



Evaluation of CRP, IL-6 and Calprotectin in Saliva of Patients Suffering from Crohn's Disease (CD)

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Abstract

Inflammatory bowel disease (IBD) is a chronic inflammatory condition of the gastrointestinal tract characterized by alternating episodes of clinical relapse and remission (Wallace, 2014), with Crohn's disease (CD) and ulcerative colitis (UC) being the two primary subtypes (Souza, 2016). The present study was achieved in a Baghdad teaching hospital from November 2021 to June 2022 to prove if the diagnostic markers of Crohn's disease (CD) (IL-6, CRP, and calprotectin) rise in saliva as well as in sera and if there is a significant difference in the levels of those markers between the Crohn's disease (CD) group and the control group. The recent study measured the levels of C-reactive protein (CRP), interleukin-6 (IL6), and calprotectin in the saliva of CD patients using the ELISA method, as those proteins have previously been proven to be elevated in the sera of CD patients. The results of the present study noted significant mean differences in salivary levels of each of CRP, IL6, and calprotectin between the CD group and the control group ($P < 0.05$). The current study proved that there is a significant positive correlation between IL-6 levels and age in all study groups (CD and control) ($P < 0.05$) and a significant positive correlation was also found between IL-6 and CRP in CD group patients ($p < 0.05$), respectively.

Keywords: Salivary IL-6, CRP, Calprotectin, CD patients.

1. Introduction

Inflammatory bowel disease, which includes both Crohn's disease and ulcerative colitis, is a chronic, progressive, relapsing disease of the gastrointestinal tract that requires long-term treatment or maintenance therapy [1]. IBD is becoming more common all across the world. It has



a complex etiology that is currently unknown; however, it is thought to be multifaceted. IBD is defined by chronic immune-mediated intestinal inflammation as well as environmental factors such as nutrition, lifestyle, antibiotic usage, and the gut microbiome. Genetics has long been thought to play a role in the initiation of inflammation [2].

Crohn's disease (CD) is one of the types of IBD, which is a gastrointestinal inflammatory illness that is mediated by the immune system as the inflammation in CD reaches all the way through the gut wall, from the mucosa to the serosa. The illness has a recurring and remitting pattern. CD can advance from mild to moderate inflammatory states to severe piercing (fistulization) or structuring illness after multiple relapses [3]. The inflammation in CD may involve any segment of the digestive tract, from the mouth to the anus, and is associated with discontinuous transmural lesions of the gut wall [4].

The current study has brought attention to the significance of inflammatory cytokines by demonstrating their essential role in IBD pathogenesis by controlling the development, amplification, and persistence of IBD. Cytokines are actively involved in the breakdown of mucosal tissue in CD [5]. Examined the relationship between salivary cytokine levels and these cytokines' plasma level was conducted. Only three variables significantly correlated with plasma levels: IL-6, IFN- γ , and MIP-1[6] was found. Proving that the oral environment, the impact of local immunity, and diurnal rhythms all have an impact on salivary cytokine levels [7]. IL-6, and TNF- α are considered a master key mediators in the inflammation process. These cytokines cause systemic effects such as increased body temperature, neutrophil mobilization, and increased lymphocyte activation [8].

IL-6 may be a sign of CD, according to the research done so far on salivary cytokines. IL-6 is a soluble mediator that affects inflammation, the immunological response, and hematopoiesis in a pleiotropic manner. IL-6 functions were initially examined and given different biologically active names, including B-cell stimulatory factor 2. Which was derived from its ability to induce differentiation of activated B cells into antibody (Abs)-producing cells [9].

A calcium-binding protein is called calprotectin. It is a complex that is made up of subunits that are mostly expressed in neutrophils and inflammatory monocytes and macrophages, but it has also been found in epithelial cells, endothelial cells, fibroblasts, keratinocytes, and osteoclasts under certain conditions [10]. Calprotectin released by keratinocytes, phagocytes, monocytes, granulocytes, and vascular cells is recognized by toll-like receptors (TLRs) to induce an inflammatory response [11].

Calprotectin can be found in a wide range of fluids, including human plasma, urine, cerebrospinal fluid, and saliva. Immune regulation, coagulation, and inflammation are only a few of the basic activities it performs [12]. It is engaged in leukocyte recruitment and cytokine release in inflammatory areas [13]. It represents the pro-inflammatory activities mostly generated by activated granulocytes. Calprotectin has also been linked to protection against pathogens, as evidenced by the fact that epithelial cells that produce calprotectin are more resistant to bacterial infections than epithelial cells that do not express calprotectin [14].

