Assessment of interferon gamma and interlukine-10 among patients with celiac disease in Karbala Province.

Zainab Majid Jasim Awadh[1*], Ahmed Abbas Hasan[2]
[1,2] Al_Furat Al-Awsat Technical University, Kufa, Iraq
*Corresponding Author Email: msc.zainabimmunologist@gmail.com

Abstract

Celiac disease (CD), also known as "celiac sprue," is a persistent inflammatory condition that involves the small intestine, with a incidence of 1% in the majority of population. This study aimed to investigate the role of some immune parameters such interlukin-10 (IL- 10) and interferon gamma (IFN-γ) among patients with Celiac Disease. This study was conducted on a total of (60) individual in different sex and age group cases (18 males + 42 females) including (30) patients with Celiac Disease and (30) healthy individuals. CD patients were recruited at Karbala Teaching Hospital for Children, through the duration of the beginning of August 2021 till the middle of January 2022. All patients diagnosed with CD by Anti-tTG IgA test. The age range of the study population was from (2-55) years with mean age 13.4 years. Blood was withdrawn from a vein, the serum was used for immunological tests including IL-10 and IFN-γ by ELISA technique. The findings revealed that the Celiac disease patients had a significantly higher mean of tTg IgA than the healthy Group, 131.00 pg\ml vs. 1.99 pg\ml, respectively. (P.value <0.05). Celiac disease patients had a significantly higher mean of IFN-γ than the healthy Group, 245.93 pg\ml vs. 52.69 pg\ml, respectively. (P.value <0.05). Celiac disease patients had a significantly higher mean of IL-10 than the healthy Group, 34.86 pg\ml vs. 13.68 pg\ml, respectively. (P.value <0.05). There was a significant direct (positive) correlation between IL-10 and IFN-γ (p<0.01).

Keywords: *Assessment of IL-10 and IFN-γ, Patients with Celiac Disease.*

1. Introduction

eliac disease is a chronic, immune-mediated enteropathy generated by gluten intake in genetically susceptible people [1]. Celiac disease affects around 1% of the world's population [2]. CD was first thought to be a childhood condition marked by diarrhea and malabsorption. As a result, it was discovered that CD may impact individuals of any age [3]. Multiple clinical trials of CD shown that non-specific symptoms or no symptoms are widespread in the Middle East [4]. Celiac disease is characterized by inflammation and damage to the small intestinal mucosa and mostly affects the upper small intestine [5]. Unfortunately, there is a clear increase in the occurrence of extra-intestinal manifestations among CD patients, such as short stature, delayed puberty, neurological symptoms, stomatitis, and dermatitis herpatiformis. Diagnosis of CD among patients with extra-intestinal symptoms demands a high level of awareness of clinical conditions that carry a high risk for underlying CD. Cytokines are critical in the immunological pathogenesis of celiac disease, as are CD4+ T cells. The T lymphocyte-mediated response produces the pro-inflammatory cytokine IFN-γ, which is essential in the development of this enteropathy. IFN-γ secretion elevates HLA-DQ2 expression on the surface of antigen-presenting cells, making peptide presentation more effective. T-cell responses will eventually concentrate on the most immunogenic and stable peptides [6]. The anti-inflammatory cytokine IL-10 is a critical immunological modulator of the intestinal tract [7]. Has previously been found to be up-regulated in celiac disease patients as compared to controls in previous studies [8]. IL-10 acts by interfering with antigen presentation and stimulates low responsiveness in gliadin specific T cells [9].

2. Materials and Methods

Patients Group

The collection of blood specimens was carried out during the period from the beginning of August 2021 till the middle of January 2022 from 30 patients with Celiac disease whose ages ranged between (2-55) patients were recruited of Karbala Teaching Hospital for Children. First, patients were interviewed directly by using an anonymous questionnaire which included the details and history of the patients. This study was in agreement with the ethics of Karbala Teaching Hospital for Children and verbal informed consent were obtained from all participants.

Healthy Group

The Healthy Group was composed of 30 randomly healthy persons with the age ranging between (4-38) years. This Healthy Group was examined by ELISA. All Healthy Group was asked to fill a questionnaire and all had no family history of disease.

