Hepatitis C virus (HCV) infection is a major worldwide public health problem. The World Health Organization (WHO) estimates that 3% of the world's populations are chronically infected with HCV, most of these cases occur in Africa^[1]

HCV has a high rate of replication with approximately one trillion particles produced each day in an infected individual. HCV also has an exceptionally high mutation rate, a factor that may help it illude the host's immune response. HCV mainly replicates within hepatocytes in the liver. It utilizes the intracellular machinery necessary to accomplish its own replication^[2]. The prevalence of chronic hepatitis C ranges from 0.1 to 5% in different countries^[3]. It is estimated that there are 4 million HCV chronic carriers in the US and 5 million in Western

Europe. The prevalence seems to be higher in Eastern Europe compared to Western Europe. In industrialized countries, HCV accounts for 20% of cases of acute hepatitis, 70% of cases of chronic hepatitis; 40% of cases of end stage cirrhosis, 60% of cases of HCC and 30% of liver transplants^[4]. Mudawi, *et al* (2007) in a cross sectional study carried out in Gezira State of Central Sudan, (an area with a high prevalence of *Schistosoma mansoni* infection), to determine the prevalence, common genotypes and risk factors for hepatitis C virus infection in Sudanese patients with hepatosplenic schistosomiasis antibodies and risks factors for HCV infection^[5].

Material and Methods:

This study aimed at the prevalence determination of the Hepatitis C Virus among blood donors attending the Central Blood Bank in Wad Medani Teaching Hospital.

According to the random stratified sampling technique, 1000 samples were selected for this study, according to blood donors. All vaccinated blood donors were excluded from the study. Five ml of venous blood were drawn from each participant into EDTA containing tube after obtaining a written informed consent. Anti-HCV antibody was screened spectrophotometrically by ELISA and the absorbancy against assay concentration was registered. Further data was collected by a questionnaire. A descriptive analysis was done by the statistical package for Social Sciences (SPSS)[®] version 16.

Results:

This study consisted of a total of 1000 blood donors from the Central Blood Bank in Wad Medani Teaching Hospital. All the selected subjects were screened for anti-HCV antibodies using both ELISA and ICT. The mean age of the

subjects was 28 years 1 ± 6.2 with a range between 18- 49 years. All of them were males. When the selected subjects were screened for HCV by ELISA, for detection of anti-HCV antibodies, only 2/1000 (2%) were positive for HCV. Those samples, which were confirmed by ICT were also positive. Both of the individuals, who tested HCV-positive, were males with an age of 36 and 47 years. One was a labourer and the other was a driver. Both of them were from the urban areas. One out of the two had previously experienced injury and the other had undergone surgery. When the 998 samples that were tested negative by ELISA, were selected for HCV screening by ICT, all of them were found negative for HCV.

When the blood donors were classified according to age groups, the majority of them were between 21-28 years, 509 (51%), followed by 29-36 years,

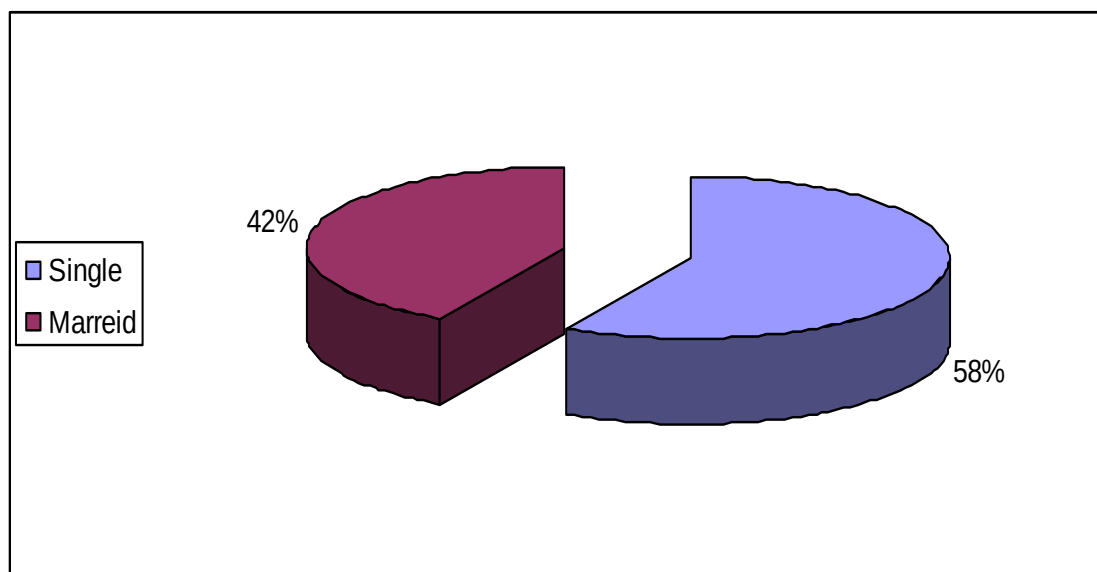
320 (32%), 37-44 years, 93 (58%), while only 419/1000 (9.2%) and 45-52, 13 (1.3%) as shown in Table (1). 1).

The majority of the study subjects were single 581/1000

Table 1: The Distribution of the Study Subjects by Age. (N=1000)

Age group	Frequency
<20	65 (6, 5%)
21-28	509 (51%)
29-36	320 (32%)
37-44	93 (9, 2%)
45-52	13(1, 3%)
Total	1000 (100%)

Figure: 1. The Distribution of the Study Subjects According to their Marital Status



Discussion:

Hepatitis C virus (HCV) infection has been reported to be a prevalent disease since the second half of the 20th century⁶. It is also a major cause of end stage liver disease in many parts of the world. Approximately 3% of the world populations, 170 million people, are chronically infected by HCV⁷. HCV is transmitted primarily by exposure to infected blood. However, in up to 50% of cases no recognizable transmission factor/route could be identified⁸. The studies on HCV infection in Sudan demonstrated similar seroprevalence ranging from 2.2% in the Gezira state, an area endemic with schistosomiasis to 4.8% in patients with schistosomal periportal fibroses^[9]. The highest prevalence of HCV infection in Sudan was noted in patients with end stage renal disease on regular hemodialysis with a seroprevalence of 23.7%. Major

risk factors for infection were the longer durations of dialysis, dialyses in multiple centers, and the age above 30 years.

These findings were again Low compared to those obtained by Abou *et al.*, (2009) in Nyala, Sudan that were reactive for anti-HCV (0.65%).^[10]

These results were Low compared to those obtained by Elsheikh who found that the prevalence of anti-HCV among pregnant women in Sudan was 0.6%^[11]. These results were inconsistent with those reported by Sirchia, in Italy^[12], who found a prevalence of (1%). Almost a bit similar to that were the results obtained by Frommel in Ethiopia (1.4%) and Aceti had reported a relatively lower result in Somalia (0.97%)^[13,14]. Studies carried out in Saudi Arabia (1992) revealed that HCV infection was more prevalent among apparently healthy individuals, who were over 50 years of age (5%).

However, studies conducted in Egypt (1993) among university student blood donors showed HCV prevalence of 9.2%, while a prevalence of 26.6% was obtained among blood donors in Cairo. The results of this study were high compared to those obtained by Abdelbagi M. Nagi, in Shandi, Northern Sudan who found that the Seroprevalence of anti HCV antibodies among blood donors was (1.3%)^[15].

Blood Donors are often victims of occupational injuries by contaminated tools or projection of contaminated fluids in to mucous membranes and are therefore at risk for transmission of HIV, HBV and HCV. Van Wijk and Pelk-Jongen^[16] stated that blood exposure incidents pose a risk for transmission of blood borne pathogens among health care workers.

Conclusion and

Recommendations:

The HCV infection is a serious menace, especially, that it can

develop easily to Chronic HCV, which might, eventually, mature into cirrhosis of the liver and hepatocellular carcinoma (HCC) both of which are fatal diseases. Notwithstanding the relatively low prevalence of HCV, locally one should bear in mind that the hovering threat of the transmission from neighboring countries. Egypt, for instance, has the highest rate of infection in the globe and the entire African states are not secure. Vaccination and proper treatment are recommended.

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Evaluation of Microalbuminuria as an Early Predictor Marker of Nephropathy in Diabetic Patients in Khartoum State

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Abstract:

Background. Untreated diabetes mellitus can cause long term complications and cause chronic kidney failure or nephropathy. The aims of this study was to assess microalbumin: creatinine ratio in diabetic patients as an early predictor marker of diabetic nephropathy.

Materials and Methods. This study was conducted at Soba hospital and East of Nile hospital in Khartoum state during the period from February to May 2016. In this study 70 urine samples were collected and analyzed with full automated chemistry analyzer (COBAS C311); 35 samples from diabetic patients and 35 samples from healthy individuals as control.

Results. There was a significant increase in the mean value of microalbumin: creatinine ratio in the diabetic patients (88.5 mg/g) compared with the mean value of microalbumin: creatinine ratio in the normal individuals (26.2 mg/g) with *p-value* (0.05). There was no correlation between microalbumin: creatinine ratio with age among diabetic patient, *p-value* (0.877).

Conclusion. From this study it was concluded that microalbumin: creatinine ratio level in diabetic patients increased and influenced by both type 2 diabetes mellitus and the duration of disease. Therefore, regular screening for microalbumin is recommended for all diabetic patients, as early diagnosis and treatment are critical for reducing cardiovascular risks and slowing the progression to late stages of diabetic nephropathy.

Key words:

Microalbuminuria, creatinine, diabetes mellitus, chronic kidney failure.

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1. Introduction:

Diabetes mellitus (DM) commonly referred to as diabetes, is a group of metabolic diseases in which there are high blood sugar levels over prolonged period (Bishop Michael L., 2010)

Symptoms of high blood sugar include frequent urination increased thirst and increased hunger, if left untreated diabetes can cause many complication; acute complications include diabetic ketoacidosis and non ketotic hyperosmolar coma Serious long term complications include cardiovascular disease, stroke, chronic kidney failure, foot ulcers and damage of the eye (Bishop Michael L.,2010).

Damage to the kidneys known as diabetic nephropathy can lead to tissue scarring urine protein loss and eventually chronic kidney disease sometimes requiring dialysis or kidney transplant (Klausen K & Borch-Johnsen K.,2007)

Patients who are at risk of kidney disease need a microalbuminuria test

check of albumin in the urine. Large amount of albumin in the urine indicate kidney damage and this can lead to chronic kidney disease. Microalbumin occur due to the loss of systemic endothelial glycocalyx-a protein rich surface layer on the endothelium. (S. C. Satchell & J. E. Tooke, 2008)

One of the functions of the kidney is to conserve plasma protein so it will not be excreted in the urine. Any failure on the part of the kidneys to excrete creatinine results in a rise of level in the blood (S. C. Satchell& J. E. Tooke.,2008).

Microalbumin and creatinine ratio act as an early indication for kidney damage and the factors that affect the microalbumin creatinine ratio include: vigorous exercise, the presence of fever, urinary infection, congestive heart failure, acute severe elevations of blood pressure or blood sugar or menstruation (S. C. Satchell& J. E. Tooke., 2008).

