Original Research Article

The bacterial pattern of diabetic foot infections among Libyan patients at Al-Jalla Hospital, Benghazi

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ABSTRACT

Background: One of the major consequences of diabetes mellitus is foot infections, which can result in gangrene , in the worst case scenario,leadingtolimb amputation. In order to prevent diabetic foot from having a poor prognosis,it is important to correctly diagnose the causing agents and determine whether they may be susceptible upon antibiotics to control this infection .**Aims:** Our purpose of this study was to analyze the demographic data, etiological bacterial agents and antibacterial susceptibility pattern of bacterial pathogens isolated from diabetic foot ulcer infections among Libyan patients at Al-Jalla Hospital,Benghazi.**Materials and Methods:** A Cross sectional study of 156 adult Libyan diabetic Patients with a confirmed diagnosis of foot ulcers,admitted to AL-Jalla hospital in Benghazi,Libya,swabs were examined for bacteria from foot lesions in addition of antibiotic sensitivity of isolates.**Results:** Diabetic foot were 81 Libyan males and 75 females, the age mean was 65 years , According to the bacteria noticed,the more common causative bacteria for diabetic foot was Pseudomonas, but the lowest percentage was S. pneumonia, synonymous to bacteria tested against antibiotics,indicates that Septrin is more resistant, also shown to be more sensitive to Imipenem and Meropenem.**Conclusion :** this research shows that the more typical cause of diabetic foot ulcers was Pseudomonas also shows that the Benghazi society abuses antibiotic, Imipenem and Meropenem were shown to be highly effective antibiotics, whereas Cefoxitin as well Fusidic acid were more resistant to the infection.

Keyword : ***Bacterial prevalence ,Antibiotic resistance, Bacterial prevalence ,Al-jalla Hospital and Benghazi.***

1. Introduction

Diabetes mellitus (**DM**) is one of the most common chronic diseases. That is, association with poor limb circulation leading to the development of diabetic foot ulcers (**DFU**),DFU is defined as a non- or poorly healing

wound, located distal to the ankle in an patient with DM,these infections are cause by Facultative anaerobic bacteria *Staphylococci*, *Streptococci*,as well as *Enterobacteriaceae*, also by Obligate anaerobes such as *Bacteroides*, *Clostridium*, *Peptostreptococci*, and *fungi*.[1]Foot infections are classified as mild, moderate and sever ,treatment is based on the extent and severity of the infection ,mild infections are treated with oral antibiotics, wound care, and pressure off-loading in the outpatient setting ,moderate infections and severe infections should be hospitalized, given intravenous antibiotics, evaluated for possible surgical intervention.[2] The common underlying causes of (**DFU**) include neuropathy, foot deformities, trauma, elevated plantar pressures, and peripheral vascular disease .[3] Diabetic foot ulcers were classified according to Wagner's Classification into

- **Grade-0:**No ulcer in a high-risk foot.
- **Grade-1:**Superficial ulcer involving the full skin thickness but not underlying tissues.
- **Grade- 2:**Deep ulcer, penetrating down to ligaments and muscles, but no bone involvement or abscess formation.
- **Grade-3:**Deep ulcer with cellulitis or abscess formation, often with osteomyelitis.
- **Grade-4:**Localized gangrene.
- **Grade-5:**Extensive gangrene involving the whole foot.[4]

Foot problems are largely preventable, and successful treatment depends on the correct evaluation of the patient, diagnosis, knowing the microbes that cause infections and proper management of infection.[1]

The current study's objective was to assess the distribution of genders, age range, and foot infection sites among Libyan patients suffering from diabetic foot ulcers, to identify and assess the bacterial agent that causes foot infections as well as the isolates patterns of antibacterial susceptibility among patients admitted to Al-Jalla Hospital. Ultimately, the aim was to improve the prognosis and cure rate of diabetic patients by choosing an efficient antibiotics.

2.Materials and methods

A cross-sectional study of diabetic adult patients took place over a period of 2 years and 8 months, from March 2019 to October 2021. This research was carried out at the Al-Jalla Teaching Hospital for Surgery and Trauma in Benghazi, Libya.

