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Original Research Article

Molecular Estimation of IL-15 Gene Effect and *H. Pylori* Prevalence in Celiac Disease

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Abstract: Remarkable positive associations across IL-15 values and the histopathological severity of celiac disease (CeD) indicate that interleukin (IL)-15 plays an essential part in the immunopathogenesis of the illness. There has been inconsistent evidence in certain research linking Helicobacter pylori to CeD. The goal of this meta-analysis was to measure the connection between CeD and H. pylori. Furthermore, the investigation examined the influence of H. pylori on the indications and categorization of CeD. This study includes 35 patients with CeD. They were fully informed of the study's goals before providing their signed consent. A group of 35 healthy blood donors made up the control group. Blood was drawn, transferred into gel tubes, and centrifuged. Serum specimens from both participants and controls have been obtained and then kept at -20°C in a deep freezer for subsequent use. It have been investigated the molecular relationship between IL-15 gene and CeD. It have been investigated the prevalence of *H. pylori* and CeD. The result of age and CeD incidence correlation indicates that there is no significance between age samples parameter and CeD incidence (control= 12.83 ± 0.4211, n=35 patients=12.23 ± 0.3358, n=35, p value=0.2692). Results of correlation between gender and CeD incidence indicates that number of male samples (24) and CeD incidence is more than that of female samples (11). Results of correlation between urban-rural residence and CeD incidence indicate that the average CDAT score for those who live in rural was higher in the urban area (good adherence to a gluten-free diet).

Keywords: IL-15 Gene, *H. Pylori*, Celiac Disease, CDAT Score, Genetic Tree.

INTRODUCTION

The immunopathogenesis of celiac disease (CeD) is particularly well-understood. The disorder is characterized by responsiveness to gluten proteins, which are present in grains, and the lesion is limited to the gut. However, the diagnosis can be made if highly disease-specific autoantibodies against transglutaminase 2 are found in the blood [1].

Ancient Greece is where celiac disease (CeD) was first documented. The Greek word koiliakós, which implies abdominal, is where the name celiac originates. But it wasn't until the 1940s that the illness was connected to wheat ingestion [2].

CeD was formerly thought to be a food hypersensitivity illness, but more research has revealed that it shares many characteristics with tissue-specific autoimmune disorders including rheumatoid arthritis and type 1 diabetes. Autoimmune disorders are typically characterized by strong correlations with the human leukocyte antigen (HLA), the production of unique autoantibodies, and immune-mediated death of a particular cell type (enterocytes). Nevertheless, CeD is distinct from other autoimmune diseases in that it develops only after being exposed to an external cause. The group of linked grain proteins known as gluten is made up of this alien pathogenic driver. Gluten technically refers to the cereal storage proteins found in wheat, but it is often used to refer to comparable proteins found in rye, barley, and oats. All of these cereals cause patients to experience negative immunological responses, with the exception of oats, which are usually regarded as safe. As of right now, the only treatment for CeD—a condition with a pooled prevalence of 1.4% that affects most communities worldwide—is the rigorous elimination of gluten from the diet [3].

The medical profession has identified several distinct clinical patterns associated with CeD. Classical CeD presents with weight loss, anemia, or failure to thrive along with malabsorption signs and symptoms include steatorrhea and diarrhea. However, malabsorption does not indicate non-classical CeD. This distinction is important because, if a doctor with a strong index of suspicion decides to investigate the source of an iron deficiency that no other etiology can account for, some patients with gastrointestinal symptoms and no clear functional criteria for CeD may be appropriately diagnosed with the disease [4].

Anemia is thought to be the most prevalent extraintestinal symptom of celiac disease, with up to 20–30% of patients exhibiting anemia. Up to 85% of people have been found to have iron deficiency anemia in some investigations. Malabsorption is believed to be caused by the ensuing atrophy of the duodenal mucosa, and a number of micronutrient and mineral deficiencies, such as those involving folate, vitamin B12, and vitamin D, may play a role in the development of anemia. Although anemia is a typical sign of celiac disease, not all occurrences of this phenomenon can be explained by dietary inadequacies; in certain cases, inflammation appears to play a role, as demonstrated by the prevalence of anemia of chronic disease in individuals with the disease [5].

