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Prospective screening for anti-gliadin IgA in Applied Medical sciences students at Taif University

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Celiac disease is an autoimmune enteropathy disease caused by an immune reaction to gliadin which is a component of gluten that affects the intestinal lamina and leads to its atrophy, which occurs when a celiac patient consumes gluten products. The symptoms are different from diarrhea, vomiting, or abdominal pain after eating gluten, however, most of them are asymptomatic. Due to the low frequency of studies regarding celiac disease among youngsters in Saudi Arabia, thegoal of this study was to screen anti-gliadin IgA among students at the College of Applied Medical Sciences at Taif University. A cross-sectional study was conducted on 182 healthy participants from students at the College of Applied Medical Sciences at Taif University from March 3, 2022, to March 26, 2022. Some participants have confirmed to have food allergy or an immune disorder such as nut allergy, systemic lupus erythema, and wheat sensitivity. The anti-gliadin IgA test was performed by ELISA to assess anti-gliadin IgA titer on the serum of the students. 9 out of 182 were anti-gliadin IgA positive test. Most of the positive participants were females, and one was male, and all were healthy and confirmed to be undiagnosed previously with celiac disease neither their relatives. Moreover, they are not shown symptoms that are associated with their gluten intake. We found an association with many parameters of AGA positivity of the participants such as gender, BMI or COVID-19 infection and vaccine. This study provides a screening analysis of anti-gliadin IgA among students at College of Applied Medical Sciences at Taif University, and our results are similar to the prevalence of celiac disorder in Saudi Arabia. However, seropositivity for anti-gliadin IgA can be a marker for other enteropathies therefore other confirmatory tests should be performed.

Keywords: Celiac disease– anti-gliadin IgA–gluten – Taif University– gliadin

INTRODUCTION

Celiac disease is an immune-mediated enteropathy caused by the consumption of gluten which a primary stored protein found in wheat, barley, and rye, causes a treatable but unrecoverable inflammation of the small intestine in genetically sensitive people that affects around 1% of the population in several regions of the world.

Patients with CD have distinct histopathologic abnormalities of the small intestine mucosa, indicating that adaptive and innate immunity response to toxic peptides found in the gliadin part of the gluten protein produce mucosal inflammation, small intestine villous atrophy, and increased gut permeability.

The damage of the small intestine can result in a variety of gastrointestinal problems. These individuals may be asymptomatic or develop symptoms such as diarrhea, constipation, anemia, osteoporosis, dermatitis herpetiformis, and malabsorption.

The genetic condition is induced in susceptible people who have DQ2 and DQ8 genotypes of human leukocyte antigen (HLA). CD is diagnosed by serological testing using certain markers such as Anti-gliadin Antibody (AGA), Anti-tissue Transglutaminase Antibody (anti-tTG) and Anti-endomysial Antibody (anti-EMA), as well as histological examination of duodenal samples (Biesiekierski and Jessica Biesiekierski, 2017; Elli et al. 2015; Jericho and Guandalini, 2018; Yu et al. 2021).

History

Celiac disease was first described by Samuel Jones Gee in 1888. Until the mid-20th century, the condition was termed "Gee-Herter" by Gee and Christian Herter. During the 1930s and 1940s, a Dutch physician Willem Dicke provided the most important and interesting historical explanation of celiac disease. The standard therapy for celiac diseases in children at that time was either banana

diet or Fanconi diet. In 1941, Dicke suggested a wheat-free diet based on discoveries he obtained when recommending families to try a variety of diets, Insufficiency of bread during the Second World War resulted in clinical benefits for many celiac disease patients of Dicke's. The return of bread has resulted in a significant recurrence of celiac disease (McAllister et al. 2019).

Epidemiology

Celiac disease affects around 1% of the global population. The prevalence of CD has been recorded in several studies, ranging from 1: 200 to 1: 100 (depending on the regions) among those at-risk people and 1:10 among close relatives. Females are frequently impacted two to three times more than males.