2.1.Patient selection

Participants who were admitted to the surgical department for assessment had their personal and medical histories collected, additionally, they were receiving treatment at the Al-Jalla Hospital .In view of selection criteria, the intrusion criteria were Libyan diabetic subjects presented with confirmed clinical signs and symptoms of diabetic foot infections, Conversely, extrusion criteria included instances involving non-Libyans, non-diabetics, and foot ulcers brought on by burns ,trauma, or complications from prior foot procedures.209 respondents were assessed, 156 cases were chosen, both genders, their ages ranged from 20-79 years old.

2.2.Sample collection and Bacterial identification

Swab culture specimens were collected from the ulcers after washing with saline. Sterile cotton-tipped swab sticks were applied to the base of the ulcer for 5 to 10 seconds. The swabs were promptly transported to the laboratory in sterile containers for microbiological analysis. Samples were sent to the microbiology lab for bacterial culture and antibiotic susceptibility testing, data quality control aseptic techniques were consistently applied during sampling and handling using sterile materials. Autoclaving was performed for solutions and equipment containing water. The sterility of the media was verified by incubating 5% of the batch at 37°C for 18-24 hours. Bacterial specimens were cultured in nutrient broth for microbial growth enhancement aerobically at 37°C overnight. The next day, sub culturing was performed on MacConkey agar, Blood agar ,Chocolate agar, and Mannitol salt agar,

(**Oxoid, Ltd., Uk**) at 37°C for 24 hours. Plates were incubated aerobically as well as in a carbon dioxide jar, and all plates were examined for microbial presence. Pure cultures were then subjected to microbial identification. Bacterial pathogen identification followed the Monica manual of systematic Bacteriology .[5]Based on the morphology of the colony, the presence of the capsule, the results of Gram staining and biochemical tests (catalase, oxidase, coagulase, etc.), isolates were identified, but anaerobic bacteria were excluded due to a lack of necessary laboratory equipment.

2.3. Antibiotics testing

The sensitivity of isolates to different antibiotics was determined using the disc diffusion method recommended by the Clinical and Laboratory Standard Institute (**CLSI**).Several antibiotic discs were applied on Muller Hinton agar inoculated with isolated organisms and inhibition zones were measured after 24 hours of incubation at 37°C.

2.4.Statistical analysis

Data were gathered and analyzed using the IBM-provided statistical package for social sciences (**SPSS-23**) software version was used to organize data between groups at a significant level of acceptance (***P***-value 0.05) as well as with a confidence interval of **95**%. Age was divided into four age groups concurrently and the mean was calculated to present the numeric variable age along with frequencies and percentage.

3.Results

These results were based on patient's personal data, medical history, clinical examination and bacteriological analyses of samples taken from subjects in this presented study.

3.1.Demographic data and infectious foot sites

Males had a higher incidence (**Figure-1),** also the average age was 65years,but according to diagnostic foot site, it showed that most commonly found in right foot in 53.2.% ,as shown in (**Table-1**).

Table-1:Demographic Data and foot ulcer sites

History			Prevalence (%)	Chi-square	P-value	Significance
Personal data	Gender	Males	81(51.9)	25.705[a]	<0.00001	Significant
		Females	75(48.1)			
	Age groups	20-30	10(6.4)			
		30-40	10(6.4)			
		40-50	30(19.23)			
		Above 50	106(67.9)			
Foot infection site	Right foot		83(53.2)	41.131[a]	<0.00001	Significant
	Left foot		73(46.8)			
Total			156(100)			

