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RIGINAL ARTICLE

Detection of Interleukin-33 in Patients with Celiac Disease

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ABSTRACT

Background: *Celiac disease (CD) is a chronic autoimmune disorder triggered by gluten ingestion in genetically predisposed individuals. Recent research highlights the potential role of interleukin-33 (IL-33), an inflammatory cytokine released by epithelial and endothelial cells, in the pathogenesis of CD.* ***Objective:*** *To evaluate serum IL-33 levels in patients with celiac disease compared to healthy individuals and assess its potential involvement in disease development.* ***Methodology:*** *A total of 128 participants were enrolled, including 64 diagnosed celiac disease patients and 64 healthy controls. The study was conducted at Al-Husainyah Hospital, Al-Hassan Al-Mojtaba Hospital, and Imam Al-Husain Medical City. Five milliliters of venous blood were collected from each participant and centrifuged at 3000 rpm for 10 minutes. Serum IL-33 levels were measured using the enzyme-linked immunosorbent assay (ELISA) method. Participants with other autoimmune or gastrointestinal disorders were excluded. Patient data were collected through structured questionnaires.* ***Results:*** *Celiac disease patients showed significantly elevated serum IL-33 levels compared to the healthy control group.* ***Conclusion:*** *The findings suggest that IL-33 may play a significant role in the immunopathology of celiac disease and could serve as a potential biomarker for disease activity.*

INTRODUCTION

Celiac disease (CD) is defined as an autoimmune-related enteropathy that is more likely to occur in genetically susceptible people, and it can be activated through the eating of gluten proteins, which are found in several different cereals like rye, barley, and wheat[1]. People with a genetic predisposition may develop inflammatory gut disease attributed to the inability of digestive enzymes in the intestines to completely degrade gliadin proteins, which are found in specific meals [2]. Gluten is a complicated collection of many closely similar proteins that are not soluble in water but are soluble in alcohol. This type of material stands out for its high amino acid concentration, which includes glutamine and proline. These amino acids contribute to the gastrointestinal tract's particular resilience to protease degradation [3]. The CD rate in Saudi Arabia is 3.2%, the highest rate was among Arab nations [4]. The worldwide prevalence of CD in the last decade elevated from 1% to 1.5% [5]. This may be attributed to alterations in environmental factors that affect the body's response to dietary gluten and advancements in diagnostic methods that have higher sensitivity [6]. The CD rate in Arab countries, especially in Saudi Arabia is 3.2%, to becoming the highest among them [4]. An independent investigation in Iraq recorded the CD prevalence among patients who suffered from irritable bowel syndrome (IBS) at 12.1%[7]. The serological analysis of celiac disease mainly includes anti-gliadin (AGA), with anti-tissue transglutaminase (anti-tTG) antibodies to both IgA and IgG, by the ELISA technique [8].

Interleukin-33 is a nuclear element that regulates the secretion of different pro-inflammatory genes, like IL-8 and IL-6 [9]. Interleukin-33, an essential cytokine from the interleukin-1 (IL-1) group, is extensively expressed in many organs, mainly in epithelial and endothelial cells [10,11]. It works as a ligand for the ST2 receptor, stimulating many immunological cells, like T-cells, natural killer cells, and memory T-cells that provide an essential role in immunological responses [12]. In 2005, Suppression Tumorigenicity-2 (ST2) was discovered to be a receptor for interleukin-33, whereas it was first recognized as the only receptor for this cytokine in 1993 [13,14]. During apoptosis, IL-33 is not released. In necrosis or tissue damage, the attachment of IL-33 to ST2 facilitates the transfer of perforin-2 from the cytoplasm into the cell membrane, resulting in opening formation and enabling the escape of IL-33 from an inner to an external cellular state [15]. IL-33, mainly called alarmin, plays a role in increasing innate immune system activation. IL-33 makes up the first molecules that indicate a break in the essential protection of the gut epithelium toward inflammation. Under stable conditions, IL-33 cannot be completely released from cells. Consequently, IL-33 serves as a crucial and starting signal for the body's immunity in response to breaks in mucosal structure resulting from the breakdown of epithelium [16].

