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Clinical, microbiological and immunological relationship in Candida induced denture stomatitis

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ABSTRACT

Background: Candida induced denture stomatitis is a common inflammatory disease and the levels of cytokines in the saliva are affected by this condition. This study was attempted to explore the relationship between the clinical forms in Candida induced denture stomatitis with Candida coloni count and immunological marker.

Patients, materials and methods: The study has enrolled 37 participants with Candida induced denture stomatitis according to Newton`s criteria. Palatal mucosa was swabbed for fungal culturing and colonies counting. Samples of unstimulated saliva were collected for immunological marker detection. Salivary level of IL-6, L-10 and IgA were performed by enzyme linked immunosorbent assay.

Results: There was no significant difference between the clinical grade and Candida coloni count on one side and with IL-6, IL-10 and IgA in other side. In addition there was no significant differences between Candida coloni count and these immunological markers.

Conclusions: The Candida coloni count was not related to the clinical staging, and there was no association between the occurrence of Candida related denture stomatitis and the concentrations of IL-6, IL-10 and IgA.

Keywords: oral candidiasis, Candida albicans, cytokines, IgA.

INTRODUCTIONS

Denture stomatitis is a common oral Candida infection or inflammatory disease mainly found underneath the removable prosthesis [(1-4)]. Clinically, the lesion can be found ranging from pin point erythema to generalised erythematous inflammation with or without papillary hyperplasia [(3,5)]. It has been reported that 11% - 67% of the otherwise healthy denture wearers have positive Candida culture and Candida albicans was found to be the predominant species in the oral cavity induce denture stomatitis [(2,3,6)].

Cytokines are soluble factors, which are mostly generated by immune cells and in turn play crucial roles in the differentiation, maturation and activation of various immune cells. Production of cytokines in whole saliva can be expected to have a major impact on the initiation of the inflammatory response. Several studies showed the controversial nature of the cytokine production in individuals with Candida related denture stomatitis [(4,6)]. In Candida associated denture stomatitis, protective immunity is mainly linked to secretory IgA antibodies and cell mediated immunity [(7)]. In patients with denture stomatitis, there is elevated level of these antibodies against C albicans. Salivary secretory IgA may be considered first line of defence against candidiasis and its level is a critical modulator of microbial aggregation, microbial clearance, and surface adherence [(8)]. The relationship among clinical criteria, Candida coloni count and immunological markers in patients having oral candidiasis was assessed in this study.

Patients, materials and methods:

The sample of the study were patients recruited from Specialist dental health centers in Baghdad city for One year period. The study was conducted after approvals of the research protocol by the local Research Scientific and Ethics

Committee was obtained. Patients fulfilled the clinical selection criteria of oral candidiasis with C. Albicans positive culture have entered the clinical trial. Patient must be in good general health and presence of other oral pathological lesion was excluded.

Clinical signs consistent with oral candidiasis in palatal mucosa were categorized according to Newton`s criteria as follows [(5)]:
0: no inflammation; Grade 1: pinpoint hyperaemia; Grade 2: generalized erythematous type and Grade 3: hyperplastic granular type.

Mucosa swab from palate of the patients was taken. The sample collected were streaked semiquantitively onto Sabouraud dextrose agar plates, incubated aerobically at 37°C for 48 hours. The possibility of C albicans being present in the culture media was identified by culture characteristics regarding shape, color and size of the yeast colonies grown on agar medium. Gram stain and germ tube was done to examine microscopically. Candida count was done for each culture after incubation period.

Unstimulated Saliva Collection was made when subjects were instructed to remove their dentures and refrain from eating, drinking, brushing their teeth or chewing gum for 1 hour prior to salivary collections, and were performed between 8:30 and 11:00 AM. Subjects were instructed first to swallow to clear the mouth of any accumulated saliva, tilted their head forward, and whole unstimulated 2 ml of saliva was allowed to pool in a disposable sterilize plastic container. Saliva sample was then centrifuged for 10 minutes at 3000 rpm, and the supernatant aliquot divided into Eppendrof tubes then stored at(-65°C) until immunological analysis. Enzyme linked immunosorbent assay (ELISA) (kits; abcam CO., USA) was done to analyse saliva to detect IL-6, IL-10 and IgA.

Statistical analysis: Data were translated into a computerized database structure using SPSS version 21. The age is described by mean, SD (standard deviation). The outcome quantitative variables were described by median and interquartile range. The difference was assessed by the non-parametric test Mann-Whitney, Kruskall-Wallis test and Spearman's rank linear correlation coefficient. P value less than 0.05 level of significance was considered statistically significant.