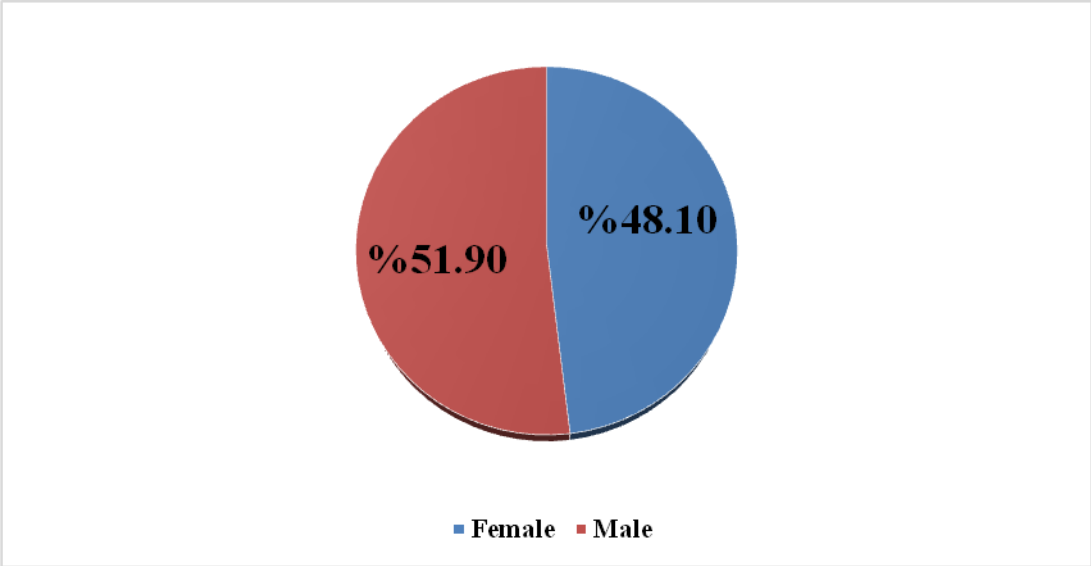

Figure- 1: Percentage of Gender of 156 Libyan subjects with diabetic foot ulcers

3.2.Results of Bacteria isolated from diabetic foot infections sites

From the sum of 156 specimens taken from foot infection sites, positive cultures were 146 (93.6%), whereas there were 10 negative cultures (6.4%),following that eleven species were recovered based on Gram stain and biochemical test, there were seven species (74.4%) from Gram negative bacteria in addition of four species (25.6%) from Gram positive bacteria, also three patterns of bacterial infections were detected in the present work as displayed in **Table-2.**

Table-2: Distribution of Bacterial isolates from diabetic foot lesions in the presented study

	Bacterium		Incidence(%)	Total occurrence (%)	Chi-square	*P*-value	Statistic significance
Monobacterial	Gram positive	*S.aureus*	13(8.5)	33(21.6)	41.131[a]	<0.0001	Significant
		S.epidermis	11(7)				
		Enteroccoci	6(3.8)				
		S.pneumoniae	3(1.9)				
	Gram negative	*Pseudomonas*	21(13.4)				
		Proteus	12(7.6)				
		Klebsiella	5(3.3)	53(34.9)			
		Acinetobacter	5(3.3)				
		E.coli	4(2.5)				
		Enterobacter	3(1.9)				
		Morganellanmorganii	3(1.9)				
Dibacterial	Gram positive	*Enterococcus+S.epidermis*	1(0.6)	46(28.7%)	104.393[a]	<0.00001	Significant
		Enterococcus+S.aureus	4(2.5)				
		Enterococcus+ Acinetobacter	3(1.9)				

	Gram positive/Gram negative	*Enterococcus+ Enterobacter*	1(0.6)				
		Enterococcus+ Pseudomonas	3(1.9)				
		S.aureus+ Proteus	1(0.6)				
		S.aureus+ Acinetobacter	1(0.6)				
		S.aureus+ Pseudomonas	1(0.6)				
	Gram negative	*Pseudomonas+ Acinetobacter*	1(0.6)				
		Pseudomonas+ E.coli	5(3.3)				
		Pseudomonas+ Proteus	4(2.5)				
		Pseudomonas+ Klebsiella	2(1.2)				
		Proteus+ M.nmorganii	3(1.9)				
		M.nmorganii+ Klebsiella	3(1.9)				
		Proteus+ Klebsiella	2(1.2)				
		Acinetobacter+ Klebsiella	3(1.9)				
		Acinetobacter+ Enterobacter	2(1.2)				
		E.coli+ M.nmorganii	3(1.9)				
		E.coli+ Klebsiella	2(1.2)				
		E.coli+ Enterobacter	1(0.6)				
Tribacterial	Gram negative	*Proteus+Pseudomonas +E.coli*	2(1.2)	14(8.4)	70.492[a]	<0.0001	Significant
		Proteus+Enterbacter+Acinetobacter	1(0.6)				
		Proteus+ Klebsiella+Acinetobacter	1(0.6)				
		Proteus+ Pseudomonas+Enterobacter	1(0.6)				
		Klebsiella+ Pseudomonas+ M.nmorganii	1(0.6)				
		Klebsiella+E.coli+Acinetobacter	1(0.6)				
	Gram negative/Gram positive	*Enterococcus+Proteus+Klebsiella*	1(0.6)				
		Enterococcus+E.coli+Acinetobacter	2(1.2)				
		Enterococcus+Proteus+ Mnmorganii	1(0.6)				
		S.aureus+ Klebsiella+ Pseudomonas	1(0.6)				
		S.aureus+E.coli+Acinetobacter	1(0.6)				
		S.epidermis+ Klebsiella+ Pseudomonas	1(0.6)				
	No-growth		10(6.4)	10(6.4)			
	Total		156(100)	156(100)			

