Journal of the Faculty of Medicine Baghdad
Print ISSN: 0041-9419, Online-ISSN: 2410-8057
DOI: https://doi.org/10.32007/jfacmedbagdad.6612031

Open Access

Research Article

Estimation of Salivary IL-6 and Calprotectin in Patients with Ulcerative Colitis

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Received: Feb. 2023
Revised: Jan. 2023
Accepted: Aug. 2023
Published: Dec. 2024

Abstract

Background: Inflammatory bowel disease is a chronic inflammatory condition affecting the gastrointestinal tract, encompassing two primary conditions: Crohn's disease and ulcerative colitis. Calprotectin, a protein released by keratinocytes, phagocytes, monocytes, granulocytes, and vascular cells, plays a key role in the body's inflammatory response. It is recognized by toll-like receptors, which trigger pathways that lead to inflammation. The chronic nature of inflammatory bowel disease presents a significant health challenge, requiring precise methods for regular assessment and monitoring of disease activity. Elevated calprotectin levels are widely recognized as a biomarker for detecting inflammation in the gastrointestinal tract, making it an essential tool in managing inflammatory bowel disease, particularly ulcerative colitis.

Objectives: To examine whether significant differences exist in the levels of interleukin-6 and calprotectin between patients with ulcerative colitis and healthy control subjects, this study analyzes and compares these inflammatory markers across both groups. suggesting that both markers could serve as potential diagnostic tools for ulcerative colitis. Furthermore, these findings highlight saliva as a non-invasive source for evaluating inflammatory markers in patients with ulcerative colitis.

Methods: The subjects included were twenty-five patients with ulcerative colitis and twenty-five healthy individuals as the control group. All of whom ranged in age from 20-55 years, and the levels of interleukin-6 (IL-6) and calprotectin in the saliva of ulcerative colitis patients were measured using the ELISA method.

Results: When compared to the control group, the current findings indicated that both (IL-6) and calprotectin levels were significantly higher in UC patients. Moreover, this study found a significant positive correlation between IL-6 levels and age in all study groups (UC and control) and between IL-6 and calprotectin in UC patients.

Conclusion: There are higher levels of IL-6 and calprotectin in the saliva of patients with UC disease, both markers could be used as diagnostic markers for UC disease

Keywords: Calprotectin; Inflammatory Bowel Diseases; Interleukin-6; Saliva and Ulcerative Colitis

Introduction:

A chronic inflammatory ailment called inflammatory bowel disease (IBD) has been linked to cytokines in terms of its pathophysiology and etiology. Ulcerative colitis (UC) and Crohn's disease (CD) are the two most prevalent clinical forms of IBD (1). In people with a genetic predisposition to IBD, leads to inflammation and intestinal ulcers (2). There are numerous Therapeutic options available for the idiopathic chronic inflammatory disease of the colon known as ulcerative colitis (3).

One type of inflammatory bowel illness that affects the colon and the rectum is ulcerative colitis. Rarely does it affect infants and young children (4). It is still unclear what causes ulcerative colitis and how it develops. The notion that a genetic element is key in the progression of the disease, however, has received attention (5). The incidence has increased in nations that have adopted an industrialized lifestyle, which refers to regions where steps have been done to enhance the state of health globally, such as. vaccination, gastrointestinal disease prevention, processed foods, etc. Exacerbations can be life-threatening and come with problems. Severe UC is diagnosed based on clinical, biochemical, and endoscopic findings Serious UC patients need to be hospitalized (6). Interleukin-6 (IL-6) is produced in acute inflammatory responses that aid in host defense. It is involved in the processes of immune response regulation, inflammation, hematopoiesis, and cancer (7). Immune responses may be disrupted if IL-6 levels are elevated as IL-6 is involved in the regulation of lymphocyte tracking through the lymph node following developmental stimulation (7). It was stated that IL-6 promotes the change from severe to chronic inflammation by secreting the monocytes chemo-attractant protein-1 (MCP-1) (8). IL-6 and TNF are regarded as the two main mediators of the inflammatory process. These cytokines have systemic effects that include raised body temperature, enhanced lymphocyte activation, and neutrophil mobilization (9).

