Global Advanced Research Journal of Microbiology (ISSN: 2315-5116) Vol. 7(4) pp. 064-072, June, 2018
Issue. Available online http://garj.org/garjm

Full Length Research Paper

Isolation of Aerobic Bacteria from Wounds of Diabetic Patients Undergoing Hyperbaric Oxygen Therapy in Khartoum State

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Accepted 07 June, 2018

Background: Diabetic wound infection is one of the complications of diabetes mellitus, hyperbaric Oxygen Therapy (HBOT) can be used as an adjacent treatment for diabetic wound infection, it involves inhalation of 100% oxygen under a pressure greater than 1 atmospheric absolute. This was a longitudinal case study, in which the wound specimens were collected from AL-Dirby Centre and further processing was done at the Microbiology Laboratory, Sudan University of Science and Technology (SUST), between January and July 2016. Objective: The aim of this study was to evaluate the effect of hyperbaric oxygen therapy (HBOT) on aerobic bacteria isolated from the wounds of diabetic patients attending Al-Dirby Center. Methodology: A total of 22 diabetic patients were involved in this study, their ages between 40 and 98 years. The majority of the patients were males 17 (77.3 %) and 5 (22.7%) were females, Insulin dependent diabetic patients were 15 (68.2%) and insulin independent diabetic patients were 7 (31.8%). A Questionnaire was used to collect data from the patients. The data was analyzed by using SPSS version (11.5). The results showed (78.1%) Gram-negative rods and (21.9%) Gram- positive cocci. The most frequent isolated bacteria were Proteus mirabilis 8(25%) followed by Pseudomonas aeruginosa 6(18.7%), Staphylococcus aureus and Escherichia coli 5 (15%), Proteus vulgaris 4(12.5%), and (Sterptococcus pyogenes, Serratia marcescens, Viridans streptococci, and Klebsiella pneumoniae) 1 (3.1%). Gram negative rods were more sensitive to Imipenem while Gram positive cocci were more sensitive to Gentamicin. Complete wound healing and bacterial eradication was observed in 17(77.3%) of the diabetic patients while incomplete wound healing and bacterial persistence was observed in only 5(22.7%). Conclusion: This study showed that HBOT could be a useful tool in woundstherapy.

Keywords: *Diabetic wound, hyperbaric Oxygen Therapy, AL-Dirby, Proteus mirabilis*

INTRODUCTION

The skin is the largest organ of the body, consisting of an external cellular layer, the epidermis, and a deep connective tissue layer called the dermis (Venus et al., 2010). The skin is critical in the body defense against injury and dehydration, as well as against foreign bodies, it is equally important in sensation and the regulation of temperature (Davies & Tovey 2014; Ford, 2010). The skin is colonized by an array of organisms which forms its normal flora (Drake et al., 2008). The relatively arid area of the fore and back arm is colonized with fewer organisms, predominantly Gram-positive bacteria and yeasts in moister areas such as the groin and the armpits, where the organisms are more numerous, more varied and include Gram-negative bacteria. The normal flora of the skin plays an important role in protecting the surface from foreign invaders (Koreck et al., 2003; Goering et al., 2013).

Skin infections may be either primary or secondary, primary skin infections are mainly caused by staphylococci or streptococci, staphylococci infection present as furuncles, carbuncles, superficial folliculitis and scalded skin syndrome (Rhody, 2000). Streptococcal infections present as impetigo, ecthyma, erysipelas and cellulitis. Secondary bacterial infections of pre-existing wounds, burn and drematitic skin are usually present (Tognetti et al.,2012).

Wound infections occur primarily beside skin and soft infection as a result of a break in the skin surface (Tille, 2014). A great variety of organisms are involved in wound infection, the bacteria most frequently isolated differ according to anatomic site and predisposing factors (Dryden, 2010). Traumatic open wound infections are caused primarily by members of the soil flora such as *Clostridium perfringes* (DiNubile & Lipsky, 2004). Surgical wound infections are usually due to *Staphylococcus aureus* (Levinson, 2008). Swabs or preferably pus obtained directly from the wound or abscess is adequate to find the causative organism (Greenwood et al.,2007).