Blood Collection

Five milliliters of venous blood sample was taken from all study groups. The blood was transferred into a Gel tube for serum separation, the blood was left for about 30 minutes in room temperature for clotting and then centrifuged at 3000 g for 2 minutes. Then the serum was collected in a sterile eppendrofe tube in three repeaters and kept frozen at -80 C for the determination of Interlukin-10 (IL-10) and interferon gamma (IFN-γ) [10].

Anti-Tissue Transglutaminase (Anti-*tTg*

doi.org/10.31838/hiv22.02.44 Received: 03.01.22, Revised: 03.04.22, Accepted: 01.08.22

IgA) Antibody principle

Microwells were coated with human recombinant tissue transglutaminase. The determination was done via an indirect enzyme-linked immune reaction, which consisted of the following steps: Specific antibodies in the patient sample bind to the antigen coated on the reaction wells' surfaces. Following incubation, a washing step was performed to eliminate unbound and unspecifically bound serum or plasma components. Following that, an enzyme conjugate was added and linked to the immobilized antibody-antigen complexes. Following incubation, a second washing step was used to eliminate unbound enzyme conjugate. Following the addition of substrate solution, the linked enzyme conjugate hydrolyzed the substrate, resulting in a blue component. The addition of an acid terminated the process and produced a yellow end product. The yellow color intensity associated with the concentration of the antibody-antigen combination and may be evaluated photometrically at 450 nm.

Interferon Gamma (IFN-γ) principle

This ELISA kit made use of the Sandwich-ELISA principle. On the micro ELISA plate included in this kit, the antibody specific for Human IFN- γ has been pre-coated. In the micro ELISA plate wells, samples (or standards) were combined with the particular antibody. Then, in each microplate well, a biotinylated detection antibody specific for Human IFN- **γ** and an Avidin-Horseradish Peroxidase (HRP) conjugate were added and incubated. The unneeded components were cleaned away. Each well received the substrate solution. Only the wells containing Human IFN-γ, biotinylated detection antibody, and Avidin-HRP conjugate will become blue. The enzyme-substrate reaction was stopped by adding stop solution, and the color changed to yellow. The optical density (OD) was measured spectrophotometrically at a wavelength of 450 nm 2 nm. The quantity of Human IFN- γ in the sample was proportional to the OD value. The concentration of Human IFN- γ in the samples was measured by comparing the OD of the samples to the standard curve

Interleukin 10 (IL-10) principle

The Sandwich-ELISA principle was utilized in this ELISA kit. This kit includes a micro ELISA plate that has been pre-coated with an antibody specific to Human IL-10. Samples (or Standards) were placed in the micro ELISA plate wells and mixed with the appropriate antibody. Then, in each microplate well, a biotinylated detection antibody specific for Human IL-10 and an Avidin Horseradish Peroxidase (HRP) conjugate were added and incubated. The unneeded components were cleaned away. Each well obtained the substrate solution. Only the wells containing Human IL-10, biotinylated detection antibody, and Avidin-HRP conjugate would turn blue. The enzyme-substrate reaction was stopped by adding stop solution, and the color changed to yellow. At a wavelength of 450 2 nm, the optical density (OD) was determined spectrophotometrically. The OD value correlated with the concentration of Human IL-10. By comparing the OD of the samples to the standard curve, the concentration of Human IL-10 in the samples was estimated.

Statistical Analysis

Data of the study participants, CD patients and Healthy group, were entered, managed and analyzed using the Statistical Package For Social Sciences (SPSS) version 25 software for windows, IBM, US, 2017. All variables were checked for errors or inconsistency prior to the analysis process.

Continuous variables included Anti-Tissue Transglutaminase (Anti-tTg IgA) Antibody, interleukin-10 and Interferon Gamma (IFN-γ) were tested for statistical normality distribution using histogram and normal distribution curves and they all appeared to follow the statistical normal distribution. (t) test used to compare mean levels of these parameters. Level of significance (P. value) of 0.05 or less considered significant. Finally, results and findings presented in tables and or figures accordingly, using the Microsoft Word application 2013 for windows [3].