Anemia can also result from an infection with Helicobacter pylori (H. pylori). It is thought to afflict around half of all people on the planet and is one of the most prevalent bacterial illnesses globally. Abdominal discomfort, nausea, bloating, and heartburn are just a few of the stomach and intestine symptoms that can be brought on by H. pylori. Due to the persistent inflammation that an H. pylori attack causes in the stomach and small intestine, bleed in the gastrointestinal system can occur. Although the bleeding is usually moderate, it can lead to over time losses of blood and malabsorption, and this can end in anemia. The most common type of anemia brought on by an H. pylori infection is microcytic anemia, which is defined by small red blood cells, low hemoglobin levels, and low blood iron levels [6].

However, only about 1% of these people go on to acquire CD, pointing to the potential influence of additional genetic variables. Human genome research has more recently revealed numerous non-HLA gene loci and particular SNP connected to the cytokines implicated in CD [7].

Interleukin (IL)-15 is one among these cytokines, and it plays a vital part in the immunopathogenesis of CD, as seen by the strong positive association found between IL-15 levels and the disease's histological severity. It is virtually nonexistent in the lamina propria and only occurs on villous enterocytes in healthy individuals. Conversely, enterocytes and lamina propria mononuclear cells enhance it when the body is in an inflammatory state [8].

A combined immune response occurring in the intestinal epithelium and lamina propria characterizes the inflammation in CD. It encompasses the immune systems that are innate and adaptive. Gluten-derived peptides attach to disease-predisposing HLA DQ2 and DQ8 molecules on antigen-presenting cells shortly after digestion and alteration by tissue transglutaminases. This results in a CD4+ T cell respond in the lamina propria, which is the main indicator of inflammation in CD patients. Interferongamma, IL-10, and IL-15 are just a few of the proinflammatory cytokines secreted as a result of this action. Together with this adaptive T cell response, there is also an innate response that activates intraepithelial lymphocytes and is primarily triggered by IL-15. The recruitment of B cells and the production of antibodies to tissue transglutaminase and deamidated gluten are driven by T cell activation [9].

The present study aimed to manifestation the molecular effect of IL-15 gene and prevalence of Helicobacter pylori in developing Celiac disease.

MATERIAL AND METHODS

Samples Collections

This study includes 35 patients with CeD. They were fully informed of the study's goals before providing their signed consent. A group of 35 healthy blood donors made up the control group.

Blood was drawn, transferred into gel tubes, and centrifuged. In order to be used later, the collected serum samples from patients and controls were stored at -20°C in a deep freezer.

ILISA Test

The IL-15 was estimated using ILISA test, for (70) samples, (35) patients and (35) control groups.

IL-15 Molecular Estimation

Genomic DNA from white blood cells (WBC_S) for both patients and group were extracted by using DNA extraction kit (favorgen) according to the leaflet of kit; (Special protocol frozen Blood). For IL-15 rs2857261 genotyping (12), a set of primers exist in table (1).

Table 1: A set of IL-15 rs2857261 primers

Primer	Sequence	Product	References
forward primer	5′ - TCTTCAATACTTAAGGATTTAC -3′	250 bp	7, 12
Reverse primer	5′- AAGAAGAGCCTATCAAGATG --3′		

***H. Pylori* Serological Estimation**

It has been investigated the prevalence of *H. pylori* and CeD. using *H. pylori* Antibody Rapid test cassette.

Analytical Statistics

To undertake genetic analysis, the Chi-square ($\chi 2$) test was used. P-values below 0.05 are taken into account. The SPSS 19 version was utilized for doing statistical analysis.