This study was done to detect the level of the monocyte chemotactic protein-1 and determine its role in the CD pathogenesis.

METHODOLOGY

The study design was a case-control study, and it was done at Al-Hussainyah General Hospital, Imam Al-Hassan Al-Mujtaba Hospital, and Imam Al-Husain Medical City. The samples and information were gathered between November 2024 to February 2025.

The study groups

The whole study included 128 participants. The research consisted of two groups: 64 healthy controls and 64 celiac disease (CD) patients. The patients were identified via a physician, either already or recently diagnosed by a doctor of the gastrointestinal tract. Control subjects were free of CD.

Inclusion criteria: This research comprised people of (18-50) ages and of both genders.

Exclusion criteria: patients having diabetes mellitus, ischemic heart disease, any autoimmune disorder, gastrointestinal problems, renal diseases, and Pregnancy before the trial were eliminated from the sample collection.

Sample collection and methods

Five milliliters of blood from controls and patients was withdrawn by venipuncture into a sterile tube. The blood was allowed to coagulate at ambient temperature before centrifugation at 3000 rpm for 5 minutes. The serum from each sample was withdrawn and kept in Eppendorf tubes, which were kept directly at -20 °C until required to carry out ELISA test.

Immunological Assays

The enzyme-linked immunosorbent assay (ELISA) approach was used to assess blood levels of IL-33, using a biochemical kit provided by Bioassay Technology Laboratory (BT LAB) company and mated Laboratory Methods.

The levels of anti-tTG IgA, anti-tTG IgG, anti-gliadin IgA, anti-gliadin IgG, and IL-33 were measured using enzyme-linked immunosorbent assay (ELISA) kits (BT Lab, Shanghai, China). The following kits and catalog numbers were used: Anti-tTG IgA (Cat. No. E1651Hu), Anti-tTG IgG (Cat. No. E1652Hu), Anti-gliadin IgA (Cat. No. E1541Hu), Anti-gliadin IgG (Cat. No. E1542Hu), and IL-33 (Cat. No. E1363Hu). All procedures were performed according to the manufacturer's instructions. Absorbance was measured at 450 nm using a microplate reader, and concentrations were calculated using standard curves.

Ethical approval

The study protocol was reviewed and approved by the Institutional Review Board of Imam Hassan Al-Mujtaba Pediatric Teaching Hospital and the Ethics Committee of Imam Al-Hussein Medical City (Approval No. IRB/2024/015). All procedures were conducted in accordance with the Declaration of Helsinki and local regulations for human subject research.

Statistical Analysis

This study's data was conducted using SPSS version 29, with results analyzed at a significance level of equal to or less than 0.05 ($p \leq 0.05$). The immune marker MCP-1 was measured with tTG IgA, tTG- IgG, Anti-gliadin- IgA, and Anti-gliadin- IgG, and these markers were compared between patients and healthy groups using analysis of variance (ANOVA). Additionally, patients and healthy individuals were compared in the serum IL-33 level using the Independent Samples Test. The correlation coefficient (R^2) was also calculated for this marker to determine the strength of the relationships between IL-33 and diagnostic anti-tTG and anti-gliadin antibodies.

RESULTS

Table 1 shows the difference between CD patients and the control group according to the level of IL-33. These findings indicated that the Patients group (681.63890± 66.79162) has a higher IL-33 concentration in comparison to the healthy group (595.70665 ± 75.66618), with a significant difference P-value < 0.001. According to the diagnostic tests anti-tTG (IgA, IgG), the result indicated an increased concentration of serum tissue transglutaminase IgA antibodies in the group of CD patients (189.80625± 24.40437) more than healthy people (2.86562± 0.31068) with its significant difference elevated (p <0.005).The result indicated an increased concentration of serum tissue transglutaminase IgG antibodies in the group of CD patients (70.90000± 13.65853), more than healthy people (3.446687± 0.29256), with its significant difference elevated (p <0.005).