Diabetic Nephropathy (DN) begins as glomerular hyper-filtration (increased GFR); GFR normalizes with early renal injury and mild hypertension, which worsens over time. Microalbuminuria, urinary excretion of albumin in a range of 30 to 300 mg albumin/day, then occurs. Urinary albumin in these concentrations is called microalbuminuria because detection of proteinuria by dipstick on routine urinalysis usually requires > 300 mg albumin/day. Microalbuminuria progresses to macroalbuminuria (proteinuria > 300 mg/day at a variable course, usually over years. Nephrotic syndrome (proteinuria $\geq$ 3 g/day) precedes end-stage renal disease, on average, by about 3 to 5 year, but this timing is also highly variable. Other urinary tract abnormalities commonly occurring with DN that may accelerate the decline of renal function includes papillary necrosis, type IV renal tubular acidosis, and UTIs. In DN, the kidneys are usually

of normal size or larger. Diabetes causes a number of changes to the body's metabolism and blood circulation, which likely combines to produce excess reactive oxygen species (chemically reactive molecules containing oxygen). These changes damage the kidney's glomeruli (networks of tiny blood vessels), which leads to the hallmark feature of albumin in the urine (called albuminuria) (Mundel P & Shankland SJ., 2004).

As diabetic nephropathy progresses, a structure in the glomeruli known as the glomerular filtration barrier (GFB) is increasingly damaged. This barrier is composed of three layers including the fenestrated endothelium, the glomerular basement membrane, and the epithelial podocytes. The GFB is responsible for the highly selective filtration of blood entering the kidney's glomeruli and normally only allows the passage of water, small molecules, and very small proteins

(albumin does not pass through the intact GFB) (Navin Jaipaul.,2013).

2. Materials and Methods:

The study comprised a total of 70 individuals of which 35 individuals with evidence of DM were included on the basis of clinical signs and symptoms of DM.

Other 35 healthy individuals were used as a control group, whose age and sex matched healthy.

Diabetic patients who were suffering from cardiovascular disease, hypertension, and liver disease were excluded from the study. Informed consent was taken from the patients and subjects who participated in the present study, and that after agreement of general managers of Soba Education Hospital and East of the Nile Hospital.

Ethical committee approval has also been obtained from Elrazi University and ministry of health.

In both groups urine albumin and urine creatinine levels were estimated and then urine albumin: creatinine ratio was obtained. The urine albumin and

urine creatinine was estimated by using full automated analyzer (cobas C311).

Statistical data: All data were expressed as mean $\pm$ SD. For the statistical significance, T test was performed using SPSS.

3. Results:

This study was conducted in soba University hospital and East of the Nile Hospital in Khartoum state. The (70) individuals were engaged in the study, (35) type 2 diabetic patient and (35) group matched normal healthy individuals. The mean of microalbumin to creatinine ratio in type 2 diabetic patients was found to be (88. 5mg/g $\pm$ 26.6) and control group (26.2mg/g $\pm$ 17.7) Table (1).The mean of microalbumin to creatinine ratio in type 2 diabetic patients according to different age group was classified as 1-5 years (76.6 $\pm$ 25.8SD) and >5 years (98.4 $\pm$ 44.5SD) Table (2).

There was no correlation of microalbumin to creatinine ratio with age P value (0.8) Table (3).

Table (3-1): Shows the mean of microalbumin to creatinine ratio in type 2 diabetic patients (case) and control group result expressed as (mean $\pm$ SD):

Parameter	Sample	Number	Mean $\pm$ SD	P. value
microalbumin to creatinine ratio (mg/g)	Case	35	88.5 $\pm$ 26.6	0.05
	Control	35	26.2 $\pm$ 17.7	

Table (3-2): Shows the mean of microalbumin to creatinine ratio in type 2 diabetic patients according to different age group classified as 1-5 years and >5 years, result expressed as (mean $\pm$ SD):

Parameter	Age group	Number	Mean $\pm$ SD	P. value
Microalbumin : creatinine ratio (mg/g)	1-5 years	16	76.7 $\pm$ 25.8	0.691
	>5 years	19	98.4 $\pm$ 44.5	

Table (3-3): Shows the correlation of microalbumin: creatinine ratio with age:

Variable	parameter	Number	Pearson correlation(r)	P. value
Age(years)	Microalbumin : creatinine ratio (mg/g)	35	0.027*	0.877

* No correlation

Discussion:

The first clinical sign of renal dysfunction is generally microalbuminuria, so the early detection of renal involvement in case of diabetes by measuring microalbumin as an early indicator becomes mandatory. This study was done to correlate between

microalbumin/creatinine ratio with duration of diabetic in Sudanese adults in Khartoum State. The study sheds light on the microalbumin /creatinine ratio in urine samples collected from diabetes type 2 patients from Soba Education Hospital. Thirty five samples were collected from different patients with different ages between 30-65 and different durations of diabetes.

As in table (3-1), the result expressed that there is a significant increase in the mean of microalbumin/creatinine ratio in diabetic patients compared with control group ($p\text{-value} =$

0.05). This finding was agreed with Shahid Ahmed (2008).

As in table (3-2), the result expressed that there is insignificant increase in the mean of microalbumin/creatinine ratio in diabetic patients according to duration of disease in the categories (1-5 years) and > 5 years ($p\text{-value} = 0.69$). This finding was disagreement with M. Afkhami-Ardekani *et al.*, (2008). They stated that the duration of diabetes has a significant contribution for the development microalbuminuria by prolonged exposure to hyperglycemia-induced advanced glycosylation end products accumulations also the control of diabetes with irregular treatment also plays a significant role in the development of diabetic nephropathy.

As in table (3-3), the present study showed that there is no correlation of microalbuminuria/creatinine

ratio with age in diabetic patients ($p\text{-value}= 0.87$). This finding was in disagreement with Jensen JS *et al* (1997). This disagreement may be contributed to spot urine sample which we made in the analysis of this type of sample, but Jensen J, S *et al.*, made analysis in 24hr urine sample.

Conclusion:

In general, albumin to creatinine ratio has been shown to have slightly better diagnostic accuracy than urine albumin concentration alone for detection of albuminuria in many populations. From this study it was concluded that microalbumin: creatinine ratio level in diabetic patients increased and influenced by diabetes mellitus type 2 and duration of disease. Therefore, regular screening for microalbumin is recommended for all diabetic patients, as early treatment is critical for reducing cardiovascular risks and slowing

the progression to late stages of diabetic nephropathy (overt proteinuria and end-stage renal disease).

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Albumin:Creatinine Ratio as Predictor Markers for Nephropathy in Sudanese Type 2 Diabetic Patients

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Abstract

Introduction: The aim was to assess albumin: creatinine ratio in type 2 DM patients and its relation to diabetic nephropathy complication.

Materials and methods: This case control study was conducted at Military hospital, (n = 205) diagnosed type 2 DM patients (94 males and 111 females), and [n 100] controls were included. Urinary Albumin: Creatinine ratio, ACR, was estimated by Cobas C-311[®] fully automated analyzer.

Results: Type 2 DM patients had higher level of albumin: creatinine ratio than controls *p*-value (0.000) No significant difference in level of albumin: creatinine ratio between males and females *p*-value (0.271). Moreover, patients, who received statin drugs showed increase in albumin: creatinine ratio level *p*-value [0.046] and there was a positive correlation between albumin: creatinine ratio level and duration of disease (r: 0.280, *P*-value 0.00).

Conclusion: The data suggested that, albumin: creatinine ratio is a useful diagnostic and predictor marker for diabetic nephropathy in type 2 DM patients.

Keywords

Albumin: creatinine ratio, nephropathy, type 2 DM, Sudan.

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Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder^[1-3]. Nearly, DM became most common chronic disorder^[4] with increasing prevalence worldwide^[5]. Diabetes affected more than 371 million adults worldwide in 2012. This number is expected to increase by 2030 to approximately 552 million adults^[6], 80% were living in low and middle income countries^[7]. In Sudan, prevalence of diabetes is rising from 9.3% in 2010 to 10.6 % in four states in 2013^[7].

Type 2 diabetes mellitus is a complex condition, result from resistance of insulin action combine with impaired pancreatic function^[8]. If left untreated, Type 2 DM can lead to chronic microvascular and macrovascular complications^[9]. Some studies reported that, Type 2 DM is frequently accompanied with chronic low grade of inflammation which plays vital role in the development and

progression of diabetic complications^[10].

One of the chronic and serious complication of type 2 DM is diabetic nephropathy^[11, 12]. It can lead to increase mortality and morbidity and decrease the life quality^[13]. The earliest clinical indication of nephropathy is the appearance of albumin in the urine^[14]. The term 'microalbuminuria' meaning 'small size', actually refers to the presence of a relatively 'small quantity' of protein in the urine^[15]. Detection of albumin: creatinine ratio (ACR) is an important screening tool because it is used as an early indicator of kidney disease progression and one of predictive factor of cardiovascular diseases, (CVD)^[16-20].

Previous studies documented that, diabetic patients undergo a subsequent increase in the level of urinary albumin^[21] that leads to activation of inflammatory pathways and progression of

renal and cardiovascular complications [22,23]. Therefore, we hypothesized that, ACR is associated with nephropathy in type 2 DM patients.

Materials and methods

In case control study we examined n=205 type 2 DM patients, [94 males and 111 females]¹. The age for both ranged from 39 to 75years old. As control 100 subjects were recruited from both age and sex that apparently match healthy individuals. DM patients with inflammatory, liver and/or renal diseases were excluded. Written consents information was obtained from each participations. This study was approved by local committee of Al-Neelain University, Sudan.

Measurement of ACR

According to manufacturer, urine albumin and creatinine were analyzed by Cobas C-311[®] fully automated analyzer. Anti-

albumin antibodies reacted with antigen in the sample to form antigen-antibody complexes, followed by agglutination, and then complexes were measured turbidimetrically. Moreover, creatinine reacted with picrate in alkaline medium to form a yellow-red adduct. The rate of the dye formation was directly proportional to the creatinine concentration in the specimen and was measured photometrically.

Measurement of BMI

Body mass index [BMI]¹ was calculated using (weight kg/hight m²) formula.

Statistical analysis

All statistical analyses were performed using the SPSS software (Statistical Package for the Social Sciences)¹ [version 17.0; SPSS Inc.). Independent *t*-test was employed to compare mean between groups. Pearson's correlation was used to correlate between study parameters and variables. Quantitative variables

were demonstrated as mean $\pm$ SD and significant differences were considered as P -value ≤ 0.05 .

Results

The study comprised of 205 Type 2 diabetic patients and 100 health apparently controls. The mean level of ACR was significantly increased in the case group (37.3 $\pm$ 29.3) compared with control group (7.27 $\pm$ 6.38) with a p -value 0.000¹ as shown in (figure 1).

The results of the study showed insignificant differences in mean of ACR between males

(30.6 $\pm$ 8.60) and females [42.9 $\pm$ 7.21¹ with (p -value 0.271) as presented in (figure 2).

Figure 3 revealed that the patients' group that took cholesterol lowering agent (statin) showed a significantly higher ACR level (44.3 $\pm$ 85.3) in contrast to untreated Patients (23.6 $\pm$ 39.1) with [p -value 0.046¹].

In correlation analysis, there was positive correlation between ACR and duration of diabetes (r : 0.56 p -value 0.000) as demonstrated by (table1).