Measuring fecal calprotectin protein is a common part of clinical practice. It is used as a proxy marker for endoscopic disease activity, which lets doctors know how a patient's intestinal inflammation is doing without having to do an endoscopy [15]. Calprotectin is detectable in high

concentrations in acute-phase inflammatory reactions and is linked to high levels of CRP [16]. Salivary calprotectin was thought to be a possible marker of active CD in a recent study because salivary calprotectin concentrations were higher in CD patients than in controls in unstimulated saliva, while another study found that calprotectin concentrations in stimulated saliva were three times higher in IBD patients than in healthy controls [16].

C-reactive protein (CRP), a classic acute phase reactant first identified by Tilled and Francis in 1930, belongs to the pentraxin family and is made up of five identical 23-kDa globular subunits [17]. CRP is involved in innate immunity and host defense in a variety of ways. It binds to certain ligands, such as phosphorylcholine, and activates the classical complement pathway by attaching to complement protein 1q [18]. The pentameric structure of CRP depends on calcium, but it can irreversibly break into monomers in denaturing circumstances, such as an acidic local environment. Despite the fact that monomeric or modified CRP may be discovered in tissues, its biological relevance is not understood and has to be further investigated [19]. Despite inflammatory activity, circulating levels of CRP are often modest in systemic lupus erythematosus. Although extra-hepatic CRP synthesis has been described, human CRP is primarily produced by primary hepatocytes after stimulation with the principal inducer interleukin (IL) 6, but is inhibited by interferon. The use of human salivary CRP as a diagnostic body fluid is growing in popularity, as is the interest in mucosal immunity in general [20]. Saliva as a diagnostic tool has the advantages of being non-invasive, generally straightforward and quick, safe, stress- and pain-free, and inexpensive because a sample may be taken without the assistance of professionals. CRP is frequently used to monitor the progression of chronic inflammatory illnesses, such as inflammatory bowel disease [21].

In the differential diagnosis of IBD in young patients, it is important to consider infective colitis, acute appendicitis, tuberculosis, and intestinal lymphoma. Ulcerative colitis and Crohn's disease cause similar symptoms. No single test can diagnose either condition.

2. Material and Method

This case-control study was conducted during the period from November 2021 to June 2022 at Baghdad Teaching Hospital. Samples collected were saliva taken from twenty-five subjects with Crohn's disease (CD) and compared with twenty-five healthy individuals as controls; their ages ranged between 22 and 55 years. Four ml of unstimulated saliva was taken from each individual using the procedure described in [30]. and placed in clean, covered, and marked containers in a cool box until they arrived at the laboratory. The samples were then centrifuged for 10 minutes at 3500 rpm/min, and the supernatant was withdrawn and distributed into three Eppendorf tubes and maintained at -70°C until the analysis of salivary biomarkers that included IL-6, H-CRP, and calprotectin. Salivary levels of IL-6, Calprotectin and H-CRP were determined using commercial ELISA kits for human IL-6, Calprotectin and H-CRP (USA), and the instructions of the manufacturer were followed. Absorbance was measured at a wavelength of 450 nm using a micro-plate reader (Huma Reader HS, Germany).

3. Result

The study sample included 25 adult patients with Crohn's disease (CD) and 25 healthy individuals as controls. All of them were outpatients at the Baghdad Teaching Hospital. All of whom underwent the collection of saliva in a sterilized container.

The mean levels of salivary interleukin-6, Calprotectin, and CRP showed highly significant differences in Crohn's disease groups when compared with healthy controls ($p < 0.05$) as seen in **Tables 1, 2**.

Table 1. The mean levels of Il6, CRP and calprotectin in study groups

Parameters	Groups	N	Mean \pm S.D.	Min.	Max.
Calprotectin	Control	25	169.800 \pm 18.867	132.784	198.777
	CD	25	273.096 \pm 9.235	143.101	458.366
CRP	Control	25	2.951 \pm 0.803	1.316	4.659
	CD	25	7.201 \pm 0.331	1.937	15.335
IL-6	Control	25	53.509 \pm 8.996	33.291	65.609
	CD	25	91.494 \pm 2.235	51.725	149.615

Table 2. A comparative F-test for Il6, CRP and calprotectin levels among the study groups.

Parameters		Sum of Squares	d.f.	Mean Square	F-test	p-value
Calprotectin	Between Groups	140327.097	2	70163.548	15.991	0.000
	Within Groups	315915.556	47	4387.716		
	Total	456242.653	49			
CRP	Between Groups	239.828	2	119.914	17.487	0.000
	Within Groups	493.735	47	6.857		
	Total	733.563	49			
IL-6	Between Groups	20867.773	2	10433.886	21.100	0.000
	Within Groups	35603.598	47	494.494		
	Total	56471.371	49			

Table 3 showed that the correlation coefficient of IL-6 with calprotectin in Crohn's disease patients was statistically non-significant ($r = 0.129$) (p -value > 0.05), but the correlation between IL-6 and CRP was positive and statistically significant as $r = 0.453$ ($p < 0.05$).

