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Il-2 Expression in Different Levels of Celiac Disease Patients

Mahmood Shaker Jabbar[1], Muslim Idan Mohsin[2]

[1] *Department of Pathological Analyses, Faculty of Science, University of Kufa, Najaf, Iraq.*
Email: mah.tech11@gmail.com
[2] *Department of Pathological Analyses, Faculty of Science, University of Kufa, Najaf, Iraq.*

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Abstract

Celiac disease (CD) is an autoimmune gastrointestinal ailment brought on by the ingestion of peptides found in wheat, rye, barley, and potentially oats in people who are genetically predisposed to developing the disease. Gluten, the cohesive protein mass generated by washing wheat dough to remove starch and other water-soluble components, has been labelled as dangerous proteins. Celiac disease is poorly known, including its method of presentation and immune response mechanism. To better understand the immunological response generated by celiac disease, this study analysed the demographic and clinical characteristics of 200 patients with whole blood and serum from the disease. ELIZA and qPCR were also used to measure the amount of IL-2 in the gene and serum at different stages of disease. The finding shows that IL-2 is an important part of the immune response at different stages of disease. The data showed that the titters of people with a new disease were much higher than those of healthy people. However, the titters of healthy people didn't change when they were treated or kept away from gluten. It seems that IL-2 may have a direct role with celiac disease not only at the first stage of diseases but also at relapsed cases.

Keywords

IL-2, CD, qPCR, Gluten and Relapsed Cases.

The autoimmune disorder known as celiac disease (CD) affects the digestive system. Gluten, which is rich in the peptide gliadin, is the culprit. Intestinal enzymes are unable to break down gliadin in the diet of genetically sensitive people. The result is an inflammatory reaction in the intestines(1). Gluten is a protein that can be broken down by alcohol but is difficult to digest due to its composition of several immunogenic peptides. Gluten's primary protein, gliadin, is a complex peptide abundant in the amino acids glutamine and proline. Because of their indigestibility, both of these components might cause an inflammatory response un the intestines, which can have serious and lasting consequences. Wheat, barley, rye, and Kamut are just few of the grains that contain gluten. (2).

The incidence of celiac disease has increased in recent decades, with a global prevalence of approximately 1-1.5%, due in part to increased sensitivity of diagnostic tools and changing environmental responses to dietary gluten (3).

Celiac disease is an autoimmune disorder, and it primarily affects females. Gluten-sensitive enteropathy, an asymptomatic inflammation of the small intestine mucosa that responds favourably to a gluten-free diet, is the definitive diagnosis. (4). The condition is characterised by both symptomatic and asymptomatic enteropathy, as well as a number of extraintestinal symptoms that can have catastrophic consequences. Celiac disease is the most prevalent autoimmune condition affecting people today(5). Due to the current state of knowledge and diagnostic possibilities, the

detection rate of diseases, especially atypical and subclinical diseases, has been continuously increasing in recent years in both industrialized and developing countries (6).

CD is diagnosed by looking at clinical, serological, and small bowel biopsy results. In suspected cases, autoantibody serological identification against targets like tissue transglutaminase (anti-TTG) and gliadin (AGA) is a good, non-invasive way to find out if someone has CD(7). (HLA-DQ2 and/or HLA-DQ8) are well-studied hereditary risk factors for gluten enteropathy. Differentiating sporadic from familial celiac disease relies on this genetic characteristic. (8). Detecting CD at an early stage of the disease can prevent its serious complications, such as iron deficiency anaemia, delayed bone growth and development, and infertility. A gluten-free diet prevents the development of small bowel adenocarcinoma as a late consequence(9) .

Celiac disease is a T cell-mediated disease in which tissue transglutaminase breaks down peptides from gliadin and antigen-presenting cells show them to T helper (Th) lymphocytes in the lamina propria. When proinflammatory cytokines are activated and released, this leads to the activation of intraepithelial lymphocytes and the histopathological changes that are typical of CD. (10). The level of IL-2 is closely related to CD activity and can be used as a reliable marker to detect minimal excess of GFD (11). Serum IL-2 levels in patients with celiac disease reflect specific immune activation in response to gluten intake. Therefore, measurement of serum IL-2 may be useful in assessing treatment response in patients with celiac disease (12). There are there are several pitfalls in surveying people with celiac disease, and there aren't enough markers for diagnosing the disease at its various stages. In this light, researchers all around the world are trying to find better ways to diagnose celiac disease and better understand the immunological response it elicits. To approve that: it was hypothesised that testing of IL-2 is induced up/down based on different cases of celiac disease which are: Relapsed, A new cases, fully treated and Gluten free diet.