RESULTS AND DISCUSSION

Correlation between CeD Development and Age

Table (2) reveled results of correlation between age and CeD incidence.

Table 2: Correlation between age and CeD incidence

Age	Control	Patients	P-value
	12.83 ± 0.4211, n=35	12.23 ± 0.3358, n=35	0.2692

The result above indicates that there is no significance between age samples parameter and CeD incidence. This result accepted with many other studied.

This result was consistent with research by Green, and Hauser *et al*., found no significant correlation between the incidence of disease and age [13]. Pezhman Alavinejad, *et al*., (2014) accepted with the present result.

It has already been discussed for numerous years and is still unclear if age at the time of a CeD diagnostic is associated with an increased chance of developing an autoimmune disorder. A prior investigation that included 1268 unaffected controls and 909 CeD patients discovered a strong correlation between an older CeD diagnostic age and a greater probability of having an autoimmune illness. Our null link between increased risk of autoimmune illness and advanced CeD diagnostic age (presumably longer gluten exposure and duration of active CeD while undiagnosed) could be explained by a number of possible causes [15].

Correlation between CeD Development and Gender

Table (3) reveled results of correlation between gender and CeD incidence.

Table 3: Correlation between gender and CeD incidence

Gender	Control	Patient
Male	20	24
Female	15	11

The result above indicates that number of male samples and CeD incidence is more than that of female samples. This result not accepted with other studied due to some reasons. One of them is that due to the race of samples or for the number of samples.

The multivariate logistic regression analysis corroborated some of these gender and sex variations. In particular, the women gender was 3.39 percent more likely than the men gender to have indicators of malabsorption and to have symptoms or signs for a longer period of time prior to the CeD diagnosis. When it comes to gastrointestinal problems, the multivariate analysis revealed that women patients were more likely than male patients to report upper GI symptoms such nausea/vomiting, dyspepsia, and constipation. On the other hand, it was discovered that men patients had a considerably higher representation of osteopenia/osteoporosis and a lower BMI than female patients [16].

In 2019, Rodrigo Ferreira Lima *et al.*, discovered that at the point of assessment, male and female with CeD had different clinical presentations. Compared to male, female showed higher clinical indications and gastrointestinal issues [17].

Dietary Compliance for Celiac Disease on the Rural–Urban Spectrum

Table (4) reveled results of correlation between Rural–Urban Spectrum and CeD incidence.

Table 4: Correlation between Rural–Urban Spectrum and CeD incidence.

Geographical distribution	Control	Patients
Urban	15	24
Rural	20	11

The purpose of this study was to look at how living in a rural or urban area affected other factors, such as dietary adherence, which is necessary for managing celiac disease. The investigation found that there was a moderate impact size difference between the dietary adherence of those living in urban and rural locations.

The current study's findings contradict those of Zanini et al.'s study, which revealed no difference in dietary adherence across urban and extra-urban areas based on geographical location [18].

In other hand our results is accepted with Amy Posterick and Candace L. Ayars, (2023), who indicate that the average CDAT score for those who live in cities was higher in the area of good adherence to a gluten-free diet. The mean CDAT score of those residing in non-urban areas indicated insufficient adherence to a gluten-free diet. Therefore, it is possible that people with celiac disease who live in rural areas have consumed enough gluten on average to increase their risk of the condition getting worse. On the other hand, those who live in cities generally seem to follow the diet sufficiently to prevent the progression of the illness and other consequences. The observed notable variation in dietary compliance may be attributed to limited availability of gluten-free food options, healthcare services, and therapies in non-urban regions.

***Helicobacter pylori* Infection Association with CeD**

The result of *Helicobacter pylori* infection association with CeD development and due to low sample number, show no significance correlation between them, as in table (5), while many studies shows a strong relationship.

Table 5: Comparison of study groups infected with H. pylori (positive & negative)

Infection result	control	Patients	Total	P-value
Positive	20	21	41	0.981
Negative	15	14	29	
Total	35	35	70	

Correlation between CeD and ELISA test

The results of ELISA test revealed that there is a strong relation between IL-15 and developing of CeD, as in figure (1).

