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Estimation of NESFATIN-1 and VEGFA Levels in Iraqi Celiac Disease Patients Infected with *Helicobacter pylori*

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Abstract

Celiac disease(CD) is an autoimmune disease. It is associated with atrophy of the intestinal villi, leading to reduced absorbed nutrients. Nesfatin-1 is a peptide that is used as a laboratory detection tool in CD detection. VEGF plays a role in the permeability of endothelial functions. *Helicobacter pylori* are colonized and cause stomach injury in adults and children. *H. pylori* are cause chronic gastritis, duodenal ulcers, and adenocarcinomas. The study estimated the NESFATIN-1 and VEGFA levels in celiac disease patients with *H. pylori.* (41) CD with *H. pylori* and (31) CD without *H. pylori*, (52) control group is included in the study. Nesfatin-1 and VEGFA were measured in the entire participant. Nesfatin-1 was higher in CD than in the control group. There was no significant difference of Nesfatin-1 levels in patients with *H.pylori* compared to patients without *H.pylori*. It was lower in men than women and lowered in persons under 35 years of age than in persons over 35 years of age. VEGF was higher in patients with CD, it was higher in men than in women, and higher in persons less than 35 years of age than in persons with more than 35 years of age; also, it was higher in less than 35 years of age than in more than 35 years of age, a high level of VEGFA was detected in CD. There was no significant difference VEGFA levels in patients with *H.pylori* compared to patients without *H.pylori* .VEGF expression change is reported in some tumors; it is unsuitable for detecting celiac disease but can be used for patients' follow-ups. CD cases have higher nesfatin-1 levels than in control group. Deficiencies of minerals and vitamins are detected in CD patients irrespective of age and gender. All CD cases had nutritional deficiencies. It was found that *H.pylori* does not affect the levels of VEGFA and Nesfatin_1 in celiac patients. The levels of Nesfatin-1 and VEGFA were measured by enzyme –linked immunosorbent assay (ELISA).

Keywords: Celiac disease, *Helicobacter pylori,* Vascular endothelial growth factor A *(*VEGFA), Nesfatin-1.

تقدير مستويات NESFATIN-1 و VEGFA في مرضى الاضطرابات الهضمية العراقيين المصابين ببكتريا الملوية البوابية

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الخلاصة

مرض الاضطرابات الهضمية (حساسية الحنطة) هو أحد أمراض المناعة الذاتية. يرتبط بضمور الزغابات المعوية مما يؤدي إلى انخفاض العناصر الغذائية الممتصة. Nesfatin-1 هو ببتيد مضاد للالتهابات يستخدم كأداة تشخيصية للكشف عن حساسية الحنطة مختبريا . يلعب VEGF دورًا في نفاذية الوظائف البطانية. تستوطن بكتريا الملويه البوابيه وتسبب إصابة في المعدة لدى البالغين والأطفال. تسبب بكتيريا الملوية البوابية التهاب المعدة المزمن ، وقرحة الاثني عشر ، والأورام السرطانية. كان الهدف من الدراسة هو تقدير NESFATIN-1 و VEGFA في مرض حساسية الحنطة مع الملويه البوابية. تضمنت(٤١) حالة مرض حساسية الحنطة مصابين *H. Pylori* و(٣١) حالة مرض حساسية الحنطة بدون *H. pylori*، و(٤٢) كمجموعة سيطرة. تم قياس Nesfatin-1 و VEGFA في كل المشاركين . كان Nesfatin-1 أعلى في مرض حساسية الحنطة مقارنة بمجموعة السيطرة. كان أقل عند الرجال منه عند النساء، كما كان أقل في الأشخاص الذين تقل أعمارهم عن ٣٥ عامًا مقارنة بالأشخاص الذين تزيد أعمارهم عن ٣٥ عامًا. كان VEGF أعلى في المرضى الذين يعانون من حساسية الحنطة، وكان أعلى عند الرجال منه عند النساء، وأعلى في الأشخاص الذين تقل أعمارهم عن ٣٥ عامًا مقارنة بالأشخاص الذين تزيد أعمارهم عن ٣٥ عامًا، كما كان أعلى في عمر أقل من ٣٥ عام مقارنة بعمر أكبر من ٣٥ عام . تم الكشف عن مستوى عال من VEGFA في مرض حساسية الحنطة. تم تسجيل تغيير تعبير VEGF في أنواع مختلفة من السرطانات ،هو غير مناسب للكشف عن مرض الاضطرابات الهضمية ولكن يمكن استخدامه لمتابعة المرضى. تحتوي حالات حساسية الحنطة على

مستويات أعلى من nesfatin-1 مقارنةً بـ مجموعةالسيطرة . يتم الكشف عن نقص الفيتامينات والمعادن في مرضى حساسية الحنطة غير المعالج بغض النظر عن العمر والجنس. كان كل مرضى حساسية الحنطة الذي تم تشخيصه حديثًا يعاني من نقص غذائي واحد أو أكثر.تم قياس مستويات Nesfatin-1وVEGFA بواسطة تقنية ELISA .