Also our results indicated an increased concentration of the serum anti-gliadin IgA antibodies in the group of CD patients (27.70312±7.21253), more than in healthy people (3.25781±0.33834) with its significant difference elevated (p <0.005), as in the table (1). The result indicated an increased concentration of serum anti-gliadin IgG antibodies in the group of CD patients (17.26093±3.48464), more than in healthy people (3.159375± 0.25255), with its significant difference elevated (p <0.005).

Table 1. Difference between the celiac patients group and the healthy group based on the anti-tissue, anti-gliadin antibodies, and IL-33.

Study parameter	Study groups	Mean ± SD	P. value
Anti-tTG IgA	Controls	2.86562 ± 0.31068	0.015
	Patients	189.80625± 24.40437	
Anti-tTG IgG	Controls	3.446687± 0.29256	0.018
	Patients	70.90000± 13.65853	
Anti-gliadin IgA	Controls	3.25781± 0.33834	0.024
	Patients	27.70312± 7.21253	
Anti-gliadin IgG	Controls	3.159375± 0.25255	0.023
	Patients	17.26093± 3.48464	
IL-33	Controls	595.70665 ± 75.66618	0.014
	Patients	681.63890± 66.79162	

Figure 1, shows a statistically significant increase in serum IL-33 levels in celiac disease patients (681.64 ± 66.79 pg/mL) compared to healthy controls (595.71 ± 75.67 pg/mL), with a P value of 0.014.

Correlation between interleukine-33 and diagnostic antibodies anti-tTG and anti-gliadin (IgA, IgG) through the Patients Group

Figure 2 demonstrates a strong positive correlation between anti-tTG IgA and IL-33 levels, with a correlation coefficient of R^2 = 0.899, indicating a significant linear relationship.

Figure 3 shows a strong positive correlation between anti-tTG IgG and IL-33 levels, with a correlation coefficient of R^2 = 0.873, suggesting a significant linear association.

Figure 4 illustrates a moderate positive correlation between IL-33 and anti-gliadin IgA levels, with a correlation coefficient of R^2 = 0.654, indicating a meaningful but less strong linear association.

Figure 5 reveals a strong positive correlation between anti-gliadin IgG and IL-33 levels, with an R^2 value of 0.846, indicating a substantial linear relationship.

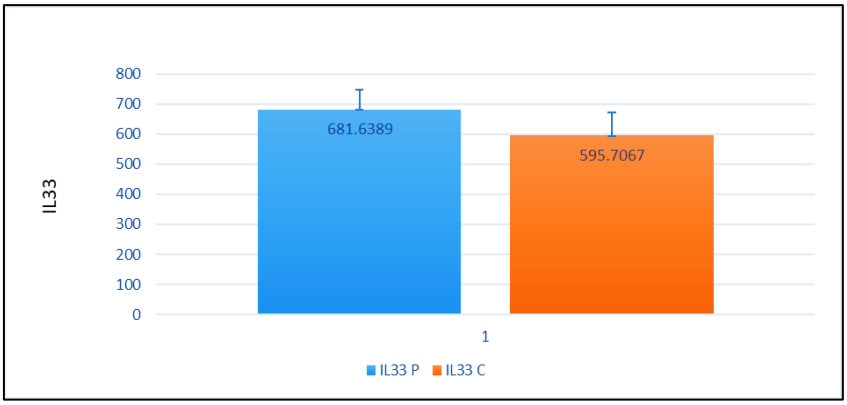

Fig. 1. Comparison between patients and healthy individuals in serum levels of IL-33.

