Title: Evaluation of the Relationship Between Celiac Disease and Refractory Epilepsy in Patients Referred to Imam Khomeini Hospital of Urmia

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Abstract

Objective: Celiac disease can be associated with other diseases, including neurological disorders. In this study, the relationship between celiac disease and refractory epilepsy was evaluated in patients referred to Imam Khomeini Hospital of Urmia.

Material & Methods: In this cross-sectional study, patients with refractory epilepsy referred to the neurology clinic of Imam Khomeini Hospital of Urmia, during the second half of 2019 and controlled epilepsy were studied as a control group. The statistical population of the present study included 50 patients with refractory seizures and 50 patients with controlled seizures. The mean age of patients was 32.96 ± 11.35 years. Five ml blood samples were taken from the patients, and a serum anti-tTG test was performed using the ELISA kit. Then, in patients with positive anti-tTG, a duodenal biopsy sample was prepared using an endoscopy.

Results: This study showed that the mean serum level of anti-tTG in patients with refractory epilepsy was higher than in patients with controlled epilepsy. Anti-tTG test results were positive in five of fifty patients with refractory epilepsy, and it was positive in two of fifty patients with controlled epilepsy. There was no significant difference between the two groups in terms of serum levels anti-tTG (p=0.14). Also, there was no significant relationship between serum level anti-tTG, age and genus (p>0.05). Biopsy results in three patients in the refractory epilepsy group and one patient in the controlled epilepsy group was in favor of a definitive diagnosis of the celiac disease. Patients in whom the celiac disease was confirmed by endoscopy had higher anti-tTG levels (p=0.006).

Conclusion: There was no significant difference between the celiac disease in the group with refractory epilepsy and controlled epilepsy.

Keywords: Celiac disease, Epilepsy, Refractory, Anti-tTG level
1. Introduction

The celiac disease or gluten-sensitive anthropopathy is an autoimmune disorder. In people genetically predisposed to celiac disease, eating gluten, which is part of the structure of many cereals, can lead to intestinal tract damage with an inadequate immune response (Gujral et al., 2012). In this disease, the intestinal villi are destroyed, and the intestinal absorption capacity is reduced. The disease has various clinical manifestations and can occur at any age, despite the prevalence of the disease in childhood and adolescence, and even adulthood, about 20% of patients are over 60 years old at the time of diagnosis (Gatti et al., 2020). The cause of the disease is gluten. Gluten is a general term for a group of water-insoluble proteins that are given different names in different grains. People genetically predisposed to DQ2, the less common DQ8 human leukocyte antigen, are more susceptible to the disease (Balakireva et al., 2016; Caio et al., 2019). The prevalence of the disease in epidemiological estimates is lower than the actual prevalence. The highest prevalence of the disease has been reported in Western Europe. For example, the prevalence of the disease in Scandinavia is 1.99%. Recent studies indicate that the prevalence of the celiac disease in the United States is comparable to that in Western Europe (Xia et al., 2020; Masood et al., 2020; Rostami et al., 2011). The four diagnostic criteria are: malabsorption due to gluten consumption in wheat and barley, atrophy of the small intestinal mucosa, the improvement of clinical symptoms of malabsorption with the improvement of atrophic symptoms of the small intestine by cutting gluten-containing foods, recurrence of clinical and histological symptoms after restarting gluten-containing foods (Rubio-Tapia et al., 2013; Rozenberg et al., 2020). Serological tests and small bowel biopsies are the most accurate diagnostic tests for celiac disease. To diagnose this disease, the initial evaluation is with serological tests. When a serological test is positive, a small bowel biopsy should be done to confirm the disease. Among the serological diagnostic tests in celiac disease, anti-tTG IgA and Ab endomysial. Anti IgA tests are the most useful and best of these tests, with high sensitivity and specificity in the diagnosis of celiac disease and provide the possibility of screening for celiac disease (Schyum et al., 2013). Anti-tTG antibodies can be easily measured by ELISA. Today, screening for the celiac disease by anti-tTG is recommended. Because EMA-IgA is a more expensive method, it is more complicated. On the other hand, tTG-IgA was able to identify all cases of false-negative EMA-IgA. The celiac disease can be associated with other diseases such as herpetic dermatitis, neurological diseases, liver, endocrine, bone metabolism, etc. (Sobhani et al., 2020). The most common neurological disorders are headache,
epilepsy, cerebellar ataxia and polyneuropathy. The relationship between epilepsy and celiac disease has been discussed in various studies. The prevalence of celiac disease among patients with epilepsy has been reported in the range of 0.5-1%. Screening is very important to prevent long-term complications of the celiac disease. Furthermore, a gluten-free diet has been suggested as a protective role in autoimmune disorders and epilepsy control (Taraghikhah et al., 2020). Since different neurological disorders such as headache, cerebellar ataxia and epilepsy have been reported in patients with the celiac disease and that the prevalence of the celiac disease in these patients with epilepsy is different in more studies, so celiac screening in patients with epilepsy can reduce long-term complications; and also due to the lack of a similar study in the country; this study aimed was to investigate the relationship between the celiac disease and refractory epilepsy in patients referred to Imam Khomeini Hospital of Urmia.

