

Salivary IgA in Patients with Ulcerative Colitis on Different Treatment Modalities

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Abstract

Background Immunoglobulin A (IgA) is the most common immunoglobulin Isotype found on mucosal surfaces, where it serves as the first line of defense against microbial invasion. **Objectives** The purpose of this study was to compare the levels of salivary IgA in patients with ulcerative colitis (UC) on two different therapeutic modalities to control subjects. **Materials and Methods** Fifty-three patients (27 male and 26 female) were diagnosed with UC, in the clinics of Gastroenterology and Hepatology Teaching Hospital in Baghdad city through random sampling, twenty five subjects were also recruited as controls with equal age and sex-matched. All study participants were subjected to intraoral examination to evaluate the oral manifestation. Unstimulated whole saliva was collected under standardized conditions by using the spitting method; the collection is made in the morning from (9-11 a.m.). The collected saliva was centrifuged at 3000 rpm for 10 minutes; the clear supernatant layer was aspirated was stored at -20°C in deep freeze until analysis. Determination of salivary IgA is done by the Enzyme Link Immunosorbent Assay. **Results** The present study revealed that the Mean of salivary IgA in patients on Azathioprine monotherapy and Combination therapy ulcerative colitis patients had a statistically non-significant difference as compared to control subjects. Also, the result revealed statistically non-significant differences between monotherapy and combination patient groups. **Conclusion** In the present study salivary IgA level increased in UC compared to apparently healthy controls and the result showed statistically non-significant differences. Also, the result revealed a statistically non-significant difference between monotherapy and combination therapy patient groups.

Keywords: Immunoglobulin A; ulcerative colitis.

Introduction

Although infection and immunological variables may be involved, the etiology and pathophysiology of ulcerative colitis are yet unknown (Kraft, 1979). Because inflammatory bowel disease affects patients' families more frequently than it does in the general population,

genetic predisposition is likely significant (Singer et al, 1971). Through interactions with the autonomic nervous system, sIgA levels in saliva change in response to physical and emotional stress (Tsujita and Morimoto, 1999; Bishop and Gleeson, 2009). Flow rates have an impact on sIgA concentrations in saliva; generally,

concentrations decline as flow rates rise. It is advisable to measure flow rates to express the sIgA secretion as a function of time (Bishop and Gleeson, 2009). The immune system uses immunoglobulin, a large Y-shaped protein, to recognize and destroy foreign substances including harmful bacteria and viruses. A specific pathogen molecule is known as an antigen is recognized by the antibody (Janeway et al, 2001). Human immunoglobulins are classified into five types: IgM, IgD, IgG, IgA, and IgE. These glycoproteins, which are composed of 82-96% protein and 4-18% carbohydrate, differ in heavy chain structure and function (Schur, 1988). Immunoglobulin A (IgA) is an antibody that is important for mucosal immunity and it is produced in greater quantities in mucosal linings than in any other types of antibody, with between three and five grams secreted into the intestinal lumen each day. This amounts to 75% of total immunoglobulin production in the body (Macpherson and Slack, 2007). Mucosal sIgA is produced through two distinct pathways, T cell-dependent and independent (Bemark et al, 2012). Immunoglobulin A is divided into two subclasses (IgA1 and IgA2) and can exist as a dimeric form known as secretory IgA. Immunoglobulin A is the main immunoglobulin found in mucous secretions such as tears, saliva, colostrum, and secretions from the genitourinary tract, gastrointestinal tract, prostate, and respiratory epithelium in its secretory form. It is also present in trace amounts in the blood. The secretory component of sIgA protects the immunoglobulin from proteolytic enzyme degradation, allowing sIgA to survive in the harsh gastrointestinal tract environment and providing protection against microbes that multiply in body secretions (Junqueira and Carneiro, 2003). Secretory IgA can also inhibit the inflammatory effects of other immunoglobulins, but it is a poor complement activator and only weakly opsonises (Holmgren and Czerkinsky, 2005). The primary functional role of IgA antibodies is to provide a first line of defense against a wide range of pathogens by preventing bacterial or toxin attachment to

epithelial cells, as well as foreign substance absorption. Secretory IgA antibodies are resistant to proteolytic enzyme cleavage and thus well suited for surface protection. Mucosal sIgA antibody responses are non-inflammatory and are induced by immunoregulatory and IgE-inhibitory cytokines such as TGF- and IL-10 (Macpherson et al, 2008). Secretory IgA antibodies inhibit antigen adherence and penetration, and high levels may theoretically prevent allergen absorption, whereas low levels of sIgA and transient IgA deficiency have been linked to an increased risk of allergy and bronchial hyperreactivity (LdviKsson et al, 2005).

