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DETERMINATION OF SOME IMMUNOLOGICAL MARKERS AMONG PATIENTS WITH CELIAC DISEASE IN DIWANIYAH PROVINCE

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Abstract

Eating gluten causes celiac disease, a systemic immune-mediated illness, in people who are genetically predisposed. Barley, the protein complex known as gluten, is present in wheat, rye, and rye. Celiac disease is characterized by a wide range of clinical signs, a specific serum autoantibody reactivity, and variable small-intestinal mucosal injury.

Aim of the study: The study aims to evaluate the role of soluble HLA-G as an immunological marker of CD and other factors in pathogenicity, including pro- and anti-inflammatory cytokines: Soluble HLA-G in patient serum, Serum levels of proinflammatory cytokines (TNF-α and IL-6) and anti-inflammatory cytokines (IL-10) in celiac disease patients and the small intestine's tissue inflammatory response. A case-control study of 65 individuals (300 suspected patients and 24 controls) aged 3–73 years was conducted at AL-Diwanyah Educational Hospital to measure serum levels of sHLA-G, IL-6, TNF-α, and IL-10. Blood samples were processed and stored at -20 °C. sHLA-G levels were measured using ELISA. The study was ethically approved by the University of AL-Qadisiyah.

Results: HLA-G, a class I molecule expressed in placental cells, showed significantly higher serum levels in patients (16.57 ± 3.33 pg/ml) compared to controls (5.88 ± 1.97 pg/ml, $P \leq 0.001$). Proinflammatory IL-6 levels were also elevated in patients (433.42 ± 40.63 pg/ml vs. 228.02 ± 27.36, $P \leq 0.016$), as were anti-inflammatory IL-10 levels (163.94 ± 28.87 pg/ml vs. 66.46 ± 17.89, $P < 0.02$).

Conclusions: Celiac disease is linked to female gender, family history, and high sHLA-G and TNF-α levels.

Introduction

One of the most prevalent autoimmune diseases, celiac disease (CD), is linked to small intestine inflammation brought on by consuming gluten-containing foods (Trynka et al., 2010). The Weight loss, fatigue, diarrhea, abdominal distention, stomach pain, and malnourishment are some of the proximal symptoms and signs of CD. Inflammation, villous atrophy, and crypt hyperplasia are the results of an immunological reaction to ingested wheat gluten and comparable rye and barley proteins. The presence or absence of gastrointestinal symptoms determines the classification of CD: "classical" or "symptomatic" CD refers to presentations that include malabsorption syndrome

and diarrhea, while a normal or asymptomatic type has no gastrointestinal symptoms (Fasano, 2005). When the characteristic enteropathy is discovered in patients who seem to be in good condition, CD is considered silent. CD refers to the likelihood of having a typical CD later in life; patients have tissue transglutaminase (TTG) and endomysial antibody (EMA) antibodies, as well as a normal or somewhat aberrant mucosa and an HLA DQ2 or DQ8 predisposing genotype (Louka and Sollid, 2003 & Kim et al., 2004).

Last but not least, refractory sprue, a particular form of CD, is characterized by significant villous atrophy and elevated intraepithelial lymphocytes (IELs) even when a strict gluten-free diet is followed (Schuppan et al., 2005). Despite significant advancements in our understanding of the illness, there haven't been many developments in treatment.

2. Materials and Methods.

2.1-Study design :

A case control study was conducted on 65 patients who have been diagnosed with. The present study was conducted on the following study group. A total of 65 individuals of both sexes, comprising 300 Suspicious Persons as the patient group with an age range of 3-73 years, furthermore 24 individuals randomly selected as the control group with an age range of 4-73 years. A questionnaire form (Appendix-1) was used to record special notes. A sample of 5 ml of blood from each individual that obtained from AL-Diwanyah Educational Hospital in Diwanyah province by puncturing a vein in a conventional, sterile tube. After allowing a blood sample to clot at room temperature, it was centrifuged for five minutes at 3000 rpm. Each sample's serum was separated into multiple 300 µl aliquots and promptly refrigerated at -20 °C until it was needed. Serum was the subject of several studies, including those on sHLA-G, IL-6, TNF-α, and IL-10.

2.2-Ethical consideration

This study, conducted at the AL- Diwanyah Educational Hospital by the University of AL-Qadisiyah, was approved by the Clinical Research Ethics Committee.

2.3- Blood sample collecting

Three milliliters of blood were extracted from each patient's vein and put in a test tube containing gel and sodium citrate for biochemistry analysis and ELISA detection of soluble HLA-G. Blood samples were centrifuged in gel tubes at 3000 ×g for 10 minutes in order to get a serum sample. The sample was then stored in three different Eppendorf tubes at -20 °C in the freezer until the study was needed.

2.3.2. Determination of Soluble HLA-G by ELISA

2.3.2.1. Serum Levels of Soluble HLA-G

Using a commercially available kit (MyBioSource USA, Cat No MB2600014), the levels of sHLA-G in serum were measured. sHLA-G ELISA is a sandwich enzyme immunoassay that uses a monoclonal anti-sHLA-G antibody to quantitatively detect the soluble forms of HLA-G in pre-coated microplate wells. It particularly measures the soluble isoform HLAG5 and the membrane-bound HLA-G1 shed.

