Comparison of the activity of IL-6 and IL22 as more inflammatory cytokines related to the pathogenesis of celiac disease between active and gluten-free diet patient

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IL-6, IL-22, Celiac Disease, Inflammatory Cytokines, Gluten-Free Diet

ABSTRACT
This study aimed to investigate the activity of anti-inflammatory IL-6 (aIL-6) and pro-regenerative IL-22 in celiac disease patients based on their dietary habits. By comparing cytokine levels between patients on active gluten-containing diets and those on gluten-free diets, we sought to elucidate the potential role of diet in modulating inflammatory responses and clinical outcomes in celiac disease. The study made use of an enzyme-linked immunosorbent assay (ELISA) to evaluate the serum levels of interleukins IL6 and IL22. This was performed on three different groups: Group 1, which consisted of 40 recently identified active celiac patients; Group 2, which included 20 patients following a gluten-free diet; and Group 3, which contained 40 apparently healthy individuals .the individuals included in the study had been previously confirmed as celiac patients through serology, specifically by detecting both anti-IgA and IgG antibodies against tTG and gliadin using indirect immunofluorescence. The findings revealed a notable difference in IL6 levels between the patient and control groups, with a P-value of 0.041. However, when the patient groups were compared using the least significant difference (LSD) method, there were no significant differences between Group 1 (G1) and Group 2 (G2) with a P-value of 0.101, and between Group 1 and Group 3 (G3) with a P-value of 0.720. Yet, a more pronounced difference was observed between Group2 and Group3,with a P-value less than 0.015.Regarding IL22 levels, there was a significant difference between the patient and control groups, with a P-value of 0.002. Further comparisons using the LSD method revealed no significant differences between Group 1 and Group 2, with a P-value less than 0.154. However, a highly significant difference was found between Group 1 and Group 3, with a P-value less than 0.01. Also, significant differences were observed between Group 2 and Group 3, with a P-value less than 0.012. In conclusion, there is important role of the inflammatory cytokines il-6 and il-22 in the inflammation of celiac disease and potential role for these cytokines in the pathogenesis and management of the disease. Adherence to a gluten-free diet appears to have a beneficial effect on modulating the inflammatory response and improving clinical outcomes in celiac disease..

1. Introduction

Celiac disease CD is an autoimmune disorder activated by consuming gluten, a protein found in wheat, barley, and rye. It primarily affects individuals who are genetically predisposed to it (1). Celiac disease is a major public health problem worldwide . The prevalence of biopsy-confirmed CD is estimated at 0.7%,while diagnosis based on serology is estimated at 1.4% of the population (2). CD can present with gastrointestinal (GI) symptoms. Typical celiac disease is characterized by GI symptoms such as chronic diarrhea, failure to thrive (in children), vomiting, constipation, abdominal distension, and anorexia. In addition to GI symptoms, celiac disease can also have non-gastrointestinal manifestations, which are more common in older patients and atypical cases. These non-GI symptoms may include anemia, osteoporosis, muscle wasting, headaches, and epilepsy (3) .Celiac disease is closely linked to an autoimmune response initiated by T-cells. When specific altered peptides, derived from gliadin, are presented to CD4+ T helper cells via HLA molecules DQ2 and DQ8, it stimulates both T-cells and B lymphocytes. This stimulation leads to the production of antibodies against gluten and tissue transglutaminase (tTG) in the lamina propria. This process ultimately results in the activation of self-reactive T helper cells (4). The activation of autoreactive Th cells in celiac disease results in the release of proinflammatory cytokines. These cytokines then activate intraepithelial lymphocytes, leading to histological changes in the small intestinal mucosa (5). Cytokines, which are produced by various types of cells in the intestines including lymphocytes,

T cells, dendritic cells (DCs), macrophages, and intestinal epithelial cells, have a crucial function in maintaining the balance of the intestines. They can trigger either a pro-inflammatory or anti-inflammatory response and impact the movement and activation of immune cells. Additionally, cytokines have the ability to regulate the function of the mucosal barrier in the intestines (6).

Among the inflammatory cytokines implicated in celiac disease, interleukin-6 (IL-6) and interleukin-22 (IL-22) have been shown to be dysregulated in patients with active disease. Interleukin 6 (IL-6) promotes the synthesis of acute-phase reactant proteins, which can contribute to inflammation and tissue damage (7) . Interleukin 6 (IL-6) is a multifaceted cytokine generated by various cell types, exhibiting both pro- and anti-inflammatory properties. In its capacity as a pro-inflammatory factor, IL-6 can trigger the production of acute-phase reactant proteins, and its excessive presence has been linked to inflammatory autoimmune disorders and uncontrolled intestinal inflammation, which is characteristic of celiac disease (CD),so there are significant relationship between IL-6 and the development of celiac disease (8). IL-22 is essential for defending mucosal barriers, promoting tissue repair, and supporting the survival and proliferation of epithelial cells. It plays a multifaceted role in various conditions, including autoimmune disease, infection, and cancer. Specifically, IL-22 acts on epithelial cells to stimulate cell regeneration and tissue repair, with a particular emphasis on the intestinal barrier (9). An abnormal adaptive immune response can lead to mucosal damage, which can be resolved by avoiding gluten in the diet (10)