In Saudi Arabia, the reported incidence of CD in risk groups varies, and the number of research is relatively limited. However, according to a cohort study published in Saudi Arabia, 2.2% of young healthy students are diagnosed with celiac disease. In reality, the frequency of CD among youngsters in Saudi Arabia was 1.5%, which is higher than the European and North American norms (Dzhumanova and Altynbekova, 2015; Al-Qefari et al. 2018; McAllister et al. 2019; Aljulifi et al. 2021).

Structure

Gluten is a mixture of two proteins, alcohol-insoluble polymeric glutenin and alcohol-soluble monomeric gliadin. It is a complicated protein with a lot of allelic variability in the genes that code for it. Different types and amounts of these compounds are produced by different genotypes. Protein, and even carbohydrate, expression in a genotype varies depending on the environment in which it was grown. which could also be influenced by growth circumstances and technological methods (Biesiekierski and Jessica Biesiekierski, 2017; Cabanillas, 2019).

Clinical presentation

CD patients may have a wide range of gastrointestinal disorders that can be classified as typical, atypical, silent, and potential. The typical form is clinical gastrointestinal symptoms related to improper intestinal absorption. Atypical celiac disease is presented with few or even no GI symptoms or signs. The silent form is characterized by abnormalities in serology and histology but no clinical signs. Individuals with positive blood serology and normal histology are referred to as potential form (McAllister et al. 2019).

Mechanisms

The destruction in the digestive tract that results in celiac disease is immune system mediated. It is mostly caused by T cells that become activated when gluten-sensitive individuals are exposed to it. Gluten exposure increases the release of zonulin, a protein that modulates the permeability of the GIT. Once gliadin gains access to the lamina propria, it is deamidated by the enzyme tissue transglutaminase. It is then further processed by antigen-presenting cells and presented most commonly by the MHC II receptor to the CD4 T helper cells. T helper cells also activate B cells that produce antibodies against gliadin, tTG, and endomysium (du Pré and Sollid, 2015; Gesualdo et al. 2021).

Treatment

The only effective treatment for CD now available is a lifelong strict gluten-free diet (GFD), in which the manifestations are cured by a diet change that results in the elimination of intestinal and extra intestinal symptoms, the absence of autoantibodies, and the repair of intestinal villi. On the other hand, the diet change is not easy. Consequently, this important change is accompanied by some disadvantages, such as a negative impact on quality of life, possible vitamin and mineral deficiencies, and often severe constipation. The majority of these CD-related problems can be avoided by getting nutritional advice from a dietitian with CD experience (Elli et al. 2015; Caio et al. 2019).

Complications

Complication due to CD It usually occurs in people that weren't diagnosed early in their life, mostly after the age of 50 and/or didn't adhere to a strict GFD. Studies show those individuals have a higher risk of mortality than other people. Also, that may occur rarely in already CD-diagnosed patients. These complications include: 1) Hyposplenism2) osteoporosis, which represents the most common complication that occurs due to defective absorption of calcium and due to vitamin D deficiency3) Intestinal T-cell lymphoma is linked to enteropathy and which is one of the most significant complications 4) Ulcerative jejunoileitis5) a collagenous sprue6) refractory CD.

Also, new evidence reveals a potentially there's a link between small bowel cancers and celiac disease. However, the pathogenic mechanisms that predispose celiac disease to malignancy are unknown, and CD may also be linked with impaired fertility in both males and females (Parzanese et al. 2017; Caio et al. 2019).

Disorders that are caused by gluten are becoming more common, and the number of people that experience symptoms after eating gluten has increased. Therefore, the goal of this study is to screen for celiac disease among students at College of Applied Medical Sciences at Taif University by using anti-gliadin IgA test.

MATERIALS AND METHODS

Study population

182 students (n=182) from the Collage of Applied Medical Science in Taif University were participating from both females and males and their ages range from 18 to

26 years old. Our sample group is 9.1% out of the total number of students attending the collage.

