Detection of specific IgG and IgA anti Epstein-Barr virus in saliva of chronic periodontitis patients and healthy subjects

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ABSTRACT

Background: Chronic periodontitis is an inflammatory disease that extends into the tissues supporting the teeth. Recent studies have demonstrated that various human herpesviruses especially Epstein-Barr virus (EBV) may play a part in the pathogenesis of human chronic periodontitis. This study aimed to detect anti EBV IgG and IgA in saliva of chronic periodontitis patients and healthy control subjects by enzyme linked immunosorbent assay (ELISA) test and to determine the differences between males and females regarding the periodontal condition and the levels of anti EBV IgG and IgA .

Materials and methods: The study sample consisted of sixty chronic periodontitis patients of both gender (32 males and 28 females) and thirty healthy control subjects of both gender (16 males and 14 females) with age ranged from 30 to 50 years. Both groups without any systemic disease.

Periodontal parameters used in this study were plaque index (PLI), gingival index (GI), bleeding on probing (BOP), probing pocket depth (PPD) and clinical attachment level (CAL). Unstimulated saliva samples were collected from all subjects and examined by ELISA test for EBV IgG and IgA antibodies detection.

Results: The results of the present study observed that there was no significant difference of PLI and GI between males and females in chronic periodntitis patients. Concerning BOP the number of bleeding sites in females was more than in males. For PPD and CAL, there was increased PPD with its different scores (0, 1, 2) in males compared with females and there was increased CAL with its different scales (scales 0, 1, 2, 3) in males than females. The percentage of control group who were positive for anti- EBV IgG was (36.7%) and in chronic periodontitis was (81.7%). Concerning IgA, 40.0% of healthy group and 68.3% of chronic periodontitis patients showed a positive reaction for anti- EBV IgA .

Conclusions: The present study showed that EBV Abs (IgG and IgA) were detectable in saliva from healthy individuals but they were significantly more frequently found in saliva of chronic periodontitis patients. The present study showed that there was no significant gender difference regarding the salivary level of anti- EBV IgG and IgA.

Keyword: chronic periodontitis, EBV, IgG,IgA. (J Bagh Coll Dentistry 2011; 23(sp. issue):125-128).

INTRODUCTION

Periodontal disease is a chronic inflammation of the gingiva and connective tissue. It is a disease attributable to multiple infectious agents and interconnected cellular and humoral host immune response.[1]. Bacterial etiology alone has not been able to substantiate various aspects such as rapid periodontal tissue breakdown with minimal plaque, phases of disease activity and quiescence, site specificity in periodontal disease and progression to advanced periodontal destruction which occurs in a fraction of a given population [2]. Since the mid 1990s, herpesviruses have emerged as putative pathogens in various types of periodontal disease [3]. Epstein-Barr virus, frequently referred to as EBV, is a distinct member of the herpesvirus family (Herpesviridae) of deoxyribunucleotide (DNA) viruses and one of the most common viruses in humans. Most people become infected with EBV, which is often asymptomatic but commonly is associated with acute infectious mononucleosis, as well as certain types of cancer, such as nasopharyngeal carcinoma and Burkitt's lymphoma. [4].

Saliva is the main vehicle for EBV transmission from individual to individual. [5]. Saliva is an accessible fluid that can easily be collected by the patient, advantages of saliva testing sample are easy and non invasive collection procedure that is neither painful nor traumatic [6]. Saliva is reliable for early detection of certain diseases and monitoring the disease course [7]. Antibodies against viruses and viral components can be detected in saliva and can aid in the diagnosis of viral infections and reactivation of infection [8]. This study aimed to detect anti EBV IgG and IgA in saliva of chronic periodontitis patients and healthy control subjects by enzyme linked immunosorbent assay (ELISA) test and to determine the differences between males and females regarding the periodontal condition and the levels of anti EBV IgG and IgA.

MATERIALS & METHODS

Human Sample

Sample population consisted of ninety males and females, age ranged from 30 to 50 years. Samples collection was started at 20th of February

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2011 till May 2011. Patients participating in the present study with chronic periodontitis (no=60, 32 males and 28 females) were recruited from the Clinic of the Department of Periodontics/ College of Dentistry/ Baghdad University.

The control group was taking from the Department of Periodontics (no=30, 16 males and 14 females) with clinically healthy gingiva, no pockets, no bleeding on probing and no evidence of bone loss.