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It was concluded that patients with IBD have significantly elevated levels of IL-6 in their plasma (10). Calprotectin is generated by phagocytes, keratinocytes, granulocytes, monocytes, and vascular cells and causes an inflammatory (11). Calprotectin, also known as the migration inhibitory factor-related proteins 8 and 14, is an acute-phase protein that regulates neutrophil migration; its quantity corresponds with neutrophil migration and indicates the intensity of inflammation in IBD. Calprotectin levels in saliva could be employed as a predictive diagnostic as well as a measure of treatment efficacy. However, doctors must keep in mind that oral inflammation, obesity, oral candidiasis, and periodontal disease all have an impact on calprotectin secretion (12,13).

This study aimed to measure IL-6) and calprotectin levels in the saliva of UC patients as those cytokines have been previously proven to be elevated in sera of UC patients but have not been proved yet to be increased in saliva.

Materials and Methods:

Four milliliters of unstimulated saliva were taken from twenty-five (UC) patients and twenty-five healthy controls. For the purpose of performing the salivary analysis of IL-6 and calprotectin, the saliva samples were centrifuged for 10 minutes at 3500 rpm/min, and the supernatant was divided into two Eppendorf tubes and kept at -70°C. Commercial ELISA kits for human IL-6 and calprotectin (USA) were used to measure the salivary levels of each marker according to the manufacturer's instructions using a micro-plate reader and the absorbance was measured at a wavelength of 450 nm (Huma Reader HS, Germany).

Statistical analysis:

The statistical analysis was done in two categories: Descriptive analysis, in which data was presented as minimum, maximum, mean, and standard deviation (SD) for quantitative variables, and frequency for qualitative variables. And inferential analysis: Inferential analysis was used to clarify valuable insights about the differences and relationships between different variables in the study community. We used parametric statistical analysis. This type of analysis is employed when dealing with continuous data and assumes that the data follows a normal distribution. The statistical tests used in the tables are the F-test, correlation coefficients, the chi-square test, or Fisher's exact test, as indicated by the p-values and independent t-tests.

Results:

The mean and standard deviations for the age of patients with UC and healthy people have the following comparable values (33.400 ± 1.0905, 30.280 ± 0.6321 ng\ ml), respectively, with no significant differences seen between both groups (*P*> 0.05) as presented in table (1).

Table (1): Distribution of study UC according to age

		Age (years)			
Group	NO	Mean	S.D.	Min.	Max.
Control	25	30.280	0.6321	23	48
UC	25	33.400 Y	1.0905 Y	20 Y	55 Y

(*p*-value UC = 0.46) (P-value control= 0.46)

The results in table (2) shown that UC patients have percentages of 48% and 52% for males and females, respectively, and the control group matches the patients' group as it has percentages of 60% for males and 40% for females with no significant difference has been observed between them (*P*>0.05).

Table (2): Distribution of subjects according to gender

Gender	Control	UC
Males	15 (60%)	12 (48%)
Females	10 (40%)	13 (52%)
Total	25 (100%)	25 (100%)

(*p*-value UC = 0.46) (*p*-value control = 0.46).

The mean levels of salivary interleukin-6 and calprotectin showed a higher concentration with a significant difference in the ulcerative colitis group when compared with the healthy controls ($p < 0.05$) as seen in Tables 3 &4.

Table (3): The mean levels of Il6 and calprotectin in study groups

Parameter	Group	No	Mean	S. D.	S.E.	Min.	Max.	*p* values
Calprotectin ng /ml	Control	25	169.800	18.867	3.773	132.784	198.777	0.000
	UC	25	241.871	6.830	13.660	135.592	378.897	
IL-6 pg\ml	Control	25	53.509	8.996	1.799	33.291	65.609	0.000
	UC	25	85.537	3.004	6.009	43.864	166.048	

Table (4): A comparative F-test for IL-6 and calprotectin levels among the study groups

Parameter		Sum of Squares	df	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			
IL-6	Between Groups	20867.773	2	10433.886	21.100	0.000
	Within Groups	35603.598	47	494.494		
	Total	56471.371	49			

Furthermore, the correlation between IL-6 and each of calprotectin and age in ulcerative colitis patients was positive and statistically significant as correlation coefficient values were (r=0.614 and r=0.405), respectively, (P <0.01) as shown in (Table 5).