Sample preparation

3 mL of venous blood was collected from 3 March to 22 March 2022from 126 females and 56 males in a plain tube and centrifuged at 3500 rpm for 10 minutes. 200µL of the serum was separated from each sample and transferred into Eppendorf tube and stored at -80°C refrigerator. The serum was thawed once immediately before the test in a water bath at 37°C for 20 minutes.

Enzyme-linked immunosorbent assay (ELISA)

The EUROIMMUN ELISA kit was used to detect anti-gliadin IgA by using an indirect semi-quantitative technique, Bio-Rad xMarkTM micro plate spectrophotometer was used for reading and incubation of the plate. 5µL of the serum was diluted in 1 ml of sample buffer (1:201). 100µL from each of the positive and negative controls, calibrator 1, calibrator 2, calibrator 3, and the diluted serum were added into the microplate wells. The plate was incubated inside Bio-Rad xMarkTM micro plate spectrophotometer at 25°C for 30 minutes. After the incubation, the wells were poured and washed three times with 300µL by the washing buffer per wash for 30-60 seconds. After that, by tapping the wells on absorbent paper, all residual wash buffer was completely removed. Each well was loaded with 100µL of enzyme conjugates and incubated inside the Bio-Rad xMarkTM micro plate spectrophotometer for 30 minutes at room temperature. Then wells were emptied and washed, as mentioned before. With the avoidance of direct sunlight inside a dark room, 100µL of chromogen/substrate solution was added to each well, and the plate was then incubated for 15 minutes inside the Bio-Rad xMarkTM micro plate spectrophotometer. At the end, 100µL of the stop solution was loaded into the wells based on the same sequence and speed of the substrate addition, the microplate was slightly shaken to ensure a homogeneous mixing of the solution prior to reading at a wavelength of 450 nm.

Statistical analysis

Microsoft for excel was used for calculating the results according to the manufacturer guidelines. Pearson's Chi-square test was applied to compared the frequency of seropositive IgA against seronegative cases.

Ethical considerations

The study has received ethical approval from the Research Ethics Committee at Taif University, Taif, Kingdom of Saudi Arabia. IRB; 40-36-0178.

RESULTS

The results were evaluated semi-quantitatively by calculating the ratio of the extinction value of the control or patient sample over the extinction value of calibrator 2 according to the next formula shows the equation for calculating the results.

$$\frac{Extinction\ of\ the\ control\ or\ patient\ sample}{Extinction\ of\ calibrator\ 2} = Ratio$$

The measured absorbance value for positive results was ratio ≥1, and for negative results was ratio <1. In Table 1, a summary of our results is illustrated.

Table 1: shows the participants characteristics and the AGA titar.

Category	Anti-Gliadin IgA titer	Frequency (%)	Chi-square
Negative control	0.149	-	-
Positive control	1.299	-	-
Calibrator 1	0.208	-	-
Calibrator 2	0.738	-	-
Calibrator 3	2.421	-	-
IgA Seronegative (mean)	0.43067	173 (95.06)	0.001
IgA Seropositive (mean)	1.77115	9 (4.94)	

Out of 182 participants, nine of them had a positive anti-gliadin IgA result (≈4.9%) (Table 2). All the positive participants were undiagnosed with a celiac disease or gluten sensitivity (them and their relatives) and have not shown symptoms that are associated with their gluten intake. The positive participants do not suffer from any chronic diseases. However, the samples collection was performed during the exams week and the all the students have confirmed to moderate to high change in their eating habits. Also, the male student has confirmed to follow a special diet to lose weight. Also, three out of the nine participants have been recovered from Covid-19 recently (Table 3).