Table 3. Correlation of IL-6 with CRP, calprotectin and age in Crohn's disease patients.

		CD		
Parameters		Calprotectin	CRP	IL-6
Calprotectin	r		-0.225	0.129
	P		0.280	0.537
CRP	r			0.453
	P			0.023

The results of **Table 4** showed that the correlation coefficient between IL-6 and each of CRP and calprotectin is positive and has values of ($r = 0.111, 0.289$), respectively, but with no significant differences in the healthy control group ($p < 0.05$).

Table 4. Correlation of IL-6 with CRP and calprotectin in control group.

		Control		
Parameters		Calprotectin	CRP	IL-6
Calprotectin	R		0.103	0.289
	P		0.623	0.161
CRP	R			0.111
	P			0.597

The mean and standard deviations for the age of patients with CD and healthy people have comparable mean \pm SD values (32.36 ± 0.8440 , 30.280 ± 0.6321), respectively, with no significant difference ($P > 0.05$) as presented in **Table 5**.

Table 5. Distribution of study individuals according to Age.

Groups	N	Age		
		Mean \pm S.D.	Min.	Max.
Control	25	30.280 ± 0.6321	23	48
CD	25	32.360 ± 0.8440	20	50

(P-value CD = 0.46) (P-value control = 0.46)

4. Discussion

IL-6 is primarily produced by T- cells and macrophages [22]. The present study selected saliva as a non-invasive and straightforward approach for IBD patients, as higher levels of IL-6 could be seen in saliva [23].

The present study found that the concentrations of IL-6 increased in the saliva of patients with CD as compared with the control group. 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 part of the digestive tract [24]. Other studies showed that the activity of IBD might be estimated from the levels in saliva as well as plasma in UC patients [25]. Furthermore, a significant positive correlation was found between the concentration of salivary IL-6 content and CRP in CD [26].

CRP levels are partially genetically regulated and have a baseline concentration of 1 mg/l. CRP levels skyrocket in the presence of acute-phase inflammation or infection. It is well known that when the inflammatory process is treated, CRP concentrations decrease rapidly [27].

The current study was found a statistically significant difference in salivary CRP levels of CD patients groups and the control groups.

As CRP was a very sensitive index of ongoing inflammation, rapidity of response, and specificity for inflammation in comparison to erythrocyte sedimentation rate (ESR) [28]. Understanding the cytokines networks leads to important developments in both the diagnostic and therapeutic phases of CD.

Correlations between CRP levels and inflammatory bowel disease activity have been studied in Crohn's disease patients, with conflicting results seen by [31]. This agrees with the findings of other authors who observed a positive relationship that is statistically significant between CRP levels and the involvement of CD [27].

Even though only a small number of previous researchers have discussed the potential use of salivary CRP as a sign of systemic inflammation [29], In addition, CRP levels in those who have UC and CD are linked with clinical and salivary activities. The inability of CRP to accurately predict activity revealed that CRP is insufficient on its own to evaluate disease activity. For these patients, combining CRP with salivary indicators might be a superior, non-invasive option to assess disease activity. The evaluation of calprotectin, a clinically validated fecal marker of IBD, in the saliva of IBD patients served as the starting point for the investigations of oral immunological manifestations by inflammatory mediators in IBD in this work. Although calprotectin is known to be expressed in saliva and increases during periodontal inflammation, its

salivary expression had never been linked to inflammatory bowel disease (IBD) before the findings of a previous study done by [32].

Who compared calprotectin levels in unstimulated saliva from IBD patients with continuous intestinal inflammation to controls after rigorous validation of the analytical techniques and protocol. Similarly, this study showed that calprotectin levels were substantially higher in the saliva of CD patients.

Saliva contains extracellular matrix components that can interfere with immunoassay tests [33]. Such interference was demonstrated in the detection of Majster et al. [16]. Who found significantly elevated levels of calprotectin in saliva from patients with Crohn's disease.

5. Conclusion

IL-6, CRP, and calprotectin are significantly elevated in the saliva samples of IBD patients, including the CD group, when compared with the control group. IL-6 levels increased as age increased in IBD subtypes (CD) and also in the control group. A significant positive correlation was found between salivary levels of both IL-6 and CRP in the CD group.

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