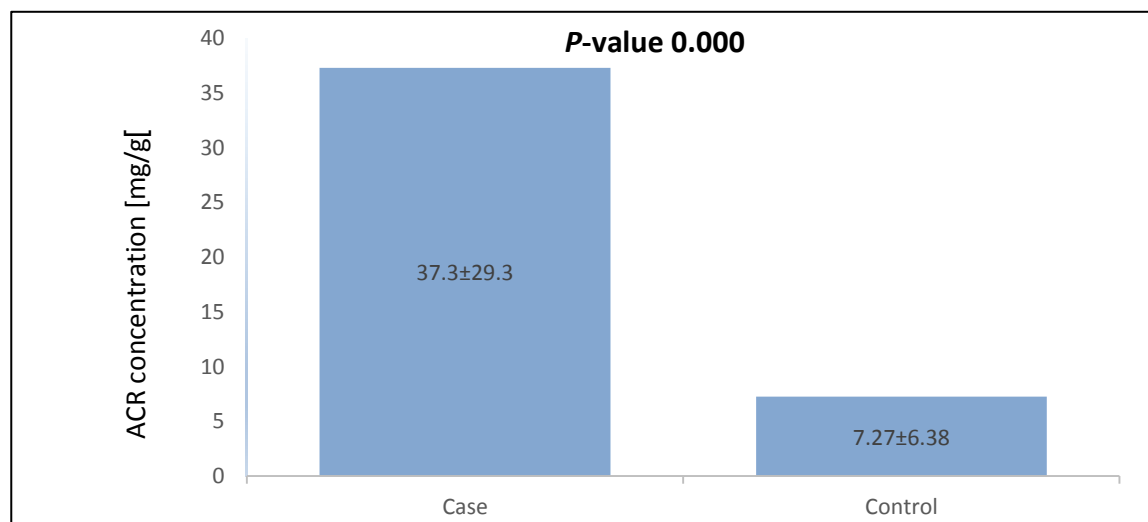


Figure (1): mean level of ACR in case and control groups

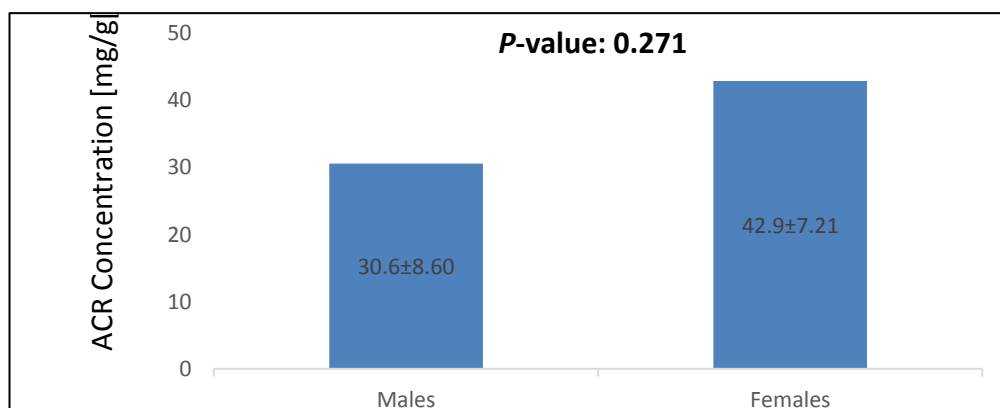


Figure (2): mean level of ACR in patients group classified according to gender

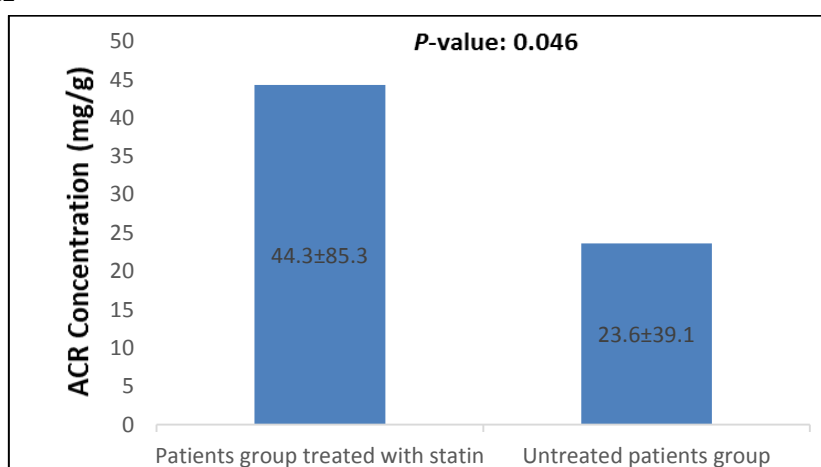


Figure (3): mean level of ACR in patients group classified according to statin treatment

Table ^[1]: Correlation of ACR with BMI, age and duration of disease

Correlation	R	P-value
ACR with BMI	0.06	0.378
ACR with age	0.28	0.688
ACR with duration	0.28	0.000**

(R): Pearson correlation test, P-value less than 0.05 was statistically considered significant.

Discussion

In this study we found that, ACR level was significantly higher in type 2 DM patients compared to control. Our results were in agreement with previous studies, which suggested that, urine albumin/creatinine ratio was significantly increased in type 2 diabetic patients ^[24, 25]. Recently, the inflammatory pathway play the curtail role in type 2 pathogenesis, development and progression of the diabetes complications ^[14]. ACR also revealed insignificant differences in males when compared with females. Indeed, our results were in line with another study that reported that the prevalence of microalbuminuria was not statistically different according to gender ^[26].

Moreover patients who received statin treatment reported higher ACR; earlier studies suggested that inhibition of mevalonate synthesis by statins resulted in

proteinuria by its action in proximal tubules ^[27].

The correlation between ACR and duration of disease reveled positive association, whereas no correlation was observed between BMI and age. Chinese type 2 DM patients showed positive correlation of ACR with course of disease and BMI and no correlation with age ^[28]. Other study observed linear relationship of ACR with age but no correlation with BMI ^[29]. The discrepancy in the findings of studies may be attributed to variation in dietary habits, life style, heterogeneity and ethnicity of selected population.

Conclusion

The study concluded that, ACR had significantly higher in type 2 DM patients and positively associated with duration of disease. Therefore, ACR might be useful diagnostic predictor marker for nephropathy in type 2 DM patients. Since, periodic monitoring should be

recommended especially for patients with long time of DM.

Acknowledgment

The authors gratefully acknowledge Dr. Nazik Mahmoud (the Specialist of Diabetes and Endocrinology) at diabetic center of Military hospital for her valuable assistance in this study.

Competing of interest

All authors have declared that, no competing of interest exists.

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Fasting C-peptide Levels as Predictor Marker for Type1 Diabetes Mellitus among Diabetic Children at Khartoum State.

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Abstract:

Background: Diabetes Mellitus (DM) is a major health problem worldwide. It can lead to life-threatening complication. So C-peptide (Cp) serves as a surrogate of pancreatic beta-cell reserve which can predict complication.

Objectives: To evaluate the clinical significance of basal Cp as a predictor of type 1 diabetes (T1D) and investigate the association of serum C-peptide level and BMI, HbA1c, age, age of onset and duration of disease in children with diabetes mellitus.

Methods: It was a descriptive cross sectional study conducted at Mohammed Elamin Hamid Pediatric Hospital during the period from November to April 2018. In this study 40 blood samples were collected and analyzed colorimetrically using ELISA technique, 40 samples (18 boys and 22 girls) from children with diabetes mellitus conducted in it. Statistical analysis: all the data was analyzed using (SPSS) version 21. The results were expressed as Mean $\pm$ SD and percentage. One sample t-test and personal correlation test used to correlate between C-peptide and age, age of onset, duration of disease, HbA1c and BMI at p-value of < 0.05 considered as significant.

Results: It demonstrated that C-peptide was significantly decreased in diabetic patients compared to the mean of normal value (0.45 ± 0.52 , 1.55), (p value = 0.001), While HbA1C was significantly increased in diabetic patients compared to the mean of normal value (10.1 ± 2.5 , 5.25). Also we found that C-peptide was weakly negative insignificant correlated with age, age of onset and HbA1C (R = -0.016, -0.016 and -0.046 respectively), (p value = 0.9, 0.9 and 0.7 respectively), while weakly positive insignificant correlated with BMI (R = 0.021), (p value = 0.8).

Conclusion: The study has concluded that the children with diabetes mellitus have significant decrease in C-peptide level and significant increase in HbA1c level in both male and female, compared to the reference value.

Keywords: Type1 Diabetes Mellitus, C-and glycemic control, C-peptides, BMI.

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INTRODUCTION:

Diabetes Mellitus (DM) is a major health problem world- wide (Jawad A.Al-Lawati, 2017). In 2017, approximately 5 million deaths worldwide were attributable to diabetes. Patients may experience acute life-threatening hyperglycemic episodes; As the disease progresses, and patients are at increased risk for the development of specific complications that cause considerable morbidity through micro vascular complications as well as premature mortality is predominantly driven by macrovascular complications. The destruction of beta cells leads to insulin deficiency and abnormalities in carbohydrate, fat and protein metabolism. Retention of cell function in patients with type 1 diabetes is known to result in improved glycemic control and reduced hypoglycemia, retinopathy,

and nephropathy, (R.Goldenberg and Z. Punthakee, 2013). Diagnostic approaches for diabetes to prevent the complications which are related to diabetes are very important to diagnose diabetes at an early stage (Ali A *et al*,2017). Until recently, the laboratory diagnosis of diabetes has been based on the presence of elevated fasting plasma glucose (126 mg/dl) or 2 h postprandial plasma glucose levels greater than 200 mg/dL (JanakaKaralliedde and Luigi Gnudi ,2016).There are several parameters which can serve as a diagnostic marker to diabetes. Some of the diagnostic markers, which can be utilized for the types of diabetes are, when a patient is suffering from blood sugar, HbA1c, c-peptide, autoantibodies, glycated albumin and glycated protein (Ali A, *et al*., 2017). C-peptide is a useful tool in the classification of diabetes. It can

help differentiate T1DM, T2DM, and MODY. C-peptide is associated with duration of disease as well as age of diagnosis. Whilst C-peptide is useful in classifying diabetes it must always be interpreted in clinical context of disease duration, comorbidities, and family history (Limei Wang *et al*, 2012).

Materials and Methods:

The study comprised a total of 40 children, who were previously, diagnosed with diabetes on the basis of clinical signs and symptoms of diabetes mellitus, and who were under exogenous insulin treatment. Their ages ranged between 7-18 years old and were classified into two groups according to duration: (30 patients) were 0-5 years of duration, (10 patients) were from 6-10 years duration with diabetes.. Subjects

who were suffering from chronic kidney disease, liver disease and obese children were excluded from the study. Informed consent was taken from the patients and subjects who participated in the present study, after the agreement of general managers of hospitals and private centers. Ethical committee approval has also been obtained from

Omdurman Islamic university, Khartoum State, Ministry of Health Research Department and from Mohammed El-amin Hamid Pediatric Hospital. In all the children, fasting serum C-peptide and glycated hemoglobin were estimated. The serum C-peptide was estimated by immune-enzymometric assay and blood glycated hemoglobin by fluorescence immunoassay.