الكلمات المفتاحية: مرض حساسية الحنطة ،بكتريا الملوية البوابيه ، عامل نمو بطانة الأوعية الدمويه(VEGFA) ،nesfatin-1 .

Introduction

Gluten consumption leads to the complex autoimmune condition known as celiac disease (CD). Its defining characteristics are the Leukocyte Antigen of the human (DQ2 and/or DQ8), a particular genetic predisposition, and ongoing exposure to gluten. These factors increase the intestine's permeability and lead to the immune response [(1)]. The clinical signs of CD include constipation, diarrhea, malabsorptive, Anemia, neurological symptoms, dermatitis, and arthritis which are additional extraintestinal symptoms that may also be present, especially in adult patients [(2)].

Inflammation of the small intestine and atrophy of the villus is the disease's hallmarks, leading to malnutrition and malignancies. The clinical signs of CD vary and include signs related to the outside gut, such as dermatitis, herpetiformis, fatigue, and Anemia [(3)].

The most recent diagnosis of CD in patients with CD clinical signs, people with abnormalities associated with CD (malabsorption), Down syndrome, and other risk groups like first-degree relatives of CD patients, T1DM, and Down syndrome [(4,5)].

The classical form of CD has chronic diarrhea and malabsorptive features, primarily affecting younger pediatric patients [(6)]. Anemia, short stature, low bone density, chronic fatigue, skin changes, depression, and chronic fatigue are symptoms from outside the gastrointestinal tract that is more prevalent in the non-classical CD, seen in children and adults [(7)]. The disease's varied clinical presentation may cause a sizable delay or even the failure to diagnose CD [(8)]. Early diagnosis of CD and adoption of a gluten-free diet (GFD) may enhance the quality of life for CD and lower the expense of the diagnosis and treatment [(9)]. Serious complications from undiagnosed CD could include oncological conditions and infertility [(10)].

The pathological changes of the intestinal mucosa in CD are included atrophy, inflammation, and infiltration of the lymphocytes. The damage degree varies between the patients, thus resulting in a wide range of clinical signs [(11, 12)].

Materials and Methods

In this study 72 samples aged (31.1 ± 10.6) were collected from patients with celiac disease diagnosed by endoscopic and serologic tests, the samples were divided into (2) groups based on *H. pylori* presence, (41) CD with *H. pylori* and (31) CD without *H. pylori*, (52) control group aged (30.3 ± 4.16) included in this study. The serum of patients was collected from Al Imam Al Sadiq Teaching Hospital in Hilla\Babel governorate and Al Yarmok Teaching Hospital in Baghdad, Iraq from November 2021 to September 2022. The blood samples dispensed in a sterile gel tubes and left for few minutes to clot and then centrifuged at 4000 r.p.m for five minutes at room temperature in order to separate serum and dispensed into Eppendorf tubes which tightly closed and stored in Deep freez at -20 c until time of analysis. Enzyme-linked immunoadsorbent assays (ELISA) were used to measure the levels of serum VEGFA and nesfatin-1 for all the samples.

Results

A total of 72 patients (41 with *H. pylori*, 31 without *H. pylori*) fulfilled the clinical and serological criteria for the diagnosis of celiac disease. A statistical comparison using the paired t-test was carried out of the data for all the parameters between subjects marked as true celiac during the study and matched controls.

The analysis of the study's demographic aspects showed that the nesfatin-1 level was higher in CD cases than in the control group (5.17 ± 0.73 ng/ml, P<0.001) (1.5 ± 0.41 ng/ml), respectively. Standard deviation (SD) was very low, so cannot be used for comparison between patients with and without *H.pylori* concerning the biochemical markers (VEGFA,Nesfatin_1).

Table 1. Serum concentration of nesfatin_1 (ng/ml) in the groups

Group	N	Mean ± SD	P value
Patient group	72	5.17 ± 0.73	< 0.001
Control group	52	1.5 ± 0.41	

SD: standard deviation

There was a lower nesfatin-1 level in men than in the women (3.96 ± 0.73 ng/ml, P < 0.001) (6.27 ± 0.71 ng/ml), respectively, in the patient group. This gender-related variation was not established in the control group, as shown in Figure 1.

