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ISOLATION AND IDENTIFICATION OF PATHOGENS FROM DIABETIC FOOT INFECTIONS FROM K. R. HOSPITAL, MYSORE

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ABSTRACT

The relative frequencies of bacterial isolates cultured from diabetic foot infections were studied. Samples were collected from 120 patients with diabetic foot infections from K. R. Hospital, Mysore, Karnataka, India, from June to November 2010. Out of 120 patients, 88 were of non-insulin dependent (NIDDM) and 32 male were insulin dependent (IDDM) among NIDDM 52 were male and 36 female. The specimens were collected from the site of the wound and cultured and identified by standards methods. From the results it is found that Staphylococcus sp. (76.66%), Pseudomonas sp. (63.33%), E. coli (56.6%), Enterobacter sp. (53.33%), Proteus sp. (50.00%), Klebsiella sp. (43.33%) and Bacillus sp. (16.66%) were the most common bacterial isolates. Whereas, Candida sp. (66.66%) was the only fungal isolate that was found.

INTRODUCTION

Insufficient utilization or production of insulin by the body is a condition known as *Diabetes mellitus* (Manikandan *et al*., 2008). It is a disease of complications popularly known as Iceberg disease (Cotron *et al*., 1994) and is the most common endocrine disorder and takes on pandemic proportions (Manikandan *et al.*, 2008). India has the largest diabetes population in the world with an estimation of 41 million people accounting to 6% of the adult population. WHO estimates that, during 2000, there are 32 million people with diabetes in India, which is projected to rise for about 80 million by the year 2030. Increase in prevalence is rapid in urban areas ranging from 2% in 1970s to 12% in 2000 and the trend is setting to increase in the rural areas also. Diabetes affects 246 million people worldwide and is expected to increase approximately to 380 million by 2025. Each year 7 million more people are expected to develop diabetes in forthcoming future. Each year 3.8 million deaths are linked directly to diabetes related causes including cardiovascular disorders which were made worse by lipid disorders and hypertension. Every 10 sec a person develop Type-1 diabetes, which predominately affects youth, which is rising alarmingly worldwide at a rate of 3% per year (Satyanarayanamma, 2010). Approximately, 15% of the people with diabetes will have foot ulcer in their life time (Mayfield *et al*., 1998).

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Foot ulceration and infection are one of the leading causes of mortality and morbidity, especially in developing countries (Sharma *et al*., 2006). There is a general consensus among clinicians that diabetic patients are at increased risk of developing infection (Braces, 2007). This special vulnerability has been attributed to impaired leukocyte function associated with vascular diseases, poor glucose control and altered host response (McMahon and Bistrian, 1995; Bhatia *et al*., 2003). Once infection occurs, it is difficult to treat since the clinical course of the infection is more fulminate and severe and posses a greater threat to the glycemic status of the patient (Louie *et al*., 1993; Beckert *et al*., 2006). The pathogenesis of diabetic foot is highly complex, including polyneuropathy, peripheral vascular disease, and compromised immunity, slower wound healing, trauma and infection.

There are several well accepted predisposing factors that place patients with diabetes at high risk for a lower-extremity amputation. The most common components in the causal pathway to limb loose include peripheral neuropathy, ulceration, infection and peripheral vascular complication for patients with diabetes. Numerous factors related to diabetes can impair wound healing, including wound hypoxia (inadequate oxygen delivered to the wound) infection, nutrition deficiencies and the disease itself (Lavery *et al*., 2007). Fluctuating blood sugar and hypoxia from poor circulation may impair the ability of white blood cells to destroy pathogenic bacteria and fungi, increasing infection

risk (Stadelmann *et al*., 1998). The aim of the present study was to screen the most common bacterial and fungal pathogens that are associated with diabetic foot ulcers in both males and females at K. R. Hospital, Mysore, Karnataka, India.

MATERIALS AND METHODS

Hundred and twenty diabetic patients with foot infection of either sex were screened by randomized method in the present study. This was performed for the duration of 6 months at K. R. Hospital, Mysore, Karnataka, India. Sterile swabs were taken from various locations of wounds from diabetic patients. One swab was used for smear examination and another for cultural purposes. Samples were inoculated on Chocolate Agar (CA), Mac Conkey Agar (MCA), Nutrient Agar (NA) and Sabourauds Dextrose Agar (SDA) media. Plates were incubated at 37°C for 24 h for bacterial and at 25°C for 5-7 days for fungal growth. Bacteria were identified by subjecting them to biochemical methods and fungi by the classic Mycological method (Fingold and Baron, 1986; Murray *et al*., 1999; Larone, 2002).