Materials and methods

This research took place between October 15, 2023, and February 15, 2024, and involved 100 participants. These individuals, who ranged in age from 7 to 65 years, were enlisted from the Digestive Center at Marjan Medical City in the Babylon Governorate.The study groups were divided into three distinct groups. Group 1 included 40 newly diagnosed active celiac patients, while Group 2 was composed of 20 patients adhering to a gluten-free diet. Group 3 consisted of 40 apparently healthy individuals who acted as controls and were chosen to correspond with the patients in terms of age and gender. Prior to the start of the study, informed consent was obtained from all participants. Blood samples were collected from members of all groups, left to clot at room temperature, and then separated using a centrifuge at 1500 g for 10 minutes. These samples were stored at -20°C until needed for further analysis.The research utilized an enzyme-linked immunosorbent assay (ELISA) to assess the serum levels of interleukins IL6 and IL22. This was done using ELISA kits from The SUNLONG Biotech Human, following the instructions provided by the manufacturer.The participants in the study had been previously diagnosed with celiac disease. This diagnosis was confirmed through serological testing, specifically by identifying the presence of both anti-IgA and IgG antibodies against tTG and gliadin via indirect immunofluorescence.

Result and Duscussion

Table 1. Distribution of demographic characteristics of celiac disease patients

		Groups						P. value
		Patients with Celiac (Diet group)		Patients with Celiac (Active Group)		Control		
Age	**Mean± SD (Range)**	26.3±15.6 (10-65)		20.6±10.6 (7-45)		23.1±9.9 (8-52)		0.201
Gender	**Male (%)**	6	30.0%	14	35.0%	10	25.0%	0.621

	Female (%)	14	70.0%	26	65.0%	30	75.0%	
BMI (kg/m^2)	**Mean± SD (Range)**	22.66±5.11 (15.12-32.05)		21.11±6.05 (8.67-35.16)		23.17±4.99 (16.46-31.02)		0.166
History of family	**Yes**	5	25.0%	7	17.5%	-	-	0.007
	No	15	75.0%	33	82.5%	40	100.0%	
Compare between percentages using Pearson, Chi-square test (X^2-test) at 0.05 level.								
Compare between means using ANOVA at 0.05 level.								

The mean ± SE serum IL6 level was 25.9540 a ± 0.618 ng/L , 29.0145 ± 1.614 ng/L and 25.2877 ± 0.377 ng/L in the diet group (G1), active group (G2) and in the control group (G3), respectively. The results a significant difference between the patients and control groups regarding the IL6 at P.value (P =0.041). Further comparisons using the least significant difference (LSD) showed no significant differences between the patient groups (G1 Vs. G2) P=0.101, also no significant differences between (G1 Vs. G3) p=0.720, but higher significant differences were observed between(G2 Vs. G3) (P-value < 0.015). As shown in Table 1.2.

Table 2. A comparison the serum levels of Interleukin 6(IL6) among the studies groups

Parameters		**Mean**	**SD**	**SE**	**F (P. value)**
IL6	Patients with Celiac (Diet group) **G1**	25.9540 a	2.764	0.618	**3.291 (P =0.041)**
	Patients with Celiac (Active Group) **G2**	29.0145 b	10.208	1.614	
	Control **G3**	25.2877 a	2.387	0.377	
	Least Significant Difference (LSD)	**Comparisons**		**P. value (between groups)**	
	3.02	(G1 Vs. G2)		0.101 NS	
		(G1 Vs. G3)		0.720 NS	
		(G2 Vs. G3)		**0.015 S**	

One- Way ANOVA; LSD as Post hoc test for equal variances ; ns : no significant at 0.01;S: significant at 0.01; G1: Diet group; G2: Active Group; G3:control The mean ± SE serum IL22 level was 39.9670 a ±0.898 pg/ml, 38.3415 ± 0.728 pg/ml and 35.9850 ± 0.576 pg/ml in the diet group (G1), active group (G2) and in the control group (G3), respectively. The results a significant difference between the patients and control groups regarding the IL22 at P.value (P =0.002). Further comparisons using the least significant difference (LSD) showed no significant differences between the patient groups (G1 Vs. G2)at (P-value < 0.154, but revealed a highly significant difference between (G1 Vs. G3) at (P-value <0.01). also significant differences were observed between(G2 Vs. G3) (P-value <0.012)As shown in Table 1.3.

