Bacterial Pathogens Associated with Periodontitis in Diabetic and Non-Diabetic Patients

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Abstract

Background
The aim of study was to evaluate that whether diabetic patient having periodontitis are more susceptible to certain classes of bacteria especially anaerobes as compared to non-diabetic. The study is clinically significant because of its implication in the treatment of bacterial periodontitis in diabetic and non-diabetic patients.

Methods
The study was conducted in the department of microbiology, Basic Medical Science Institute, (BMSI), Jinnah Postgraduate Medical Center Karachi (JPMC) & Abbasi Shaheed Hospital. 30 samples were collected from JPMC and 70 samples from Abbasi Shaheed Hospital from 2012 to 2014 This study was carried out on pus sample taken from periodontal pocket of diabetic (n- 50) and non-diabetic (n- 50) patients. Samples were collected from each patient using a sterile cotton swab at the site of lesion in the oral cavity and processed in aerobic and anaerobic condition. Bacteria were isolated and identified by standard laboratory methods.

Result
Most (97%) samples yielded a positive culture; no bacteria were isolated in 3 samples, 57 samples were positive for anaerobic bacteria; 40 samples had mixed growth of aerobes and facultative anaerobic bacteria.

Conclusion
The findings of the present study indicate that anaerobic pathogens are more common in diabetic patients with periodontitis. Among them *Prevotelladenticolla* and *Petostreptococcusanerobicus* were more frequent isolates.

Keywords
Periodontitis, bacterial flora, diabetes mellitus,

Introduction
Periodontitis is one of the most widespread diseases in the world affecting the oral cavity, and is highly prevalent in both developed and developing countries. Periodontitis is a chronic inflammatory disorder affecting the gingivae and the periodontal tissue initiated by bacteria.[1]

Periodontitis is a common chronic bacterial infection of the supporting structures of the teeth. The host response to this infection is an important factor in determining the extent and severity of the disease. Several systemic diseases, such as diabetes mellitus, may increase the prevalence, incidence, or severity of gingivitis and periodontitis.[2]

The risk of cardiorenal mortality (ischaemic heart disease and diabetic nephropathy combined) is three times higher in diabetic people with severe periodontitis than in diabetic people without severe periodontitis.[3]

The prevalence of periodontal abscess is relatively high, which is often the reason why a person seeks dental care. Periodontal abscess accounts for 6% - 14% of all dental emergencies. It is the third most common dental emergency among all emergency dental conditions; periodontal abscesses represent approximately 8% of all dental emergencies in the world, and up to 14% in the USA.[4]

Analysis of periodontal status in people with type 1 or type 2 diabetes from a population-based German study has demonstrated an association between both types of diabetes and tooth loss. These data suggest that the inflammatory response to infection in people with type 2 diabetes is more severe than in non-diabetic subjects. This may be explained by a lack of ability to produce functional antibodies against bacteria in periodontal infection.[5]

Diabetes mellitus is a growing public health problem and various inflammatory diseases and soft tissue pathologies in oral cavities are associated with diabetes mellitus. Periodontal diseases have been proposed as the sixth most prevalent complication of diabetes mellitus following the other diabetic complications.[6]

Of the various local and systemic risk factors for chronic periodontitis, diabetes is considered to be one of the most well-established and validated, aside from tobacco usage lead to discomfort from extensive mobility of a tooth (resulting from

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bone loss), or fetid odor.[7]

Recent studies in periodontal medicine suggest that there is a link between mild to moderate periodontal disease in humans and certain systemic disorders such as diabetes mellitus, pneumonia, heart disease and preterm birth. The environmental conditions in the necrotic root canal are promotive for the establishment of microbiota conspicuously dominated by anaerobic bacteria.[8]

Solid data have corroborated the hypothesis thatperiodontal diseases are more prevalent among diabetics than non-diabetics.[9]

Aggressive periodontitis has been postulated to be frequently associated with *Aggregatibacter actinomycetemcomitans* (*A.actinomycetemcomitans*) and *P. gingivalis*, *Bacteroidesforsythus* (*B. forsythus*) and *Prevotellaintermedia* (*P. iintermedia*).[10]

Therefore, the purpose of present study was to determine the frequency of periodontal pathogens and to identify and compare the different types of bacteria in diabetic and non- diabetic patients.

Methodology
This was a cross sectional study done at outpatient department of Jinnah Postgraduate Medical College and Abbasi Shaheed Hospitals during Jun 2012- 2014. All patients clinically diagnosed with periodontitis in either both diabetic and non-diabetic patients of any age group were included in the study. Patients were excluded if they were on any antibiotic and if they had dentures. The study population included both diabetic and non-diabetic patients attending oral medicine and periodontics.

A total of 100 patients including 50 diabetic and 50 non-diabetics were included in the study. Samples were collected from each patient using a sterile cotton swab at the site of lesion in the oral cavity. The samples were transported in transport medium to the department of microbiology, BMSI, JPMC Karachi and processed within 24 hours, for isolation of periodontal bacteria. The swabs were inoculated on appropriate culture media aerobically and anaerobically on selective and non-selective agars for a various groups of bacteria.

Aerobic inoculation: each sample cultured on blood agar and MacConkey agar for recovery of Aerobes and facultative anaerobes.

Carbondioxide (5-10%) enriched incubation; each sample cultured on Chocolate agar, incubated in candle jar at 37^0C. Anaerobic incubation done on Brain heart infusion blood agar and Thyoglycolate broth.