RESULTS

There were 37 patients participated in this study. (12 males, 25 females) (Table 1). Female to male ratio was 2.03.Their age ranges between 30-75 years. The mean age ± SD was 55.7± 12.24.

Table 1: gender distribution:

Gender	N	%
Male	12	32.4
Female	25	67.5
Total	37	100

Most patients of Candida induced denture stomatitis were symptomless in general, however 15 patients complaining from mild burning sensation. Only two of those patients attending to the dental clinic where pain was the chief complain, while the others were attending for prosthodontic reasons. Most patients with denture stomatitis had grade 2 diffuse erythema (17 patients), followed by papillary hyperplacia (11 patents) and the least were categorized as grade one pin point erythema (9 patients) (Table 2). One patient had psedomembranous candidiasis associating denture stomatitis.

Eight patients (21.6%) were having angular cheilitis in addition to stomatitis.

Table 2: clinical criteria:

Clinical criteria (Grade)	No	%
Pin point erythema	9	24.32
Diffuse erythema	17	45.94
papillary hyperplasia	11	29.72
Total	37	100

Comparing the clinical criteria of patients, number of Candida colonies observed, and immunologic parameters are shown in Table 3.

Mucosal coloni was increased when clinical criteria increased from pin point (grade 1) to erythema (grade 2) and papillary hyperplasia (grade 3).

However, clinical criteria did not follow ascertain way with the median and mean rank of coloni number. All parameters in this table showed no significant differences with clinical observation on the patients except in IL-10 which is slightly increased when clinical criteria increased, however there was no significant difference between them (P=0.05).

IgA and other salivary immunological markers: were divided into four quartiles to test the relation with the other parameters. There was no significant difference when compared with coloni count and amount of salivary IgA concentration. In addition there was no obviously link between IgA and IL-6in one hand and between IgA with IL-10 in other hand (Table 4).

Table 5 showed there were no relation between IL-10 concentration and microbiological measurements. In Table 6, Candida coloni and the salivary IL-6, has shown no significant difference between them (P=0.08).

Table 3: The difference in medians of selected microbiologic and immunologic measurements with clinical criteria in the samples with denture stomatitis

	Clinical criteria			
	Pin point erythema	**Diffuse erythema**	**papillary hyperplasia**	**P**
Mucosal coloni count				0.73[NS]
Range	(0 to 283)	(0 to 140)	(0 to 40)	
Median	2	6	6	
Interquartile range	(0 to 6)	(2 to 18)	(0 to 7)	
N	9	17	11	
Mean rank	16.9	20.4	18.5	
r=0.044 P=0.8[NS]				
Salivary IL-6 pg/ml				0.19[NS]
Range	(0.64 to 35.77)	(0.44 to 82.07)	(3.79 to34.39)	
Median	15.42	2.23	9.18	
Interquartile range	(4.71 to 21.23)	(0.71 to 16.78)	(5.78 to 12.48)	
N	6	10	11	
Mean rank	16.7	10.4	15.8	
r=0.054 P=0.79[NS]				
Salivary IL-10 pg/ml				0.05[NS]
Range	(0.35 to 1.48)	(0.38 to 50)	(0.59 to 3.8)	
Median	0.42	1.105	0.91	
Interquartile range	(0.38 to 0.54)	(0.44 to13.11)	(0.73 to 3.4)	
N	6	10	11	
Mean rank	7.1	16.1	15.9	
Salivary Ig-A ng/ml				0.43[NS]
Range	(19.2 to 160.5)	(6 to 154.5)	(6 to 174.5)	
Median	94.45	24.85	93.1	
Interquartile range	(23.2 to 139.5)	(21.6 to 129.9)	(35.9 to 135.2)	
N	6	10	11	
Mean rank	14.8	11.5	15.9	
r=0.115 P=0.57[NS]				

Table 4: The difference in medians of selected microbiologic and immunologic measurements with Salivary IgA ordered categories in the samples with denture stomatitis