Materials and Methods

This study compares the salivary level of IgA in patients with ulcerative colitis (UC) on two therapeutic modalities to control subjects.

Ethical approval

This research was approved by a Research Ethics Committee/ University of Baghdad/ College of Dentistry according to a decision report (Ref. Number: 440/ Date 3-1-2022).

The sample

Seventy-eight participants were enrolled in this study, of which fifty-three were endoscopically and histologically diagnosed with ulcerative colitis, where twenty-five subjects were healthy and were age and gender-matched control group. The inclusion criteria according to which ulcerative colitis patients were enrolled in this study were based on therapeutic options. In this study, 53 patients were selected and divided into 2 groups according to the treatment. The study groups are:

1. Group 1: Twenty-six ulcerative colitis patients on oral immunosuppressant therapy (Azathioprine 50mg twice daily).
2. Group 2: Twenty-seven ulcerative colitis patients on the combination therapy of intravenous infusion of anti-TNF- α (Infliximab 5mg/kg every 8 weeks) plus oral immunosuppressant

(Azathioprine 50mg twice daily).

3. Group 3: Twenty-five, healthy-looking, subjects without any signs and symptoms of any systemic diseases.

The exclusion criteria for all participants, including, any underlying systemic diseases (diabetes mellitus and hypertension), pregnant women and a history of radio or chemotherapy. All subjects were selected from the gastroenterology and hepatology teaching hospital in Baghdad City from 29 December 2021 to 7 April 2022. The laboratory works of salivary IgA were done in Alnadaer clinical lab.

Estimation of salivary IgA

Determination of salivary IgA is done by the Enzyme Link Immunosorbent Assay (Valdimarsdottir and Stone, 1997).

Principles of the assay

The microtiter plate provided in this kit has been pre-coated with an antibody specific to sIgA. Standards or samples were then added to the appropriate microtiter plate wells with a biotin-conjugated antibody specific to sIgA. Next, Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. After tetramethyl benzidine (TMB) substrate solution is added, only those wells that contain sIgA, biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in colour. The enzyme-substrate reaction is terminated by the addition of sulphuric acid solution and the color change is measured spectrophotometrically at a determined by comparing the optical density (O.D). Of the samples to the standard curve.

Statistical analysis

Analysis of data was carried out using the available statistical package of SPSS-28 (Statistical Packages for Social Sciences- version 28). Data were presented in simple measures of frequency, percentage, mean, standard deviation, and range (minimum-maximum values). The significance of the difference of

different means (quantitative data) was tested using Students-t-test for the difference between two independent means or Paired-t-test for the difference between paired observations (or two dependent means), or the ANOVA test for the difference between more than two independent means. The significance of the difference of different percentages (qualitative data) was tested using the Pearson Chi-square test (t-test) with the application of Yates's correction or Fisher Exact test whenever applicable. Statistical significance was considered whenever the P value was equal to or less than 0.05.

Results

The mean age \pm SD of UC patients on monotherapy was 35.3 ± 9.2 years, and the range of age was (20-49) years, on combination therapy was 30.7 ± 8.1 years, the range of age was (20-49) years and in healthy control subjects the mean age \pm SD was 27.2 ± 7.9 years, the range of age was (20-46) years (Table 1). The numbers of female patients were 26 (48.8), while the male was 27 (51.2%), a non-significant difference was found (Table 2). There was a non-significant difference in the mean age and male-to-female percentage between the two groups of UC patients and the controls (Table 1) and (Table 2), respectively. The present study revealed that the (mean \pm SD) of the salivary IgA in patients on Azathioprine monotherapy was (268.7 ± 144.0) μ g/ml, the range was (80.29-511.4) μ g/ml and in combination therapy ulcerative colitis patients was (235.7 ± 146.8) μ g/ml, the range was (68.04-499.8) μ g/ml, while in control group was (217.5 ± 125.0) μ g/ml, the range was (41.05-397.2) μ g/ml. The result revealed a statistically non-significant difference in ulcerative colitis patients as compared to control subjects as shown in Table (3) and Figure (1). Also, the result revealed statistically non-significant differences between monotherapy and combination patient groups.

Table (1): Mean age and standard deviation of the three study groups.

		Monotherapy "Azathioprine"		Combination "Azathioprine & (Infliximab)"		Healthy control		P-value
		No	%	No	%	No	%	
Age (years)	20-29	8	29.6	13	50.0	16	64.0	0.102
	30-39	10	37.0	8	30.8	7	28.0	NS
	40-49	9	33.3	5	19.2	2	8.0	
	Mean±SD (Range)	35.3±9.2 (20-49)		30.7±8.1 (20-49)		27.2±7.9 (20-46)		

Mean age ±SD of UC patients 33±8.9, range (20-49)
NS: non-significant P>0.05.