RESULTS

Out of 120 diabetic mellitus patients, 32 were insulin dependent and 88 were noninsulin dependent patients. Insulin dependent patient were male and among noninsulin dependent patients, 52 were males and 36 were females. Age and sex of all the diabetic patients revealed that among 120 patients 20, 8 and 8 females fall between the age group of 40-45, 45-50 and 50-55, respectively. Among male patients 4, 4, 16, 20, 16 and 24 fall between the age group of 40-45, 50-55, 55-60, 60-65, 65-70 and 70-75, respectively. It has been noticed in the present study that maximum number of male patients (24) fall between the age group of 70-75 years. In the age group of 40-45 there were no male patients but specifically it was found between the age group of 40-55 years (Table 1). Parameters such as diabetes type, family history, disease history (past diabetic history) and duration of foot infection in percentage were found out. The results showed that 26.6% male patients recorded type-I diabetes. In the type-II diabetes 43.3% and 30% were found in both males and females respectively. Type-I diabetes revealed 100% family history, type-II diabetes showed 46.15% and 22.22% were males and females, respectively. Studies revealed that in past diabetic history 36.66% males are diabetic more than 10 years and in less than 10 years 33.33% and 30% males and females respectively were recorded.

Duration of foot infection data revealed 20% males had infection more than one month. Less than one month of foot infection was found in 50% and 30% male and female respectively (Tables 2a, b, c & d). The present study of bacteria and fungi revealed 76.66%, of *Staphylococcus* sp. infection, which was found to be the highest among the bacterial infection when compared with all the other bacterial species and *Bacillus* sp. found to be the least of 16.66% and among the fungi 66.66% of *Candida* sp. found to be more (Table 3). In the present study, the percent incidence of Diabetic mellitus type-I and II revealed that the single and double pathogens were not present, but three and more than triple pathogens were found in either of sexes. This accounted up to 66.66% of type II and in male patients more than three pathogens accounted for 65%.

DISCUSSION

The prevalence of bacterial infection among IDDM and NIDDM diabetic patients were analyzed in the present study. Age, sex, Diabetic type I, type II, past diabetic history, duration of infection, pathogens isolated from diabetic foot ulcer of infection, pathogens isolated from diabetic foot ulcers were taken as criteria in the present investigation. Out 120 patients, 84(100%) and 36(100%) males and females were found to be infected by many pathogens respectively as all of them were found to be diabetic and their age ranged from 40 to 80 years. The supportive data was seen in (Ahmed and El-Tahawy, 2000; Sharma *et al*., 2006; Hena and Growther, 2010; Hayat *et al*., 2011) as they indicate males dominate in having diabetes with foot infections when compared to females. During the study, the history of diabetic patients were analyzed and found out type I diabetic was accounted for 38.1% among males and nil in females. Whereas type II, was seen in 61.9% males. Males exhibited 61.9% than females which were found to be dominant. The results were corroborative to the findings of Alsaimary (2010). Where in 38 males number of cases were 61.6% and female number was 38.8% in type I and in type II 33.3% were males and 66.6% were females. Whereas males were found more susceptible for both type I and II compared to females (Zubair *et al*., 2010).

These results explain that males become more susceptible for type I diabetic and females found to be more susceptible to type II diabetic mellitus among the patients examined in the present investigation draws the attention of the past diabetic history. The varied days, months and years of prevalence of diabetic history and the rate of infection demanded to investigate the past diabetic history. During the study, female patients had developed diabetes less than 10 years; but male patients were seen in both less and more than 10 years. Similar examination was carried out by Zubair *et al*. (2010). Were male patients showed the development of diabetic less than 10 years and more than 10 years with this it can be concluded that environmental factors may influence the susceptibility of the individuals for diabetes. In females foot infection was seen to be within the month of infection whereas in males it was observed to be within and above one month. The results were supported by Zubair *et al*. (2010). This indicates males were more susceptible for the foot ulcers when compared to females. Microorganisms were isolated from the patients with foot infection (Table 3).

Patients with the history developed foot ulcer caused by *Staphylococcus, Pseudomonas, E. coli, Enterococcus, Proteus, Klebsiella, Bacillus* and *Candia*. Similar organisms were isolated by Sharma *et al*. (2006) and Zubair *et al*. (2010) on foot ulcers. Among all *Staphylococcus* was found to be most common isolate among the patients tested, wereas *Pseudomonas* was the next common pathogen that was found in the diabetic foot ulcer (Ahmed and El-Tahawy, 2000; Hayat *et al*., 2011), *E. coli* was found in the third position common in foot ulcers (Rouhipour *et al*., 2012). *Enterobacter* was seen the next among the pathogens of foot ulcer (Ahmed and El-Tahawy, 2000). *Proteus* was the next among the isolates (Sharma *et al*., 2006; Hayat *et al*., 2012). *Klebsiella* and *Bacillus* were found to be less common, their presence were

found to be a rule than exception. Diabetic individuals who had foot ulcers exhibited *Candidial* infection among fungal organisms. The results were found to be similar with Missoni *et al*. (2005) and Nair *et al*. (2007).