Clinical examination

Periodontal examination consisted of plaque index (PLI),gingival index (GI), bleeding on probing(BOP, probing pocket depth (PPD) and clinical attachment level (CAL) at 4 sites for all teeth except 3rd molar on (mesial, midvestibular, distal, midlimgual), using a calibrated periodontal probe (Michigan O probe). Patients with chronic periodontitis had periodontal pockets equal or greater than 4mm with clinical attachment loss. All subjects participated in this study without any systemic diseases, had not received previous periodontal treatment and had not used antibiotics in the past 3 months. Patients were excluded if pregnant and smokers.

Collection of saliva samples

All participants were instructed not to eat or drink (except water) at least 1 hour prior to donation of saliva, the subject should sit in a relaxed position and samples containing blood should be discarded. Saliva was collected between 9-12 am. After the subject rinse his mouth several times by sterilized water and then wait for 1-2 minutes for water clearance, 5ml of whole unstimulated mixed saliva was collected into polyethylene tubes using a standardized method according to. [9]. Saliva then centrifuged at 10000 rpm for 10 minutes; this was done within 1hour after collection to eliminate debris and cellular matter, the supernatants were aspirated immediately into two pre labeled Eppendorf tubes and stored frozen at (-20 ºC) until they were assayed. Specific anti-Viral Capsid Antigen (VCA) IgG and anti Epstein-Barr Nuclear Antigen (EBNA-1) IgA antibodies to EBV in saliva samples of both patients and control groups were detected by enzyme linked immunosorbent assay using commercial kits (Human / Germany-Ref. No. 51204 & Demeditic / Germany-Ref. No DE-EBN02).

Statistical Analysis

The data were processed and analyzed using the statistics package for social sciences (SPSS Inc., version 17 for windows XP and excel 2007). Both descriptive and inferential statistics were used.

1. Descriptive Statistics; included mean, number (No.), Percentage, standard Deviation (SD), Standard error (S.E.) and statistical tables.
2. Inferential Statistics; included

- Student t-test.
- Z-test

In the statistical evaluation, the following levels of significance are used:

$P > 0.05$ Non-significant (NS)
$0.05 \geq P > 0.01$ * Significant (S)
$P \leq 0.01$ ** Highly significant (HS)

RESULTS

Descriptive statistics and genders difference. The mean and standard deviation of plaque index and gingival index in chronic periodontitis group are shown in table 1. There was no significant difference was seen between genders. The percentage of healthy sites (score 0) and bleeding sites (score 1) in chronic periodontitis group was shown in table 2. The result was highly significant gender difference. The percentage distribution of sites according to different probing depth scales in chronic periodontitis group was shown in table 3. The result was highly significant gender difference. The percentage distribution of sites according to different CAL scales in chronic periodontitis group was shown in table 4. The result was highly significant gender difference. The mean of anti EBV-IgG salivary levels in chronic periodontitis group was 13.89±9.97 and in control group was 10.04±2.34. The result showed highly significant difference ($P<0.01$) as shown in table 5. There was no significant gender difference regarding anti EBV-IgG in chronic periodontitis group as shown in table 5. The mean of anti EBV-IgA salivary levels in chronic periodontitis group was 20.89±19.08 and in control group was 7.86±6.54. The result showed highly significant difference ($P<0.01$) as shown in table 6. There was no significant gender difference regarding anti EBV- IgA in chronic periodontitis group as shown in table 7. The count and percentage of anti- EBV IgG and IgA of positive subjects in chronic periodontitis were higher than in control group and the results showed highly significant difference ($P<0.01$) as shown in table 8.

DISCUSSION

In this study there was no significant difference in PLI and GI between genders among chronic periodontitis patients. These results agree with Zhang *et al* regarding GI but disagree with his results regarding PLI as he found the males PLI values were significantly higher than that of