Identification was based on cell morphology, Gram stain reaction, biochemical and enzymatic tests including catalase, oxidase, indole hydrolysis, esculin hydrolysis, gelatin hydrolysis, urea hydrolysis and fermentation of sugars.[11]

Results
A total of 100 patients including 50 diabetic and 50 non-diabetics. 97 samples were positive for bacterial growth whereas no growth observed in three samples. Distribution of Periodontatis cases in Diabetic and Non Diabetics patients given in Table 1. The age range from 10 – 80 mean ages is 45 year. Male to female ratio is 2: 1.

Overall frequencies of periodontal pathogen in study population are shown in Figure 1. These bacterial infections were mostly polybacterial, with predominance of anaerobes.

It was observed that the anaerobic bacterial isolates were predominant. *Provotella* and *Peptostreptocus* groups of bacteria were the most common (Table 2). Mix growth of facultative anaerobic and aerobes were recovered from 40% of cases. Most isolates were members of *S.Viridans* & *S. aureus*.

Discussion
The results of present study show the diversity of mix growth, facultative and strict anaerobic composition of the bacteria.

Table 1. Distribution Of Periodontitis Cases In Diabetic And Non- Diabetic Patients

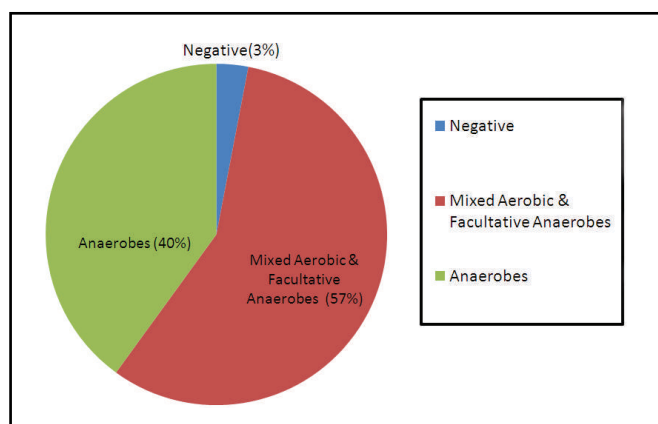| Age Groups	Diabetic		Non Diabetic	
	Male	Female	Male	Female
10-40 year	2	7	8	5
41-60 years	18	19	16	13
61-80 years	2	2	7	1
	22	28	31	19

Fig 1: **Distribution of Isolated Bacterial Pathogens**

Table 2. Comparisons of bacteria isolated from diabetic and Non-diabetic patients.

Isolated Bactria	Diabetic group		Non- Diabetic group	
	N	%	N	%
Prevotelladenticolla	9	5.9	3	0.93
Prevotellaoralis	7	4.62	2	0.62
Prevotellaoris	4	2.64	1	0.31
Prevotellaloescheii	2	1.32	2	0.62
Prevotellamelaninogenica	3	1.98	-	-
Veillonella spp.	4	2.64	1	0.31
Fusobacteriumnucleatum	2	1.32	1	0.31
Petostreptococcusanerobicus	6	3.3	1	0.31
Peptostreptococcus micros	5	3.3	-	-
Tanerellaforsythensis	3	1.98	1	0.31
Streptococcus mutans	3	1.98	3	0.93
S. anginosus	1	0.66	3	0.93
S.mitis	4	2.64	5	1.55
S. aureus	4	2.64	3	0.93
Acinobacillus	4	2.64	1	0.31
Lactobacillus	1	0.66	2	0.62
Actinomyces Israeli	4	2.64	1	0.31
Klebsiella	1	0.66	1	0.31
Total	66		31	

Various studies from Pakistan, India and other countries showed almost same results.[8,11,12,13,21,23]

The polymicrobial pattern of infection is routinely encountered in periodontitis. In the present study polymicrobes were found in 40% cases. We identified aerobics anaerobe, Gram positive and negative microorganisms that range from more virulenceanaerobic to moderate and low virulent facultative and aerobic bacteria. The study was consistent with other studies done by various researchers. [8, 11, 12]

Periodontitis is a common problem in patients of diabetes mellitus. However, differences in the putative periodontal pathogens in subjects with DM compared to non-DM subjects are still inconclusive. A comparative study of diabetic and Non-diabetic patients show the bacterial flora are higher in diabetic patients as compared to Non-diabetic patients.[11, 14, 15] This study revealed that anaerobic bacteria aremore common in diabetic patients. Our results are in agreement with that of previous studies, in which the number of anaerobic isolates were more in diabetic subjects.[11'16'17]

Our results differ from those showing, commonest isolation was Streptococcus salivarious.[15] In another study the putative periodontal pathogens were *P. gingivalis*, *T. forsythia* and *T. denticola* showed a higher prevalence in the periodontitis group as compared to other gram-negative anaerobe *Actinobacillus*, *actinomycetemcomitan*s, *Bacteroidesoralis*, *Staphylococcus aureus* and *Streptococcus mutans* which is well in agreement with several other existing data.[18]

In non-diabetic patients, the commonest isolate were *S.mutans*, *S. aureus* and *Serratia sp.*[10]

Our study indicated, the red complex, which includes *P. oris* (10%) & *Peptostreptococcus* (6%) encompasses the most important pathogens in diabetic cases few studies also reported the same groups of organisms in their comparative studies.[8,19,20,21] In contrast study done by reported *Porphyromonas gingivalis* predominant pathogen in diabetic patients.[9,11,22,23]

Conclusion
Data of the present study shows the diversity of anaerobic and facultative anaerobe bacteria in chronic periodontitis. The frequency of periodontitis is more in diabetic .This should be considered in the treatment strategy of the patients considering the scarce data on microbial flora in the Pakistan population further studies for assessment of microbial profile in various

forms of periodontitis should be carried out.

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