Normal wound healing occurs rapidly, after sequential phases including; homeostasis, inflammation, remodeling of granulation tissue and re-epithelialization (Rao et al., 2016). Healing process could be delayed or fail due to oxygen deficiency as a result of diabetes mellitus, irradiation, small vessel atherosclerosis or chronic infection (Ekmektzoglou, & Zografos, 2006). Diabetic patients with foot ulcers are prone to infection by multidrug resistant microorganisms due to; inadequate treatment, chronic nature of the wounds or frequent hospital admissions (Perim et al., 2015). Oxygen is needed in all stages of healing process; proliferation, angiogenesis and remodeling. Oxygen therapy has been applied as a treatment of wounds since1960s (Rao et al., 2016). Hyperbaric oxygen therapy (HBOT) is the therapeutic administration of 100% oxygen at pressure higher than 1absolute atmosphere (ATA) (Bhutani, & Vishwanath, 2012). It is administered by placing the patient in a multiplace or mono place chamber where the vessels are pressurized to 1.5-3.0 ATA for a period between 60-120 minutes once or twice a day.In the monoplace chamber the patient breathes the oxygen directly from a chamber but, in the multiplace chamber this is done through a mask (Barata et al., 2011). Hyperbaric oxygen therapy decreases wound tissue hypoxia by enhancing perfusion, reducing edema, down regulating inflammatory cytokines, promoting fibroblast proliferation and collagen synthesis (Benjamin, 2010). HBOT has been used as a potent tool in increasing the oxygen content of blood. HBOT has been advocated for the treatment of various ailments including air embolism, carbon monoxide poisoning, wound healing and ischemic stroke (Yutsis, 2003). Despite being useful in treating many ailments hyperbaric oxygen therapy has several side effects and complications with varying degree of seriousness (Huang et al., 2015). The most frequent side effects include middle ear barotraumas, progressive myopia and pulmonary dyspnea with cough and aspiratory pain (Camporesi, 2014).

MATERIALS AND METHODS

Study design

This was a longitudinal case study.

Study area

This study was conducted in Khartoum State. The specimens were collected from patients admitted to Al-Dirby Centre where initial processing was done.

Study duration

The study was carried out between January and July 2016

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Study population

Twenty-two male and female diabetic patients with wound infections attending Al-Dirby Centre, Khartoum were enrolled in this study. Sampling was repeated weekly duringHBOT.

Ethical consideration

The study was approved by the ethical committee of the College of Medical Laboratory Science- Sudan University of Science and Technology (SUST) and Al-Dirby Centre. Verbal consent was taken from all patients before collection of the specimens.

Inclusion criteria

Males and females diabetic patients with wound infections

Exclusion criteria

Males and females diabetic patients without wound infections

Data collection

A structured questionnaire was used for collection of data from thepatients.

Collection and processing of specimens

Wound swabs were collected from each patient attending *AL-Dirby* Centre before dressings. The swabs were inoculated onto Amies transport slopes for transport to the laboratory of Microbiology Department, College of Medical Laboratory Science, SUST for isolation, identification and sensitivity testing of the clinical isolates. Swabs were inoculated onto basic and selective media, then the isolates were identified through conventional methods (Mandell et al., 2010) and the identified isolates were preserved in nutrient agar slopes.

Antimicrobial Sensitivity test

This test was done to determine the sensitivity and resistance of the clinical isolates to selected antibiotics following the Kirby-Bauer (Mandell et al., 2010). The following antibiotics were selected for sensitivity testing of wound pathogens including Ampicillin, Co-trimoxazole, Erythromycin, Penicillin, Tetracycline, and Gentamicin for Gram-positive isolates. Co- trimoxazole, Tetracycline, Gentamicin, Amikacin, Ciprofloxacin, Imipenem, Ceftriaxone, and Amoxycillin were used for Gram-negative isolates. Standard strains of *Escherichia coli* ATCC (25922) and *Staphylococcus aureus* ATCC (29213) were used for quality control.

Data analysis

All data were analyzed using Statistical Package of Social Science (SPSS) (version 11.5; corp, college station, Tax),using Chi square test, *P*. value <0.05 considered as significant, then data were presented in tables and graphs using Excel.

