Role of Selective Cytokines in the Pathophysiology of Patients with Celiac Disease

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Abstract

Background and Aims: Inflammatory cytokines levels may be elevated in patients with celiac disease (CD). But little known about the association of these cytokines and some immunological parameters used for diagnosis of celiac disease. This study aimed to evaluate the levels of some inflammatory cytokines in patients with celiac disease and compare it with healthy individuals and then their association with some immunological markers used for diagnosis of celiac disease.

Methods: A total of 60 patients with celiac disease and 40 healthy persons were enrolled in 2019. The levels of Anti-tissue transglutaminase levels were measured for patients and healthy groups, also Tumor necrosis factor Alpha (TNF-α), Interleukin-6 (IL-6) and Interleukin-8 (IL-8) were measured using enzyme-linked immunosorbent assay techniques. Then compare these parameters between groups and the correlation between these parameters were assessed.

Results: The mean levels of Tumor necrosis factor Alpha (TNF-α), Interleukin-6 (IL-6) and Interleukin-8 (IL-8) give a highly significant difference between patients and healthy groups. Anti-tissue transglutaminase levels correlated and were show statistically significant with these cytokines.

Conclusions: TNF-α, IL-6 and IL-8 play a role in the pathophysiology of celiac disease.

Keywords: *Celiac disease, Anti-tissue transglutaminase, TNF-α.*

Introduction

Celiac disease (CD) consider as a type of an autoimmune disease affecting about 1% of the population and many studies were found that increase in the community[1]. Celiac disease (CD) has a wide range of clinical manifestations as a multi-component and immune-mediated intestinal disorder[2]. It was well known that gluten consumption, found mainly in wheat, rye, and barley, is the primary external CD cause in predisposed individuals[3]. Celiac disease is the product of dysregulation of the innate and adaptive immune system, Adaptive immune system activation means that gliadin (the toxic element of gluten) crosses the epithelium of the intestine[4]. It has been hypothesized that an early occurrence in CD pathogenesis is increased intestinal permeability[5]. Many cytokines in CD were more effective mediators than others to intensify the immune response[6]. Th2 cells, B cells, monocytes, macrophages, endothelial, epithelial, fibroblast cells secrete interleukin-6 (IL-6), the acute-phase response is an active inducer, th1 produce TNF-α, some Th2 and some CTL phenotypes are also producing this cytokine[7]. This stimulates the development of nitric oxide and activates, among other biological actions, microvascular endothelium; researchers found that TNF-α is the most potent inducer of TG2 transcription, working synergistically with IFN-α[8].

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Material and Methods

Serum samples

Sixty serum samples are collected from active CD patients and 40 healthy persons as a control group. Tissue transglutaminase (TTG) IgA and IgG serological antibody tests are diagnosed to all patients and the control group. Serum was isolated from peripheral blood collected from patients after informed consent and processed at 8 ºC before examination by the gastroenterologist[9]. All specimens are obtained from Al-Hussain Medical City in Karbala.

2.2. Measurement of anti-TTG TNF Alpha, IL6, IL8 antibody:

The levels of both the human TNF Alpha ELISA Kit, Anti-Human Tissue Transglutaminase (anti-tTG) ELISA Kit, Interleukin 6 ELISA Kit (IL6) and interleukin 8 ELISA Kit (IL8) are estimated based on standard sandwich enzyme-linked immunosorbent assay technology, for the quantitative or semi-quantitative determination in human serum (antibodies-online GmbH, Aachen, Germany).

Statistical Analysis

Using the statistical software package SPSS 23, the findings are analyzed. The likelihood of (Pentr 0.05) has been found in statistically significant[10].

Results

Measurement data for certain serum cytokines in patients with CD, as well as corresponding controls are given in table1. The detection of the autoantigen of CD ((tissue transglutaminase (tTG)) as anti-TTG IgA antibodies in the CD serum has become an important tool for examining this disease. The Th1 serum cytokine levels of IFN-α were much higher in patients with coeliac disease compared to controls as compared with patients with CD. A correlation seemed to exist between anti-tTG IgA serum mean levels and mean cytokine serum levels tested. positive associations with statistically significant have been reported between the (anti-TTG IgA, anti-TTG IgG) rates and all cytokines IL-6, IL-8 but the mean TNF-α levels was excepted.

