Short Communication

Prevalence of Black Pigmented *Bacteroides melaninogenicus* Species in Severe Periodontitis among Diabetic Patients.

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Introduction

Periodontitis is the major cause of tooth loss in adult human (Macphee and Cowley 1981). But, tooth loss is 15 times higher in diabetic patients than non-diabetic groups (Kataz et al 1991). Diabetic patients suffer from an increased prevalence of perio-dontitis compared to a normal population (Sandler and Stahl 1960). In severe periodontitis, deep gingival and periodontal pockets with anaerobic microenvironment are developed. Microbiological exa-mination of plaques from these pockets in rapidly destructive perio-dontitis have shown the predomi-nance of different gram negative rods. Among these, black pigmented *Bacteroides melaninogenicus* species are found most frequently in deep gingival sulci which agravate the severity of the disease (Kelstrup 1966). However, several attempts had been made in the past to determine the prevalence of black pigmented *Bacteroides melaninogenicus* spp. in periodontitis among general population. But, the reports on the association of this organism with the diabetic patients are not known so far. Therefore, it was thought worthwhile to study this bacterial species among the diabetic patients with different age, sex and nutritional groups with severe periodontitis.

Materials and Methods

A total number of 55 diabetic patients with severe periodontitis and 15 without periodontitis as controls were selected for the study. This study was performed at the Dental department of Bangladesh Institute for Research and Rehabilitation in Diabetes, Endocrine and Metabolic Disorder (BIRDEM). The bacteriological analysis of the study was performed in the microbiology laboratory of the Institute of Nutrition and Food Science, University of Dhaka.

Data about patients' age, sex, history of diabetes etc. were collected after

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the interogation through a prepared questionnaire. According to WHO-Standard age grouping (Macphee and Cowley 1981), the patients were divided into four classes, eg. 15-19, 20-29, 30-44 and 45-64 years. They were selected from both the sexes. Nutritional status of the patients was determined by measuring their Body Mass Index (BMI). The value of Percent Body Mass Index (PBMI) was assessed according to the formula of West (1980).

The severity of periodontitis was detected using "Community Periodontal Index of Treatment Needs" (CPITN) recommended by WHO (Ainamo et al 1985). While scoring the Periodontal Index, six teeth (Ramfjord teeth) (Marthaler et al 1971) were taken as representative of the entire dental arch, which were 16, 21, 24, 36, 41 & 44. If one of these teeth is missing, its distal neighbour (17, 11, 25, 37, 42 or 45 respectively) was substituted.

Sample of the periodontal plaques were collected from the selected diabetic patients using a sterile periodontal curette (Macphee and Cowley 1981). The sample was suspended in 1 ml of 0.9% sterile Nacl solution in a test tube and the suspension was added in 9 ml of 0.9% Nacl solution. Thus several ten fold dilutions were prepared. 0.1 ml of suspension from the 10^{-4} dilution was spreaded on the surface of agar media. Heart Infusion Agar (GIBCO) was used as the culture media, enriched with 10% laked human blood and 5 micro gram per ml of menadione (Gibbons and Macdonald 1960). The inoculated plates were introduced into the anaerobic jar and incubated at 37°C for 5-14 days. The intended use of laked blood was to enrich the media for the growth of black pigmented Bacteroides spp. and menadione to inhibit the growth of other bacteria and contaminants and thus made the media highly selective for black pigmented Bacteroides spp.

After incubation, the plates were examined for the presence of the black pigmented colonies, which are usually darker in the centre, edges are grey to light brown with 0.5-2.0 mm in diameter, circular, entire, convex and shiny. The turbidity and the nature of the sediment in broth culture differentiate these Bacteroides spp. (Bergey 1975). For this purpose, Peptone Yeast Glucose broth was used. The broth culture of black pigmented *Bacteroides melaninogenicus* spp. are usually turbid with smooth or stringy sediment. The gram stained slides from broth culture were prepared and observed microscopically.

Results

Out of 55 study population, the plate and the broth cultures showed *Bacteroides melaninogenicus* spp. positive in 34 cases, i. e. the percent incidence was 61.82%. Controls showed no growth of the bacteria.

The prevalence of the bacteria was 5.46% in case of the ages between 15-19 years. It was 12.73% in 20-29 years and 18.19% in 30-44 years. The incidence was however, recorded as 25.45% at the ages between 45-64 years. The results of the distribution of bacteria have been shown in Table-1.

The incidence of the bacteria was recorded as 40% in case of male patients whereas it was 21.82% in female (Table-1). The patients with PBMI < 80 representing the lean body build, showed 36.36% prevalence of the Bacteroides spp., whereas in the normal body build with PBMI between 80-119 it was 25.46%. Table-1 shows the results.

In all the cases mentioned above, the control groups showed no growth of the bacteria.

Discussion

From the results, it is evident that, the prevalence of black pigmented Bacteroides spp. is more (61.82%) in the periodontitis of diabetic patients than those of the patients without periodontitis. Hammens et al (1942) in their reports, showed lesser incidence of the bacteria from non-diabetic patients (28.26%) with periodontal disease which indicate the susceptibility of diabetic patients to Bacteroides infection.

The severity of periodontitis with increased prevalence of the bacteria correlated with the gradual increase of ages. The old age is more vulnerable to Bacteroides infection than the young age as evidenced from this study.

Table 1. Prevalence of *B. melaninogenicus* spp. in different age, sex and PBMI groups.

B. melaninogenicus	Age (Years)				Sex		PMBI	
species	15-19	20-29	30-44	45-64	Male	Famale	< 80	80-119
Positive	3	7	10	14	22	12	20	14
	(5.46%)	(12.73%)	(18.19%)	(25.45%)	(40%)	(21.82%)	(36.36%)	(25.46%)
Negative	3	4	6	8	14	7	9	12
	(5.46%)	(7.27%)	(10.91%)	(14.54%)	(25.46%)	(12.72%)	(16.36%)	(21.82%)
Total	6	11	16	22	36	19	29	26
	(10.91%)	(20%)	(29.09%)	(40%)	(65.46%)	(34.54%)	(52.73%)	(47.27%)

The results of the present study and that of Kelstrup (1966) also showed that, there is no significant difference in the incidence of the bacteria among the age groups in diabetic as well as non diabetic patients. Kelstrup, however, worked with the non-diabetic patients.

The black pigmented Bacteroides spp. was found more prevalent in male diabetic patients than those of female with severe periodontitis. Previous reports of Alam and Hoque (1991-92) showed the increased prevalence of periodontitis in male but that in case of non-diabetic patients.

Since the present study was aimed to determine the prevalence of Bacteroides spp. in diabetic patients with periodontitis and not to study the systematic analyses of the bacteria, hence only the cultural characters with black pigmentation on the selective media, broth culture with turbidity and smooth sedimentation and cellular morphology were considered for the identity of the bacteria.

However, it is evident from the study that, the diabetic patients are more prone to infection by the black pigmented Bacteroides spp. causing severe periodontitis.

Summary

Total 55 diabetic patients with periodontitis and 15 diabetic patients without periodontitis as controls were studied to determine the prevalence of black pigmented *Bacteroides melaninogenicus* spp. for their age, sex, and PBMI. Among the study population, the prevalence of this bacteria was found positive in 61.82% cases. It was found increased in the patients of higher age groups than those of lower ages. The prevalence was also higher in male than female. Observation showed from this study that, the prevalence of black pigmented Bacteroides spp. was higher in the PBMI group of < 80 representing the lean body build and comparatively lower in the normal body build with PBMI between 80-119.

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