Table 2: shows the participants characteristics and the AGA titar. (M = Male, and F = Female)

Participant no.	Gender	Height (cm)	Weight (kg)	BMI (kg\m2)	IgA titar
1	F	153	57	24.35	1.890243902
2	F	159	40	15.82	1.094850949
3	F	163	51	19.20	3.453929539
4	F	165	80	29.38	1.123306233
5	F	156	52	21.37	1.238482385
6	F	159	55	21.76	1.447154472
7	M	169	45	15.76	1.159891599
8	F	149	34	15.31	1.422764228
9	F	155	52	21.6	3.109756098

Table 3: shows the date of sample collection, the last dose of the COVID-19 vaccine, and the date of COVID-19 infection of participants.

Participant no.	Gender	Date of sample Collection (Year 2022)	Date of last dose of COVID-19 vaccine	Date of COVID-19 infection
1	F	20 March	20-1-2022	-
2	F	21 March	4-1-2022	1-7-2020
3	F	21 March	13-3-2022	6-2-2022
4	F	22 March	29-7-2021	-
5	F	22 March	3-8-2021	16-1-2022
6	F	22 March	31-12-2021	-
7	M	22 March	22-8-2021	-
8	F	6 March	28-3-2022	20-1-2022
9	F	8 March	12-2-2022	-

DISCUSSION

Gliadin, which are storage proteins found in wheat grains, are one of the most critical antigens in the pathophysiology of celiac disease. In some conditions, gliadin could penetrate into the intestinal lumina propria and stimulate the immune system after its interaction with immuno competent cells (Jiskra et al. 2003).

According to the data, 23% of participants are allergic to different types of food, such as nuts, eggs, bananas, non-celiac gluten sensitivity, and lactose intolerance. However, none of the participants with positive results have allergies or other health issues. Another set of data was obtained from positive participants to link out factors that might have affected the results that caused the AGA test to be positive among them.

In this study, we found an association with many parameters of AGA positivity of the participants. As it shown in Table 2, gender is a very noticeable factor, most of the positive results are females eight out nine participants. Following that, we have linked this factor with reproductive and\or menstrual disorders. These female participants either had irregular menstrual cycle (participant 2) or were menstruating during the sample collection (participant 1,3 and 8).

Although the discrepancy in the results could be related to participants' BMI, of which three of the positive participants were underweight (BMI <18.5). It is noteworthy to say that these three participants were overweight (BMI >25). All these participants followed strike diet to lose the weight, however, their eating habits have changed during the exams' week.

It is possible that different viral and celiac biological components interact, depending on prior study by Elli et al. (2020), who reported a link between COVID-19 and celiac disease patients. There has been a lot of conflicting information on whether COVID-19 have an impact on immune response to immunoglobulin production. According to that, we observed three participants were recently infected with COVID-19 two months ago (Table 3), and that is possibly have impact on the anti-gliadin IgA and lead to increase its level. Similarly, most of the participants got booster doses of the COVID-19 vaccine recently.

Therefore, the positivity results of AGA (Table 2) could be an indicator of non-celiac enteropathy among the students. It is compatible with Bizzaro and Tonutti (2007) in which they demonstrate that AGA are detected in CD patients' blood. However, it could also be detected in healthy people and those with other gastrointestinal illnesses such as non-celiac gluten sensitivity. Our previous studies among people living in high-altitude have shown significant findings specially among patients with chronic diseases (Almehmadi *et al.* 2018, 2019, 2020; Almehmadi, 2019). Further investigation should be considered specially after infection such as Covid19 due to possible correlation between increase in the part of gamma-globulin zone following these infections (Almehmadi, 2022).

CONCLUSION

The current study provides a screening investigation for anti-gliadin IgA among the students at an applied medical science college at Taif University. Change in eating habits during exams may have an effect among anti-gliadin IgA levels, positive anti-gliadin IgA participants were mostly females, and only one male student. It is also more prominent in underweight students. Furthermore, some of them were recently infected with COVID-19 and others had a history of menstrual disorders. In addition, some of them have recently received the COVID-19 vaccine.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

All authors have equally contributed in this work.

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