3. Results and Discussion

Anti- tissue transglutamiase *IgA* (*tTg IgA*)

Celiac disease patients had a significantly higher mean of tTg IgA than the healthy Group, 131.00 pg\ml vs. 1.99 pg\ml, respectively. (P.value <0.05).

Table 1: Comparison of mean values of tTg IgA, IFN-γ and IL-10 CD patients and healthy

groups	Control		Patients		T-test value	p.v
	Mean	S. D	Mean	S. D		
IFN-γ	52.69	40.14	245.93	196.96	5.27	.000
IL-10	13.68	12.43	34.86	23.26	4.40	.000
tTg IgA	1.99	0.72	131.00	143.07	4.94	.000

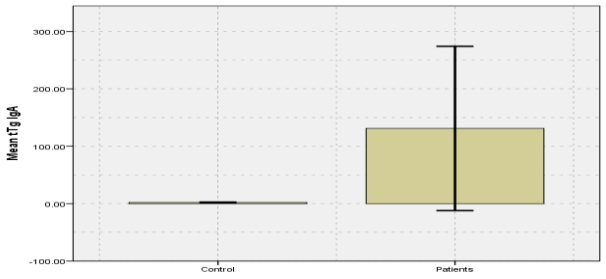

Figure 1: Graphical comparison of the mean tTg IgA level of CD patients and Healthy Group

Anti-tissue transglutaminase antibodies (Anti-tTg IgA) were shown to be higher in CD patients [11]. According to the findings of this investigation, strong anti-tTG titers not only confirm the diagnosis of CD but also reflect the degree of mucosal injury [12].

Interferon Gamma (IFN-γ)

Celiac disease patients had a significantly higher mean of IFN-γ than the healthy Group, 245.93 pg\ml vs. 52.69 pg\ml, respectively. (P.value <0.05).

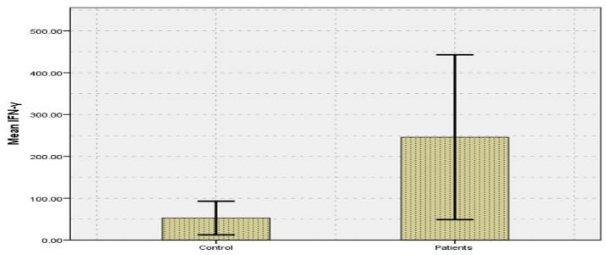

Figure 2: Graphical comparison of the mean IFN-γ level of CD patients and Healthy Group

The increased blood levels of IFN-γ, which have been proven to be high in the gut mucosa, indicate that CD causes IFN-γ production and systemic activation [13]. IFN-γ is known to stimulate CD4 + T-cell development toward a profile of TH1-cytokines, while inhibiting the TH2 immunological response and regulatory T-cell survival [14]. Previous research has shown that T cell activation in the small intestine mucosa promotes interferon-gamma (IFN-γ) generating Th1 cells in the active form of CD [15].

Interleukin-10

Celiac disease patients had a significantly higher mean of IL-10 than the healthy Group, 34.86 pg\ml vs. 13.68 pg\ml, respectively. (P.value <0.05).

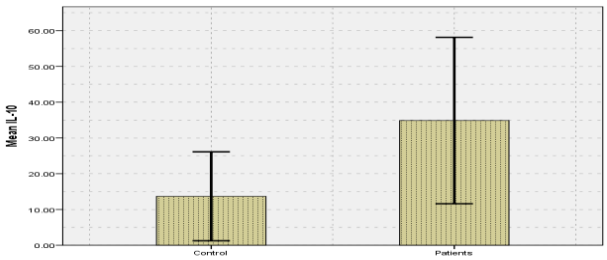

Figure 3: Graphical comparison of the mean IL-10 level of CD patients and Healthy Group

Anti-inflammatory cytokine IL-10, recognized as a significant immunomodulator in the intestinal tract, has been both up-regulated and unchanged in patients with celiac disease relative to controls in previous trials [16]. Production of IL-10 in duodenum during ACD has been confirmed, suggesting that post-challenge development of IL-10 could be part of a typical celiac reaction [9]. Adaptive immune response starts when the antigen-presenting cells (APCs) identify the primary antigen (gliadin peptides) deamidated by the transglutaminase enzyme, handle this antigen, and present it to T CD4 + lymphocytes in conjunction with class II major histocompatibility complex (MHC) molecules. CD4 Th2 lymphocytes direct their humoral immune response by secreting interleukin 10 (IL-10) which induces the spread and differentiation of B lymphocytes [17].