Tab 1: demographic and clinical characteristics in patients with celiac disease.

Characteristic	N	Percentage (%)
No of patients	60	100
Gender		
Male	23	38
Female	37	62
Age(years)		
(1-10)	31	52
(10-20)	19	32
(20-30)	10	16
duration of disease		
>10	17	28
<10	43	72

Tab 2: Levels of Anti-tissue transglutaminase in patients and healthy group.

Parameters	Patients (N=60)	Healthy group(N=4)	P. Value
	Mean±SD	Mean±SD	
Anti-tissue transglutaminase IgG	97.345±76.457	11.789±6.092	≤ 0.05
Anti-tissue transglutaminase IgG	50.752±42.123	13.098±4.178	≤ 0.05

Table 3: Mean levels of selective cytokines in patients and a healthy control group.

Parameters	Patients	Healthy group	P-value
	Mean±SD	Mean±SD	
TNF-α	11.431±2.082	1.634±1.011	≤ 0.05
IL-6	72.098±4.864	2.001±1.121	≤ 0.05
IL-8	104.324±2.981	3.542±2.032	≤ 0.05

Table 4: Correlation of three parameters among asthmatic patients according to Anti-TT-A and Anti-TT-G.

			Anti-TTG- A	Anti-TTG-G
	TNF-α	Pearson Correlation	0.156	0.855**
		Sig. (2-tailed)	0.051	0.000
		N	60	60
	IL-6	Pearson Correlation	1	0.716**
		Sig. (2-tailed)		0.000
		N	60	60
	IL-8	Pearson Correlation	0.669**	0.517*
		Sig. (2-tailed)	0.001	0.061
		N	60	60

* P≤0.05, **P≤0.01, P≤0.001.

Discussion

Analysis findings through this particular effort indicated that higher concentrations of Th-1, Th-2 cytokines in CD patients reflect this disease's inflammatory response, with unique serum cytokine elevations from non-intestinal sources such as those in the small intestinal mucosa and others[11]. The Th-1 response to dietary gluten in the small intestinal mucosa is likely to cause infiltration of lymphocytes and monocytes into the lamina propria. Even if they overlap in their role[12], [13]. The Th-1 response is an increase in cell-mediated immunity and pro-inflammatory responses, whereas the Th-2 cytokines predominantly affect the humoral immune response and play a role in inflammatory down-regulation Both responses were observed in the CD[14–17]. Many studies have shown that elevations of serum immunoglobulins against particular autoantigens, such as endomysia antibodies (EMA) and TTG-antibodies are primarily IgA isotypes in CDs[18–27]. Cytokines at the mucosal level support the production of plasma cells containing IgA while the degree of inflammation or systemic involvement cannot be determined by tests performed at a given point in time, our findings suggest roles for certain cytokines in generating or maintaining the humoral response in CD except for TNF-α [14], [28], [29]. IL-8 was execrated in neutrophilic infiltrated tissue and plays a major role in neutrophilic-mediated inflammatory responses, In our research serum levels of IL-8 are increased relative to controls in all CD patients compared to other APC-derived cytokines[30], [31]. variety of cells, including T cells, B cells, fibroblasts, endothelial cells, monocytes, keratinocytes, mesangial cells, and certain tumour cells play a role for released of a pleiotropic cytokine (IL-6) for Inflammation, immune control, hematopoiesis, and oncogenesis, IL-6 plays a key role[28], [32–36]. As with our results, serum levels of IL-6 in patients with ACD are significantly increased.

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Conflict of Interest: Non

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References

[1] D. Kaswala, G. Veeraraghavan, C. Kelly, and D. Leffler, "Celiac Disease: Diagnostic Standards and Dilemmas," *Diseases*, 2015, doi: 10.3390/diseases3020086.