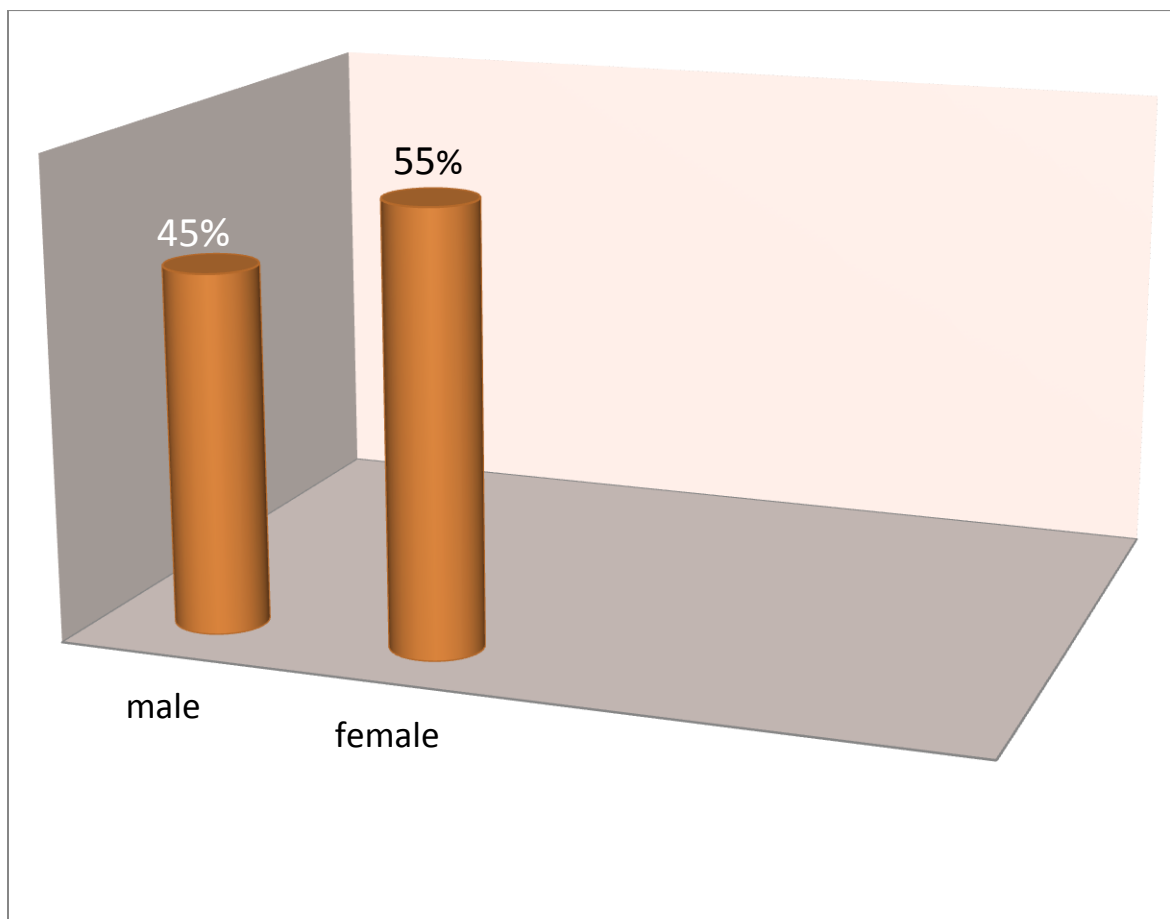
Statistical data:

All the data were expressed in Mean and Standard deviation. For the statistical significance, One sample T test was performed using SPSS software.

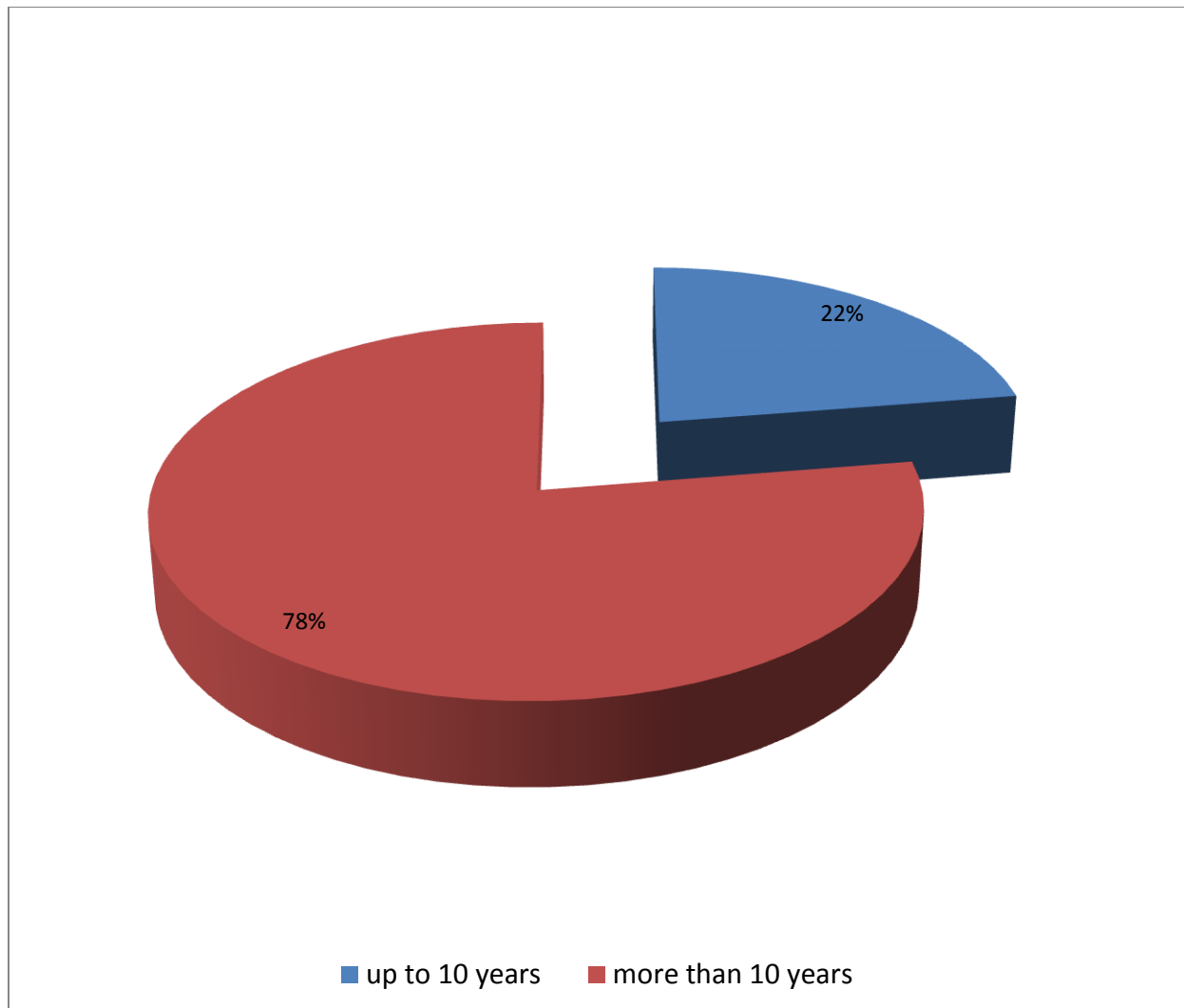
Results:

Our result demonstrated that C-peptide was significantly decreased in diabetic patients compared to the mean of normal value (0.45 ± 0.52 , 1.55), (p value = 0.001), While HbA1C significantly increased in diabetic patients compared to the mean of normal value (10.1 ± 2.5 , 5.25). Also we found that C-peptide was weakly negative and insignificantly correlated with age, age of onset and HbA1C ($R = -0.016$, -0.016 and -0.046 respectively), of (p value = 0.9 , 0.9 and 0.7 respectively), while weakly positive insignificantly correlated with BMI ($R = 0.021$), (p value = 0.8). The disease was more common

in patients with history of DM (55%) compared to those with no family history of DM (45%). Also the results revealed that frequency of disease in male was 45% and in females 55% which indicates predominance of disease in female more than male. Among children of study there was 55% of them with normal weight and 40% underweight, while 5% of them appeared over weight. The frequency of disease in patients with age up to 10 years was 22% and 78% in patients with more than 10 years of age.

Figure 1: frequency of disease among gender.

The figure revealed that frequency of disease in male 45% and female 55% which indicates predominance of disease in female more than male.

Figure 2: frequency of disease among age group

The figure shows the frequency of disease in patients with up to 10 years of age 22% and 78% in patients with age more than 10 years.

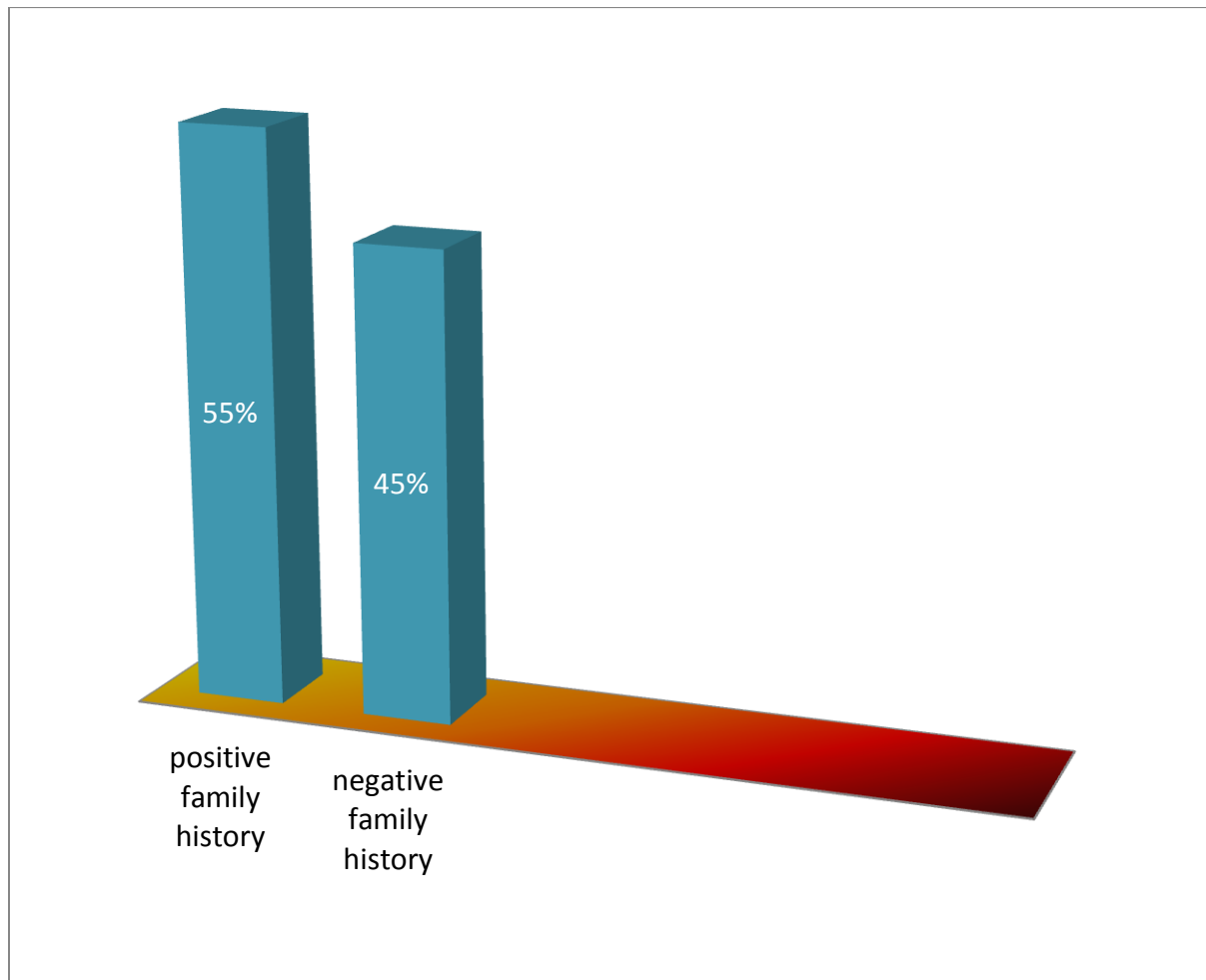
Figure 3:

Figure 3: Frequency of disease in family history of DM. It was obvious that disease was more common in patients with history of DM (55%) in contrast to those with no family history of DM (45%).