2. Material & Methods

In this cross-sectional study, patients with refractory epilepsy referred to the neurology clinic of Imam Khomeini Hospital of Urmia during the second half of 2019 and controlled epilepsy were studied as a control group. Resistant epilepsy was defined as uncontrolled epilepsy despite treatment with two drugs, with an appropriate dose for six months. This study aimed was to determine the relationship between celiac disease and refractory epilepsy in patients referred to Imam Khomeini Hospital of Urmia in the second half of 2019. The present study after obtaining permission from the committee Ethics of East Azerbaijan University of Medical Sciences and management of Imam Khomeini Hospital of Urmia. After completing the written consent form by the patients participating in the study, five ml of blood samples were taken from them, and the serum level of tissue transglutaminase (anti-tTG) IgA was assessed using the ELISA test. Then, if the above test is positive in patients, a duodenal biopsy specimen was prepared using an endoscopy and the biopsy specimen was examined by a pathologist without knowing the serological results and using Marsh criteria (Table 1).
Table 1. Biopsy Marsh criteria

| Type | Definition |
|------|------------|
| 0    | Natural and normal biopsy |
| I    | Normal appearance of mucosa with increased lymphocytes in mucosal epithelium |
| II   | hyperplastic crypts and natural villi of the intestine |
| IIIa | hyperplastic crypts and relative atrophy of intestinal villi |
| IIIb | hyperplastic and almost complete atrophic crypts of intestinal villi |
| IIIc | hyperplastic crypts and complete atrophy of intestinal villi |

2.1. Inclusion criteria

Patients older than eighteen years with refractory epilepsy; Resistant epilepsy were defined as uncontrolled epilepsy despite treatment with two drugs and appropriate doses for six months.

2.2. Exclusion criteria

Exclusion criteria were included structural disorders of the brain and electrolyte disorders.

2.3. Measurement of anti-tTG serum levels

After obtaining written consent to participate in the study, five ml of blood was taken from the participants in the study (the group of refractory epilepsy and controlled epilepsy) after centrifugation and separation of serum, it was transferred to two vials and immediately at negative temperature. Serum levels of tissue IgA tran-glutaminase (anti-tTG) were assessed using ELISA and recombinant human anti-tTG was used as the antigen (AU-tTG IgA, Erospital, Trieste, Italy). According to manual of kit, values of more than seven international units/ml were considered positive.

2.4. Duodenal biopsy

The tissue view of small bowel biopsy in people with the celiac disease has special morphological characteristics. Typically, the inner surface of the intestine has villi. In people with the celiac disease, this condition disappears and the intestinal surface becomes smooth. Histological changes are made to diagnose the celiac disease based on marsh classification definitively. In marsh I, the normal appearance of the mucosa is seen with an increase in lymphocytes within the epithelium.
of the villi. Marsh III is also seen with hyperplastic crypts and moderate to severe reduction of villi. Most patients with celiac disease at the time of diagnosis fall into the marsh III category. Marsh III is also based on the severity of the conflict in three classes IIIa, IIIb and IIIc are classified; in marsh IIIa you have a relative atrophy of the villi in such a way that the ratio of the villus to the crypt be less than three to one. In marsh IIIb we have subtotal villous atrophy and in marsh IIIc we have atrophy of the complete villi so that the small intestinal mucosa is completely similar to the colon mucosa. If the pathology is consistent with the celiac disease, a definitive diagnosis of the celiac disease is made.

2.5. Specifications of data collection tools and how to collect it

Patients participating in the study will receive five ml of blood at the beginning of the study to assess serum anti-tTG levels. If the antibody is positive, they are asked to refer to a duodenal mucosa sample.

2.6. Statistical analysis of data

After collecting information, the data were entered into SPSS 21 and statistically analyzed. Then the mean and standard deviation were used to describe the quantitative data and tables and graphs were used for the qualitative data. Independent sample test (or its nonparametric equivalent) was used to compare quantitative variables in the two groups and a Chi-square test was used to compare qualitative variables in the two groups. If the distribution is abnormal, it was evaluated using the Spearman test and p-value level less than 0.05 was considered statistically significant.