Table 1. Male and female ratio of diabetic foot ulcers with age

			Gender		Total
Ages			Male	Female	
		Count	4	20	24
	<50	% of GENDER	4.8%	55.6%	20.0%
	51-55	Count	0	8	8
		% of GENDER	.0%	22.2%	6.7%
	56-60	Count	4	8	12
		% of GENDER	4.8%	22.2%	10.0%
	61-65	Count	16	0	16
		% of GENDER	19.0%	.0%	13.3%
	66-70	Count	20	0	20
		% of GENDER	23.8%	.0%	16.7%
	71-75	Count	16	0	16
		% of GENDER	19.0%	.0%	13.3%
	76-80	Count	24	0	24
		% of GENDER	28.6%	.0%	20.0%
Total		Count	84	36	120
		% of GENDER	100.0%	100.0%	100.0%
CC= .658			P= .ooo		

AGE					
	N	Mean	Std. Deviation	Minimum	Maximum
Male	84	68.71	7.744	48	78
Female	36	50.44	3.350	46	56
Total	120	63.23	10.762	46	78

Table 2a: Diabetes mellitus gender

Crosstab					
			GENDER		Total
			Male	Female	
		Count	32	0	32
	Type I	% of GENDER	38.1%	.0%	26.7%
		Count	52	36	88
DM	Type II	% of GENDER	61.9%	100.0%	73.3%
		Count	84	36	120
Total		% of GENDER	100.0%	100.0%	100.0%
CC=.367				P=.000	

Table 2b: Family history

Crosstab					
			GENDER		Total
			Male	Female	
PH	N	Count	28	28	56
		% of GENDER	33.3%	77.8%	46.7%
	Y	Count	56	8	64
		% of GENDER	66.7%	22.2%	53.3%
Total	Count	84	36	120	
	% of GENDER	100.0%	100.0%	100.0%	
	CC= .378			P= .000	

Table 2c: Past diabetic history

DURATION * GENDER Cross tabulation					
			GENDER		Total
			Male	Female	
DURATION	<10 y	Count	52	36	88
		% of GENDER	61.9%	100.0%	73.3%
	>10 y	Count	32	0	32
		% of GENDER	38.1%	.0%	26.7%
Total		Count	84	36	120
		% of GENDER	100.0%	100.0%	100.0%
CC= .367			P= .000		

Table 2d: Duration of foot infection

Duration of infection* GENDER Cross tabulation					
			GENDER		Total
			Male	Female	
INF	< 1 m	Count	48	36	84
		% of GENDER	57.1%	100.0%	70.0%
	> 1 m	Count	36	0	36
		% of GENDER	42.9%	.0%	30.0%
Total		Count	84	36	120
		% of GENDER	100.0%	100.0%	100.0%
CC= .394				P=.000	

Table- 2a, b, c and d: Ratio of Type-I, Type-II diabetes mellitus, family history and past diabetic history with duration of foot infection of both male and female.

Table-3 Percent incidence of bacterial and fungal infection in diabetic foot ulcer patients

Sl. No.	Bacterial and Fungal types	Frequency (Percent)	X^2	p
1.	Staphylococcus sps	92 (76.7)	34.133	.000
2.	Pseudomonas sps.	76 (63.3)	8.533	.003
3	E. coli	68 (56.7)	2.133	.144
4	Enterobacter sps.	64 (53.3)	.533	.465
5	Proteus sps	60 (50.0)	.000	1.000
6	Klebsiella sps.	52 (43.3)	2.133	.144
7	Bacillus sps.	20 (16.7)	53.333	.000
8	Candida sps.	80 (66.7)	13.333	.000

Conclusion

In the present investigation male patients dominated with the age of 70-80 years duration. Type I males were found susceptible than females in Type II. Less than 10 years female dominated more than 10 years males; whereas foot infection was more in one month males when compared to less than one month female. In general, among male and female patients *Staphylococcus* dominated followed by *Pseudomonas*. *Candida* was found dominating among fungal infection. With this both males and female are found to be exposed to the stressed environmental conditions with their history to develop Type I and Type II diabetic which leads to foot ulcers. Which is found to be fatal that leads to gangrene. Organisms that were isolated are all triple pathogens, single, double was not found in the present study.

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