the females. [10].In this study the number and percentage of bleeding sites in females were more than in males, the explanation for this finding may be due to hormonal changes in females. Regarding PPD &CAL there was increase in severity in males than females. Males usually exhibit poorer oral hygiene than females, the reason for these gender differences have not been explored in detail, but are thought to be more related to oral health, less positive attitudes towards oral health, and dental-visit behavior among males than to any genetic factor. [11]. In this study ELISA results revealed that the percentage of anti-EBV IgG +ve saliva of chronic periodontitis was 81.7% while in control group was 36.7%. Concerning IgA, the percentage of anti-EBV IgA+ve saliva of chronic periodontitis was 68.3% and in healthy control was 40.0%. Idesawa *et al* [12] detected EBV in saliva of 49% periodontitis patients and 15% in saliva of healthy subjects. There is a study reported in 2008 [13] where they detected EBV in 37.5% of saliva samples in chronic periodontitis. Hochman *et al* [14] detected antibodies (Abs) against EBV in 32% of samples from 34 study sites of chronic periodontitis lesions, these Abs were of the immunoglobulin A (IgA) isotype and of immunoglobulin G (IgG). The reasons for variation in EBV occurrence among studies may include differing EBV detection techniques, dissimilar periodontitis disease states studied, and true geographic variation in EBV prevalence (5), or may be due to diagnostic difficulties and a natural fluctuation of periodontal EBV (15).

Although EBV Abs were detectable in saliva from healthy individuals, they were significantly more frequently found in saliva of chronic periodontitis patients, which suggested that EBV infection might be associated with the pathogenesis of chronic periodontitis [16,17].

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Table 1: Mean and SD of PLI and GI of Chronic periodontitis group by gender.

Chronic periodontitis	PLI		t-test	P-value		GI		t-test	P-value
	Mean	SD				Mean	SD		
Males	1.27	0.37	-0.323	0.748	Males	1.18	0.18	1.125	0.265
Females	1.31	0.46			females	1.24	0.24		

Table 2: Percentage distribution of sites and level of significant according to the presence or absence of bleeding on probing among chronic periodontitis group by gender.

Score	Males (2888)		Females (2468)		Z-test	P-value
	No.	%	No.	%		
0	2320	80.3	1883	76.3	3.52	0.0004**
1	568	19.7	585	23.7	3.52	0.0004**

Table 3: Percentage distribution of sites according to different probing depth scales with statistical difference among chronic periodontitis group by gender.

PPD scores	Males (2888)		Females (2468)		Z-test	P-value
	No.	%	No.	%		
0 (1-3mm)	2349	81.3	2148	87.0	5.62	P<0.0005**
1 (4-5 mm)	432	15.0	256	10.4	4.97	P<0.0005**
2 (≥6mm)	107	3.7	64	2.6	2.2	0.027*

Table 4: Percentage distribution of sites according to different CAL scales and significant difference among chronic periodontitis group according to gender.

CAL scales	Males (2888)		Females (2468)		Z-test	P-value
	No.	%	No.	%		
0 no attachment loss	1741	60.3	1731	70.1	7.46	P<0.0005**
1 mild (1-2 mm)	395	13.7	281	11.4	2.48	0.013*
2 moderate (3-4 mm)	406	14.1	297	12.0	2.23	0.025*
3 severe (≥5mm)	346	11.9	159	6.5	6.7	P<0.0005**

Table 5: The difference in mean of anti EBV- IgG salivary level among study group.

EBV IgG (HU/ml)	Chronic Periodontitis	Control
Count	60	30
Mean	13.8992	10.0416
Standard Deviation	9.97031	2.34269
Standard Error of Mean	1.23	0.29
Minimum	5.97	6.45
Maximum	56.07	14.90
P-value	0.0001**	

Table 6: ELISA results of anti-EBV IgG among chronic periodontitis patients by gender.

Gender	Total No.	EBV IgG +ve	Z-test	P-value
Males	32	26 (81.3%)	-0.25	0.799
Females	28	23 (82.1%)		

Table 7: The difference in mean of anti-EBV IgA among study groups.

EBV IgA (U/ml)	Chronic Periodontitis	Control
Count	60	30
Mean	20.8902	7.86
Standard Deviation	19.08422	6.54
Standard Error of Mean	2.39	1.19
Minimum	3.27	1.05
Maximum	69.82	19.75
P-value	0.0001**	

Table 8: ELISA results of anti-EBV IgA among chronic periodontitis by gender.

Gender	Total No.	EBV IgA +ve	Z-test	P-value
Males	32	18 (56.3%)	1.870	0.062
Females	28	23 (82.1%)		

Table 9: Count and percentage of anti- EBV IgG and IgA of positive and negative subjects among chronic periodontitis and control groups.

	Chronic Periodontitis		Control		P-value
	Count	%	Count	%	
EBV IgG (HU/ml) Positive (≥8)	49	81.7	11	36.7	0.0001**
Negative (<8)	11	18.3	19	63.3	
EBV IgA (U/ml) Positive (≥8)	41	68.3	12	40.0	0.0001**
Negative (<8)	19	31.7	18	60.0	