RESULTS

A total of twenty-two patients were sampled, of whom 17 (77.3%) were males and 5 (22.7%) were females. Insulin dependent diabetic patients were 15 (68.2%), and Insulin independent were 7 (31.8%). Frequencies of gender with IDDM and INDDM were presented in Table (4.1). Patient's age groups presented in Figure (4.1). The study revealed that the wounds of 20 (90.9%) of the patients were in feet, and only 2 (9.1%) wounds were in the hands. A total of 32 bacteria representing 9 different bacteria were isolated as shown in Table (4.3). Patients with single bacterial infection (monomicrobial) during the course of HBOT were 13 (59.1%) while patients infected with more than one bacterium(poly microbial) were 9 (41.9%).The antimicrobial sensitivity tests were done to evaluate the sensitivity and resistance patterns of isolates against selected antibiotics and the results presented in Tables (4 and 5) respectively.

The outcome of Hyperbaric Oxygen Therapy on isolated bacteria in this study was statistically significant with *P*. value <0.05. Bacterial eradication and wound healing was successful in 17 (77.3%) patients, while bacterial persistence was observed in 5 (22.7%) patients, 4 of them had amputation Table (4.6).

DISCUSSION

Frequency of gender with Insulin dependent diabetes mellitus and Insulin independent diabetes mellitus in this study is similar to that reported by Al-Saimary, (2016) in Iraq. The category which was frequently affected with

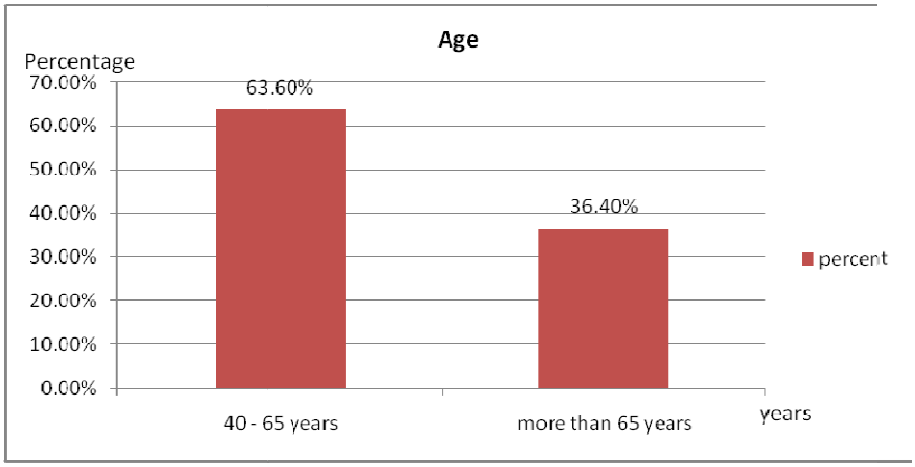

Figure (4.1): Distribution of enrolled patients according to age groups

Table (4.1): Frequency of IDDM and NIDDM among male and female patients

		IDDM	NIDDM	Total
Sex	Male	12 (54.5%)	5 (22.7%)	17 (77.3%)
	Female	3 (13.6%)	2 (9.1%)	5 (22.7)
	Total	15 (68.2%)	7 (31.8%)	22 (100%)

Table(4.2):DistributionofbacterialisolatesaccordingtotheirGramreaction

Type of Bacteria	Frequency
Gram positive cocci	7 (21.9%)
Gram negative rods	25 (78.1%)
Total	32 (100%)

Table No (4.3): Distribution of bacteria isolated from infectedwounds

Bacterial isolates	**Frequency (%)**
Proteus mirabilis	8 (25.0%)
Proteus vulgaris	4 (12.5%)
Staphylococci aureus	5 (15.6%)
Pseudomonas aeruginosa	6 (18.7%)
Escherichia coli	5 (15.6%)
Klebsiella pneumoniae	1 (3.1%)
Streptococcus pyogenes	1 (3.1%)
Serratia. Marcescens	1 (3.1%)
Viridans streptococc*i*	1 (3.1%)
Total	32 (100%)