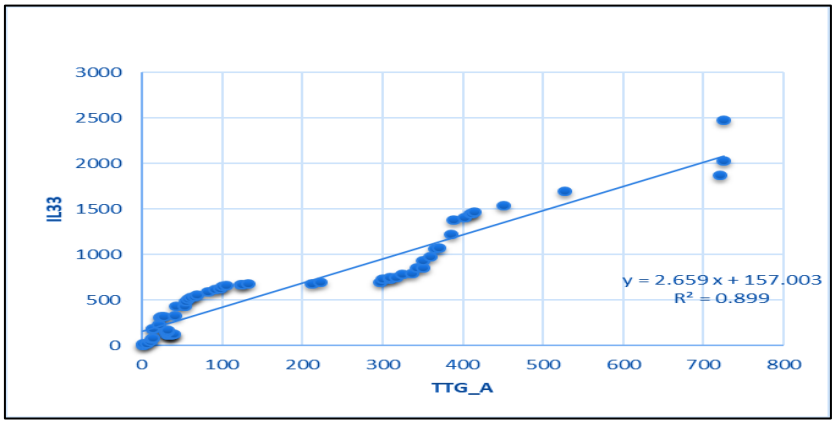

Fig. 2: Correlation between anti-tTG IgA and IL-33.

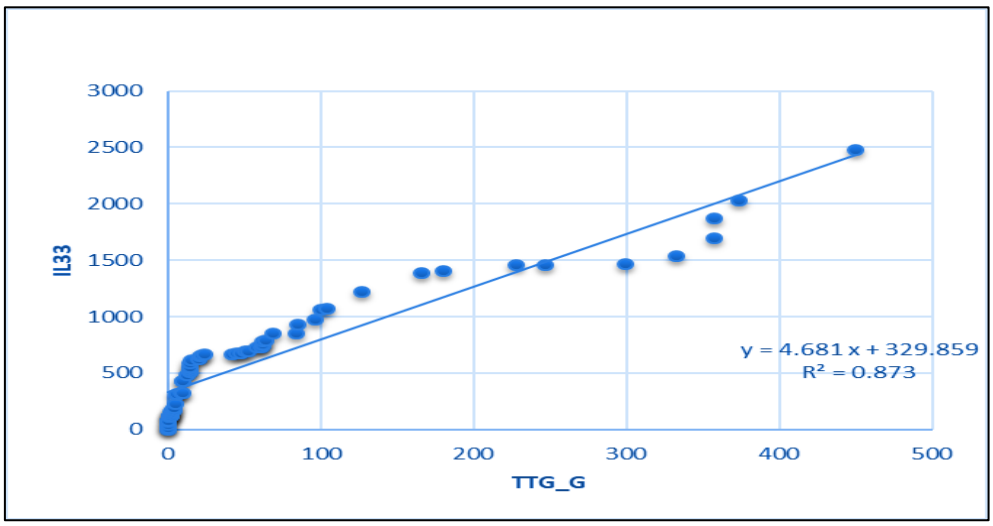

Fig. 3: Correlation between anti-tTG IgG and IL-33.

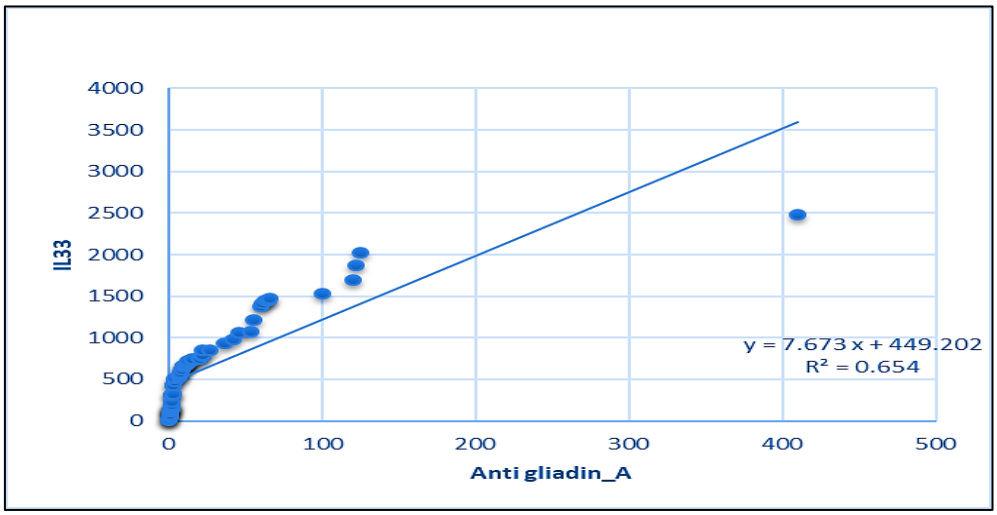

Fig. 4: Correlation between IL-33 and anti-gliadin IgA.