4. Correlation Analysis Among il-10 and ifn-γ

The results of correlation analysis showed that there is as significant correlation is a direct (positive) between IL10 and INF-γ (p<0.01).

Table 2: Correlation analysis among the immunological parameters in the study

Group	Parameter	IFN-γ
CD patients (n=30)	IL-10	.367*
Healthy group (n=30)	IL-10	-.001
*. Correlation is significant at the 0.05 level (2-tailed).		

Several studies have found Gluten is delivered to TCD4+ cell and activates and causing the secretion of of IL-10, and IFN-γ in patients with CD as compared with healthy population [18]. Cytokines are implicated in both enhancing and suppressing immune responses through their influence on T-cells and other immune effectors. INF-γ activate T helper type 1 (Th-1) lymphocytes, while IL-10 lead to activation of T helper type 2 (Th-2) cell [2].

A previous study aimed to understand the local immune reaction by determining which intraepithelial T cell subsets produce the different cytokines. In active CD, CD8+αβIELs showed a significant increase in expression levels of both IFN-γ and IL-10. Interestingly, IL-10 was increased also in CD4+αβIELs. Cytokine levels were low in γδIELs. 'Classical' CD94−CD8+αβ T cells within the epithelium are responsible for the excessive production of IFN-γ, believed to drive the formation of intestinal lesions in active CD. Production of IL-10 may be a common feature of IELs producing pro-inflammatory cytokines, thereby attempting to limit inflammation in an autocrine fashion [9].

5. Ethical Approval

All authors hereby declare that all actions have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

6. Conflict of Interests

The authors did not declare any conflict of interest.

7. Acknowledgments

We gratefully thank Karbala Teaching Hospital for Children staff for supporting and agreeing to perform this work in their Immunity Lab and in particular MSC. Al-

Fadhel Hassanein Khalil.