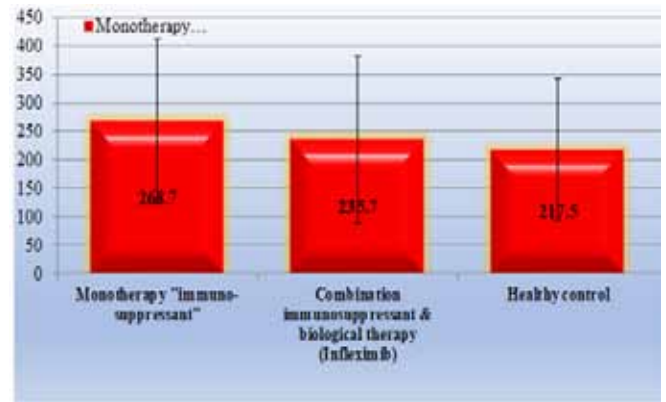


Figure (1): The mean of salivary IgA of three study groups.

Table (2): Gender distribution of the study groups.

		Monotherapy "Azathioprine"		Combination Azathioprine & (Infliximab)		Total		Healthy control		P-value
		No	%	No	%	No	%	No	%	
Gender	Male	10	37.0	17	65.4	27	51.2	16	64.0	0.065
	Female	17	63.0	9	34.6	26	48.8	9	36.0	NS

NS: non-significant P>0.05.

Table (3): Mean±SD of salivary IgA with ANOVA-test between study groups.

		Monotherapy "Azathioprine"		Combination Azathioprine & Infliximab		Healthy control		P-value
		No	%	No	%	No	%	
IgA (µg/ml)	(<70)	-	-	1	3.8	3	12.0	0.389
	(70-300)	18	39.3	18	81.3	14	56.0	
	(>300)	11	40.7	9	34.6	8	32.0	
	Mean±SD (Range)	268.7±144.0 (80.29-511.4)		235.7±146.8 (69.04-499.8)		217.5±125.0 (41.05-397.2)		0.408

NS: non-significant P>0.05.

Discussion

Only a few studies have investigated the saliva of UC patients, and only a small number of studies have investigated salivary IgA in UC, with inconclusive results, so IgA in the saliva and serum will be discussed. Salivary immunoglobulin can be used to detect general oral inflammation. Salivary IgA is thought to be the host's first line of defense against pathogens in saliva by binding to soluble and particulate antigens and inhibiting various enzymes and bacterial colonization on oral hard surfaces (Proctor and Carpenter, 2001). Salivary immunoglobulin (IgA) reflects the glands' functional capacity. Increased concentrations of this component are typically indicative of a poor overall condition (Teeuw et al, 2004; Pink et al, 2009; Hopcraft and Tan, 2010). According to the logistic regression analysis, the patient's age, the number of concomitant diseases, and the low salivary flow rate values are the variables that explain the highest tertiles of salivary protein concentrations (Janket et al, 2010). In the present study, although salivary IgA level was higher in UC compared to apparently healthy control, it doesn't reach a significant level. This result agrees with Morris et al, (1981); who studied salivary IgA in thirty-six patients with ulcerative colitis and thirty-six normal control, and found a non-significant difference between the patients with ulcerative colitis and the control

group. Secretory component deficiency results in normal serum levels of IgA but greatly diminished levels of secretory IgA in secretions such as saliva and jejunal fluid (Strober et al, 1976). Engstrom et al study in 1978, reported that salivary IgA concentration did not differ between UC and healthy subjects, Salivary IgA was normal in the affected family members and unrelated patients with UC. However, the free secretory component of salivary IgA was absent or markedly depressed in family members, as well as in unrelated patients with ulcerative colitis. This deficiency of the secretory immune system appears to characterize more frequently ulcerative colitis than Crohn's disease and may compromise mucosal host defenses in Inflammatory bowel disease (Engstrom et al, 1978) and this result agrees with this present study. The study of Week and Jarnum, (1971), also agrees with this result, the study dealt with serum in 36 patients with ulcerative colitis, and the results were compared with 78 healthy persons as matched controls and found that; the mean level of the serum IgA did not deviate significantly from the normal mean. Soltoft et al, (1973) also found normal salivary levels of IgA in UC patients. However, another study reported a significant negative correlation between disease activity and the concentration of IgA in whole saliva (Crama-Bohbouth et al, 1989). Therefore, further studies on the salivary IgA concentration and its possible role in oral health problems in UC seem warranted. The results of this study showed that the mean age of UC patients was 33.0 years with an age range of (20-49) years. These results disagree with the study of Al-Mudhaffer and Abdul-Ghafoor, (2013), which found salivary interleukin-6, c-reactive protein and albumin in ulcerative colitis patients in relation to oral findings. Another study by Al-Ghurabi et al, (2009), reported that serum carcinoembryonic antigen and IL-6 levels in serum patients with ulcerative colitis, which included 35 patients with UC, the mean age of (41.4± 15.7) years, ranged between (18- 83 years). Also, in the Iraqi study done by (Hussein, 2019) who study ulcerative colitis Patients, the