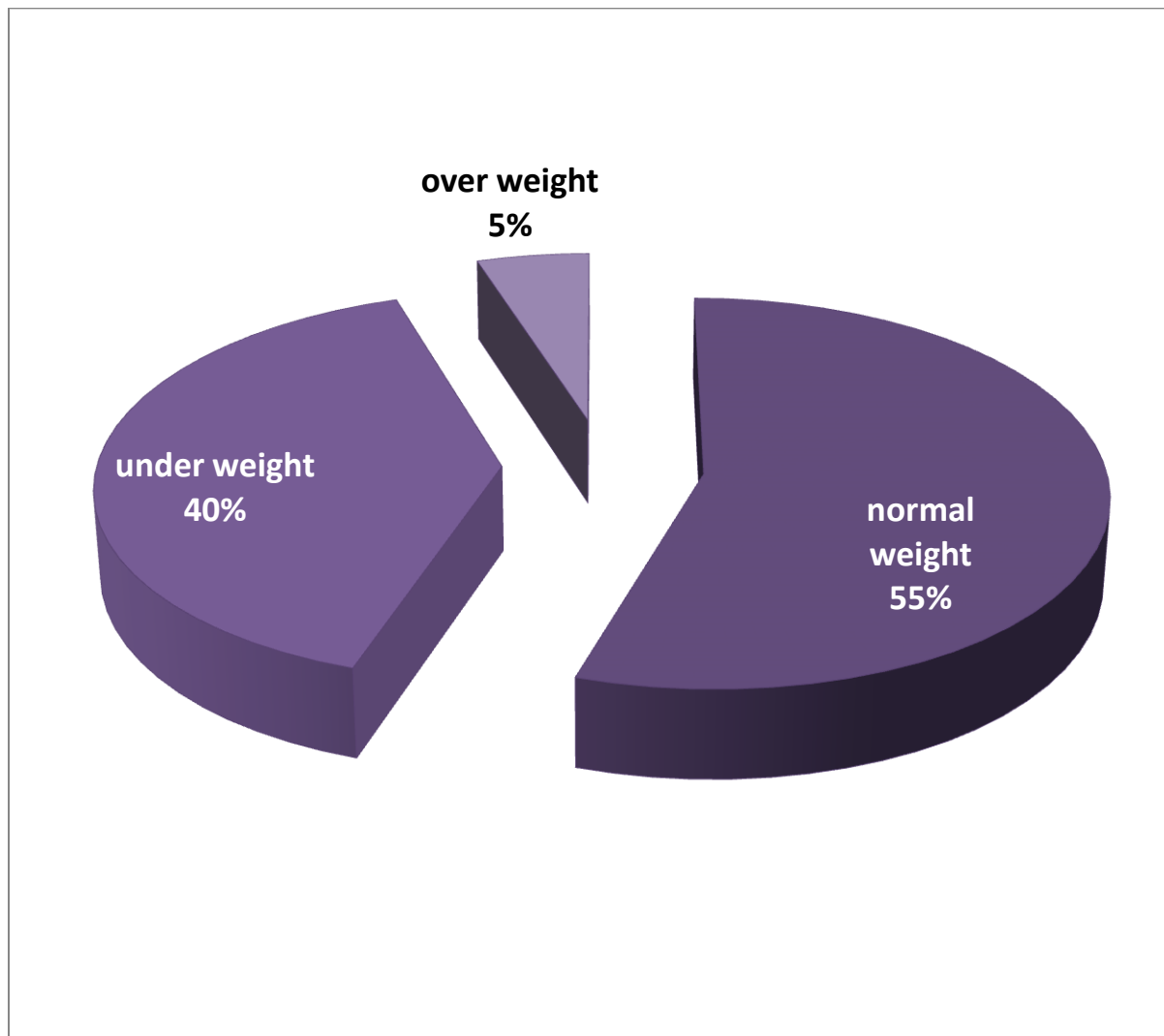


Figure 4: BMI frequency among children. The figure shows that among children of study there are 55% of them with normal weight and 40% underweight, while 5% of them appeared over weight.

Table 1: Comparison of HbA1C and C-peptide with mean of normal value (result expressed as Mean $\pm$ SD). our result demonstrated that HbA1C was significantly increased in diabetic patients compared to the mean of normal value (10.1 ± 2.5 , 5.25), (p value = 0.000), while C-peptide was significantly decreased in diabetic patients compared to the mean of normal value (0.45 ± 0.52 , 1.55), (p value = 0.001).

Parameters	Mean $\pm$ SD	Mean of normal value	P value
HbA1C %	10.1 ± 2.5	5.25	0.000
C-peptide ng/ml	0.45 ± 0.52	1.55	0.001

Table 2: Shows the correlation of C- peptide with age, age of onset, duration of Disease, BMI and HbA1C. The study revealed that C-peptide was weakly negative insignificantly correlated with age, age of onset and HbA1C (R = -.016, -.016 and -.046 respectively), of (p value = 0.9, 0.9 and 0.7 respectively), while weakly positive insignificant correlated with BMI (R = 0.021), (p value = 0.8).

Parameter	R and P value	Age	duration	age of onset	BMI	HbA1C
C peptide	R value	-.016	.056	-.016	.021	-.046
	P value	0.9	0.7	0.9	0.8	0.7

Discussion:

Type1diabetes results from an immune mediated destruction of pancreatic beta cells that begins long before, and is believed to continue long after, the clinical diagnosis of type1diabetes (Palmer JP 2009). So measurement of C-peptide is a well-accepted method for the quantification of endogenous insulin secretion, beta cell function (Jones AG and Hattersley AT 2013; Kulkarni CM and Patil S 2016) also to differentiate between type1 diabetic patients and MODY being misdiagnosed as type1diabetes. (Maria Thunander *et al.*, 2012); especially in children. This study showed that C-peptide level was significantly decrease in diabetic children compared to the mean of normal value (0.45 ± 0.52 , 1.55), (p value =0.001) that was agreed with studies were done

by Emma Leighton, Landin-Olsson M, Jung Won Hwang and Kuhreiber (Landin-Olsson M *et al.*, 1990; Kuhreiber *et al.*, 2015; Emma Leighton *et al.*, 2017; Jung Won Hwang *et al.*, 2017). While the HbA1c level was significantly increased in diabetic children compared to the mean of normal value (10.1 ± 2.5 , 5.25), (p value =0.000) in this study. According to new ADA criteria for diagnosis of diabetes, a study done by Aljabri KS and Bokhari SA 2013 in Saudi Arabia. (Aljabri KS and Bokhari SA 2013).

C-peptide was insignificant correlated with Age, Age of onset, HbA1c and BMI, this study opposite to study done by Lauria, who found significant correlation when the C-peptide level decrease (Lauria *et al.*, 2015), this disagree due to; low sample size used in our study, difference in

classification groups according to duration ,age group

intravenous glucagon.
Diabetologia; 13: 615-9

Conclusions:

From the results of this study it was inferred that: the level of fasting c-peptide is decrease in the type 1 diabetes mellitus and poor glycemic control in those patient. There is no correlation between level of c-peptide and age, age of onset, glycosylated hemoglobin and body mass index.

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Alum Hematoxylin and Eosin Stain, Comparison between Bluing in Running Tap water and Ammonia Solution

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Abstract:

Background: Hematoxylin together with eosin (H&E) stain has been the most universal and traditional staining method. The accurate nuclear reaction is essential for tissue evaluation. **Objective:** is to compare between bluing agent of hematoxylin in running tap water at different pH values (6.1, 6.7, and 7.7) against ammonia solution as the standard pH [8.9]. **Materials and methods:** The study included 72 formalin fixed different tissues stained with Mayer's hematoxylin and treated with tap water at different pH values.

Results: the study showed that the nuclear reaction that yielded excellent results was 70%. When compared the nuclear results, with respect to the pH value, there was no difference between the standard and pH 6.1, yet the pH 6.7 and red sea water of pH 7.7 gave a minor reaction quality. No significant difference was observed between the quality of nuclear and cytoplasmic reaction; (68%) of cytoplasm gave excellent results. **Conclusion:** As a conclusion all sections, to some extent, gave acceptable results of hematoxylin bluing reaction. The variation in drinking water has no significant variable result of nuclear hematoxylin reaction.

Keywords: Hematoxylin, Nuclear reaction, bluing

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Introduction:

Hematoxylin together with eosin is a widely used histological stain. Its popularity is based on simplicity and ability to demonstrate clearly an enormous number of different tissue structures ^[1,4].

The staining method involves application of hemalum, which is a complex formed from aluminium ions and oxidized Hematoxylin. This colors nuclei of cells blue ^[6].

Hematoxylin component stains the cell nuclei blue/black with good intra nuclear details; eosin stains the cytoplasm by pink color ^[1,4].

Hematoxylin and eosin is used to identify inflammation, Chronic inflammatory, vasculitis or other infectious and inflammatory conditions ^[10].

Eosin is the most common dye for staining the cytoplasm in histology. It is an acidic dye that binds to basic components of the

cell, mainly proteins located in the cytoplasm.

Alum Hematoxylin is chemically ripened with sodium iodate. It can be used as an aggressive stain like any alum hematoxylin, however it is also useful as a progressive stain, particularly in situations where a nuclear counter stain is needed to emphasize a cytoplasmic component which has been demonstrated by special stain and where the acid alcohol differentiation might destroy or de-color the stained cytoplasmic component ^[3].

During staining alum hematoxylin are usually passed on to a neutral or alkaline solution to neutralize the acid and form an insoluble blue aluminum hematin complex; this procedure is known as bluing.

The bluing solution causes the mordant dye-lake to reform in the tissue and become more permanent.

When tap water is not sufficiently alkaline or is even acidic, a few drops of strong alkaline solution are used ^[5].

The biggest objection to Mayer's hematoxylin as used in the past has been that stained slides often fade after 1 to 3 years. This problem was eliminated,

however, when the slides were washed, after the Hematoxylin application, with running water for a minimum of 20 minutes ^[7].