Table 3. A comparison the serum levels of Interleukin 22(IL22) among the studies groups

Parameters		**Mean**	**SD**	**SE**	**F (P. value)**
IL22	Patients with Celiac (Diet group) **G1**	39.9670 a	4.016	0.898	**6.952 (P =0.002)**
	Patients with Celiac	38.3415 a	4.607	0.728	

	(Active Group) **G2**				
	Control **G3**	35.9850 b	3.645	0.576	
	Least Significant Difference (LSD)	**Comparisons**		**P. value (between groups)**	
	1.86	(G1 Vs. G2)		0.154 NS	
		(G1 Vs. G3)		**<0.01 S**	
		(G2 Vs. G3)		**<0.012 S**	

One- Way ANOVA; LSD as Post hoc test for equal variances ; ns : no significant at 0.01;S: significant at 0.01; G1: Diet group; G2: Active Group; G3:control IL-6 plays a significant role in the body's response to inflammation and tissue damage. It is produced by different types of cells, such as T and B cells, monocytes, fibroblasts, and endothelial cells. This cytokine, is involved in regulating the growth and differentiation of various cell types, particularly in the immune system, and inflammation (11). The current study shows an increase in IL-6 levels in active patients compared to those following a gluten-free diet (GFD) and healthy controls The increase in IL-6 in active celiac disease is likely due to immune response triggered by the ingestion of gluten in individuals with celiac disease. Gluten consumption leads to the activation of the immune system in the small intestine, resulting in the production of pro-inflammatory cytokines like IL-6 which act as acute phase protein .while, in patients under a strict gluten-free diet have lower levels of IL-6 as the inflammation and immune response are reduced when gluten is removed from the diet so, there are active role of IL-6 in the pathogenesis of celiac disease. The current study agrees with (12), where found the concentration of IL-6 was significantly elevated in the active group in comparison to the diet and control groups. Furthermore, a correlation was observed between IL-6 and the serological marker anti-TTG. This suggests that IL-6 can be effectively used as a dependable marker for monitoring disease activity. Also, Romaldini et al., 2002, found the levels of IL-6 in the blood of untreated CD patients were significantly higher compared to the control group. However, there was no significant difference in the IL-6 values between treated CD patients and control patients. This suggests that measuring IL-6 levels could be a non-invasive way to assess the activity of CD and the response to treatment. In a local study conducted by (14)., it was found that Iraqi celiac disease patients had significantly higher levels of IL-6 in their blood compared to apparently healthy individuals ($P < 0.01$). This significant increase in IL-6 levels suggests its important role in inflammation and its contribution to the development of celiac disease. IL-6 production has diverse biological effects that can be both pro-inflammatory and anti-inflammatory. This emphasizes the important role of IL-6 in activating and modulating immune responses. Additionally, IL-6 is known to activate the STAT3 signalling pathway, which further contributes to its biological impacts (15). IL-22 plays a crucial role in protecting the mucosal barrier, promoting tissue repair, and supporting the survival and multiplication of epithelial cells. Recent studies have also highlighted the dual nature of IL-22, showing that it can have both beneficial and harmful effects in various situations, such as autoimmune disorders, infections, and cancer (16). The current study shows there is an increase in patients with active celiac disease compared to healthy controls, and further elevated in celiac disease patients adhering to a gluten-free diet (GFD) in comparison to both active and control. This dysregulation of IL-22 in celiac disease suggests a potential role for this cytokine in the pathogenesis and management of the disease and also may be a compensatory mechanism to promote mucosal healing and restore intestinal barrier function in response to gluten withdrawal. A recent study by Vorobjova et al., 2019 discovered that patients with Celiac disease had notably higher levels of IL-22 circulating in their blood compared to individuals with healthy mucosa. This increase in IL-22

levels is likely a compensatory mechanism of the body. Moreover (17) found In patients with Crohn's disease, the level of IL-22 expression increases during intestinal inflammation. This suggests that this cytokine system plays a significant role in preserving the intestinal barrier by promoting the movement of intestinal epithelial cells, indicating its vital role in managing intestinal inflammation and aiding in wound healing (18).

Conclusion

In conclusion, there is important role of the inflammatory cytokines il-6 and il-22 in the inflammation of celiac disease and potential role for these cytokines in the pathogenesis and management of the disease. Adherence to a gluten-free diet appears to have a beneficial effect on modulating the inflammatory response and improving clinical outcomes in celiac disease. Future research should focus on elucidating the underlying mechanisms by which dietary interventions influence cytokine activity and exploring potential therapeutic strategies targeting aIL-6 and IL-22 in the management of celiac disease.

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