age of the studied patients was ranging between 18-58 years with a mean age group of 36 (± 9.9) years. The results of this study showed that the gender distribution of ulcerative colitis patients was 27 (51.2%) male and 26 (48.8%) female, this result agrees with the study of Al-Khazraji, (2008), which showed that in ulcerative colitis patients, in which the male-to-female ratio of this study was 1:1. The present study disagrees with Crama-Bohbouth et al, (1989) study which measured the concentrations of IgA in whole and parotid saliva with the ELISA technique in 20 patients with ulcerative colitis (UC) and 19 healthy control subjects, noticed that IgA predominated in whole and parotid saliva in both patients and controls; and in both groups, whole saliva had higher levels of IgA than did parotid saliva. IgA levels were significantly elevated in the whole saliva of UC patients as compared with the controls. No correlation was found between immunoglobulin levels and age, sex, duration of the disease, and extent of colitis. A significant negative correlation was found between the activity of the disease and the concentration of IgA in whole saliva.

Conclusions

The results of this study showed that salivary IgA level was higher in UC patients than in healthy control but statistically was non-significant.

References

- Al-Ghurabi, B.H., Al-Hassan, A.A. and Ghazi, H.F., 2009. Serum CEA and IL-6 levels in patients with ulcerative colitis. *Al-Mustansiriyah J. Sci*, 20, pp.1-7.
- Al-Khazraji, K.A., 2008. Prevalence of autoimmune hemolytic anemia in ulcerative colitis. *Journal of the Faculty of Medicine Baghdad*, 50(3), pp.273-281.
- Al-Mudhaffer, M.H. and Abdul-Ghafoor, S.H., 2013. Salivary assessment of Interleukin-6, C-reactive protein and albumin in ulcerative colitis patients in relation to oral findings. *Journal of Baghdad College of Dentistry*, 325(2204),

pp.1-5.

Bemark, M., Boysen, P. and Lycke, N.Y., (2012). Induction of gut IgA production through T cell-dependent and T cell-independent pathways. *Annals of the New York Academy of Sciences*, 1247(1), pp.97-116. <https://doi.org/10.1111/j.1749-6632.2011.06378.x>

Bishop, N.C. and Gleeson, M., 2009. Acute and chronic effects of exercise on markers of mucosal immunity. *Front Biosci*, 14(2), pp.4444-56.

Brandtzaeg, P., (2009). Mucosal immunity: induction, dissemination, and effector functions. *Scandinavian journal of immunology*, 70(6), pp.505-515. <https://doi:10.1111/j.1365-3083.2009.02319.x>

Crama-Bohbouth, G., Pena, A.S., Verspaget, H.W., Biemond, I., Weterman, I.T. and Lamers, C.B., 1989. Immunological findings in whole and parotid saliva of patients with ulcerative colitis and healthy controls. *Hepato-gastroenterology*, 36 (4), pp.185-187.

Engstrom-Heg, R., Colesante, R.T. and Silco, E., 1978. Rotenone tolerances of stream-bottom insects. *New York Fish and Game Journal*, 25(1).

Holmgren, J. and Czerkinsky, C., (2005). Mucosal immunity and vaccines. *Nature medicine*, 11(4), pp.S45-S53. <https://doi.org/10.1038/nm1213>

Hopcraft MS, Tan C. Xerostomia: an update for clinicians. *Aust Dent J*. 2010 Sep;55(3):238-44; quiz 353. <https://doi: 10.1111/j.1834-7819.2010.01229.x>

Janeway, C.A., Travers, P., Walport, M. and Capra, D.J., (2001). *Immunobiology* (p. 600). UK: Garland Science: Taylor & Francis Group. ISBN-10: 0-8153-3642-X

Janket S, Meurman JH, Baird AE, Qvarnström M, Nuutinen P, Ackerson LK, Hong J, Muthukrishnan P, Van Dyke TE. Salivary immunoglobulins

and prevalent coronary artery disease. *J Dent Res*. 2010 Apr;89(4):389-94. <https://doi:10.1177/0022034509359884>

Junqueira, L.C. and Carneiro, J., (2003) *Basic Histology Text and Atlas*, Lange. Wolter Kluwer, Lippincott Williams and Wilkins, pp.332-344.