3.3. Antibiotic susceptibility and resistance pattern of isolated bacteria

3.3.1. The pattern of antibiotics sensitivity against Gram –Positive bacteria isolated from patients with Diabetic foot infections

From the results of our study,Vancomycin was very effective in treatment of Gram-positive bacterial infection ,because ithad the highest susceptibility(100%) ,followed by Ciprofloxacin (92.3 %) ,but but the highest resistance was seen for Cefoxitin and Fusidic acid(100 %),as shown in **table-3** and **figure-2.**

Table-3: Susceptibility of antibiotic against isolated Gram-positive bacteria based on percentage

Antibiotic Name	Disc code	Potency	Susceptibility of antibiotic against Gram positive bacteria	
			S	R
Amikacin	AK	30 mcg	31.2%	68.8%
Augmentin	AM	10mcg	69.9%	30.1%
Cefoxitin	FOX	30 mcg	0	100%
Ciprofloxacin	CIP	5 mcg	92.3%	7.7%
Clindamycin	DA	2 mcg	90.9%	9.1%
Daptomycin	DAP	30 mcg	47.3%	52.7%
Erythromycin	E	15mcg	78.3%	21.7%
Fusidic acid	FD	10 mcg	0%	100%
Gentamicin	CN	10 mcg	53.8%	46.2%
Imipenem	IPM	10 mcg	90.8%	9.2%
Kanamycin	K	30mcg	15.4%	84.6%
Levofloxacin	LEV	25 mcg	77.3%	22.7%
Linezolid	LNZ	10mcg	92.3%	7.7%
Meropenem	Mem	30 mcg	88.7%	11.3%
Septrin	SXT	5 mcg	25.1%	74.9%
Tetracycline	TE	30mcg	72.1%	27.9%
Vancomycin	VA	10mcg	100%	0%

[S: sensitive R: resistant]

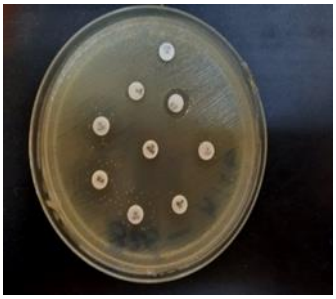

Figure-2: Antibiotic resistances of *S.aureus* to different antibiotics from a 66 years old Libyan male case.

3.3.2. The frequency of antibiotics sensitivity against Gram-negative bacteria isolated from 156 patients with Diabetic foot ulcers

All the Gram negative isolates have sensitivity approaching (93.2%) to Meropenem, followed by Imipenem (77%), whereas the highest resistance antibiotic to Gram Negative bacteria was to Septrin(86.3%) as presented in**Table-4** and **figure-3**

Table-4: Incidence of antibiotics Sensitivity against Gram-negative bacteria from patients with diabetic foot in the study as stated in percentage.

Antibiotic	Disc code	Potency	Susceptibility of antibiotic against Gram Negative	
			S	R
Amikacin	AK	30 mcg	71.8%	28.2%
Augmentin	AM	10mcg	26.4%	73.6%
Ceftriaxone	CRO	30 mcg	41.7%	58.3%
Ceftazidime	CAZ	30 mcg	71.8%	28.2%
Ciprofloxacin	CIP	5 mcg	64.3%	35.7%
Colistin	CT	10 mcg	46.3%	53.7%
Imipenem	IPM	10 mcg	77%	23%
Levofloxacin	LEV	5 mcg	56.9%	43.1%
Meropenem	Mem	10mcg	93.2%	6.8%
Septrin	SXT	25 mcg	13.7%	86.3%

[S: sensitive R: resistant]