Methodology

Collection of the specimens

Between September 5, 2022, and January 15, 2023, researchers collected approximately 200 samples from celiac disease patients and healthy controls of varying ages and sex. Where they were separated into five categories: new patients, patients with recurrent disease, those who needed a gluten-free diet, therapy patients, and healthy patients. Each patient had two samples taken, one of serum and one of whole blood, and both were frozen immediately after collection; however, triose was added to the whole blood samples to protect the RNA before freezing.

Real time RT-qPCR

Whole blood samples from -ve (control), relapsed patients, gluten-free diet, and treated cases are processed with GB-Bio RNA Mini Kits to isolate the mRNA. At a rate of 200xg for 5 minutes, whole blood cells were separated. For the qPCR, we used a Reverse Transcription Kit to generate complementary DNA (cDNA). The PCR was run with Primer Design Precision's 2x qPCR SYBR green Master Mix. Plates with sets of primers and probes for the genes of interest and for use as a quality assurance measure in the lab. The 7900HT Abi Prism equipment was used to conduct 40 cycles of qPCR on the plate. The fluorescence emission from the product can be used to pinpoint the cycle point using qPCR. Target IL-2 levels are proportional to the Ct value.IL-2 primers are used F: CAACTGGAGCATTTACTGCTGG, R: TCAGTTCTGTGGCCTTCTTG and GAPDH primers are used F: TGCACCACCAACTGCTTAGC and R: GCATGGACTGTGGTCATGAG. These primers were purchased from the South Korean company macrogen. Relative expression was normalised by comparison to established reference genes. When comparing relative fold expression differences, the Ct technique was utilised.

The Estimation of Human IL-2

All of the samples were tested for IL-2. We got the samples ready according to the plan. Brief centrifugation of the serum to filter out debris before an instantaneous assay. After reconstituting the standard with 1.0 mL standard sample diluent to create a stock solution of 500 pg/mL, 500 ul of standard sample diluent is pipetted into a 250 pg/mL tube and the remaining tubes. initial steps included adding 100 l of standard and/or sample to each well. It was incubated at 37 degrees for 90 minutes with the plate covered. Three times, 1 x wash buffer is aspirated and used to wash each well. After a 30-minute incubation at 37 degrees Celsius, a 100 l working solution of biotin-conjugated anti-human interleukin-2 antibody is added to each well. Three times 1x Wash buffer is used to wash each well. After replacing the adhesive strip on the micro titter plate, 100 l of HRP-avidin working solution is poured into each well. After washing three times with 1 x wash buffer, the mixture was incubated for 30 minutes at 37°C. After adding a 100ul TMB substrate to each well and gently mixing, the plates are placed in a dark incubator at 37°C for 15-20 minutes. Each well had 50 l of stop solution put to it to halt the color change. Within 30 minutes, the optical density of each well is measured with a micro plate reader at 450 nm. After compiling all of the data, the IL-2 concentration in the mystery sample could be estimated.

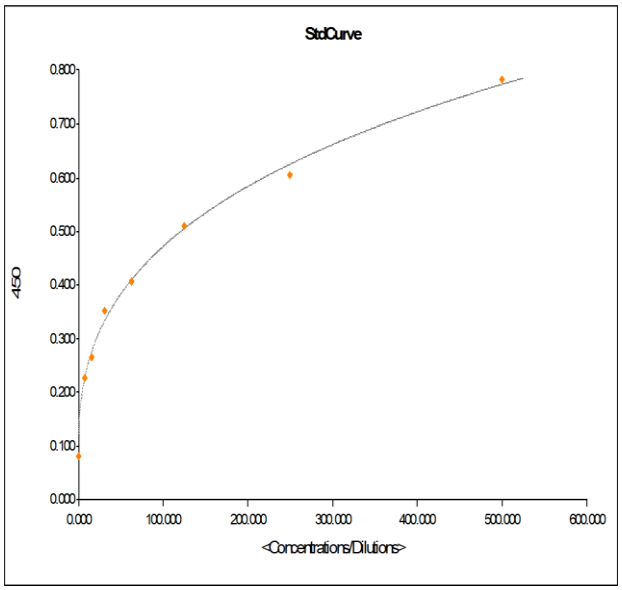

Figure 1: The standard curve of Interleukin **2**concentration (mg/dl)

Statistical Analyses

All graphs and statistical analysis were created and analysed using GraphPad prism.9. One- and two-way analysis of variance (ANOVA) for multiple comparisons were used to identify the samples with statistically significant differences. The data is displayed as a mean standard error of the mean.