Table (5): Correlation coefficient of IL-6 with calprotectin and age in ulcerative colitis patients

UC			
Parameter		Calprotectin	IL-6
Age	r	-0.282	0.405
	P	0.172	0.039
Calprotectin	r		0.614
	P		0.001

The correlation coefficient between IL-6 and calprotectin in healthy control group was positive non-significant correlation (P>0.05) but the correlation between IL-6 and age was a significantly positive (r = 0.421) (p<0.05) as shown in table (6).

Table (6): Correlation coefficient of IL-6 with calprotectin and age in control group

Control			
Parameter		Calprotectin	IL-6
Age	r	0.017	0.421
	P	0.936	0.036
Calprotectin	r		0.289
	P		0.161

Discussion:

Furthermore, this work showed no significant differences in exposure rate to UC between both genders (p>0.05) whereas an epidemiological survey from East Asian countries notably Japan and China showed lower incidence in females than males (28). According to data from twelve Asian–Pacific countries, it was demonstrated a male predominance of UC from adolescence till age of 65 years, after which UC incidence rates were similar between females and males (30).

The ages of UC patients in present study ranged from 20-55 years with a mean value of 33.4±1.09 years. Similarly, the results of Nijakowski et al(2021)that was carried out in 2021 showed that the UC group has an age range between 24 -40.5 years with a mean age value of 32 years. Also, comparable results were seen by a cross-sectional study conducted at the Kurdistan center for gastroenterology and hepatology of the teaching hospital in Sulaymaniyah, Iraq, which included 101 patients who had previously been diagnosed with inflammatory bowel disease that showed that UC patients have a mean age value of 45.74years (16, 17). In general, females and males showed similar incidence of UC before age 45; however, above age 45 years, males demonstrated higher risk of UC incidence than females (29).

This study selected saliva collection as a straightforward and non-invasive approach for UC patients. It was observed that there was a statistically significant difference in salivary IL-6 levels between UC patients and the control group whereas other studies have also revealed that unstimulated saliva of IBD patients has higher levels of IL-6 (32). Other studies have previously demonstrated that IL-6 levels are increased in patients with inflamed, non-adhesive intestinal mucosa of IBD (33).

The patients with UC had higher IL-6 concentrations in their saliva. Because the cells that produce saliva are components of the digestive system, this may suggest that the inflammatory process in the intestine induces a significant release of IL-6 in the saliva (22). Another study illustrated that the activity of IBD might be estimated from the levels in saliva as well as plasma in UC patients (23).

After thorough validation of our analytical methods and protocol, the current study compared calprotectin levels in unstimulated saliva from UC patients with ongoing intestinal inflammation to controls. The calprotectin levels were substantially higher in UC patients' saliva than the control group. The findings of this investigation have been supported by another previous study which also observed significantly elevated levels of calprotectin in saliva of patients with ulcerative colitis as compared with control group (24).

Calprotectin is mostly present in neutrophils and, to a lesser extent, in other cells, calcium-binding protein in reactive macrophages and monocytes (25). Plasma calprotectin has been reported to increase 5- to 40-fold in inflammatory and infectious circumstances, and it has bacteriostatic and fungi-static characteristics. Stool contains calprotectin, and fecal calprotectin concentration is approximately six times that of normal plasma. Patients with intestinal irritation had feces with noticeably higher amounts of calprotectin (26).

This is the first study that evaluated calprotectin level in saliva. It was noted that calprotectin had a significant higher levels in UC group when compared with control group whereas previous researchers looked at calprotectin in feces and proved that fecal calprotectin levels in gastrointestinal disorders, such as gastritis, gastric ulcer, gastric carcinoma, duodenitis, ulcerative colitis, have significantly higher levels than the controls (27). Compared to the control group, the concentrations of calprotectin and myeloperoxidase in saliva were significantly lower both in CD patients and in UC patients (31).

Conclusion:

This study concluded that levels of interleukin-6 and calprotectin in saliva are higher in patients with ulcerative colitis compared to healthy individuals (control group), suggesting that both markers could serve as potential diagnostic tools for ulcerative colitis. Furthermore, these findings highlight saliva as a non-invasive source for evaluating inflammatory markers in patients with ulcerative colitis. Further research is recommended to explore the relationship between these marker levels and disease progression, which may enhance patient care and guide therapeutic strategies.

