CORRELATION OF SALIVARY LEVELS OF ANTI EPSTEIN-BARR VIRUS IGG, IGA AND SEVERITY OF CHRONIC PERIODONTITIS [+]

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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 herpes viruses especially Epstein-Barr virus (EBV) may play a part in the pathogenesis of human chronic periodontitis and show correlation with periodontal disease severity.

Aims of the study: To correlate anti EBV IgG and IgA levels in saliva of chronic periodontitis patients with the severity of the disease.

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 were without any systemic disease.

Periodontal parameters used in this study were bleeding on probing (BOP), probing pocket depth(PPD) and clinical attachment level(CAL). Unstimulated salivary samples were collected from all subjects and examined by enzyme linked immunosorbent assay (ELISA test) for EBV IgG and IgA antibodies detection.

Results: The results of the present study showed that the mean of anti-EBV IgG and IgA salivary levels in chronic periodontitis patients were 13.8992 HU/ml, 20.8902 U/ml respectively. In this study there was no significant correlation between EBV IgG and IgA Abs levels and BOP in chronic periodontitis patients. For PPD, there was a significant positive correlation with score 1 and 2 with EBV IgG salivary level, while EBV IgA has a significant positive correlation with score 1 and 2. Concerning CAL, there was a positive significant correlation with score 2 and highly significant positive correlation was found between EBV IgG and IgA and score 3.

Conclusions: The present study showed that there may be an association between EBV infection and the severity of chronic periodontitis and thus coinfection with EBV may play a part in destruction of periodontal tissue.

Key words:- chronic periodontitis, EBV, IgG,IgA.

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العلاقة بين المستويات اللعابية المضادة لفيروس ابشتاين بار نوع ، ج و أ مع شدة التهاب النساغ المزمن

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المستخلص:

الخلفية: التهاب النساغ المزمن هو مرض التهابي يمتد إلى الأنسجة الداعمة للأسنان وقد أثبتت الدراسات الحديثة أن . فيروسات قوباء المختلفة وخاصة فيروس ابشتاين بار قد تلعب دورا في التسبب في التهاب النساغ المزمن ويظهر ارتباطا مع شدة أمراض اللثة.هدف الدراسة هو ايجاد العلاقة بين مؤشرات التهاب اللثة وشدة الالتهاب مع المستوى اللعابي لمضادات فيروس ابشتاين بار.

المواد والطرق: تألفت عينة الدراسة من ستين مصابا بالتهاب الأنسجة الداعمة المزمن من الجنسين(32من الذكور والإناث 28)وثلاثين شخصا من الأصحاء من الجنسين (16)من الذكور والإناث (14)مع مستوى العمر يتراوح بين 30إلى 50 سنة .كلا الفريقين دون أي أمراض جهازية. ومعلمات اللثة المستخدمة في هذه الدراسة تشمل النزف عند البحث (BOP)، سبر عمق الجيب (PPD)و مستوى الارتباط السريري .(CAL) تم جمع عينات من اللعاب Unstimulatedمن جميع المرضى وفحصها الفحص المناعي انزيم مرتبط (ELISA test)لمفتش وايغا EBV وكشف الأجسام المضادة.

النتائج: أظهرت نتائج الدراسة أن متوسط المضادة للمفتش EBV ومستويات ايغا اللعابية في المرضى الذين يعانون التهاب اللثة المزمن هو 13،8992 HU / مل و 20،8902 U / مل على التوالي. في هذه الدراسة لم يكن هناك ارتباط كبير بين مستوى مضادات EBV اللعابية وBOP في المرضى الذين يعانون التهاب اللثة المزمن.بالنسبة لPPD، كان هناك ارتباط كبيرو إيجابية قوية مع درجة 1 و 2 مع مستوى مضادات EBV اللعابية، في حين EBV ايغا لديه علاقة إيجابية هامة مع درجة 1 و 2. بشأن CAL، كان هناك ارتباط إيجابي كبير مع مقياس 2 و إيجابية ارتباط كبير مع مقياس 3.

الاستنتاجات: أظهرت هذه الدراسة أنه قد يكون هناك وجود ارتباط بين الإصابة ب EBVو شدة التهاب اللثة المزمن وبالتالي عدوى مرافقة مع EBVقد تلعب دورا في تدمير الأنسجة اللثوية.

Introduction:

Periodontal diseases are bacterial infections of the gingiva ,bone &attachment fibers that support the teeth & hold them in the jaw[1] . It is a disease attributable to multiple infectious agents and interconnected cellular and humoral host immune responses[2]. 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 [3].Chronic periodontitis is a slowly progressing periodontal disease that at any stage may undergo an exacerbation resulting in additional loss of

attachment apparatus[4]. Since the mid-1990s, herpes viruses have emerged as putative pathogens in various types of periodontal disease[5]. 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[6]. The general term of saliva referes to the fluid that surrounds oral hard &soft tissues & composed of all fluids found in the oral cavity [7].