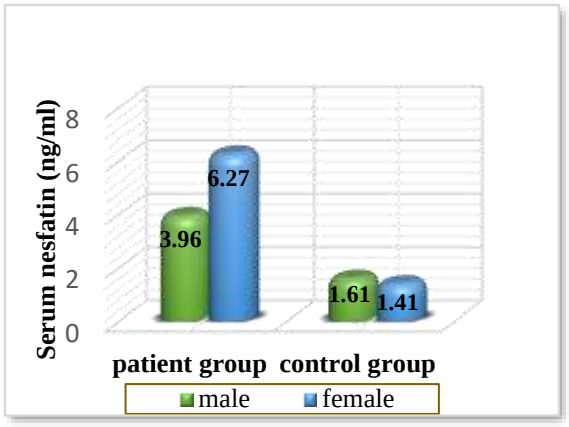

Figure 1. Comparison of the serum nesfatin-1 level (ng/ml) between males and females in the study groups

There was a significantly lower serum level of nesfatin-1 in individuals less than 35 years of age compared to individuals more than 35 years of age (4.1 ± 0.76 ng/ml, P < 0.01) (6.3 ± 0.65 ng/ml) in the patient group. This age-related variation was not established in the control group, as shown in Figure 2.

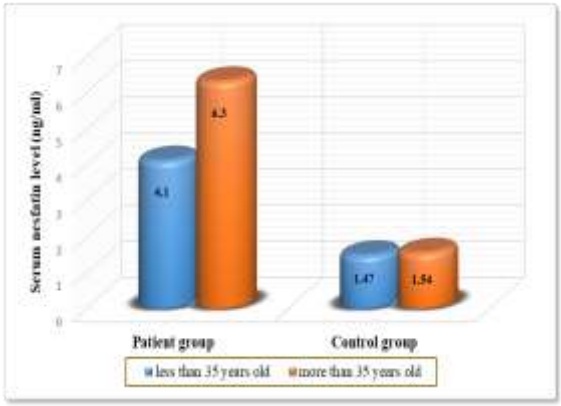

Figure 2. Comparison of the serum nesfatin-1 level (ng/ml) according to the age of individuals in the study groups

The serum level of VEGFA was higher in CD patients than in the control group (598.6 ± 182.4 pg/ml, P<0.001) (136.8 ± 23.3 pg/ml, P<0.001) respectively, as shown in Table (2)

Table 2. Serum concentration of vascular endothelial growth factor-A (pg/ml) in the study groups

Group	N	Mean ± SD	P value
Patient group	72	598.6 ± 182.4	< 0.001
Control group	52	136.8 ± 23.3	

SD: standard deviation

There was a significantly higher serum level of VEGFA in men compared to women (691.1 ±182.5 ng/ml, P < 0.001) (552.4 ± 165.5 ng/ml), respectively, in the patient group. This gender-related variation was not established in the control group, as shown in Figure 3.

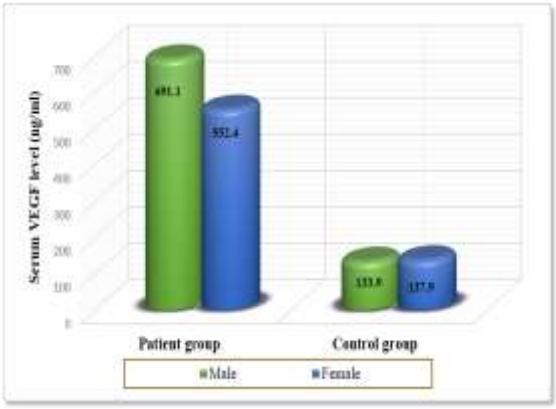

Figure 3. the serum VEGFA level (ng/ml) between males and females in the study groups

There was a significantly higher serum level of VEGFA in individuals less than 35 years of age compared to individuals more than 35 years of age (654.2 ± 204.6 ng/ml, P < 0.01) (511.2 ± 88.8 ng/ml) in the patient group. Also, there was a significantly higher serum level of VEGFA in individuals less than 35 years of age compared to individuals more than 35 years of age (140.9 ± 23.2 ng/ml, P < 0.01) (114.7 ± 8.5 ng/ml) in the control group, as shown in Figure 4.

