Salivary assessment of Interleukin-6, C-reactive protein and albumin in ulcerative colitis patients in relation to oral findings

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ABSTRACT

Background: Ulcerative colitis disease is a chronic inflammatory condition that affects the gastrointestinal tract. In regulation of this inflammatory process, Interleukin-6, C-reactive proteins and albumin have a major role. Overproduction of IL-6 by immunocompetent cells contributes to activate the liver to produce CRP, transudation of plasma albumin and development of the inflammatory condition. Elevated levels of IL-6 in saliva could be expected, because the saliva-producing cells are part of the digestive system. The purpose of this study was to assess salivary IL-6, CRP and albumin in ulcerative colitis patients in relation to oral findings.

Materials and methods: Forty eight saliva specimens collected from three groups of subjects (sixteen newly diagnosed UC patients, sixteen UC patients on medication and sixteen healthy subjects). The specimens were centrifuged and stored at -20°C then three ELISA kits were used for estimating the three variables.

Results: There was a significant elevation of salivary IL-6, CRP and albumin level in both newly diagnosed and on medication groups in comparison to healthy persons. There was a significant elevation differences of salivary IL-6, CRP and albumin level between newly diagnosed and on medication groups. The prevalence of aphthus ulcer was highly significant in the newly diagnosed group in comparison to the other groups. Twenty five percent of patients on medication complain from candidiasis and only one patients with tempromandibular joints problem (hard clicking).

Conclusions: Salivary IL-6, CRP and albumin are elevated simultaneously in UC patients, in both newly diagnosed and on medication groups, but the mean of variables in second group was lower than in the newly diagnosed group. There are no correlation between salivary IL-6, CRP and albumin with oral findings.

Key word: Interleukin-6, CRP and albumin, Ulcerative colitis, Aphthus stomatitis. (J Bagh Coll Dentistry 2013; 25(1):105-109).

INTRODUCTION

The human body is a complementary unit physically and biochemically, its parts are interrelated one with another and with the body as a whole. The oral cavity and its contained structures are important parts which serve as an indicator of the general health status of the whole body [(1)]. The mouth and its contained structures comprise the first part of gastrointestinal tract, the GI diseases leaves their shadows on the oral cavity, they affect oral tissues and saliva. Saliva is a unique biological fluid, with an important role in the oral physiology. It is a major player in the process of oral and general health maintenance [(2)].

Patients with inflammatory bowel disease **(IBD)** are at increased risk of developing dental caries and oral infections. Mucosal changes of ulcerative colitis (**UC)** in the oral cavity include stomatitis, glossitis, cheilitis, aphtous ulcerations, and pyostomatitis vegetans. The latter represents a specific marker of ulcerative colitis [(3)]. Cytokines play a key role in the initiation, augmentation, and perpetuation of the disease, since they are directly responsible for the mucosal injury. The role of cytokines involved in UC pathogenesis is characterized by a Th2 atypical immune response, with high levels of IL-6, IL-10, and IL-13, beside the classical pro-inflammatory cytokines [(4)].

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MATERIAL AND METHODS

This cross-sectional study in which forty eight volunteers from (40-50) years old were recruited and divided into three groups; each contains sixteen subjects. All of them were selected from patients attending al-Sadder hospital in Najaf city and Baghdad teaching hospital.

Group1: Newly diagnosed Ulcerative Colitis patients not yet on medication
Group2: Ulcerative colitis patients on medication.
Group3: Healthy control subjects.

Inclusion criteria:

1. All patient were selected (newly diagnosed and on medication) from proctosigmoditis type of Ulcerative colitis.
2. All patients in group 2 on sulfasalazine anti-inflammatory drug.
3. The mean age of diagnosis was 45 years.
4. All subjects were selected from male gender.

Exclusion criteria:

Patients were selected with no sign and symptoms of any other systemic disease, smoking and gingivitis or periodontitis.

Method

Method of examination

Oral examination

All the patients have been examined by single examiner, under standardized conditions; the oral cavity has been examined by artificial light by using a mouth mirror. The procedure of examination of oral soft tissue was done in

sequence according to direction suggested by the **W.H.O. (1987):** the examination would begin with the lip, upper and lower sulcus, retro-molar area, upper and lower labial mucosa, buccal mucosa, then hard and soft palate, dorsal margin and inferior surface of the tongue, floor of the mouth were also examine. In case of oral mucosal lesion; the duration, size, clinical description, location of lesion, and finally the clinical diagnosis was stated.