Saliva is the main vehicle for EBV transmission from individual to individual [8].Saliva is an accessible fluid that can easily be collected from the patient . Advantages of saliva testing sample are easy and non-invasive collection procedure that is neither painful nor traumatic[9].Saliva is reliable for early detection of certain diseases and monitoring the disease course [10] .Antibodies against viruses and viral components can be detected in saliva and can aid in the diagnosis of viral infections and reactivation of infection[11] .This study aimed to detect anti EBV IgG and IgA in saliva of chronic periodontitis patients by enzyme linked immunosorbent assay (ELISA) test and to show the correlation of EBV antibodies with the severity of chronic periodontitis[12].

Materials & Methods:

Human Sample:

The population sample consisted of ninety subjects, age ranged from 30 to 50 years. This study was carried out between February & May 2011. Patients participating in the present study were informed about the purpose of the study & they agreed to participate.The sample was collected from patients attending perio-clinic in the department of Periodontics/ College of Dentistry/ Baghdad University seeking for periodontal treatments. The sample was consisted of 60 patients(32 males &28 femals) with chronic periodontitis ,in addition to them , thirty healthy patients as a control group (16 males and 14 females) .They were free of any signs & symptoms of periodontal diseases. All patients in this research were with no history of any systemic disease, had not received previous periodontal treatment . Patients were excluded if pregnant and/or smokers.

Clinical examination:

Periodontal examinations & measurements were done by the same examiner. The following periodontal parameters were measured: 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.For ease of estimation , a scale was designed to measure the PPD &it included the following scores : score 0 =1-3 mm , score 1=4-5 mm , score 2 = equal or greater than 6 .Also another scale was designed to measure CAL & as follows : score 0 = no attachment loss , score 1=1-2 mm ,score 2 =3-4 mm & score 3 = equal or more than 5.

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 rinsed his mouth several times by sterilized water and then waited for 1-2 minutes for water clearance, 5ml of whole unstimulated mixed saliva was collected into polyethylene tubes using a standardized method [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.mean, SD, percentages,S.E,t-test,z-test,were used where indicated.
In the statistical evaluation, the following levels of significance were used:

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

Results:

The percentage distribution of sites according to different scales of BOP,PPD,CAL,were shown in table (1).Descriptive statistics for EBV IgG antibodies level were shown in table (2). The mean of anti-EBV IgG salivary level in chronic periodntitis patients was (13.8992 HU/ml) and in control group was (10.0416 HU/ml). The results showed statistically highly significant differences ($P<0.01$). Descriptive statistics for EBV IgA were shown in table (3) The mean of anti-EBV IgA salivary concentrations in chronic periodontitis patients was(20.8902 U/ml) and in control group was(7.86 U/ml). The results showed statistically highly significant differences ($P<0.01$). The percentage of both anti EBV IgG and IgA salivary levels in study groups was shown in figure (1). Correlations between levels of IgG and IgA anti- EBV and BOP and PPD are seen in table (4). The results of this study show no significant correlation with BOP and both IgG and IgA. Concerning PPD a significant positive correlation with IgG anti-EBV salivary level and score 1 and 2 was found.The correlation of IgA anti-EBV level with PPD was a significant positive correlation with score 1 and 2 as shown in this table. The coefficient of correlations of salivary levels of IgG and IgA anti-EBV with clinical attachment level (CAL) were seen in table (5). There was no significant correlation between IgG level and CAL score 1. A significant positive correlation was found with CAL score 2 and a highly significant positive correlation was found between IgG level and CAL score 3 at p value of 0.01 level of significance as shown in this table. Concerning IgA levels, no significant correlation with score 1 was found, while a significant positive correlation was found between IgA level and CAL score 2 and a highly significant positive correlation between IgA level and score 3 was found at 0.01 level of significance as shown in this table.

Discussion:

In this study, there was no significant correlation between EBV IgG and IgA antibodies salivary levels and BOP in chronic periodontitis patients. EBV can occur with minimal gingival bleeding as seen in some chronic periodontitis lesions [13,14].The results of the present study were in agreement with those of Ling *et al*.(2004)[14] ,who found that there was no significant association between the prevalence of EBV infection and BOP. Idesawa *et al*. (2004) [15] indicated that the frequency of EBV is associated with BOP. Concerning PPD, there was highly significant correlation with PPD score 1 and 2 and with anti EBV IgG, while EBV IgA had positive significant correlation with score 1 and 2. These results agree with Saygun *et al*. (2005) [16] who observed a positive correlation between salivary counts of EBV and periodontal pocket depth, he suggested that periodontitis lesions were a source for salivary EBV.

Konstantinidis *et al*. (2005)[17] observed no differences in probing pocket depth between EBV positive and EBV negative chronic periodontitis patients.