References

1. Ludvigsson JF, Leffler DA, Bai JC, Biagi F, Fasano A, Green PHR, Hadjivassiliou M, Kaukinen K, Kelly CP, Leonard JN, Lundin KEA, Murray JA, Sanders DS, Walker MM, Zingone F, Ciacci C. The Oslo definitions for coeliac disease and related terms. Gut. 2013;62(1):43-52. https://doi.org/10.1136/gutjnl-2011-301346
2. Parzanese I, Qehajaj D, Patrinicola F, Aralica M, Chiriva-Internati M, Stifter S, Elli L, Grizzi F. Celiac disease: From pathophysiology to treatment. World J Gastrointest Pathophysiol. 2017;8(2):27-38. https://doi.org/10.4291/wjgp.v8.i2.27
3. Al-Rawi Kh. M. Entrance to Statistics, 2ed ed. Babylon, Iraq, . 2000;19(8):993–100.
4. Al-Shami SA. Prevalence of Celiac Disease in Developing Countries. Iraqi Journal of Medical Sciences. 2017;15(4):324-6. Available from: https://www.iasj.net/iasj/article/141560
5. Koehler P, Wieser H, Konitzer K. Celiac disease and gluten. Multidisciplinary Challenges and Opportunities. 2014;1:96. https://doi.org/10.1016/C2013-0-13353-3
6. Dahal-Koirala S, Risnes LF, Sollid LM. Pathogenesis of coeliac disease–a disorder driven by gluten-specific CD4+ T cells. In: Coeliac Disease and Gluten-Related Disorders. Elsevier, 2022. p. 41-68. https://doi.org/10.1016/B978-0-12-821571-5.00007-6
7. Veenbergen S, Samsom JN. Maintenance of small intestinal and colonic tolerance by IL-10-producing regulatory T cell subsets. Current opinion in immunology. 2012;24(3):269-76. https://doi.org/10.1016/j.coi.2012.03.004
8. Manavalan JS, Hernandez L, Shah JG, Konikkara J, Naiyer AJ, Lee AR, Ciaccio E, Minaya MT, Green PH, Bhagat G. Serum cytokine elevations in celiac disease: association with disease presentation. Human immunology. 2010;71(1):50-7. https://doi.org/10.1016/j.humimm.2009.09.351
9. Forsberg G, Hernell O, Hammarström S, Hammarström M-L. Concomitant increase of IL-10 and pro-inflammatory cytokines in intraepithelial lymphocyte subsets in celiac disease. International immunology. 2007;19(8):993-1001. https://doi.org/10.1093/intimm/dxm077
10. Bonetti TCdS, Salomao R, Brunialti M, Braga D, Borges Jr E, Silva IDCGd. Cytokine and hormonal profile in serum samples of patients undergoing controlled ovarian stimulation: interleukin-1β predicts ongoing pregnancy. Human Reproduction. 2010;25(8):2101-6. https://doi.org/10.1093/humrep/deq171
11. Cerqueiro Bybrant M, Udén E, Frederiksen F, Gustafsson AL, Arvidsson CG, Fureman AL, Forsander G, Elding Larsson H, Ivarsson SA, Lindgren M. Celiac disease can be predicted by high levels of tissue transglutaminase antibodies in children and adolescents with type 1 diabetes. Pediatric Diabetes. 2021;22(3):417-24. https://doi.org/10.1111/pedi.13165
12. Alessio MG, Tonutti E, Brusca I, Radice A, Licini L, Sonzogni A, Florena A, Schiaffino E, Marus W, Sulfaro S, Villalta D, for the Study Group on Autoimmune Diseases of the Italian Society of Laboratory M. Correlation Between IgA Tissue Transglutaminase Antibody Ratio and Histological Finding in Celiac Disease. Journal of Pediatric Gastroenterology and Nutrition. 2012;55(1). Available from: https://journals.lww.com/jpgn/Fulltext/2012/07000/Correlation_Between_IgA_Tissue_Transglutaminase.9.aspx
13. Heydari F, Rostami-Nejad M, Moheb-Alian A, Mollahoseini MH, Rostami K, Pourhoseingholi MA, Aghamohammadi E, Zali MR. Serum cytokines profile in treated celiac disease compared with non-celiac gluten sensitivity and control: a marker for differentiation. Journal of Gastrointestinal & Liver Diseases. 2018;27(3). https://doi.org/10.15403/jgld.2014.1121.273.hey
14. De Re V, Caggiari L, Tabuso M, Cannizzaro R. The versatile role of gliadin peptides in celiac disease. Clinical biochemistry. 2013;46(6):552-60. https://doi.org/10.1016/j.clinbiochem.2012.10.038
15. Meresse B, Chen Z, Ciszewski C, Tretiakova M, Bhagat G, Krausz TN, Raulet DH, Lanier LL, Groh V, Spies T. Coordinated induction by IL15 of a TCR-independent NKG2D signaling pathway converts CTL into lymphokine-activated killer cells in celiac disease. Immunity. 2004;21(3):357-66. https://doi.org/10.1016/j.immuni.2004.06.020
16. Weiss JM, Subleski JJ, Wigginton JM, Wiltrout RH. Immunotherapy of cancer by IL-12-based cytokine combinations. Expert opinion on biological therapy. 2007;7(11):1705-21. https://doi.org/10.1517/14712598.7.11.1705
17. Bizzaro N, Tozzoli R, Villalta D, Fabris M, Tonutti E. Cutting-edge issues in celiac disease and in gluten intolerance. Clinical reviews in allergy & immunology. 2012;42(3):279-87. https://doi.org/10.1007/s12016-010-8223-1
18. Björck S, Lindehammer S, Fex M, Agardh D. Serum cytokine pattern in young children with screening detected coeliac disease. Clinical & Experimental Immunology. 2015;179(2):230-5. https://doi.org/10.1111/cei.12454