	Salivary Ig-A ng/ml			
	First (lowest) quartile (<= 23.9)	**Average (24.0 - 135.2)**	**Fourth (highest) quartile (135.3+)**	**P**
Mucosal coloni count				0.46[NS]
Range	(0 to 27)	(0 to 140)	(0 to 18)	
Median	0	6	3.5	
Interquartile range	(0 to 8)	(1 to 25)	(1 to 7)	
N	7	14	6	
Mean rank	11.1	15.6	13.6	
r=0.15 P=0.45[NS]				
Salivary IL-6 pg/ml				0.31[NS]
Range	(0.44 to35.77)	(0.48 to 34.39)	(0.64 to 2.07)	
Median	4.23	9.425	11.475	
Interquartile range	(1.76 to 5.78)	(3.79 to 21.23)	(6.61 to16.78)	
N	7	14	6	
Mean rank	10.1	14.9	16.3	
r=0.298 P=0.13[NS]				
Salivary IL-10 pg/ml				0.58[NS]
Range	(0.35 to 3.62)	(0.38 to 13.11)	(0.42 to 50)	
Median	1.09	0.76	2.105	
Interquartile range	(0.41 to 1.48)	(0.44 to 1.04)	(0.54 to44.57)	
N	7	14	6	
Mean rank	13.6	12.9	16.9	
r=0.26 P=0.19[NS]				

Table 5: The difference in medians of selected microbiologic and immunologic measurements with Salivary IL-10 ordered categories in the samples with denture stomatitis

	Salivary IL-10 pg/ml			
	First (lowest) quartile (<= 0.44)	**Average (0.45 - 3.40)**	**Fourth (highest) quartile (3.41+)**	
Mucosal coloni count				0.4[NS]
Range	(0 to 27)	(0 to 40)	(0 to 140)	
Median	6	5	12	
Interquartile range	(1 to 13)	(0 to 7)	(1 to 37)	
N	7	14	6	
Mean rank	14.5	12.3	17.4	
r=0.169 P=0.4[NS]				
Salivary IL-6 pg/ml				0.86[NS]
Range	(0.48 to 26.49)	(0.44 to 35.77)	(0.71 to 82.07)	
Median	11.91	7.73	10.285	
Interquartile range	(1.76 to 21.23)	(4.23 to 11.04)	(2.7 to 34.39)	
N	7	14	6	
Mean rank	14.6	13.2	15.2	
r=0.104 P=0.61[NS]				

Table 6: The difference in medians of selected microbiologic measurements with Salivary IL6 ordered categories in the samples with denture stomatitis.

	Salivary IL-6 pg/ml			
	First (lowest) quartile (<= 2.70	**Average (2.71 - 18.93)**	**Fourth (highest) quartile (18.94+)**	**P**
Mucosal coloni count				0.08[NS]
Range	(0 to 140)	(0 to 7)	(0 to 40)	
Median	13	3	12	
Interquartile range	(0 to 27)	(0 to 6)	(1 to 37)	
N	7	14	6	
Mean rank	17.7	10.8	17.3	
r=-0.029 P=0.89[NS]				

DISCUSSION

Candida associated denture stomatitis is a pathological reaction characterized by inflammation of the palatal mucosa in contact with the maxillary denture and is the most common form of oral Candidal infection with Candida albicans being the principal etiological agent [1-5, 9].

The age range of this study was wide, which varies between adult and elderly. Yassen study founded that there was no significant relation between age and Candida carriage, [10]. However, other study showed that the mean ages of the Candida-infected elderly denture stomatitis patients were significantly higher than those of the adult Candida infected denture stomatitis participants [9]. In gender distribution, the female was more than male, the same result was shown in Bakhshi et al study [11].

According to Newton's criteria, type II denture stomatitis was found a greater in frequency, this was in agreement with the literature, which reports that denture stomatitis type II is the most common clinical type found among patients [12].
Angular cheilitis may be a complication of Candida infection induced by denture stomatitis [3,13] it has been noticed in 21.6% of this study samples.

In discussing the relationship among clinical criteria and microbiological measurements, median of mucosal coloni was increased when grade of the clinical criteria type increased from pin point to erythema grade 2 and papillary hyperplasia grade 3. This is explained to be due to accumulation of yeast biofilm on the denture underlying mucosa [1-3, 13]. Barros study [6] was detected more Candida coloni count in grade 2 erythematus rather than grade 3 papillary hyperplasia lesions and in saliva of control healthy subjects. Histopathologically, in papillary hyperplasia clinical form, Candida albicans doesn't invade the epithelium. The presence of factors like mechanical irritation and allergic reaction to the denture base may be involved in the initiation of the lesion [5,13].

Clinical criteria did not follow ascertain way with the numbers of colonies that swabbed from palatal mucosa. Statistically, there was no significant differences between clinical grade and number of coloni in this study. This agree with Al_Tarawneh et al [2] and with other research done on 128 denture stomatitis patients, while found there were no correlation between the presence of denture stomatitis in the elder and the quantity of Candida infection [9]. Studies suggested that none invasive Candida may grow in the culture medium (even a positive culture, with a high number of microorganisms) is not necessarily indicate the presence of the pathogenic form [12].