Bluing solution, practically-wise, includes diluted ammonia water, lithium carbonate and Scott's tap water ^[8]. These chemical methods are classically used in the bluing step for nuclear hematoxylin reaction. Most of laboratories in recent years use running tap water as bluing agent instead of the chemical solution, yet the source of water may vary according to the salt ingredients; therefore this study aims to compare between the results of

chemical and tap water with different pH values.

Material and Methods:

The study was conducted in Khartoum State; as a descriptive study. Seventy two (72) tissue sections were prepared from human organs (Liver, intestine and kidney) fixed in 10% formalin. Mayer's hematoxylin applied on all sections and subsequently treated with the solutions of different pH values, as bluing agents and then stained with Eosin as counter stain and then mounted with DPX mounting media. Microscopic evaluation for all slides performed.

Data analysis Statistical Package for Social Science SPSS computer software was used for data analysis. (Significance level was $P < 0.05$).

Results: The total specimens were 72 sections, equally distributed between liver, kidney and intestine of human organs.

The sections treated with different pH values during bluing step (pH 6.1, pH 6.7, pH 7.7 and 0.05% ammonia standard solution of value pH 8.9).

Nuclear reaction of all sections in different pH: 51 (70%) were excellent, 15 (20%) were Good, 4 (7%) were fair and 2 (3%) gave poor results (Table 1). About description of nuclear reaction according to the pH, the standard and pH 6.1 gave excellent 16 (89%) and 2 (11%) good. The pH value 6.7 distributed as 9 excellent, 5 good, 3 fair and 1 poor. pH 7.7 with a result of 10 excellent, 6 good, 1 fair and 1 poor show (table 2).

Cytoplasmic reaction of all sections in different pH: 49

(68%) were excellent, 7 (10%) were fair, 6 (8%) were poor and 10 (14%) yielded good results (Table 3). The distribution of the 51, classified as excellent, were the nuclear reactions according to tissue type, at variable pH values. Those included, in descending order, the kidney 24 (100%), the intestine 17 (70%), and the liver 10 (40%), vide (table 4).

The distribution of the 49 excellent cytoplasmic reaction at different pH values, according to tissue type, were (kidney 24 (100%), intestine 17 (70%) and the liver 8 (33%), as shown in (Table 5).

Table (1): Nuclear reaction of all sections in different pH

Nuclear Reaction	Frequency	(%)
Excellent	51	70.0
Good	15	20.0
Fair	4	7.0
Poor	2	3.0
Total	72	100.0

Table (2): The nuclear reaction according to the pH

pH	Nuclear reaction				
	Excellent	Good	Fair	Poor	Total
Standard	16	2	0	0	18
6.1	16	2	0	0	18
6.7	9	5	3	1	18
7.7	10	6	1	1	18
Total	51 (70%)	15(20%)	4(7%)	2(3%)	72(100%)

P-value 0.000

Table (3): Cytoplasmic reaction of all sections in different pH

Cytoplasmic Reaction	Frequency	(%)
Excellent	49	68.0
Good	10	14.0
Fair	7	10.0
Poor	6	8.0
Total	72	100.0

P-value 0.001

Table (4): Distribution of 51 excellent nuclear reaction according to tissue type at different pH values.

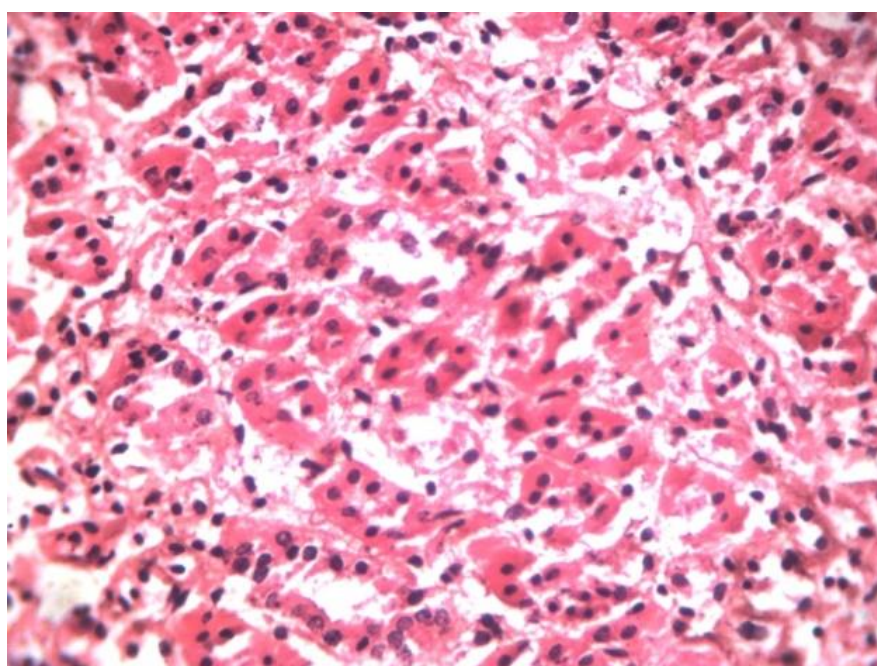
Nuclear Reaction	Type of tissue			Total
	Kidney	Intestine	Liver	
Excellent	24 (100.0%)	17 (70.8%)	10 (41.7%)	51
Good	0 (0.0%)	5 (20.8%)	10 (41.7%)	15
Fair	0 (0.0%)	0 (0.0%)	4 (16.7%)	4
Poor	0 (0.0%)	2 (8.3%)	0 (0.0%)	2
Total	24 (100.0%)	24 (100.0%)	24 (100.0%)	72

P-value 0.000

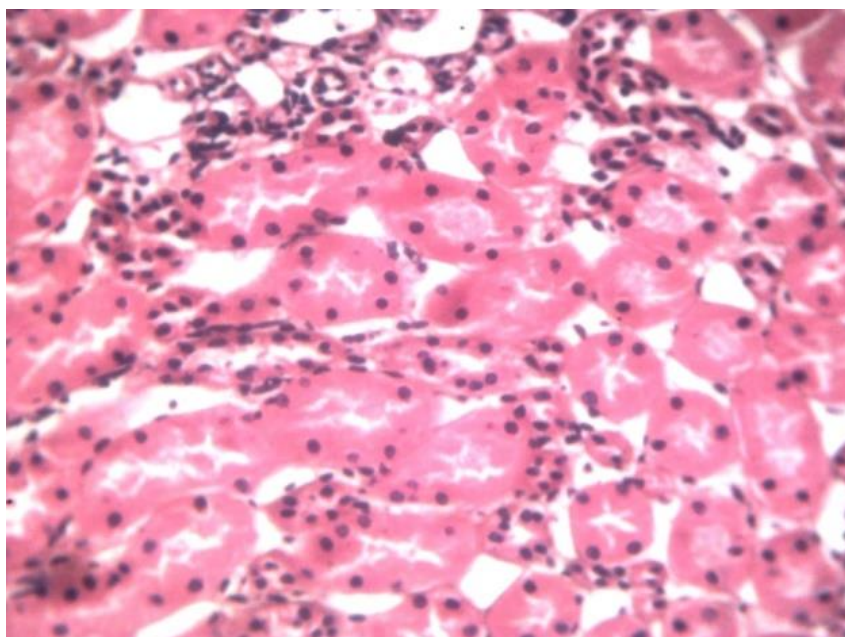
Table (5): Distribution of 49 Excellent cytoplasmic reaction according to tissue type in different pH

Cytoplasmic Reaction	Tissue types			Total
	kidney	Intestine	Liver	
Excellent	24 (100.0%)	17 (70.8%)	8 (33.3%)	49
Good	0 (0.0%)	5 (20.8%)	5 (20.8%)	10
Fair	0 (0.0%)	0 (0.0%)	7 (29.2%)	7
Poor	0 (0.0%)	2 (8.3%)	4 (16.7%)	6
Total	24 (100.0%)	24 (100.0%)	24 (100.0%)	72

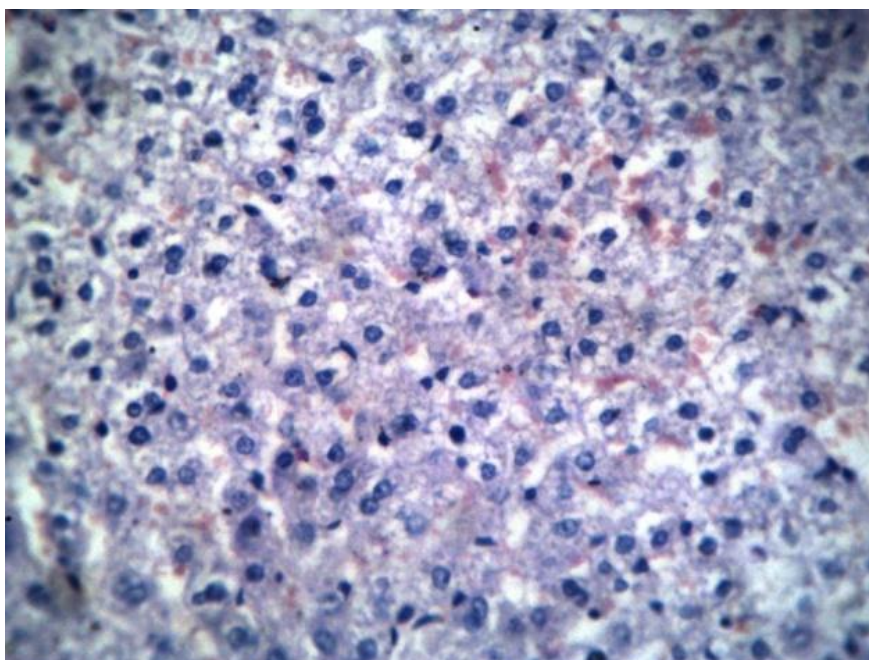
P-value 0.001



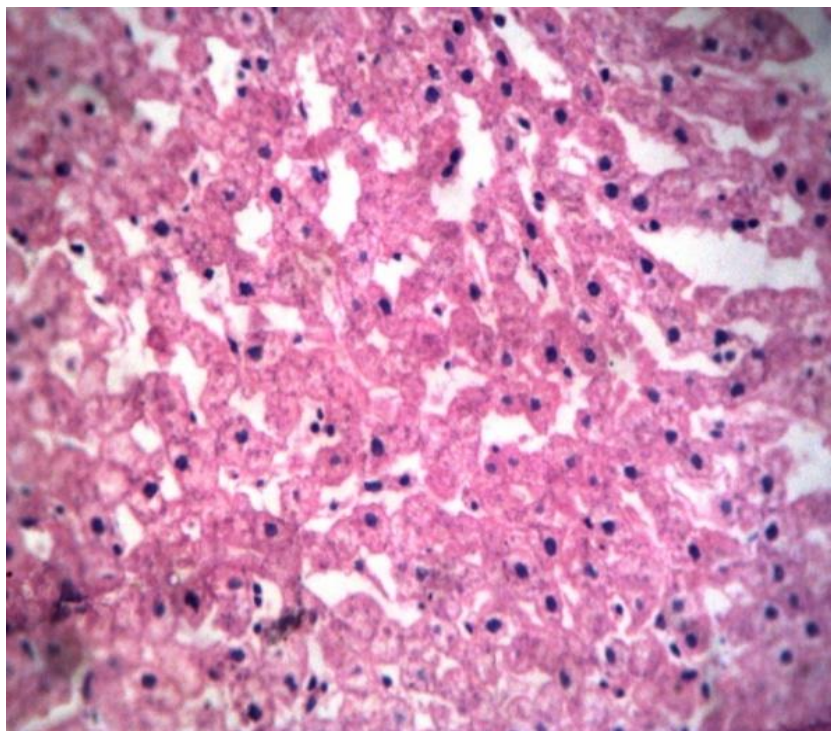
Microphotograph (1): Kidney in Ammonia Solution with Good nuclear and cytoplasm reaction, H&E x40