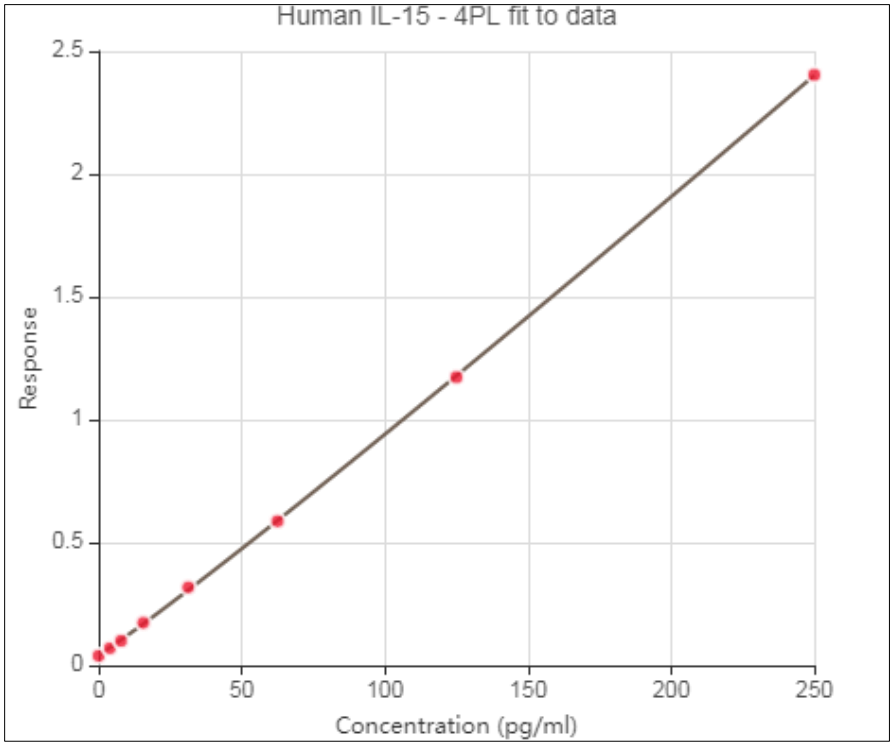

Figure 1: Correlation between CeD and IL-15 ELISA test

Table (6) indicates the high significance of IL-15 and CeD development, which give 0.0001 *P-value*.

Table 6: Correlation between CeD and ELISA test

Patients	Control	P-value
40.42 ± 1.651****, n=35	17.61 ± 0.533, n=35	0.0001

Lack of a standardized test to correctly sample for IL-15 amounts in blood, small sample size, and brief research period and tissues that had been exposed to an anti-IL-15 antibody were the study's primary limitations. Furthermore, it was not possible to rule out selection bias, and further research with a bigger sample of CeD patients over a longer period of time is necessary to confirm the encouraging results.

The study concludes that IL-15 induced inflammatory activation plays a significant role in the development of celiac disease.

These results accepted with (Stefan L. Popa and Giuseppe Chiarioni, 2020) and Paola Sindaco, *et al*., (2023) [20,21].

Prolonged overexpression of cytokines can set off biological processes that result in immune cell hyperproliferation and malignant transformation. In addition to its contentious involvement in the etiology and progression of different hematological malignancies, the cytokine interleukin-15 (IL-15) plays a critical role in providing a cytotoxic boost to tumoral cells [21].

Relationship of *IL-15* Gene and Disease Development

Figure (2) indicates the genetic tree of *IL-15* gene between patients and control groups.