Results and Discussion

The aim of study to find a new technique for diagnostic the celiac disease in different cases. First of all, it was collected 200 samples based on different criteria. Samples have to meet the following criteria between September 5, 2022 and January 15, 2023: new cases, relapsed cases, cases treated with medication, cases treated with a gluten-free diet, and healthy cases. Anti-tissue transglutaminase (IgG and IgA) and anti-gliadin (IgG and IgA) antibodies have been used to test all of the samples. The ELISA (Enzyme-Linked Immunosorbent Assay) method lies at the heart of the chorus gadget. The antigen has become embedded in the solid state. Incubation with diluted human serum results in the binding of specific immunoglobulins to antigens. The unbound protein is washed away, and then the sample is treated with an anti-human immunoglobulin antibody that has been coupled to horseradish peroxidase. Unbound conjugate is removed and peroxidase substrate is added. The colour produced is directly proportional to the concentration of the specific antibody present in the serum sample. Disposables contain all reagents to perform tests in the Chorus/Chorus TRIO instrument. Results are expressed in arbitrary units (AU/mL), device for diagnosis a celiac disease. The result shows that the percentage of relapsed disease is about 24%, while a new disease 22.5%, and Gluten free diet samples were about 22%. However, the treated samples were the less percentage from all collected samples which was 16.5% as demonstrated in (Figure 2). It seems that the percentage of relapsed and new diseases were highest than other groups. It could be return to our condition for collection or the celiac disease were increased at these years. This result was in agreement with(13). have find the percentage of celiac disease increasing at these

days. Furth more,(14) have also find at these days the celiac disease was increased. However,(15).

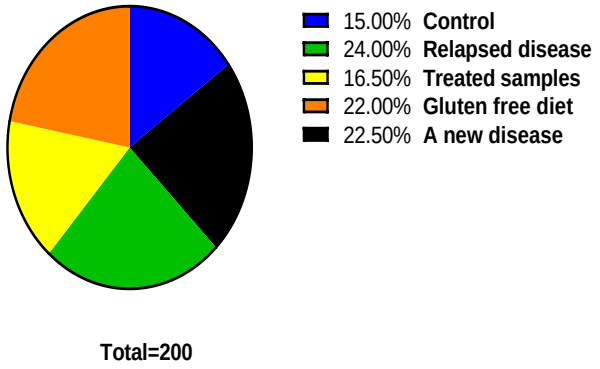

Figure 2: The percentage of the different types of celiac disease samples that were taken from different people.

The results show the percentage of 200 samples from different cases of celiac disease that were isolated in different labs in Al-Najaf province. Anti-tissue transglutaminase and anti-gliadin tests were used to check all of the samples for celiac disease.

The Changes of IL-2 genes in response to celiac disease

The goal of this part was to see if celiac disease changed how IL-2 was turned up or down. In short, the RNA was taken out, and a reverse transcriptase kit was used to make cDNA. After looking at the qPCR results, the equation 2^(-ΔΔCt) was used to figure out the fold change. The results show IL-2 were mostly increased with new disease and relapsed disease rather than treated cases or Gluten free diet as demonstrated in **figure 3**. Interestingly, The IL-2 were significantly increased with a new disease group P-value 0.0001 **** more than relapsed disease group, but still increase with both of these two groups. These results were agreed with others researchers' findings shown by (16) However, IL-2 were no changed at mRNA levels in both groups treated with drug and Gluten free diet as show in figure 4.2. It seems that the IL-2 can use as good marker for detect the celiac disease. These results were similar with another result obtained by(17).

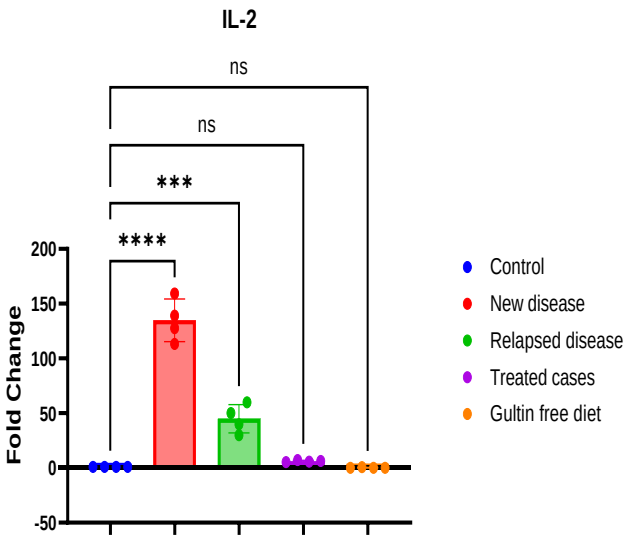

Figure 3 The IL-2 gene expression has changed in response celiac disease with different cases.

qPCR has been used to measure the amount of **IL-2** in patients with new celiac disease, relapsed celiac disease, cases that have been treated and are on a gluten-free diet, and cases that are healthy. After figuring out the expression of the housekeeping gene **GAPDH**, the 2-Ct method was used to measure the expression of IL-2 in both healthy and sick people. Whole blood cells changed because of IL-2. One-way ANOVA was used to see how important the changes were, where ***** = p0.0001, *** = p0.001, and ns = not significant. The data are the averages of three different experiments with 200 samples each.