2.7. Ethical Notes

At the beginning of the study, written consent was received from all patients. No fee was charged to patients for participating in the study and performing the necessary tests. In this study, the personal characteristics of the patients were not recorded in any written document. Also, the implementation stages of the project did not begin before receiving the code of ethics from the ethics committee of West Azerbaijan University of Medical Sciences.
2.8. Executive limitations of the plan and how to reduce them

One of the limitations of the present study is the possibility of patients’ unwillingness to participate in the study. To address this issue, they were told that participating in the study would not be an unproven treatment for them, would not be harmful, and would not be costly.

3. Results

3.1. Demographic profile

The study was performed on fifty patients with refractory epilepsy and fifty patients with controlled epilepsy as a control group with the aim of determining the relationship between the celiac disease and refractory epilepsy in patients referred to Imam Khomeini Hospital in Urmia in half a year. The second took place in 1398. The mean age of patients was 32.96±11.35 years. Independent t-test showed no significant difference between the two groups in terms of age (P=0.57). Genus distribution in the two groups were significant; In the group with refractory epilepsy 46% and in the group with controlled epilepsy 62% were women (P = 0.001) (Table 2).

| Demographic variable | percentage or mean ± standard deviation | Significant level | Statistical |
|----------------------|-----------------------------------------|-------------------|-------------|
|                       | Resistant epilepsy                      | Controlled epilepsy|             |
| Age                  | 32.30±1.54                             | 33.60±1.67        | 0.57        |
| genus                | Female                                  | 46%               | 62%         | 0.001       |
|                      | Man                                     | 53%               | 38%         |

3.2. Evaluation of serum level of tissue anti-transglutaminase (anti-tTG)

The serum level of tissue anti-transglutaminase was assessed by the ELISA method. The mean serum level of the anti-tTG in patients with refractory epilepsy was 4.6 AU / ml, and in the group with controlled epilepsy was 3.0 AU / ml (Figure 1).
Figure 1. Serum levels of tissue anti-transglutaminase in two groups of refractory and control epilepsy

Anti-tTG test results were positive in five of fifty patients with refractory epilepsy, and it was positive in two of fifty patients with controlled epilepsy. Independent t-test showed a statistically significant difference. There was no difference between the two groups in terms of serum anti-tTG level ($P = 0.14$) and no significant relationship was found between serum anti-tTG level and age ($P > 0.05$) and genus ($P > 0.05$).

3.3. Pathological examination of duodenal biopsy

In patients with anti-tTG levels higher than 7 AU/ml, upper endoscopy and a duodenal biopsy were performed for pathological examination. Out of fifty patients with refractory epilepsy, the duodenal biopsy was performed in five patients and out of fifty patients with controlled epilepsy, in two patients. The result of biopsy in three patients in the group resistant to epilepsy and one patient in the group with controlled epilepsy favored of a definite celiac disease diagnosis. The Chi-square test showed that there was no statistically significant difference between the two groups in terms of involvement with celiac disease ($P > 0.05$) (Table 3).
Table 3. Comparison of duodenal pathology in the group with refractory epilepsy and controlled epilepsy

| Pathology of duodenal biopsy | Resistant epilepsy | Controlled epilepsy | Significant level | Statistical |
|-----------------------------|-------------------|---------------------|-------------------|-------------|
| Positive                    | 3 (60%)           | 1 (50%)             |                   | 0.74        |
| Negative                    | 2 (40%)           | 1 (50%)             |                   |             |
| Total                       | 5                 | 2                   |                   |             |

The characteristics of four patients with duodenal biopsy in favor of a definitive diagnosis of celiac disease are described in Table 4.

Table 4. Characteristics of 4 patients with duodenal biopsy in favor of definitive diagnosis of celiac disease

| Patient | Type of seizure         | genus | age | Ant-tTG (AU/ml) |
|---------|-------------------------|-------|-----|-----------------|
| 1       | Controlled              | women | 33  | 22              |
| 2       | Resistant to treatment  | man   | 19  | 45              |
| 3       | Resistant to treatment  | women | 53  | 59              |
| 4       | Resistant to treatment  | women | 28  | 76              |

In the present study, no significant relationship was found between the diagnosis of celiac disease and age, genus and refractory epilepsy (P > 0.05). The mean serum level of anti-tTG in patients with pathological diagnosis of the celiac disease was 50.5 ± 11.42 AU / ml and in other patients with the positive anti-tTG test was 11.16 ± 1.76 AU / ml. Comparison of serum anti-tTG levels in patients with pathological diagnosis of the celiac disease and other patients who underwent endoscopy by independent t-test showed that the level of anti-tTG in patients with pathological diagnosis of celiac disease was significantly higher than other patients (P = 0.006) (Figure 2).
1. Discussion