Authors' Declaration:
We here by confirm that all the Figures and Tables in the manuscript are ours. The project was approved by the local ethical committee in College of Dentistry/ University of Baghdad, Iraq.
Conflicts of Interest: None
Funding source: None

Authors' Contributions:
Study conception & design: (Maha A. Mahmood). Literature search, Data acquisition, Data analysis, interpretation & Manuscript preparation:(Fadhel A. Abed). Manuscript editing & review:(Maha A. Mahmood).

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How to Cite this Article
Abdullah Abdullah FA, A. Mahmood M. Estimation of Salivary IL-6 and Calprotectin in Patients with Ulcerative Colitis. J Fac Med Baghdad. 2024;66(4):419-24. Available from: https://iqjmc.uobaghdad.edu.iq/index.php/19JFacMedBaghdad36/article/view/2031

تقدير الإنترلوكين اللعابي 6 وكالبروتكتين في مرضى التهاب القولون التقرحي

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الخلاصة
مرض التهاب الأمعاء هو التهاب مزمن في الجهاز الهضمي ويشمل مرض كرون والتهاب القولون التقرحي ، وقد يتسبب الالتهاب لفترات طويلة في غزو الطبقات المتعددة من جدران الأمعاء مما يؤدي إلى تلف الجهاز الهضمي.
الهدف : أجريت الدراسة الحالية من أجل توضيح ما إذا كانت العلامة التشخيصية لـ IBDوهيIL6 وcalprotectin ترتفع في اللعاب كما هو الحال في الأمصال وما إذا كانت هناك فروق ذات دلالة إحصائية في المستويات من تلك العلامات بين مرضى التهاب القولون التقرحي وموضوعات المراقبة الصحية.
طريقة البحث :أجريت الدراسة الحالية في مستشفى بغداد التعليمي ومستشفى أمراض الجهاز الهضمي من تشرين الثاني (نوفمبر) 2021 إلى أيار (مايو) 2022. شملت الدراسة خمسة وعشرين مريضا يعانون من التهاب القولون التقرحي وخمسة وعشرون من الأفراد الأصحاء يمثلون المجموعة الضابطة. تراوحت أعمار كل منهم بين 20-55 سنة.
تم قياس مستويات Interleukin-6 وcalprotectinفي لعاب مرضى التهاب القولون التقرحيUCباستخدام طريقة ELISA في الدراسة الإحصاء تم إجراء التحليل الإحصائي على فئتين: التحليل الوصفي: تم تقديم البيانات على أنها الحد الأدنى والحد الأقصى والمتوسط والانحراف المعياري (SD) للمتغيرات الكمية وتكرار المتغيرات النوعية. والتحليل الاستنتاجي: تم استخدام التحليل الاستدلالي لتوضيح رؤى قيمة حول الاختلافات والعلاقات بين المتغيرات المختلفة في مجتمع الدراسة. استخدمنا التحليل الإحصائي البارامترية. يتم استخدام هذا النوع من التحليل عند التعامل مع البيانات المستمرة ويفترض أن البيانات تتبع التوزيع الطبيعي. الاختبارات الإحصائية المستخدمة في الجداول هي اختبار F ، ومعاملات الارتباط ، واختبار مربع كاي ، أو اختبار فيشر الدقيق ، كما يتضح من قيم p واختبارات t المستقلة.
النتائج :عند المقارنة بمجموعة التحكم ، أشارت النتائج الحالية إلى أن كلا من (IL-6) ومستويات calprotectin كانت أعلى بشكل ملحوظ في مرضى التهاب القولون التقرحي ($P = <0.05$) .علاوة على ذلك ، وجدت هذه الدراسة ارتباطا إيجابيا معنويا بين مستويات IL-6 والعمر في جميع مجموعات الدراسة (التهاب القولون التقرحي والتحكم ($P <0.05$) وبين IL-6 وcalprotectinفي مرضى التهاب القولون التقرحي ($P <0.05$)
الاستنتاج :خلصت هذه الدراسة إلى أن هناك مستويات أعلى من IL-6 و calprotectin في لعاب المرضى الذين يعانون من مرض UC مقارنة بالأشخاص الأصحاء (مجموعة التحكم) ونتيجة لذلك ، يمكن استخدام كلا الواسمتين كواسمات تشخيصية لمرض UC.
الكلمات المفتاحية: انترلوكين -6, كالوبروتكتين , مرضى التهاب القالون التقرحي.