Cytokines are regulatory proteins produced by immune cells and other cells of the body. Cytokines may exert proinflammatory and anti-inflammatory effects. The abnormalities of various cytokines may reflect the imbalance among different immune cell subsets contributing to pathogenesis of disease [13, 14].

The cytokines investigated in this study were chosen based on the fact that they represent important member of proinflammatory (IL-6) and anti-inflammatory cytokines (IL-10). In addition, IgA is antibody molecule playing an important role in cell-mediated immunity and considered first line of defence against Candida [6-8,15].

The correlations between IL-6, IL-10 and IgA and the associated clinical conditions were not statistically significant. This agree with the results of IL-6 observation in non treated control group as represented in Simunovic-Soskic et al study [16].

Salivary levels of IL-6 are elevated in elderly denture stomatitis [4]. The concentrations of this cytokine in saliva are physiologically relevant, and forming shield over the oral mucosa against colonizing microbes [9, 14, 17]. In current study, statistically, there was no association found between the occurrence of Candida related denture stomatitis and the concentrations of IL-6, this agree with Barros et al and Pesee studies [6, 9]. However Barros et al in the same study founded a significant association of elevated level of salivary IL-1 and IL-8 with increased C. albicans salivary count.

Antiinflammatory cytokine IL-10, which inhibits the secretion of proinflammatory cytokines and impairs anti fungal effect or functions by phagocytes, increases the innate antifungal resistance [18,19]. IL-12 and IL-10 are negatively affects the innate antifungal response, but is required for the induction of optimal adaptive immune response to C. albicans [20]. In vitro, C albicans induces immunosuppression in which increased IL-10 production by macrophages [21]. In this study, there was no significant association between the Candida infection in denture stomatitis patients and the concentrations of salivary IL-10, this agree with a result of Pesee and Arpornsuwan study [9].

IgA is the main immunoglobulin appears in saliva. In patients with Candida induced denture stomatitis, there is elevated level of these antibodies against C albicans [8,15]. During low grade inflammation, induced by Candida infection, cytokines may provide a regulatory link among secretory immunoglobulin production [14]. Studies examining salivary IgA antibody levels in patients have reported contradictory findings showing either elevated or reduced anti Candida IgA in saliva of patients with oral candidiasis [15, 22]. In current study, there was no significant different between counts of Candida induced denture stomatitis and salivary IgA concentration. This disagree with study that concluded significantly higher IgA antibody in Candida infected HIV patients than that in subjects whom no Candida count were isolated [22].

Although Candidal carriage appears to be related to salivary Candida antibody levels, the presence of large numbers of infecting yeast might conversely adsorb out antibodies in the oral cavity, leading to artificially lowered antibody concentrations in whole saliva. The degradation of IgA by candidal proteases production or the secretion of specific IgA proteases by various oral bacteria may also affect antibody levels [22-24]. Other explanation of various IgA concentrations was the extent to which candidal antigens contribute to the inflammation is not known, this is explained as the inflammatory response in Candida induced denture stomatitis represents a complex immune response both a humoral and cellular immune reaction to the plaque deposits on the lining mucosa. A close-fitting denture may impair access of antibodies to the denture-epithelial interface [7].

In many investigations, there was lowering in salivary antibodies concentration, although Candidal carriage appears to be related to salivary Candida antibody levels, the presence of large numbers of infecting yeast might conversely adsorb out antibodies in the oral mucosa. Also, the production of candidal proteases able to degrade IgA or the secretion of specific IgA proteases by various oral bacteria may similarly affect antibody levels [22-24].

Although this clinical trial selected patients with similar study protocols for oral candidiasis, there were some limitations of the findings when reviewing the literatures. A lot of controversies were found on the relation between salivary cytokine concentration and oral candidiasis which could explain the great variability found in the concentration of inflammatory mediators from one patient to another in this study. This is because of the many factors that cause limitations as nutritional status [25], stress [26], age, rate of salivary flow, bacterial colonization, presence of periodontitis, hormonal, individual genetic influence and methodology [16, 19] which could explain the great variability in the concentration of inflammatory mediators from one patient to another noticed in this investigation.

In conclusions**,** the Candida level was not related to the clinical staging of denture stomatitis. Also there were no association found between the occurrence of Candida related denture stomatitis and the concentrations of IL-6, IL-10 and IgA.

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