Analysis of the findings of the present study showed that the mean age of patients was 32.96 ±11.35 years. Genus distribution in the two groups was statistically significant. In the group with refractory epilepsy 46 % and in the group with controlled epilepsy 53 % were women (P = 0.001). The mean serum level of anti-tTG in patients with refractory epilepsy was 4.6 AU / ml, and 3.0 AU / ml in the group with controlled epilepsy. Anti-tTG test was positive in five patients with refractory epilepsy and positive in two out of fifty patients with controlled epilepsy. Independent t-test showed no statistically significant difference between the two groups in terms of serum anti-tTG level (P = 0.14). Also, no significant relationship was found between serum anti-tTG level and age (P> 0.05) and genus (P> 0.05). Biopsy in three patients in the refractory seizure group and one patient in the controlled seizure group favored a definitive diagnosis of celiac disease. Statistical tests also showed that there was no statistically significant difference between the two groups in terms of involvement with celiac disease (P> 0.05). Also, comparing serum anti-tTG level in patients with pathological diagnosis of celiac disease and other patients undergoing scapularity in independent t-test showed that anti-tTG level in patients with pathological diagnosis of celiac disease was significantly higher than other patients (P = 0.006). Karimzadeh et al. designed a cross-sectional

Figure 2. Mean serum anti-tTG level in patients with pathological diagnosis of the celiac disease and patients with positive anti-tTG test.
study in 2020 in Tehran to investigate the relationship between celiac disease and refractory epilepsy. Therefore, 155 children with idiopathic epilepsy were included in the study. The results of this study showed that the mean age of patients was 6.7 ± 3.3 years. In our study, adult patients were included in the study and the mean age was 32.96 ± 11.35 years. In the study of Karimzadeh et al., the level of tTG antibody was positive in 7 patients with epilepsy. A study by Dai et al. (2014) examining the prevalence of temporal lobe epilepsy and celiac disease also showed that 2.22% of the 90 children with temporal lobe epilepsy were found in two patients (serum tTG). A study by Vieira et al. (2013) found that out of 100 children with epilepsy, 3 had anti-tTG, and in a study by Antigoni et al. 255 children with epilepsy and 280 healthy children showed that anti-tTG positive was detected in five children with epilepsy. In our study, anti-tTG was positive in two out of fifty patients with controlled epilepsy. In the study of Karimzadeh et al., which was conducted to investigate the association between celiac disease and refractory tuberculosis, seven children were positive for anti-tTG and the biopsies of three of them were positive (Karimzadeh et al., 2011). In the study of Dai et al., where the level of anti-tTG was high in two children, the pathological examination of the biopsy specimens also showed evidence of celiac disease in the biopsy specimens of these two children (Dai et al., 2014). A study by Vieira found that out of 100 children with epilepsy, three had anti-tTG, with normal duodenal mucosa in two cases and lymphocytic infiltration in one case. It was inside the epithelium (Vieira et al., 2013). A study by Antigoni et al. on 255 children with epilepsy and 280 healthy children showed that anti-tTG positive was detected in five children with epilepsy. Histological changes were found in the biopsy of all five children with epilepsy (Antigoni et al., 2007). A study by Fois et al. (1993) in Italy showed that celiac antibodies were positive in nine children, and a biopsy confirmed the diagnosis of celiac disease (Fois et al., 1994). In our study, biopsy in three patients in the refractory seizure group and one patient in the controlled seizure group favored a definitive diagnosis of celiac disease. The results of this study showed that the mean age of patients was 32.96 ± 11.35 years. Genus distribution in the two groups was statistically significant; In comparison with the uncontrolled group, a higher percentage of the controlled group was women. 46% in the group with refractory seizures and 53% in the group with controlled seizures were female (53 vs 46%, P = 0.001). The mean serum level of anti-tTG in patients with refractory epilepsy was higher than patients with controlled epilepsy. Anti-tTG test was positive in five patients with refractory epilepsy and positive in two patients out of fifty patients with controlled epilepsy. There was no statistically significant difference between the two groups in
terms of serum anti-tTG levels ($P = 0.11$). No significant relationship was found between serum anti-tTG level and age ($P > 0.05$) and genus ($P > 0.05$). Biopsy in three patients in the refractory seizure group and one patient in the controlled seizure group favored a definitive diagnosis of celiac disease. Also, there was no statistically significant difference between the two groups in terms of involvement with celiac disease ($P > 0.05$). Compared with patients who had a positive anti-tTG level, the level of anti-tTG was higher in patients who had biopsy confirmed by biopsy ($P = 0.006$).

2. Conclusion

According to the results of the study the relationship between celiac disease and refractory epilepsy in adult patients not proved, therefore using gluten free diet dose not effect on the ability to control epilepsy particularly in refractory cases.

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Ethical Approval and Consent to participate

This study was executed under supervision of ethical committee of Urmia University of Medical Sciences, Urmia, Iran.

Consent for publication

Not applicable

Availability of supporting data

The data achieved and analyzed during this study are available from the corresponding author on reasonable request.

Competing interests

The authors have no conflicts of interest.
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