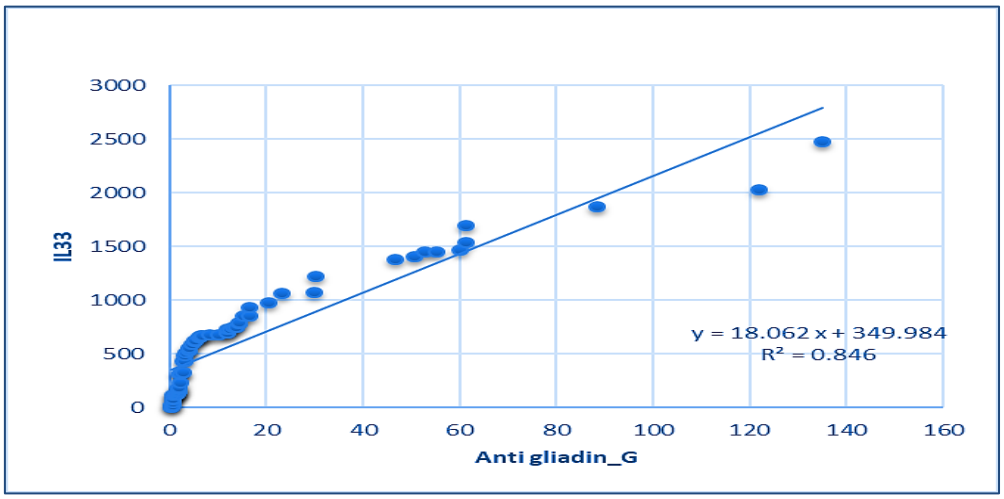

Fig. 5: Correlation between anti-gliadin IgG and IL-33.

DISCUSSION

The current study results showed a highly significant statistical difference in interleukin-33 concentration in the patients group compared to the healthy group (681.63890 ± 66.79162) versus (595.70665 ± 75.66616) and (p. value 0.014). The findings agree with the study of López-Casado, *et al.* [17], which also indicated a substantial association between the level of IL-33 and biological markers associated with CD, as well as anti-tissue transglutaminase Ab titer. Other studies done by Komai-Koma, *et al.* [18] had compatible results with this current study, indicating that increased IL-33 occurs during celiac disease pathogenesis.

An important study done by Perez et al. indicated an increased level of interleukin-33 in active patients of celiac disease more than in healthy people; these findings show the possible role of IL-33 in increasing inflammation in CD pathogenesis [19].

However, another study was also done by Schmitz et al., who demonstrated the same results as this current study, as an increased level of serum IL-33 in patients of CD [13].

The reason for high IL-33 in those patients is due to the intestinal Epithelial Damage. Gluten ingestion in individuals with CD triggers immune-mediated damage to the small intestinal epithelium. Damaged epithelial cells release IL-33 as a danger signal to stimulate the immunological responses. IL-33 is known to activate innate lymphoid cells (ILC2s), dendritic cells, and mast cells, contributing to the inflammatory cascade. Interleukin-33 enhances Th2 and Th17 responses and promotes the activation and differentiation of Th2 and Th17 cells to exacerbate inflammation in the gut mucosa. An important elevated function of IL-33 in Fibrosis and Tissue Remodeling, often seen in advanced celiac disease. Targeting the IL-33/ST2 pathway is being investigated as an option to reduce inflammation and improve mucosal healing in celiac disease.

CONCLUSIONS

The current study results showed that the elevated serum level of IL-33 is linked with the early stages of celiac disease.

Declarations:

Consent for publication: Not applicable

Availability of data and material: Data are available upon request.

Competing interests: The author(s) declare no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

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