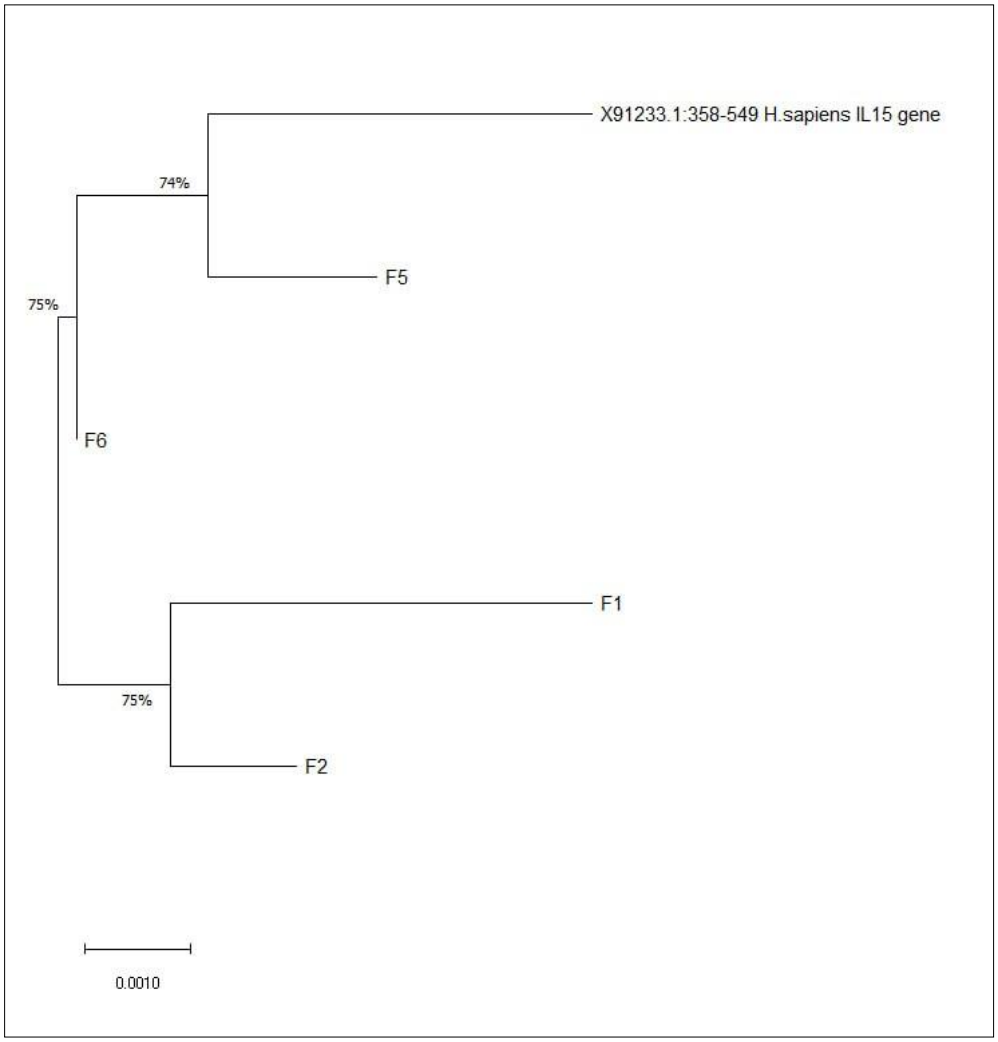

Figure 2: The genetic tree of *IL-15* gene between patients and control groups

According to Manoochehr Rasouli *et al*., (2013), there is some genetic diversity in the association between patient samples, as indicated by the above finding [22]. Based on this study, it is possible to hypothesize that these gene variations, which likely have an impact on IL-15 production, are the variables determining VL in the Iranian population. However, more research is advised to elucidate the relationship between these polymorphisms and the IL-15 level.

CONCLUSION

The result *of Helicobacter pylori* Infection Association with CeD development and due to low sample number, show no significance correlation between them.

Ethical Approval

This paper was confirmed to the ethical approval and rules, and it was reviewed and ensured by a local ethics committee which numbered by number 1742 on November 24, 2022.

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