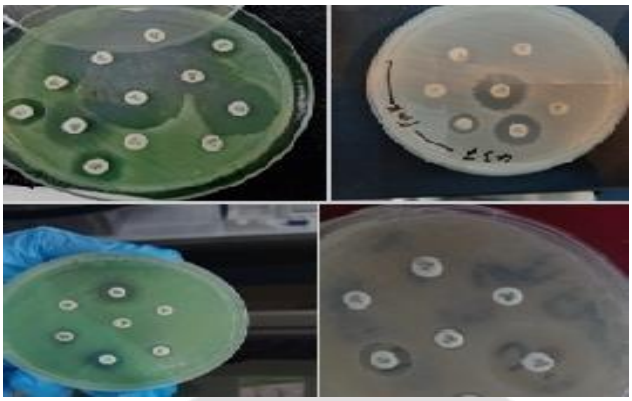

Figure-3: Antibiotic Sensitivity of Gram-negative bacteria isolates to different antibiotics from several pateints in this study

4.Discussion

The World Health Organization estimates that, by 2030, there will likely be 245,000 diabetics in the country. Local epidemiological research conducted in Libya revealed that 3.8% of known diabetes individuals over the age of 20 had the disease.[6]

According to the head of Al-Jallia's biostatics and documentation office, the staff has finished 5047 cases between 2019 to the most current date, with an average of 103 cases per month, continuously gathering up to 1236 cases annually.

The majority of the 156 patients in the present research, 51.9%, were male. The frequency of female patients was 48.1%. These results were similar to those of studies by Wei et al., [7]and Khalifa et al., [8] where the occurrence of female patients was 36.3%, 35%, and the incidence of male patients was 63.7%, 65%. It might be because there are not many female smokers with in Libyan society, which contrasts with Jaafar, [9] where 30% of cases are male and 70% are female. The lowest age was 20 in just one case (0.7%), while the peak was between 60 and 70 years old (29.7%).

Our study revealed an abundance of Gram negative bacteria, with Gram negative bacilli being more prevalent in patients (33.9%) than Gram positive cocci (21.2%).These findings correlated well with those of Wei et al.,[7] who reported that 54.9% of the organisms that were isolated were Gram negative bacilli, and with a study conducted in Kuwait, [8] which reported that more Gram-negative pathogens (51.2%) were isolated than Gram-positive pathogens (32.3%).Additionally, a study carried out by Mahrous et al.,[10]demonstrated that Gram negative bacilli were more popular (60%) than Gram positive cocci (40%), as well as results from a study by Olunjuwon et al.,[11] presenting the greater number of Gram-negative bacteria than Gram-positive bacteria associated with (60%) than Gram positive cocci (40%),Based on findings from a study by Olunjuwon et al.,[11] there were more Gram-negative bacteria than Gram-positive bacteria related to diabetic foot infections in Nigerian hospitals. The percentage of Gram-negative bacteria which occurred was 53%, while the percentage of Gram-positive bacteria was 47%.The reason for an increase in the proportion of Gram-negative bacteria could be their tough outer shell, which makes them more resistant to antibiotics and difficult to destroy. When their cell wall is compromised, Gram-negative bacteria begin to release endotoxins, which worsens the situation.

This study indicated that 86 (55.1%) of the patients had a monomicrobial cause, and 60 (38.5%) had a polymicrobial etiology. These findings are comparable to those of a study published by Hefni et al.,[4]which found higher percentages of monomicrobial infections (52%), while polymicrobial infections (40%). They are also comparable to a study executed in by Saada et al.,[12]which found higher percentages of monomicrobial infections (97%), as well as a study carried out in Malaysia by Nur et al.,[13] which found (79.2%) patients had monomicrobial infections and (13%) patients had polymicrobialinfections.Conversely, research performed in Kuwait by Khalifa et al., [8] and Nigeria by Ofonime et al.,[14] has shown that polymicrobial infections exceed

monomicrobial infections. It was found that most mild infections are monomicrobial, while most severe infections are typically polymicrobial.

This suggests that the cause of monomicrobial infections was more common in our patients as a result of the relationship between the types of infections (mild, moderate,severe) and the number and types of organisms that caused wound infections. In the present study, the highest bacterial infection rate for diabetic foot ulcers was recorded (39.6%) for *P.aeruginosa,* followed by *S.aureus* with 39.4%,these findings correlated well with study Miyan et al.,[15]Wei et al .,[7]and Khalifa et al.,[8]who reported *P . aeruginosa* and *S.aureus* as the predominant pathogen, which comprised 17.4%, 23.1 % and17.4 % of their isolates respectively for *P. aeruginosa* and 11.8%, 19.2% and18.5% respectively for *S.aureus***,**On the contrary, study by Olorunjuwon et al.,[11] revealed *S.aureus*had the highest occurrence of 22.03%, next to *P. aeruginosa*16.95% and other study in Khartoum by Waiel et al ., [16]found *E.coli* was higher incidence isolate (18.6%),followed by *S. aureus* (10.8%), followed by *P. aeruginosa*(8.3%),it might be due to *P. aeruginosa* may grow and spread in a variety of environments, including soil, food, skin contact, and contaminated medical devices, it was more commonly encountered in this study.

