The Impact of Interleukin-15 Serum level and Gene Expression in Celiac Disease for Sample of Iraqi Patients\

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Abstract

Background and Aims: The inflammatory cytokine IL-15 has a significant role in celiac disease (CD) development. This research aims to determine the impact of serum levels of interleukin-15 and its relationship to gene expression in patients with celiac disease.

Materials and Methods: "Sixty patients with celiac disease and thirty healthy-looking controls had peripheral blood samples obtained. IL-15 serum levels were determined by an enzyme-linked immunosorbent assay (ELISA) technique. Total RNA was extracted, and the mRNA expression levels of the IL-15 gene were investigated by the quantitative real-time polymerase chain reaction (qRT-PCR) method".

Results: There was a statistically significant ($p \leq 0.000$) increase in IL-15 serum levels between CD patients and seemingly healthy controls. The IL-15 gene expression was also significantly higher in patients than in the healthy control group ($p =\leq 0.000$).

Conclusion: The findings indicated that IL-15 could be a valuable and sensible marker for distinguishing celiac disease patients from healthy controls. However, further study is needed to comprehend Interleukin-15's diagnostic value properly.

Keywords: *Celiac disease (CD), Interleukin-15, Gene expression, qRT-PCR.*

INTRODUCTION

Autoimmune diseases such as Celiac disease (CD), psoriasis, and vitiligo appeared with high prevalence in Iraq in the last decade (1,2). Gluten proteins (found in wheat, rye, and barley) trigger an immunological response in genetically people prone to developing “celiac disease (3). About 1.4% of the world's population suffers from CD”, a common auto-immune disease that affects the small intestine and can strike at any age and affects women more frequently than men 2:1 (4,5). The onset and progression of CD can be traced back to environmental and genetic factors (6). It is widely acknowledged that T-cell-mediated celiac disease is caused by “gliadin-derived peptides that are deaminated by tissue trans-glutaminase and presented to lamina propria T- helper lymphocytes by antigen-presenting cells (7). After being stimulated, the latter, in addition to macrophages, secrete pro-

inflammatory cytokines, most notably IL-15 (8). It leads to the invigoration of intraepithelial lymphocytes, resulting in the CD's distinctive histologic changes (9). Interleukin-15 performs various biological functions critical for the retention and employment of various cell types; its up-regulation has been reported in many organ-specific autoimmune disorders, despite regulating its expression. The increase of IL-15 expression in the intestinal mucosa has become a telltale sign of CD, an intestinal inflammatory disorder caused by gluten intake (10). Interleukin-15 is overexpressed in both the lamina propria and the gut epithelium, and it acts on almost every cell type of the innate and adaptive immune systems", influencing several immunological pathways to disturb immune homeostasis in the gut, it increases epithelial cell death via apoptosis and causes atrophy in the villous and crypt hyperplasia, resulting in a decrease in intestinal absorptive area. However, because the small intestine contains a reserve functional capacity, the clinical manifestations of this inflammation are somewhat variable. Symptoms may be absent or severe, depending on nutritional status (11). Increased stool frequency, stomach pain, malabsorption, fatigue, and weight loss are the most prevalent symptoms in adults. Often subtle, these symptoms may not be recognized for quite some time after they first appear. Abnormal clinical symptoms are pretty uncommon. There are examples of severe protein and energy deficiency due to widespread malabsorption, characterized by abdominal distention, steatorrhea, and malnutrition. A lack of nutrients is a common cause of anemia and inadequate bone mineral density (12).

MATERIALS AND METHODS

This research was carried out between the 15th of November 2021 and the 15th of February 2022 on sixty celiac disease Iraqi patients (17 males and 43 females) with ages ranged 7-76 years who were clinically diagnosed by a consultant medical staff in the Hilla Teaching Hospital Babylon/ Iraq, Central Public Health Laboratory (CPHL) Baghdad/ Iraq, in addition to thirty healthy individuals (control group) who had been randomly selected to be matched with the patients regarding age and gender. Blood samples from the patient and control groups were obtained to obtain sera. Before usage, all samples were kept at -70°C. "The serum level of IL-15" was determined by using ELISA Kit (My BioSource, USA).The RNA extraction was also assessed using a NanoDrop device. "According to the manufacturer's instructions, a fixed quantity of RNA was reverse-transcribed into complementary DNA (cDNA)" using the QubitTM RNA HS Assay Kit (Q32852), ProtoScript® First Strand cDNA Synthesis Kit (E6300S), and QubitTM dsDNA HS Assay Kit (Q32851, USA, UK, and USA). Using the Luna Universal qPCR Master Mix (M3003S), "2 mL of each cDNA was amplified in a competitive reverse-transcription polymerase chain reaction (Rt-pcr)" to normalise each reaction (UK). "This real-time quantitative polymerase chain reaction was carried out using the Applied Biosystems 9600 real-time PCR system with the following temperatures and times: denaturation for 30 seconds at 95 °C, annealing for 34 seconds at 57 °C, and extension for 34 seconds at 72 °C". Primers used in this research came from IDT® (Belgium). The table provides the name and sequence (1). The relative quantitation of interleukin-15 gene expression and the average cycle of threshold values for each

sample were computed using the Livak formula.

"ΔCT (Patient)= CT (Patients gene) - CT B2M (House Keeping Gene)"

"ΔCT (Control)= CT (Control gene) - CT B2M (House Keeping Gene)"

"ΔΔCT = ΔCT (Patients) - ΔCT (Control). Fold of IL-15 gene expression = 2- ΔΔCT"

Table (1): Name and Sequence of Primers

Primer	Sequence	References
IL-15	Forward-- 5'-TTAAGGTGGCGCATCTGGAG-3' Reverse-- 5'-ATCCATCCACAATGCCTCCG-3'	Newly Designed
B2M	Forward-- 5'-CTGGGTTTCATCCATCCGACA-3' Reverse--5'-TCAGTGGGGGTGAATTCAGTG-3'	

MORAL ENDORSEMENT

Research ethics committees at the University of Baghdad's Institute of Genetic Engineering and Biotechnology, all participants and the hospitals where samples were taken provided the necessary approvals for the study to proceed.