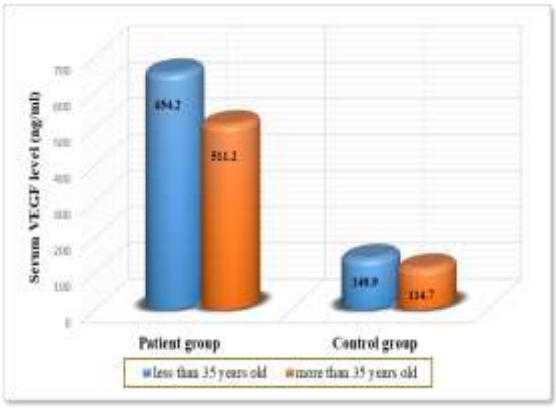

Figure 4. the VEGFA level according to the age of individuals in the study groups

Discussion

The gastrointestinal system bears the central effect of celiac disease due to the damaging effects of ingested gliadin, and therefore gluten-sensitive enteropathy is presently a preferred term. Most of these patients suffer from malabsorption of essential nutrients. B-vitamins deficiency is thought to be absorbed in the small intestine, which is the prominent site affected in the CD [13].

The current study reflects that the serum level of nesfatin_1 was higher in CD than in the control group (5.17 ± 0.73 ng/ml, P<0.001) (1.5 ± 0.41 ng/ml) respectively; these results were in agreement with another study that shows Nesfatin-1 were higher in the CD than in the IBS-D group and the control. Nesfatin-1 was higher in the IBS-D than in the control group. Nesfatin-1 can differentiate between CD cases and IBS-D cases. The histopathological reports are required to determine the nesfatin-1 role in the CD diagnosis [(14)].

In this study, there was lower nesfatin-1 in men than in the women (3.96 ± 0.73 ng/ml, P < 0.001) (6.27 ± 0.71 ng/ml), respectively, in the patient group. Male mice receiving a high-fat diet showed a decrease in serum nesfatin-1 levels [(15)], whereas this diet had no effect on female mice's gastric expression. This suggests that gender differences may be at play [(16)]. In women with anorexia nervosa or obesity, there was a positive correlation between circulating nesfatin-1 levels and anxiety, whereas this relationship was inverted in obese men [(18)]. Similar to other studies that included no differences in nesfatin-1 concentration detected between the female subjects and healthy males, this gender-related variation was not established in the control group [(19)].

The current study found significantly lower serum levels of nesfatin_1 in individuals less than 35 years of age compared to individuals more than 35 years of age (4.1 ± 0.76 ng/ml, P < 0.01) (6.3 ± 0.65 ng/ml) in the patient. This result was in agreement with [(20)] found that a healthy human (47.3 years) demonstrated a higher fasting nesfatin-1 level than the young healthy (19.4 years) [(20)].

The VEGFA was higher in the CD (598.6 ± 182.4 pg/ml, P<0.001) than in the control group (136.8 ± 23.3 pg/ml, as shown in table (3.3), vascular lesions develop in CD patients due to high levels of angiogenic factors like VEGF. There is proof that CD patients have overexpression of mucosal VEGA [(21)]. However, the serum level of VEGF remains unchanged [(22)] despite the patients' increased rectum and descending colon VEGF perfusion. Since VEGF expression has been linked to a number of illnesses, including various cancers, it cannot be used to diagnose celiac disease or as a specific biomarker, but it can be used to monitor patients.

There was a significantly higher serum level of VEGF in men compared to women (691.1 ±182.5 ng/ml, P < 0.001) (552.4 ± 165.5 ng/ml), respectively, in the patient group. There is no article or study about this current study.

In this study, there was no significant gender-related variation between males and females regarding the serum concentration of ferritin, vitamin D3, and vitamin B12 in both patient and control groups. This result agrees with another study that didn't demonstrate a marked difference in minerals and vitamin levels between women and men. However, the vitamin administrated before the disease diagnosis was more prevalent in women than men (30% vs. 13%) (152).

The present study showed significantly higher serum levels of VEGF in individuals less than 35 years of age compared to individuals more than 35 years of age (654.2 ± 204.6 ng/ml, P < 0.01) (511.2 ± 88.8 ng/ml) in the patient group. There is no article or study about this current study.

Also, there was a significantly higher serum level of VEGF in individuals less than 35 years of age compared to individuals more than 35 years of age (140.9 ± 23.2 ng/ml, P < 0.01) (114.7 ± 8.5 ng/ml) in the control group. There are no articles that agree with the current study.

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Conflict of Interest

The authors have no conflict of interest to declare.

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Author Contribution

Farah H. Mahdee**:** contributed to data gathering, analysis, practical (follow the procedure) and written parts of the study. Shurooq R. Kadhim and Wassan A. Abbas final approval and agreement for all aspects of the study, supervision, revision ,and rearrangement.

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