[2] M. Sierra, N. Hernanz, and I. G. y. L. Alonso, "Celiac disease," *Med.*, 2020, doi: 10.1016/j.med.2020.01.002.

[3] M. M. Leonard, A. Sapone, C. Catassi, and A. Fasano, "Celiac disease and nonceliac gluten sensitivity: A review," *JAMA - Journal of the American Medical Association*. 2017, doi: 10.1001/jama.2017.9730.

[4] M. V. Barone and K. P. Zimmer, "Endocytosis and transcytosis of gliadin peptides," *Mol. Cell. Pediatr.*, 2016, doi: 10.1186/s40348-015-0029-z.

[5] P. Singh *et al.*, "Global Prevalence of Celiac Disease: Systematic Review and Meta-analysis," *Clin. Gastroenterol. Hepatol.*, 2018, doi: 10.1016/j.cgh.2017.06.037.

[6] G. Losurdo, M. Principi, A. Iannone, E. Ierardi, and A. Di Leo, "The Interaction between Celiac Disease and Intestinal Microbiota," *J. Clin. Gastroenterol.*, 2016, doi: 10.1097/MCG.0000000000000682.

[7] B. Jabri and L. M. Sollid, "T Cells in Celiac Disease," *J. Immunol.*, 2017, doi: 10.4049/jimmunol.1601693.

[8] S. Yoosuf and G. K. Makharia, "Evolving therapy for celiac disease," *Frontiers in Pediatrics*. 2019, doi: 10.3389/fped.2019.00193.

[9] M. M. Jawad, A. N. Aldujaili, and M. H. Homady, "ASSESSMENT STUDY OF ALPHA-FETOPROTEIN LEVEL AFTER TREATMENT WITH URTICA DIOICA PHENOLIC EXTRACT IN MALE RAT INDUCED BY CARBON TETRA-CHLORIDE," vol. 18, no. 1, pp. 410–414, 2018.

[10] J. J. Albright and D. M. Marinova, "Estimating multilevel models using SPSS, Stata, SAS, and R," *Inf. Technol. Serv. Indiana Univ. (http//www. indiana. edu/ statmath/stat/all/hlm/)*, 2010.

[11] A. Kaur, O. Shimoni, and M. Wallach, "Celiac disease: from etiological factors to evolving diagnostic approaches," *Journal of Gastroenterology*. 2017, doi: 10.1007/s00535-017-1357-7.

[12] G. Goel *et al.*, "Serum cytokines elevated during gluten-mediated cytokine release in coeliac disease," *Clin. Exp. Immunol.*, 2020, doi: 10.1111/cei.13369.

[13] R. Mandile *et al.*, "Lack of immunogenicity of hydrolysed wheat flour in patients with coeliac disease after a short-term oral challenge," *Aliment. Pharmacol. Ther.*, 2017, doi: 10.1111/apt.14175.

[14] I. Comino *et al.*, "Identification and molecular characterization of oat peptides implicated on coeliac immune response," *Food Nutr. Res.*, 2016, doi: 10.3402/fnr.v60.30324.

[15] M. Uhde *et al.*, "Intestinal cell damage and systemic immune activation in individuals reporting sensitivity to wheat in the absence of coeliac disease," *Gut*, 2016, doi: 10.1136/gutjnl-2016-311964.

[16] Z. P. de Camargo and L. E. Cano R., "Humoral Immunity," in *Paracoccidioidomycosis*, 2018.

[17] B. Lebwohl, D. S. Sanders, and P. H. R. Green, "Seminar Coeliac disease," *Lancet*, 2018, doi: 10.1016/S0140-6736(17)31796-8.

[18] C. Tiberti *et al.*, "Detection of four diabetes specific autoantibodies in a single radioimmunoassay: An innovative high-throughput approach for autoimmune diabetes screening," *Clin. Exp. Immunol.*, 2011, doi: 10.1111/j.1365-2249.2011.04479.x.