Sample collection

Saliva sample

Unstimulated (resting) whole saliva was collected, under resting conditions between 8.0-11.0 a.m. Patients were asked to avoid any oral hygienic procedure and rinse their mouth with water and to generate saliva in their mouth and to spit into a wide test tube [5].

Estimation of Salivary Interlukin-6 (IL-6) Levels

For Interleukins-6 quantitative determination, the method of ELISA was used in duplicate procedure. The solid phase antibody was prepared by purified human IL-6 antibody which has been pre-coated onto a microplate, standards reagent and samples containing IL-6 were added into the wells. After washing away any unbound substances, a horse reddish peroxidase (HRP)-labeled-IL-6 antibody was added in the form of a complex of antibody-antigen-enzyme labeledantibody. After washing to remove any unbound antibody-enzyme reagent, tetramethylbenzidine (TMB) substrate was added. TMB substrate produced blue color when was catalyzed by the HRP enzyme. After reaching desired color intensity, the reaction was terminated by adding an acidic stop solution which changed the solution color from blue to yellow. The absorbance (OD value) was determined at wave length (450) by microplate reader and the concentrations of IL-6 were calculated according to a standard curve

RESULTS

Oral findings:

Chi-square was used to assess the significant difference between newly diagnosed and on medication Ulcerative colitis groups **(Table 1)**. About eighty percent of the newly diagnosed group complain from apthus stomatitis and only twenty five percent from on the medication group complain from candidiasis, and only one patient with tempromandibular joint problem (hard clicking) as in **figure (1, 2)**.

Table 1: Chi-square comparison between Ulcerative colitis groups.

Chi-square	With oral findings	Without oral findings	P-Value
Newly diagnosed	13 (81.2%)	3 (18.8%)	0.004 **
On medication	4 (25%)	12 (75%)	

** = highly significant difference

Variables difference

Descriptive statistics and analysis of variance for salivary markers among study groups

The mean, standard deviation, standard error, maximum, minimum and P- values for Interleukin-6, C-Reactive and albumin are shown in **Tables (2, 3, 4) and figures (3, 4, 5).** For all groups saliva sample were taken from subjects of age range from (40-50) years to estimate IL-6, CRP and Albumin levels, It was found that these markers increased in Ulcerative colitis patients with newly diagnosed in comparing with healthy control subjects. Ulcerative colitis patient groups taking anti inflammatory medication showed that their salivary markers levels were lower than newly diagnosed group but remain higher than healthy control subjects by comparing the means of variables.

One way ANOVA test was used to examine the differences among study groups. Analysis of variance between and within groups showed highly significant differences at *P- value = 0.000* in three markers tested in saliva (IL-6, CRP and Albumin).

Table 2: The Descriptive statistic and ANOVA analysis of salivary IL-6 among study groups.

Groups	*Mean Pg/ ml*	*SD*	*P-Value*
1	7.89	3.63	0.000 ***
2	0.03	0.79	
3	-1.97	1.80	
Total	1.98	4.8	

*** = Very highly significant difference.

Table 3: The Descriptive statistic and ANOVA analysis of salivary CRP among study groups.

G.	*Mean Pg/ ml*	*SD*	*P-Value*
1	1484.37	235.94	0.000 ***
2	893.78	160.51	
3	539.05	92.25	
T	972.40	428.91	

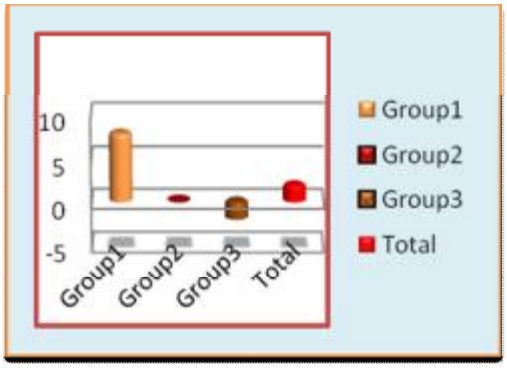

Figure 1: The mean of saliva IL-6 for all groups

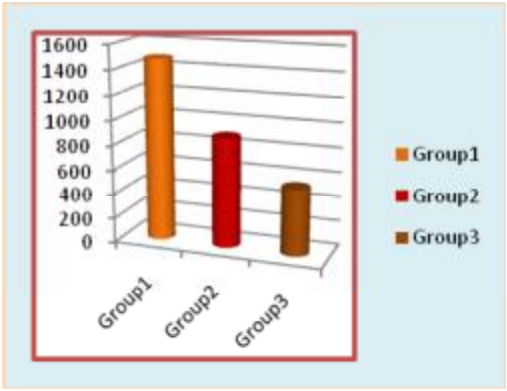

Figure 2: The mean of saliva CRP for all groups

Table 4: The Descriptive statistic and ANOVA analysis of salivary albumin among study groups.