STATISTICAL ANALYSIS

The Statistical Program for Social Science (SPSS) was employed to assess the impact of several research parameter components. T-test was used to compare percentages and the least significant difference. Statistically substantial and highly significant values were regarded as less than (0.05)* and (0.01)**, respectively

RESULTS

Detection of IL-15 serum level and gene expression in the patients and control groups

"Serum level measurement of IL-15 in the patients as compared with control clarified that the mean ± SD of IL-15 level was 39.01 ± 9.89 pg/ml in the patient group while it was 19.49 ± 3.02 pg/ml in the healthy controls, through the results, it was noted that the mean serum levels of IL-15 are significantly higher in patients with CD than in healthy controls with a significant difference (p-value≤ 0.000) as shown in Table" (2).

"Table (2): Serum IL-15 levels in Celiac disease patients compared with healthy control group".

Parameter		Number	Mean±SE	P-Value
IL-15 (pg/ml)	Patient	60	39.01 ± 1.27	0.000**
	Control	30	19.49 ± 0.55	

GENE EXPRESSION OF INTERLEUKIN-15

The expression level of the IL-15 gene was examined between 30 patients with Celiac disease and 9 healthy controls using real-time reverse transcription polymerase chain reaction and the relative quantification method. "Table (3) showed high expression of the IL-15 gene in 18 (60%) mean ± SD (1.74 ± 1.75) of the celiac disease patient group, whereas regular gene expression appeared in the 9 (100%) mean ± SD (0.98 ± 1.50) healthy controls with a highly significant difference.

(p=0.000).

Table (3): Gene Expression of Interleukin-15 in celiac disease patients

IL-15		Number ( )%	Fold of expression ($2^{-\Delta\Delta CT}$) Mean±SE	P.value
Patients	High gene expression	18 (60) %	1.74 ± 0.65	0.000**
Control		9 (100) %	0.98 ± 0.26	

DISCUSSION

Different autoimmune diseases are associated with levels of cytokines and may lead to serious health complications (13). This analysis aimed to evaluate IL-15 expression in celiac disease on both the mRNA and serum levels. Untreated CD patients had considerably increased IL-15 expression in their peripheral blood compared to healthy controls. IL-15 is a pro-inflammatory cytokine with multiple biological functions in CD pathogenesis (14). T cells overproduce cytokines when exposed to gluten, setting off an inflammatory response and revving up B-lymphocytes, which in turn leads to the creation of particular antibodies and the activation of autoimmune processes (15). It is thought that IL-15 plays a significant role in mucosal damage and the persistent inflammatory state. Dendritic cells, macrophages, and intestinal epithelial cells contribute to the production of IL-15, which boost the cytotoxicity of intestinal epithelial lymphocytes and modify apoptotic signals that eliminate damaged cells to maintain a healthy intestinal epithelium. In diseases like active CD, linked to cell-damaging agents, IL-15 is overexpressed by enterocytes and lamina propria mononuclear cells (LPMCs). (16). As the celiac disease progresses, intestinal epithelial cells (IECs) increase IL-15 expression levels and their role in conventional CD4+ T cell biology, where the IL-15 affect the initial T cell activation, which has been documented (17). Compared to healthy controls, Aghamohamadi et al. found that IL-15 gene expression in CD patients with Marsh II was elevated in biopsy specimens (18). IL-15 overexpression was also clarified in patients with active CD by Mention et al. (19). Many organ-specific autoimmune disorders have been linked to IL-15 upregulation. Intestinal lamina propria and epithelium both have elevated levels of IL-15. The expansion of IELs with a cytotoxic nature in CD patients depends on IL-15 expression by IECs. By acting on certain cell types and regulating specific immunological pathways, IL-15 disturbs intestinal immune homeostasis. While initially discovered as a T-cell growth factor, IL-15 has since been found to influence virtually every type of cell in the immune system, both innate and adaptive (20). Intraepithelial CD8+ cytotoxic T cells (IE-CTLs) need adaptive anti-gluten immunity and IL-15 upregulation in IECs to become fully activated killer cells that can cause tissue

damage. In addition, activating CD8 T cells and producing tissue damage requires the cooperation of IL-15 and CD4+ T cells. Epithelial cell death can be triggered by exposure to non-immunodominant gluten peptides and has been linked to the activation of CD4+ T lymphocytes specific for gluten peptides and the generation of IL-15 by innate immune cells (21). Patients with active CD exhibit increased IL-15 levels in the lamina propria and the intestinal epithelium compared to healthy controls and inactive CD patients (22). Intriguingly, CD patients with active, latent, or GFD express IL-15 differently (23). Increased levels of IL-15 in the intestinal mucosa were identified in patients with active CD in a previous investigation (24). Furthermore, Abadie and Jabri (25) validated the present finding that IL-15 levels were considerably more significant in individuals with untreated CD compared to control group which confirmed the result of the present study.

CONCLUSION

Increased mRNA expression and IL-15 serum levels were seen in celiac disease patients compared to healthy controls, suggesting that "IL-15 serum levels" and gene expression might be helpful and reasonable indicators for identifying CD patients from apparently healthy control.

DECLARATIONS

I attest that the paper's authors have all been approved for publication.

Data and materials are readily available.

The authors report no competing interests about the submission of this paper for publication.

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