3-Results

3.2. Immunological study of soluble human leukocyte antigen g (sHLA-G) serum levels

The HLA class I heavy chain paralogues include HLA-G. Beta-2 macroglobulin is a class I molecule that is a heterodimer made up of a heavy chain and a light chain. The membrane serves as an anchor for the hefty chain. Placental cells generated from fetuses express HLA-G. Its gene has eight exons, and the heavy chain is roughly 45 kDa. The leader peptide is encoded by exon 1, the alpha1 and alpha2 domains are encoded by exons 2 and 3, both of which bind the peptide; the alpha3 domain is encoded by exon 4, the transmembrane region is encoded by exon 5, and the cytoplasmic tail is encoded by exon 6.

Highly significant differences ($P \leq 0.001$) between patients (16.57±3.33) and control (5.88± 1.97) were recorded in serum sHLA-G (Table 3.2).

Studied groups	No.	Mean ± S.D Pg/ml	Min.-Max.	*P* value
Patients	65	16.57± 3.33	(10.98-21.1)	0.001
Control	24	5.88± 1.97	(2.62- 9.49)	

3.2.2 Determination of proinflammatory cytokines

3.2.2.1 Evolution of Interleukin 6 (IL-6) Serum Levels

IL-6 in disease, immunology, and inflammation. A cytokine with pleiotropic properties, IL-6 causes hepatocytes to produce acute phase proteins such as CRP, serum amyloid A, fibrinogen, and hepcidin while suppressing albumin synthesis. By promoting the formation of antibodies and the development of effector T cells, IL-6 also has a significant impact on the acquired immune response. Furthermore, IL-6 can encourage the growth or differentiation of a number of nonimmune cells. Numerous disorders are brought on by the dysregulated, ongoing production of IL-6 due to its pleiotropic action. VEGF is vascular endothelial growth factor; RANKL is a receptor activator of nuclear factor κB (NF-κB) ligand; and Treg is a regulatory T cell.

According to the data (Table 3.3), patients with celiac disease had higher levels of IL-6 (433.42±40.63) than controls (228.02±27.36), with a significant difference ($P \leq 0.016$).

Table (3.3) Concentration of IL-6 in patients and control groups.

Studied groups	NO.	Mean ± S.D Pg/ml	Min.-Max.	*P* value
Patients	65	433.42±40.63	(364.0-493.7)	0.016
Control	24	228.02±27.36	(187.6- 284.8	

3.2.3. Determination of anti-inflammatory cytokines

3.2.3.1 Evolution of Interleukin 10 (IL-10) Serum Levels

According to recorded data, there was a significant difference ($P < 0.02$) between the IL-10 levels of celiac disease patients (163.94±28.87) and control patients (66.46±17.89) (Table 3.5).

Studied groups	NO.	Mean ± S.D Pg/ml	Min.-Max.	*P* value
Patients	65	163.94±28.87	(111.6-212.9)	0.02
Control	24	66.46±17.89	(40.7- 93.9)	

4. Discussion

4.1. 4.1. Study Characterization

Gluten consumption causes damage to the small intestine in those with celiac disease, an autoimmune condition. Since primary prevention is impossible for celiac disease, the discussion surrounding mass screening ought to be revived (Arend et al., 2005; Briani et al., 2008).

4.1.1. Age Distribution

There is no age-related group associated with celiac disease. It can manifest at any age. For example, if person tests negative for celiac disease at age 50, it's likely that they will experience symptoms at age 65 because gluten intolerance can develop at any age. (Crespo-Escobar et al., 2017), This is consistent with recent age-related findings that showed no discernible variations in patient age. The majority of celiac disease (gluten-sensitive enteropathy) patients, according to the current study, were between the ages of 21 and 30. This finding is consistent with numerous local and international studies (Ducharme et al., 2013; Benchimol, 2014; Cohen et al., 2014; Mullen et al., 2014; Namatovu et al., 2014). According to reports, sixty percent of instances of celiac disease that were documented were in people aged twenty-five. They mentioned that CD rose with age and could be caused by a variety of factors, such as lifestyle choices, radiation exposure, hormones, and heredity.

Comparable to the age reported in other research (Krigel et al., 2016; Ahlawat et al., 2017; and Smith et al., 2017), Globally, women are more likely than men to have CD. Although the exact cause is unknown, it might have something to do with the hormonal changes that females with celiac disease experience. Additionally, males with celiac disease are far more likely to acquire autoimmune illnesses than men without the condition (Ludvigsson et al., 2016).

4.2. Immunological study

4.2.1. Soluble Human leukocyte antigen G(sHLA-G)

Every case chosen for this investigation exhibits CD symptoms. According to the statistical analysis, patients' sHLA-G levels differed significantly from controls' ($P \leq 0.001$). The findings of the present investigation are consistent with those of research by Carosella et al. (2008), Chen et al. (2010), Provatopoulou et al. (2012), and Castelli et al. (2014), which identify the primary risk factor for the development of disease.

As anticipated, patients who refuse a gastroscopy and those who are currently following a gluten-free diet but are reluctant or unable to participate in a gluten challenge. When evaluating family members of individuals with celiac disease, sHLA-G testing may be helpful (Murray et al., 2008).

4.2.2. Interleukine 6 (IL-6)

When compared to controls, patients with active celiac disease had significantly higher serum levels of IL-6 ($p \leq 0.016$), suggesting that IL-6 may be a key target for cytokine-specific treatments (Jericho et al., 2017).

Similarly, it has been demonstrated that individuals with CD had significantly higher serum levels of IL-6, which were positively connected with the disease's inflammatory activity (Nenna et al., 2016). Celiac illnesses have been linked to IL-6 in a number of studies (Lionetti et al., 2010; Kapoor et al., 2013; Ertekin et al., 2014).

Lastly, scholars interested in using these data to study celiac disease may find this work useful. Nonetheless, this data can be very useful tools for expanding relevant medical knowledge.

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