Table (4.4): The results of the sensitivity tests of Gram negative rods

Bacteria frequencies and percentages	Antibiotic discs															
	Ciprofloxacin		Gentamicin		Amoxycillin		Co-Trimoxazole		Amikacin		Ceftriaxone		Imipenem		Tetracycline	
	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R
P. mirabilis	3 37.5%	5 62.5%	6 75%	2 25%	3 37.5%	5 62.5%	2 25%	6 75%	8 100%	0 0%	2 25%	6 75%	8 100%	0 0%	0 0%	8 100%
Ps. aeruginosa	5 83.3%	1 16.7%	5 83.3%	1 16.7%	Not tested		Not tested		6 100%	0 0%	2 33.3%	4 66.6%%	Not tested		Not tested	
E.coli	3 60%	2 40%	4 80%	1 20%	Not tested		Not tested		4 80%	1 20%	4 80%	1 20%	Not tested		Not tested	
P. vulgaris	1 25%	3 75%	3 75%	1 25%	Not tested		Not tested		4 100%	0 0%	0 0%	4 100%	Not tested		Not tested	
S. marcescens	0 0%	1 100%	0 0%	1 100%	0 0%	1 100%	0 0%	1 100%	1 100%	0 0%	1 100%	0 0%	1 100%	0 0%	0 0%	1 100%
K. pneumoniae	1 100%	0 0%	1 100%	0 0%	0 0%	1 100%	0 0%	1 100%	1 100%	0 0%	1 100%	0 0%	1 100%	0 0%	0 0%	1 100%

Key: S: Sensitive, R: Resistant

Table (4.5): The results of sensitivity tests of Gram positive cocci

Bacteria	Antibiotic discs									
	Gentamicin		Erythromycin		Penicillin		Tetracycline		Cefixime	
	sensitive	Resistant	sensitive	resistant	sensitive	resistant	sensitive	resistant	sensitive	resistant
S. aureus	5 100%	0 0%	5 100%	0 0%	0 0%	5 100%	1 20%	4 80%	Not tested	
St. pyogenes	Not tested		1 100%	0 0%	1 100%	0 0%	Not tested		1 100%	0 0%
Viridans streptococci	1 100%	0 0%	0 0%	1 100%	0 0%	1 100%	Not tested		0 0%	1 100%

Table (4.6): Distribution of bacterial species before and after Hyperbaric Oxygen Therapy (HBOT)

Bacterial isolates	**Total No. before therapy**	**Total No. after therapy**
Proteus mirabilis	8	2
Proteus vulgaris	4	0
Staphylococcus aureus	5	2
Pseudomonas aeruginosa	6	1
Escherichia coli	5	1
Klebsiella pneumoniae	1	0
Streptococcus pyogenes	1	0
Serratia marcescens	1	0
Viridans streptococci	1	1
Total number	32	7

P .value ≤0.05

Table (4.7): Outcome of HBOT in relation to age group

Age / year	**Eradication of bacteria**	**Persistence of bacteria**	**Total**
40-65	12 (85.7%)	2 (14.3%)	14 (100%)
More than 65	5 (62.5%)	3 (37.5%)	8 (100%)
Total	17 (77.3%)	5 (22.7%)	22 (100%)

Table (4.8): Hyperbaric oxygen therapy (HBOT) in diabetic patients in relation to the duration of Diabetes Mellitus

Duration of DM/years	Eradication of bacteria	Persistence of bacteria	Total
Less than 10	8 (88.9%)	1 (11.1%)	9 (100%)
11-15	4 (66.7%)	2 (33.3%)	6 (100%)
16-20	4 (100%)	0 (0%)	4 (100%)
21-25	0 (0%)	1 (100%)	1 (100%)
26-30	1 (50%)	1 (50%)	2 (100%)
Total	17 (77.3%)	5 (22.7%)	22 (100%)

Table (4.9): Treatment outcome of diabetic wounds after Hyperbaric Oxygen Therapy (HBOT)

Description	Frequency (%)
Complete Healing	17 (72.7%)
Incomplete Healing	5 (27.3%)
Total	22 (100%)

wound infections was those of age group 40- 65 years old, this result is similar to those reported by Ahmed, (2003) in Sudan. The commonest site of infection in this study was the feet 90.9% infection, which is similar to that reported by Mahgoub and Omer, (2015) in Sudan which was 98.7%.There was bacterial growth from all the swabs; probably this because diabetic patients are more prone to infections. Moreover, the increased incidence of infections could be related to impairment of the immune response in the diabetic patients (Ahmed, 2003).