The Changes of IL-2 Protein in response to celiac disease

The aim of this section was to approve the IL-2 also are changed with protein levels as changed with gene expression within different cases of celiac disease. To do this, all samples are collected and classified according of our criteria. The serum was collected and kept at -20 c from healthy and non-healthy people. The IL-2 Eliza kit (solorbio-China) was used according to manufacturer instructions using sandwich Eliza technique, and the results were analysed using standard curve technique. The results showed a significant increased with a new disease compared with healthy cases titers, However, there is non-significant changes with other group of our criteria which are old a new

disease, treated and gluten a free diet cases compared with healthy people, as demonstrated in figure (**4**). These results were agreed with others researchers' findings shown by (16) At 4 and/or 6 hours after a matched gluten-free challenge, IL-2 levels were considerably higher than they were in patients with newly diagnosed illness. Responder analysis found that no patients who had taken the gluten-free food challenge were responders to any cytokine, based on the threshold level established in sham-challenged participants as it had been for the injection of gluten peptides. It is consistent with activated CD4+ T lymphocytes being the driver of cytokine release in both circumstances because the serum cytokine profile after ingestion of gluten is less prominent but qualitatively similar and over a matching time course to that after injecting gluten peptides(17). and Patients on a gluten-free diet and those being treated may benefit from a gluten challenge accompanied by assessments of symptom intensity and serum IL-2 levels, as was shown in the current study. A positive IL-2 result would provide strong evidence for a CD diagnosis, while a negative IL-2 result may help pinpoint individuals who require further diagnostic workup for CD. The severity of symptoms and the extent to which IL-2 levels rise in response to a routine challenge may help stratify patients with confirmed CD into those most likely to benefit from the supplementary pharmacological treatments under investigation (18).

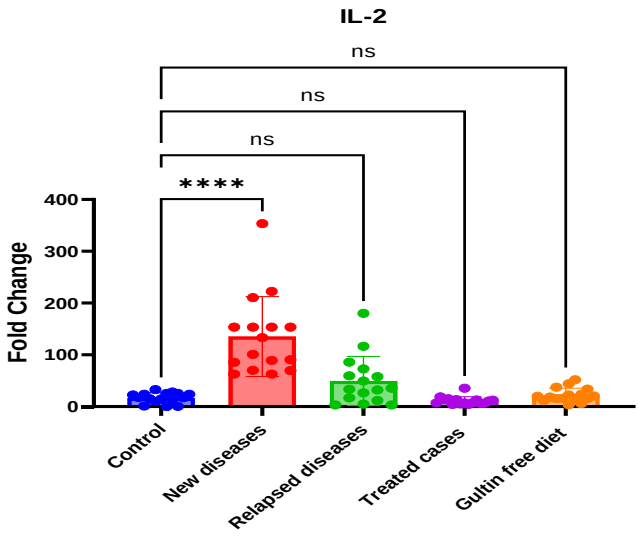

Figure 4 The IL-2 protein expression has changed in response celiac disease with different cases.

Eliza has examined IL-2 protein expression in people who have been diagnosed with celiac disease at various stages, including those who are newly diagnosed, those who have relapsed, those who have been treated with a gluten-free diet, and those who are healthy. Standard curve estimation with manufacturer data was used to determine IL-2 expression in healthy and unhealthy instances. Changes in IL-2 were seen in WBCs. One-way ANOVA was used to test for statistical significance between groups, yielding the following results: ***** p0.0001 significant; ns=non-significant. Means and standard deviations are presented for three trials involving a total of 200 replicates.

In conclude, celiac disease is considered one of the most important and widespread diseases currently that affects all ages of adults and children and causes them health problems, especially children, causing them poor growth and wasting. IL-2 is up-regulated at mRNA levels with a new infection, old a cute infection. IL-2 is changed only with a new infection at protein level compare with control and our criteria which are acute old infection, treated cases and Gluten free diet.

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Declaration

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Ethics Clearance

This article does not contain any studies with human participants or animals performed by any of the authors

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