Kazemi-Shirazi, L., Gasche, C.H., Natter, S., Gangl, A., Smolen, J., Spitzauer, S., Valent, P., Kraft, D. and Valenta, R., 2002. IgA autoreactivity: a feature common to inflammatory bowel and connective tissue diseases. *Clinical & Experimental Immunology*, 128(1), pp.102-109. <https://doi:10.1046/j.1365-2249.2002.01804.x>

Kraft, S.C., 1979. *Inflammatory bowel disease (ulcerative colitis and Crohn's disease)* (pp. 95-128). Edinburgh London New York: Churchill Livingstone.

Lúðvíksson BR, Arason GJ, Thorarensen O, Ardal B, Valdimarsson H. Allergic diseases and asthma about serum immunoglobulins and salivary immunoglobulin A in pre-school children: a follow-up community-based study. *Clinical & Experimental Allergy*. (2005) Jan;35(1):64-9. <https://doi:10.1111/j.1365-2222.2005.02141.x>

Macpherson AJ, Slack E (2007). «The functional interactions of commensal bacteria with intestinal secretory IgA». *Current Opinion in Gastroenterology*. 23 (6): 673–678. <https://doi:10.1097/MOG.0b013e3282f0d012>

Macpherson, A.J., McCoy, K.D., Johansen, F.E. and Brandtzaeg, P., (2008). The immune geography of IgA induction and function. *Mucosal immunology*, 1(1), pp.11-22. <https://doi:10.1038/mi.2007.6>

Morris, T.J., Matthews, N. and Rhodes, J., 1981. Serum and salivary immunoglobulin A and free secretory component in ulcerative colitis. *Clinical & Experimental Allergy*, 11(6), pp.561-564. <https://doi:10.1111/j.1365-2222.1981.tb02175.x>

Pink, R., Simek, J., Vondrakova, J., Faber, E., Michl, P., Pazdera, J. and Indrak, K., 2009. Saliva as a diagnostic medium. *Biomedical Papers of the Medical Faculty of Palacky University in Olomouc*, 153(2). [https://doi: 10.5507/bp.2009.017](https://doi.org/10.5507/bp.2009.017)

Proctor, G.B. and Carpenter, G.H., 2001. Chewing stimulates secretion of human salivary secretory immunoglobulin A. *Journal of dental research*, 80(3), pp.909-913. [https://doi: 10.1177/00220345010800031201](https://doi.org/10.1177/00220345010800031201)

Schur, P.H., (1988). IgG subclasses. A historical perspective. *Monographs in allergy*, 23, pp.1-11.

Singer, H.C., Anderson, J.G., Frischer, H. and Kirsner, J.B., 1971. Familial aspects of inflammatory bowel disease. *Gastroenterology*, 61(4), pp.423-430.

Söltoft, J., Binder, V. and Gudmand-Höyer, E., 1973. Intestinal immunoglobulins in ulcerative colitis. *Scandinavian Journal of Gastroenterology*, 8(4), pp.293-300. <https://doi.org/10.1080/00365521.1973.12096709>

Strober, W., Krakauer, R., Klaeveman, H.L., Reynolds, H.Y. and Nelson, D.L., 1976. Secretory component deficiency: a disorder of the IgA immune system. *New England Journal of Medicine*, 294(7), pp.351-356.

Teeuw, W., Bosch, J.A., Veerman, E.C. and Nieuw Amerongen, A.V., 2004. Neuroendocrine regulation of salivary IgA synthesis and secretion: implications for oral health. [https://doi: 10.1515/BC.2004.147](https://doi.org/10.1515/BC.2004.147)

Tsujita, S. and Morimoto, K., 1999. Secretory IgA in saliva can be a useful stress marker. *Environmental health and preventive medicine*, 4(1), pp.1-8. <https://doi.org/10.1007/BF02931243>

Valdimarsdottir, H.B. and Stone, A.A., 1997. Psychosocial factors and secretory

immunoglobulin A. *Critical Reviews in Oral Biology & Medicine*, 8(4), pp.461-474. <https://doi.org/10.1177/10454411970080040601>

Weeke, B. and Jarnum, S., 1971. The serum concentration of 19 serum proteins in Crohn's disease and ulcerative colitis. *Gut*, 12(4), pp.297-302. [https://doi: 10.1136/gut.12.4.297](https://doi.org/10.1136/gut.12.4.297).