Monomicrobial infection was observed in (59.1%) of the cases, while polymicrobial infection was observed in (41.9%) of the cases, these results disagree with those reported by Mahgoub and Omer,(2015) in Sudan, in which monomicrobial and polymicrobial infection were (77.3%) and (22.7%) respectively.Monomicrobial infection was close to that reported by Sugandhi and Prasanth,(2014) in India which was (56%) unlike polymicrobial infection which was (44%). Moreover, it was less than the rate of monomicrobial (83.5%) and of polymicrobial (16.4%) reported by Turhan *et al*., (2013) in Turkey. In contrast to monomicrobial infections polymicrobial infection is reported in serious infections that fail to respond to previous antibiotic therapy. Gram negative isolates were more than Gram positive isolates as they were (68.1%) and (21.9%) respectively. These results disagree with those reported by Ali, (2005) in Sudan, where Gram negative isolates were (57.8%) and Gram positive isolates were (42.8%). However, these results are similar to those reported by Mahgoub and Omer in Sudan, where Gram negative isolates were (77.3%) and Gram positive isolates were (22.7%).

The most common bacteria isolated during this study was *Proteus mirabilis* (25%), which is more than those reported by Abbass, (2009) in Sudan which was (19%). In another study in Iran by Akhi *et al., S. aureus* was the predominant isolate(28%)(2015). The variation between the most frequent isolate in different studies and percentages of Gram positive and Gram negative bacteria could be attributed to the source of infection, severity of infection and even the immune status of diabetic patients. Moreover, the high incidence of Gram negative isolates compared to Gram positive isolates among diabetic wound patients could be justified by the complexity of Gram negative cell walls which makes them more resistant to antimicrobial therapy. In this study *P. mirabilis* was more frequent than *P. vulgaris*, and *S. marcescens* which was also found in diabetic wound infection, this is similar to that reported by Ali, (2005) in Sudan. Moreover, *Proteus species* were resistant to many antibiotics and this resistance pattern was also reported byMordi and Momoh, (2008) in Nigeria. This observation could be explained by the wide spread of plasmid resistance genes among *Proteus* species (Mordi & Momoh,2009).

In this study, Gram negative bacteria were sensitive to Imipenem, this is similar to those reported by Sugandhi and Prasanth, (2014), Mohanasoundaram, (2012) in India, and Turhan *et al.,*(2013) in Turkey. In this study all Gram negative isolates were resistant to Tetracycline; this is similar to that reported by Abbas, (2009) in Sudan, and Mordi and Momoh, (2008) in Nigeria. The most effective antibiotic against *Ps.aeruginosa* was Amikacin,

this is similar to that reported by Abdel Wahab *et al.,*(2013) in Sudan.All *S. aureus* in this study were sensitive to Erythromycin, which was similar to that reported by Mohanasoundaram, (2012) in India. Furthermore, all *S. aureus* were resistant to penicillin which is similar to that reported by Ahmed, (2003) in Sudan. The routine use of antibiotics in both medical and veterinary medicine has led to wide spread of antibiotic resistance and development of antibiotic resistance genes particularly within the Gram negative organisms (Mordi& Momoh,2008).

The results of pre-and post-procedural wound cultures were statistically significant at *P*.value<0.05, which is similar to those reported by Doctor, (1992) in India. The study revealed that, the frequency of cases that showed healing were (77.3%), which is close to those reported by Ong, (2008) in Singapore, in which the success rate after HBOT was(71%).

CONCLUSIONS

In conclusion, diabetic wound infections continue to cause problems particularly to the feet where they affect more than 90% of the patients in this study. The wound infections are caused by Gram positive and Gram negative bacteria where they were 7 (21.9%) and 25 (78.1%) respectively. The most common pathogen that caused diabetic wound infections in this study was *P. mirabilis* (25) %. All Gram negative rod isolates were sensitive to Imipenem, and all of them were resistant to Tetracycline. All Gram positive cocci isolates were sensitive to Gentamicin. Complete healing and bacterial eradication was observed in 17(72.7%), while persistence and incomplete healing was observed only in 5 (27.3%)after HBOT.HBOT is an effective enhancement tool of antimicrobial therapy against aerobic bacteria that cause diabetic wound infections.

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