Groups	*Mean Pg/ ml*	*SD*	*P Value*
1	11.82	1.49	0.000 ***
2	7.67	0.69	
3	6.15	0.85	
Total	8.55	2.63	

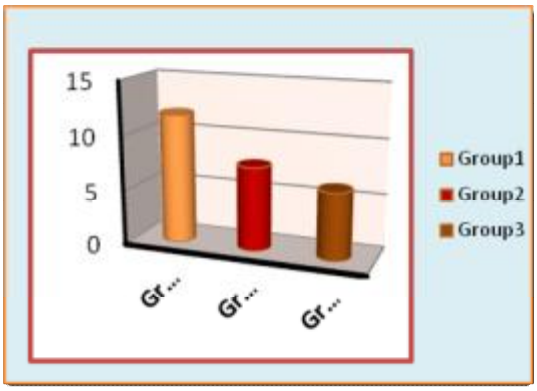

Figure 3: The mean of saliva albumin for all groups

Least significance difference among study groups (LSD) test:

The LSD test carried out between three groups separately that it was named group (I) and group (J), group (I) represented one of the study group that used to compare the differences with two other groups.

Multiple comparison (LSD) test for three dependent variables saliva IL-6, CRP and Albumin were showing significant difference at P-value = (0.021- 0.000) and mean differences (MD) at the 0.05 level **(Tables 5)**.

Table 5: Least significance difference (LSD) of saliva IL-6 among groups.

Group(I)	Group(J)	MD(I-J)	P-Value
1	2	7.85	***
	3	9.86	***
2	1	-7.85	***
	3	2.01	**
3	1	-9.86	***
	2	-2.01	**

The Pearson correlations between variables (r):

To examine the interrelationship between variables among three groups, simple scattered correlation were carried out between these measures. Pearson correlation coefficient revealed significant correlation between variables at 0.01 level as in **table (6)** and **Figures (4, 5, 6).**

Table 6: The Pearson correlations between variables in all group.