[19] A. Al-Toma *et al.*, "European Society for the Study of Coeliac Disease (ESsCD) guideline for coeliac disease and other gluten-related disorders," *United European Gastroenterology Journal*. 2019, doi: 10.1177/2050640619844125.

[20] R. Comerford, J. Kelly, C. Feighery, and G. Byrne, "IgG anti-tTG responses in different autoimmune conditions differ in their epitope targets and subclass usage," *Mol. Immunol.*, 2015, doi: 10.1016/j.molimm.2015.06.026.

[21] J. Woodward, "Coeliac disease," *Medicine (United Kingdom)*. 2015, doi: 10.1016/j.mpmed.2015.01.011.

[22] J. Wolf *et al.*, "Antibodies in the diagnosis of coeliac disease: A biopsy-controlled, international, multicentre study of 376 children with coeliac disease and 695 controls," *PLoS One*, 2014, doi: 10.1371/journal.pone.0097853.

[23] G. J. Kahaly and M. P. Hansen, "Type 1 diabetes associated autoimmunity," *Autoimmunity Reviews*. 2016, doi: 10.1016/j.autrev.2016.02.017.

[24] E. A. Aranda and M. Araya, "Tratamiento de la enfermedad celíaca. ¿Cómo medir adherencia a la dieta libre de gluten?," *Rev. Chil. Pediatr.*, 2016, doi: 10.1016/j.rchipe.2016.01.007.

[25] R. Tortora *et al.*, "The presence of anti-endomysial antibodies and the level of anti-tissue transglutaminases can be used to diagnose adult coeliac disease without duodenal biopsy," *Aliment. Pharmacol. Ther.*, 2014, doi: 10.1111/apt.12970.

[26] K. Lindfors *et al.*, "Coeliac disease," *Nature Reviews Disease Primers*. 2019, doi: 10.1038/s41572-018-0054-z.

[27] B. Lebwohl, D. S. Sanders, and P. H. R. Green, "Coeliac disease," *The Lancet*. 2018, doi: 10.1016/S0140-6736(17)31796-8.

[28] N. Saligrama *et al.*, "Opposing T cell responses in experimental autoimmune encephalomyelitis," *Nature*, 2019, doi: 10.1038/s41586-019-1467-x.

[29] T. Van Gils, P. Nijeboer, R. L. Van Wanrooij, G. Bouma, and C. J. J. Mulder, "Mechanisms and management of refractory coeliac disease," *Nature Reviews Gastroenterology and Hepatology*. 2015, doi: 10.1038/nrgastro.2015.155.

[30] P. Biancheri *et al.*, "Abnormal thymic stromal lymphopoietin expression in the duodenal mucosa of patients with celiac disease," *Gut*, 2016, doi: 10.1136/gutjnl-2014-308876.

[31] D. Kocsis *et al.*, "Prevalence of inflammatory bowel disease among coeliac disease patients in a Hungarian coeliac centre," *BMC Gastroenterol.*,

2015, doi: 10.1186/s12876-015-0370-7.

[32] W. Y. Tseng *et al.*, "TNFR signalling and its clinical implications," *Cytokine*, 2018, doi: 10.1016/j.cyto.2016.08.027.

[33] M. Kuwana, "Endothelial progenitor cells," in *Systemic Sclerosis*, 2016.

[34] B. Lebwohl, P. H. R. Green, and R. M. Genta, "The coeliac stomach: Gastritis in patients with coeliac disease," *Aliment. Pharmacol. Ther.*, 2015, doi: 10.1111/apt.13249.

[35] V. Vaira *et al.*, "microRNA profiles in coeliac patients distinguish different clinical phenotypes and are modulated by gliadin peptides in primary duodenal fibroblasts," *Clin. Sci.*, 2014, doi: 10.1042/CS20130248.

[36] A. Zhernakova, S. Withoff, and C. Wijmenga, "Clinical implications of shared genetics and pathogenesis in autoimmune diseases," *Nature Reviews Endocrinology*. 2013, doi: 10.1038/nrendo.2013.161.