The relationship of EBV Abs levels with deep probing depths suggest a positive relationship between EBV periodontal infection and clinical severity of chronic periodontitis. According to the results of this study, there was a positive significant correlation with scale 2 and highly significant correlation was found between IgG and scale 3 of CAL.

Concerning EBV IgA, there was a positive significant correlation with scale 2 and highly significant correlation with scale 3, these results agree with Wu *et al*. (2007)[18] and disagree with Wu *et al*. (2006)[19] who found no statistical differences between EBV positive patients and CAL.

The presence of EBV Abs in saliva of chronic periodontitis indicate the correlation of EBV with periodontal disease severity. A periodontal dual infection of EBV and pathogenic bacteria gives rise to enhanced cytokine release and immune signaling dysregulation and tends to be associated with more severe periodontitis than a periodontal infection involving solely bacteria [20].

Table (1): Percentage distribution of sites and level of significance according to the presence or absence of bleeding on probing , PPD scores & CAL scores among chronic periodontitis group by gender.

BOP	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**
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*
CAL scores	**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 (2): The difference in mean of anti EBV-IgG salivary level among study groups.

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 (3): 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**	

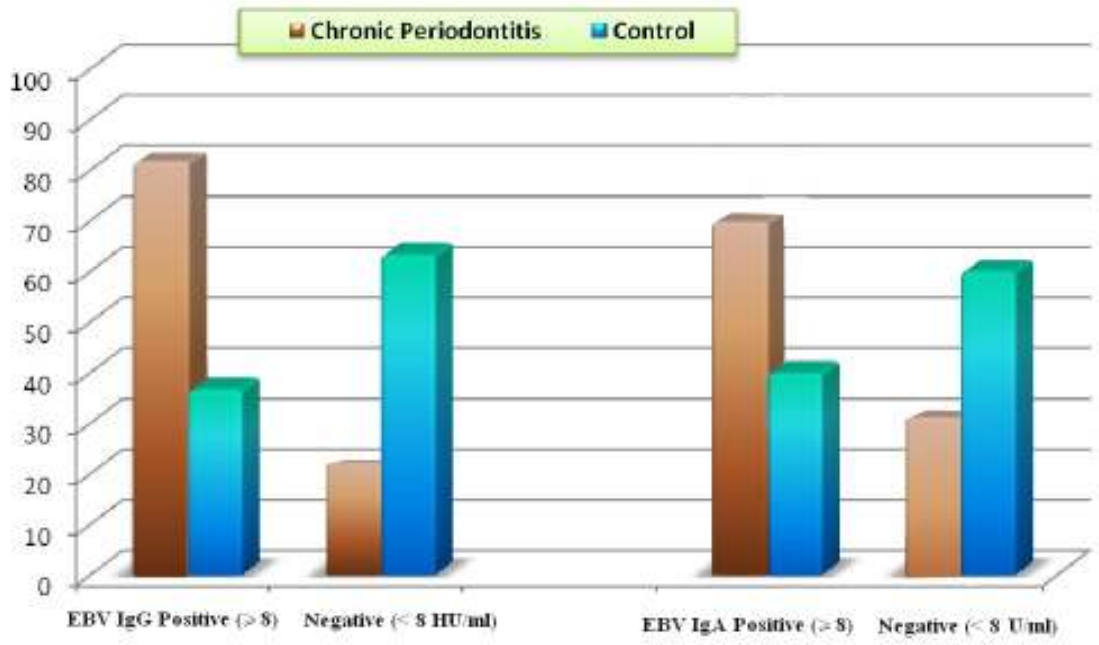

Figure (1): The bar chart of both EBV IgG and EBV IgA salivary levels in chronic periodontitis and control groups

Table (4): Correlation coefficient of IgG and IgA anti-EBV with BOP and PPD in chronic periodontitis group.

Scores	EBV IgG (HU/ml) Chronic Periodontitis		EBV IgA (U/ml) Chronic Periodontitis	
	r	sig	r	sig
BOP score 0	-0.177	NS	-0.176	NS
BOP score 1	0.024	NS	-0.005	NS
PPD score 0 (1-3mm)	-0.682**	HS	-0.545**	HS
PPD score 1 (4-5mm)	0.535**	HS	0.341*	S
PPD score 2 (≥ 6mm)	0.579**	HS	0.377*	S

Table (5) Correlation coefficient of IgG and IgA anti-EBV with CAL in chronic periodontitis group.

CAL scales	EBV IgG (HU/ml) Chronic Periodontitis		EBV IgA (U/ml) Chronic Periodontitis	
	r	sig	r	sig
CAL score 0 no attachment loss	-0.580**	HS	-0.617**	HS
CAL score 1 (Mild 1-2mm)	-0.019	NS	-0.074	NS
CAL score 2 (Moderate 3-4mm)	0.374*	S	0.427*	S
CAL score 3 (Severe ≥5mm)	0.717**	HS	0.752**	HS

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