Resistance to bacteria can spread to humans, hospitalized patients, alongside the surrounding environment from a variety of sources, such as animals, plant-based diets, fish, poultry, and other industries where antibiotics are used for a variety of purposes. This can result in the emergence of resistant bacterial strains, [11]a combination of factors including the fact that diabetic foot infections are frequently polymicrobial, these infections also increase the risk of limb amputation by increasing the association between diabetic foot ulcers and multi-drug resistant organisms (**MDROs**). Furthermore, infections with MDROs lengthen hospital stays, increase management costs, and increase morbidity and mortality rates among diabetic patients.[10]

Inference from the study's findings,vancomycin was very effective at treating Gram-positive bacterial infections because it had a maximum susceptibility(100%), after Ciprofloxacin (92.3%), Linezolid (92.3%), imipenem (90.8%), and meropenem (88.7%). In contrast, all Gram Negative isolates had sensitivity approaching 93.2%) to meropenem, accompanied by imipenem (77%), and amikacin (71.8%). Remarkably, imipenem as well meropenem were very effective at handling Foot ulcers, which were caused by both Gram Positive and Gram Negative isolates. likewise Gram-positive bacterial infections had the highest resistance to Cefoxitin (10%), Fusidic acid (10%), Kanamycin (84.6%), and Septrin (74.9%), while the foremost resistance antibiotic to Gram Negative isolates was Septrin.These findings indicate that the Libyan population is using antibiotics appropriately .In an analysis performed by Waiel et al.,[16]at a Tanzanian hospital, most isolates were susceptible to 100% meropenem, 100% imipenem, 81.8 % vancomycin, 55.6 % clindamycin, and 53.6% ciprofloxacillin. These findings align with those of this study. Analogous to Khalida et al.,[8]found that Amikacin, Imipenem, Piperacillin-tazobactam, and Vancomycin were the most effective treatments for Gram-negative bacteria, whereas vancomycin was the most effective treatment for Gram-positive bacteria,increased resistance to many medication classes that has been reported (**MDRO**). This is consistent with our findings, which showed that isolates resistant to three or more antibiotic groups (**MDRO**) were recognized. Further, multidrug resistance was found in more Gram negative bacterial isolates than Gram positive isolates. A comparable high percentage of MDR organisms in Gram negative bacteria have been documented in the majority of other worldwide studies.[10]

5.Conclusion

Recognizing the bacterial pathogen that causes foot ulcers, choosing appropriate antibiotic and early diagnosis play an important role to control, management and treatment to have successful outcomes. The implications of the study's results Diabetic foot was more common in Libyan men than women, higher incidences in between 60 and 70 years old and really rare in 20 years old since there was only one case in the study, according to foot site, it had more prevalence in the right foot than the left foot among the patients.

Based on the bacteria found during the current study, monomicrobial infections were significantly more prevalent than polymicrobial infections. The more prominent causative bacteria for diabetic foot was *Pseudomonas* additionally reveals that the bacteria that were least likely to be caused by *S. pneumonia*, meanwhile according to different antibiotic tests, showed a higher sensitivity to imipenem and morepenem while having greater resistance to cefoxitin and fusidic acid. The increase in antibiotic resistance, which will result in higher health care costs and even the loss of a lot of lives.

6.Ethics statements

The team had verbal consent from participants.

7.Supporting and Sponsoring financially

Non

8.Conflicts of interest

The authors declare no conflicts of interest.

9.Authors contribution

The following contributions to the work have been confirmed by the authors: Huda F. Elfeituri designed and conceptualized the study; Huda F.Elfeituri had collected the data. Fatma Mohamed Ali handled the data analysis and interpretation; she also prepared the draft written content. After reviewing the findings, each author gave their approval to the manuscript's final draft.

10.Acknowledgments

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