Microphotograph (2): Kidney in pH 7.7 with fair nuclear and cytoplasm reaction, H&E x40



Microphotograph (3): Liver in pH 6.7 with Good nuclear reaction and poor cytoplasm reaction, H&E x40



Microphotograph (4): Liver in Ammonia Solution with an excellent nuclear reaction, H&E x40

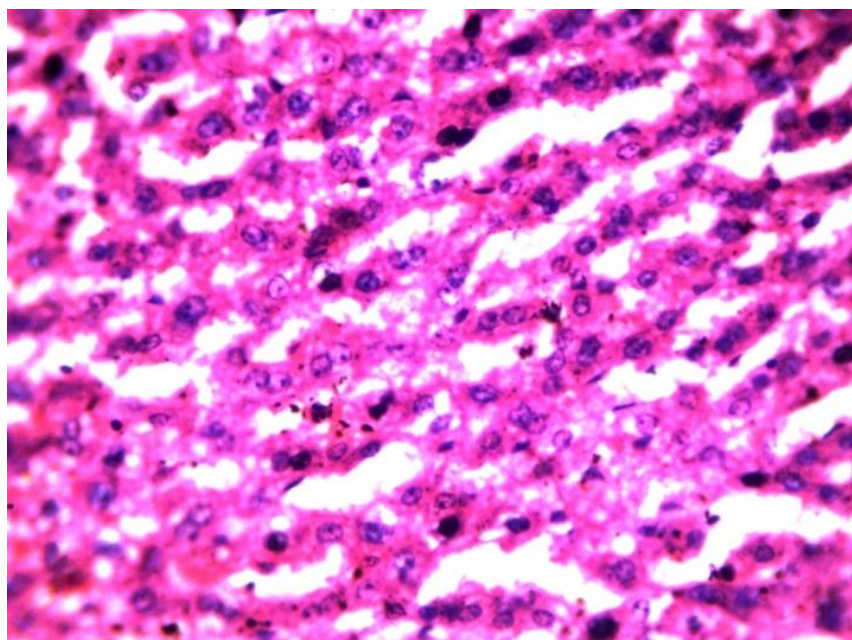


Figure (5): Liver in pH7.7 with poor nuclear and cytoplasmic reaction, H&E x40

Discussion:

There are numerous formulations of bluing reagents available. The active ingredient in any bluing reagent is the alkaline material- either ammonia or alkaline salts such as lithium or magnesium carbonate. The choice of whether to use tap water as a bluing reagent will depend upon the quality of the local tap water ^[8].

This study was conducted in Khartoum state-Omdurman Islamic University during the period from January to May 2018, to evaluate water bluing of hematoxylin nuclear reaction in different pH values.

The study included seventy two[72] formalin paraffin sections from different tissue types [liver, kidney, and intestine] .the study used Mayer's hematoxylin with eosin stain. The sections subsequently after hematoxylin staining were treated with variable media solutions of different pH values,

as bluing step. Those comprised [diluted ammonia solution as standard of pH value 8.9, pH6.1, pH6.7, and red sea water with pH7.7]

All the sections were evaluated microscopically, the result showed that the nuclear reaction with excellent were 70% at the level of *p-value* 0.001. When compared the nuclear results according in terms of the medium, only at the pH 6.7 and red sea water [pH7.7] gave less quality reaction. Although the pH 6.1 was considered acidic, yet it yielded good quality reaction almost similar to the standard. This might be due to the other factors like fixation and processing, because the sections were prefixed and processed. Regarding the cytoplasmic reaction no differences were observed between nuclear and cytoplasmic reaction. The quality of cytoplasmic scored (68%) ,

and was classified as an excellent result.

As far as the reaction according to tissue type, the study showed that kidney tissue scored a perfect result followed by the intestine and then the liver. This might be attributed to the variations in tissue processing.

As conclusion all sections to some extent gave acceptable results of hematoxylin bluing reaction. The variation in drinking water has no significant variation on the result of nuclear Hematoxylin staining reaction.

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Prevalence Rate of Galactorrhea among Sudanese Hyperprolactinemic Infertile Women, Who Attended Reproductive Health Care Center –Khartoum (2005-2010).

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ABSTRACT

This was a prospective study conducted at Reproductive Health Care Center in Khartoum State during the period from 2005 to 2010 to determine prevalence rate of galactorrhea, among infertile women with hyperprolactinemia. The study protocol was approved by the local research Ethics Committees of the Sudan Fertility Association Care, Project of Reproductive Research. No data existed about incidence and prevalence of hyperprolactinemia and galactorrhea in Sudanese infertile women. For this purpose the study targeted infertile women visiting center. The women were screened, to determine the prevalence of hyperprolactinemia among infertile women, which was carried out in Reproductive Health Care Center Khartoum state from 2005 to 2010. The investigation was based on determination of prolactin levels and the prevalence of hyperprolactinemia in the infertile women within the reproductive age (18-45 year). An overall hyperprolactinemia was represented in 29% of total number of 14102 infertile women included in this study. The prevalence of hyperprolactinemia among women with galactorrhea was 74.8%. During the last year of study of 49 women from 105 hyperprolactinemic women with Amenorrhea, 27 (50%) were suffering galactorrhea, and were treated by bromocriptine. Another 22 (43%) women from 51 treated by Cabergoline contracted galactorrhea, during the monitoring and follow up of hyperprolactinemic women. The duration of Galactorrhoea disappearance per weeks was insignificant while the dosage of subsidence of galactorrhoea was highly statistically significant with a P-value = 0.00. Galactorrhea disappeared in all women 100% having galactorrhea in all study groups. These findings raise interesting questions about etiology of infertility and disease course of hyperprolactinemia that induces galactorrhea.

KEYWORDS: Prevalence, Hyperprolactinemia, Amenorrhea, Galactorrhea, Bromocriptine, Cabergoline

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INTRODUCTION:

Galactorrhea is a discharge of milk or milk-like secretion from the breast in the absence of pregnancy or more than 6 months postpartum, without breastfeeding (David and Slovik, 2009). The secretion may be intermittent or persistent, free-flowing or expressible, and is more common in women aged 20–30 years. It may or may not be accompanied by hyperprolactinemia (Leung and Pacaud, 2004). Galactorrhea refers to the secretion of a milky fluid from the breast, occurring either spontaneously or with manual expression, in the absence of pregnancy or the postpartum state in a non-breast feeding woman Galactorrhea, or inappropriate lactation, is a relatively common problem that occurs in approximately 20 to 25 percent of women and unilateral or bilateral (Gibney, *et al* 2005). Idiopathic galactorrhea is a

diagnosis of exclusion, and patients may have normal or elevated levels of prolactin. In such situations, the mechanism of milk production may be an increased prolactin release in response to stimuli, with a normal basal prolactin rate. The differential diagnosis of galactorrhea is extensive. However, patients can be reassured that this condition is not associated with breast cancer. In fact, one study found that idiopathic galactorrhea was associated with a reduced risk of breast cancer (Sanfilippo 1999). The patient should be asked about symptoms of an intracranial mass, such as visual-field defects, cranial nerve palsy and headache (Gillam, *et al* 2006). It is also important to inquire about symptoms of systemic diseases, including hypothyroidism and Cushing's disease. An accurate list of all medications, including over-the-

counter OTC drugs and illicit substances, herbs and other supplements, is essential (Schade, *et al* 2007). The physical examination includes an evaluation of the patient's visual fields, thyroid gland, breasts and skin. If the type of nipple discharge is in doubt, because prolactin levels are influenced by stress and breast stimulation, blood should not be drawn immediately after a breast examination. Rather, it should be drawn at least one hour after the examination and when the patient is relaxed (Mishra, *et al* 2002). If the initial prolactin level is borderline, the level should be repeated one or two times because of the great fluctuation in prolactin levels throughout the day. A level greater than 200 ng per mL (200 µg per L) is almost always associated with a prolactinoma or other prolactin-secreting tumor (Gillam, *et al*, 2006).

Serum cortisol, growth hormone and insulin-like growth factor levels should be obtained if the patient has signs or symptoms of Cushing's disease or acromegaly (Barkan, *et al* 2010). Imaging studies are also important in the evaluation of abnormal lactation. If the patient has symptoms suggestive of an intracranial mass, galactorrhea with amenorrhea, or an elevated prolactin level (greater than 20 ng per mL), magnetic resonance imaging (MRI) of the brain is indicated to detect a pituitary tumor or other intracranial lesion (Aron, *et al* 2007). If the patient has normal menses and a normal prolactin level, the risk for pituitary adenoma is low, and imaging is not necessary. However, if a patient has galactorrhea associated with amenorrhea or oligomenorrhea, even with a normal prolactin level, the risk of a pituitary adenoma is still significant, and

an imaging study of the gland warranted (Vartej, *et al* 2001). The prevalence of galactorrhea in women has been reported to range from 0.1-32%. Much of this variability can be attributed to differences in examination techniques; the investigator's definition of galactorrhea (Molitch, 2005). Oral Contraceptive, OC, associated galactorrhea is a frequent diagnosis in patients with non-puerperal lactation and has been implicated in 10-14% of cases (Friedman and Goldfien, 1996). The condition is more common in women who are 20 to 35 years of age and in previously parous women; it is less common in children and nulligravid women. Galactorrhea also can occur in men. In children, galactorrhea is more common in infants and teenage girls (Mishra, *et al* 2004). Isolated galactorrhea, with normal menses and normal serum prolactin levels, estimated

to occur in up to 20% of women at lactation (Eftkhari, *et al* 2009). In hyperprolactinaemic women, the incidence of galactorrhea is 30 to 80% of cases, depending on the diligence with which galactorrhea and the estrogen status of the patient is sought (Biller 1999 and Serri, *et al* 2003). Hyperprolactinemia is found in one-third of women with amenorrhea, and in 75% of women who have both amenorrhea and galactorrhea (Serri, *et al* 2003). Measurement of serum prolactin levels indicated in all cases of galactorrhoea (Mah and Webster 2002). The prevalence of galactorrhea, the etiology of hyperprolactinemia and/or galactorrhea, pituitary tumor, parapituitary lesions, oral contraceptive (OC) associated galactorrhea, drug induced galactorrhea, hypothyroidism, neurogenic stimulation,