Variables		N	r	P-Value
IL-6	CRP	48	0.798	0.000 ***

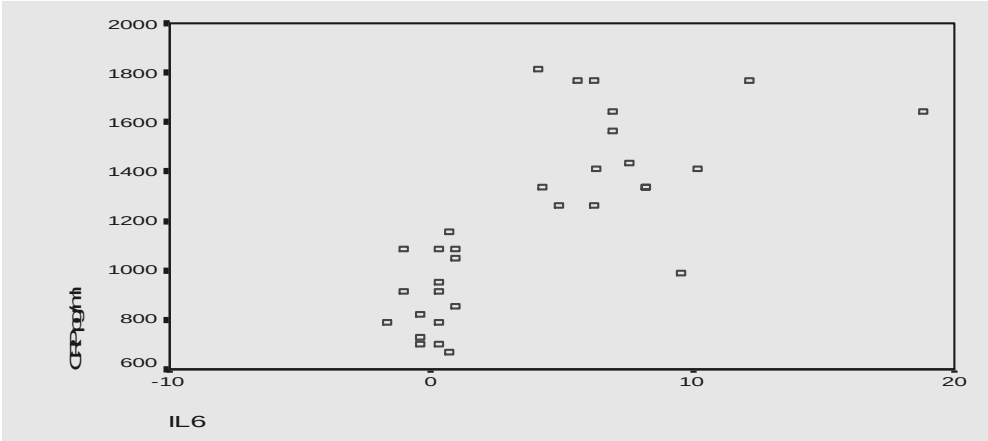

Figure 4: Correlation between saliva CRP and saliva interleukin-6 variables

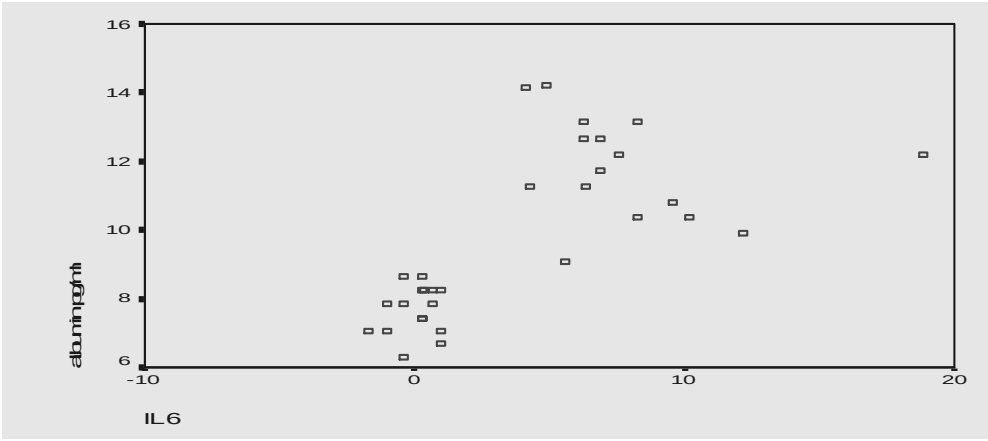

Figure 5: Correlation between saliva interleukin-6 and saliva albumin variables

DISCUSSION

Oral Findings

During examination of patients who gave saliva samples, found about eighty percent of the newly diagnosed Ulcerative colitis patients complain of recurrent aphthus stomatitis (RAS) and twenty five percent of the on medication group complain from candidiasis and tempromandibular joint clicking. This complication agrees with [3,6-9].

Temporo-mandibular joint problem (hard clicking). One of the patients on medication group (6.25%) complains from hard clicking. This may be muscular changes or bony changes [10].

Bony changes may be secondary to ulcerative colitis disease. This comes into agreement with Daley and Armstrong [9].

Finally, It was difficult to determine whether oral manifestations were expressions of Ulcerative colitis, represent preexisting, coincidental findings, or as a direct result from medical treatment.

Variable differences

Newly diagnosis group

The present study found the concentrations of IL-6 increased in saliva patients with UC. This may indicate that the inflammatory process in the bowel causes a high release of IL-6 in the saliva, because the saliva-producing cells are a part of the digestive tract [15].

The activity of IBD might be estimated from levels of IL-6 in plasma and albumin in CD patients and levels of IL-6 in saliva as well as plasma and albumin in UC patients [14].

Salivary CRP increased in patients with Ulcerative colitis. The elevation of salivary CRP was not surprising in inflammatory bowel disease, this come in agreement with [11].

The CRP was a very sensitive index of ongoing inflammation, rapidity of response and specificity for inflammation in comparison to erythrocyte sedimentation rate (ESR) [12, 13].

The understanding of the cytokines networks leads to important developments in both diagnostic and therapeutic phase of UC [4].

Variables level in patients on medication group

The most common drugs used in the treatment of UC (sulfasalazine, oral and topical 5-aminosalicylic acid, systemic and topical corticosteroid and immunosuppressors)

Corticosteroids are potent anti-inflammatory agents for moderate to severe relapses of

Ulcerative colitis. They act through inhibition of several inflammatory pathways suppressing interleukin transcription, induction of IkB that stabilizes the NFkB complex, suppression of arachidonic acid metabolism, and stimulation of apoptosis of lymphocytes within the lamina propria of the gut and maintained barrier function and decreased vascular permeability [17].

Therefore Albumin, CRP and IL-6 level were decreased in saliva Ulcerative colitis patients on medication group than patients without medication but still higher than that in healthy control, these salivary level depending on dose taking and indicated that these variables high sensitive mediator to any inflammatory process [17].

Variables correlation

There were significant correlations between IL-6, CRP and Albumin level in saliva of Ulcerative colitis patients. This comes into agreement with [18,19].

The microalbuminuria level in urine patients with Ulcerative colitis may result from the increased renal microvascular permeability in response to increased circulating cytokines [18].

The Interleukin-6 is the chief stimulator of the production of most acute-phase proteins [19].

Variables in relation to oral findings

No statistical difference was observed between T cells secreting IL-5 or IL-6 in patients with RAS and controls [20].

So these studies revealed that there is no any relation between oral findings and salivary IL-6, CRP and albumin level

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