idiopathic galactorrhea, clinical features of galactorrhea, laboratory evaluation, neuroradiologic evaluation, hypocyctoidal tomography, carotid angiography, pneumoencephalography (PEG), computerized tomography (CT), management, and therapy (Sakiyama and Quan 1983). OC discontinuation can also produce galactorrhea. Pharmacologic agents are a common cause of hyperprolactinemia and/or galactorrhea. A variety of forms of neurogenic stimulation have been linked to hyperprolactinemia and/or galactorrhoea (Holtz 1982). Idiopathic galactorrhea is a diagnosis of exclusion and is applicable to 40-50% of patients with nonpuerperal lactation (Molitch 2005). In patients with galactorrhea, the presence of the abnormal, milky breast secretion may be the sole symptom or it

may be associated with other pertinent clinical findings. All patients with galactorrhea should have measurements of thyroid stimulating hormone and serum prolactin performed (Mah and Webster 2002). The condition may result in patient anxiety and physician concern and can signify a serious underlying disorder. and can be confirmed if necessary by visualizing fat droplets in secretions using low power microscopy (Luciano 1999). Galactorrhea is rarely seen in intrinsic breast diseases, which are usually associated with purulent, clear, yellow (serous), multicoloured or sanguinous discharge (Luciano 1999). Depends on prolactin stimulation of an estrogen-primed breast and may be physiological due to peaks of prolactin secondary to episodic secretion, cyclic menstrual hormonal variations, pregnancy, post-lactational state, and many

other influences such as sleep, exercise, stress or high protein meals (Luciano 1999). Pathological causes include most commonly pituitary tumours and drugs, and less commonly primary hypothyroidism, chronic renal failure or hepatic disease, and neurogenic stimulation from breast manipulation and chest wall lesions (Gongsakdi and Rojanasakul 1986). Although galactorrhoea is not associated with breast cancer, neoplastic processes in the brain and pituitary gland can cause it. Fortunately, most of these tumors are benign. Approximately 20 percent of women with galactorrhoea have radiologically evident pituitary tumors, and the prevalence increases to 34 percent in women who also have amenorrhea (Luciano 1999). The most common tumor resulting in hyperprolactinemia is the pituitary prolactinoma, a benign

growth of the prolactin-secreting cells of the anterior pituitary gland (Schlechte 2003). Clinical signs and symptoms include headache, galactorrhoea, amenorrhea, defects in peripheral vision, hirsutism, acne, and hypogonadism presenting as decreased libido, decreased fertility or decreased bone density (Mah and Webster 2002). Craniopharyngiomas and other tumors, infiltrative diseases, pituitary-stalk resection and empty-sella syndrome may disrupt the delivery of dopamine to the pituitary gland (Vallette-Kasic 2002). Systemic diseases must also be considered in the differential diagnosis of galactorrhea. The most common is hypothyroidism (Casanueva, *et al* 2006). Galactorrhea and symptoms of hypothyroidism abate with thyroid hormone replacement therapy (Biller 1999). Chronic renal failure may cause galactorrhea as a result of

decreased clearance of prolactin by the kidneys. Hypersecretion of cortisol (Cushing's disease) or growth hormone (acromegaly) may also have associated hyperprolactinemia (Cola, *et al* 2008). Numerous medications and some herbs can cause galactorrhea (Mah and Webster 2002). About 15 percent of patients report galactorrhea within seven to 75 days after starting antipsychotic medication (Bankowski and Zacur 2003). Estrogen and progesterone, found in oral contraceptive formulations and the medroxyprogesterone contraceptive injection (Depo-Provera), may cause lactation. Possible mechanisms include direct actions on the breast tissue or effects on gonadotrophins (Biswas, *et al* 2005). The dosages of estrogen and progesterone used in postmenopausal hormonal replacement therapy are

generally not high enough to cause galactorrhea (Schade, *et al* 2007). However, some patients with hyperprolactinemia may not have symptoms if they are estrogen deficient. Once hormone replacement therapy started, the breast tissue is primed, and galactorrhea may then occur. Chest Wall Irritation galactorrhea can occur because of chest wall irritation from clothing or ill-fitting brassieres (Cola, *et al* 2003). Breast surgeries, including implant placement and reduction mammoplasty, can cause postoperative galactorrhea (Biller 1999).

The goals of galactorrhea treatment include decreasing or eliminating the patient's symptoms, curing any identified underlying cause, preventing bone loss, relieving the patient's anxiety and fears, and, when desired, maintaining the patient's fertility and ability to lactate

(Hatim, *et al* 2006). Patients with idiopathic or physiologic galactorrhea and normal prolactin levels should be reassured. All patients with galactorrhea advised to avoid excessive breast stimulation, including repeated self-examinations or excessive nipple manipulation during sexual activity (Hatim, *et al* 2006). If galactorrhea caused by a medication, the agent should be discontinued if possible (Sakiyama, and Quan 1983). The prevention of osteoporosis is a concern in any patient with hyperprolactinemia. High prolactin levels, through their effect on gonadotropins and resulting low estrogen levels, decrease bone density and thereby increase the risk of osteoporosis (Vartej, *et al* 2001). This risk can be reduced with medical therapy using dopamine agonists (e.g., bromocriptine [Parlodel], cabergoline

[Dostinex]), even in the absence of a tumor (Schlechte, *et al* 2003). Treatment of galactorrhea directed at the underlying cause. If possible, galactorrhea-inducing medications replaced with safe, alternative agents. Hypothyroidism should be treated with thyroid hormone replacement therapy (Goswami, *et al* 2009). An algorithm for managing women with prolactinoma presented in (Schlechte 2003). Patients with isolated galactorrhea and normal prolactin levels do not require treatment if they are not bothered by the galactorrhea, do not wish to conceive, and do not show evidence of hypogonadism or reduced bone density (Luciano 1999). Prolactin levels measured periodically in these patients. In patients with hyperprolactinemia, prolactin levels monitored, and MRI performed every two years (ono, *et al* 2008) (more often if a

pituitary tumor suspected). Treatment goals include suppressing prolactin secretion and its clinical and biochemical consequences, reducing the size of the prolactinoma, and preventing its progression or recurrence (Bankowski and Zacur 2003). The true incidence of galactorrhea is unknown, but it is estimated that 20 to 25 percent of women experience this problem at some time in their life (Luciano 1999).

METHODS

This is a retrospective study conducted at Reproductive Health Care Center – Khartoum during the period 2005-2010.

Inclusion criteria:

Infertile hyperprolactinemic women with and without galactorrhea

Exclusion criteria:

Infertile females without hyperprolactinemia

Data collection:

Clinical and demographic data of the participants was obtained from Statistical Department of Journal of Natural and Medical Sciences (JNMS) vol. **15** (2) 2014. ISSN 1858-6805 e-ISSN 1858-6813 88. Reproductive Health Care Center, Khartoum. Prevalence rate of infertile women, who visited the Reproductive Health Care Center during the period from January 2005 to December 2010 was calculated according to the Kars *et al* (13) using period prevalence rate which is calculated according to the following formula:

$$\text{Period prevalence rate} = \frac{\text{Every instance of the investigated disease within a defined time period}}{\text{Average population (at risk) during the same time period}}$$

Results:

The mean prevalence of hyperprolactinemia among women with galactorrhea was 74.8% from total population at risk (1348) yielding an overall prevalence 64.4%. The frequency of hyperprolactinemia associated with galactorrhea was ranged 63.4 to 74.2% during the study period. Table (3.1) showed that highest prevalence of hyperprolactinemia was 81.6% showed in 2007, while the frequency of normoprolactinemia in women with galactorrhea was increased sharply to 52.8% in the last year of study period. Before therapy, in the last year of study 50% of 54 women, who were treated by Bromocriptine with galactorrhoea for 13 months of duration, while in the 51 women, treated by Cabergoline 22

(43.1%) women had galactorrhea. The presence of galactorrhea was highly significant with group B. Galactorrhea disappeared in all women (100%) having galactorrhea in both groups. However, which had disappeared usually within ten weeks after the start of treatment at dose 10 mg/day with Bromocriptine group, and in weeks twelve after start of treatment at dose 0.5 mg/2weeks with Cabergoline group, respectively, which can be shown in Table (3.2).

Table (3.1): The prevalence of galactorrhoea according to prolactin level

years	No of women with Normal PRL $\leq 400\text{mIU/L}$	No of women with High PRL $>400\text{mIU/L}$	Total No of women	Prevalnce Per 100
2005	45 (27.4%)	119 (72.6%)	64 (100%) ¹	72.6
2006	56 (26.5%)	155 (73.5%)	211 (100%)	73.1
2007	53 (30.5%)	121 (69.5%)	174 (100%)	81.6
2008	54 (26.2%)	152 (73.8%)	206 (100%)	79.5
2009	58 (30.9%)	130 (69.1%)	188 (100%)	77.4
2010	214 (52.8%)	191 (47.2%)	405 (100%)	64.4
Total	480 (35.6%)	868 (64.4%)	1348 (100.0%)	Overall 64.4

Table (3.2): galactorrhoea present, duration of disappearance and Dose at disappearance during study group.

Drugs	Galactorrhoea present	Duration of disappearance Galactorrhoea(weeks)	Dose disappearance galactorrhoea
Bromocriptine N (54)	n 27 Percent 50%	10.4 ± 2.07	11.8 ±3.11(mg/day)
Cabergoline N (51)	n 22 Percent 43%	12 ± 6.56	0.64±.28 (mg/week)
P-value	001.0	0.383	0.000

Discussion, Conclusion and Recommendation

The frequency of normoprolactinemia with galactorrhea was 32.8% occurred in the last year of analysis. The mean prevalence of hyperprolactinemia in women with galactorrhea was 74.8%. The highest prevalence of hyperprolactinemia associated with galactorrhea in infertile women was 81.6% observed in 2007. These

results were higher than that reported by (Luciano 1999) who estimated that 20 to 25 percent of women experience this problem, those were similar to reported by (Ezzat, *et al* 2004), which estimated, of 25 per cent among women with galactorrhoea,. Normoprolactinemia occurred in 32.8% in women experienced galactorrhea this indicated that many women with galactorrhea, not associated with

hyperprolactinemia. On the other hand Greer, *et al* (1980) in women with reproductive disorders, hyperprolactinaemia observed in approximately 15% of women with anovulation and 43% of women with galactorrhea and anovulation (Greer, *et al* 1980). Eftkhari, *et al* (2008) showed more of 46% of patients with hyperprolactinemia-experienced galactorrhea and found that galactorrhea, with normal menses and normal serum prolactin levels, estimated to occur in up to 20% of women at lactation. These results were similar to that reported by Serri, *et al* (2003) found that there was a significant association between hyperprolactinemia and galactorrhea 46% of patients with galactorrhea and 75% of patients without galactorrhea had a high level of prolactin. However, (Biller 1999 and Serri, *et al* 2003) also shown highly prevalence of galactorrhea of the total women 30 - 80% with hyperprolactinemia. Table (3.1) showed that the prevalence

was dropped in the last year of analysis, thus, high serum prolactin levels was not indicated in all cases of galactorrhea. However, over the last years, have noticed an increased number of patients complaining of galactorrhea with hyperprolactinemia presents a gynecological problem accompanied by galactorrhea or without galactorrhea and cannot be a certain index for hyperprolactinemia. Prevalence rate of infertile females with hyperprolactinemia associated with galactorrhea increased throughout the screening period particularly the last years. Screening of all underlying causes of hyperprolactinemia is very important.

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