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RESEARCH ARTICLE

Evaluation of AGA and tTG Antibodies in Serum of Celiac Disease Patients

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ABSTRACT

Celiac disease is one of the commonest chronic digestive diseases, which is associated with immune system -mediated, and genetically predisposition. The disease is caused by exposure to foods that contain the gluten proteins which found in wheat, barley, maize and other food. This study was aimed to evaluation the levels Anti-gliadin antibodies (AGA) and Anti-tissue transglutaminase antibodies (tTG) in serum of 58 CD patients and 27 healthy individuals as control group.Our results showed the levels of AGA-IgA, AGA-IgG, tTG-IgA, tTG –IgGantibodies concentrationwere significantly increase (P0.05) in CD patients comparing with control group.This findings indicated that AGA and tTG play important role in initiation and development of CD.

Key words: Celiac disease, gluten, Anti-tissue transglutaminase antibodies, Anti-gliadin antibodies.

INTRODUCTION

Celiac disease (CD) is associated inflammatory condition of the small intestine, triggered by the bodily function of gluten. It's the one most typical genetically food-based intolerance, the foremost common chronic diseases in childhood (1,2).In the 1980s, a brand new era in celiac disease research began with the identification of specific antibodies circulating in plasma of untreated patients. IgA and IgG against gliadin that bind native gliadin, were related to the disease, however known patients with celiac disease with low levels of sensitivity and specificity, creating them obsolete (3).antigliadin antibodies (AGA) test by enzyme-linked immunosorbent assay (ELISA) constitutes a valuable tool in the decision for intestinal biopsy(4 ,5) Anti-tissue transglutaminase (tTG)antibodies are the most effective strategy for serologic diagnosing of CD patients' serum by enzyme-linked immunosorbent assays . These antibodies show sensitivity higher than 97%, specificity around 96%, and an accuracy of 98%, while IgA anti-

endomysial (IgA EMA) antibodies are used as a supportive check in tTGA positive cases thanks to their high specificity (approximately 100% vs 91% of tTGA).IgA tTG measure is that the initial test of choice and is highly sensitive and specific for the diagnosis of CD. Conversely, the older antibodies to native gliadin antibodies don't seem to be sensitive or specific enough for the diagnosis of CD (6).Brandtzaeg (2006) reported that IgA-producing plasma cells induce anti-transglutaminase in the small intestinal mucosa (7).

MATERIALS AND METHODS

Patients and control group

This study involved 58 suspected patients infected with CD in Wasit - Province/Iraq. These patients were 27 males (46.55%) and 31 females (53.45%),their age were ranged between one year to 30 years ,with mean (14.63) years, the patients were compared with control group.The control group are involving twenty seven apparently healthy individuals who had no pathological state at time of this study, all of these individuals were matched to patients, in age group and gender.Three ml of venous blood were obtained from patients and control group ,then allowed to clot in gel tubes naturally at room temperature, then centrifuged at 1500 rpm for 10 minutes to get blood serum.Serum was placed in 1.5 ml eppindrofe tubes. All samples were tagged by a serial number and the person's name, and then right away frozen at -32C for additional process, once thawed, refreezing was avoided.ELISA technique was used to detection the levels of antigliadin-IgA - IgG , and Anti- tissue-transglutaminase IgA - IgG antibodies in serum of patients and control group .

Principle

An ELISA test of antigliadin-IgA- IgG , and Anti- tissue-transglutaminase IgA - IgG antibodies werebased on the double antibodies technique. These tests were done in stepsaccording to instructions of manufacture company (Aeskulisa)

Interpretation of the results

The testsare considered positive if the sample result's over eighteen U/ ml, while the testsare considered negative if the result's less than twelve U/ ml and thought of as equivocal if the sample result's between these values.

Statistical analysis

The statistical analyses of results were carried out by the help of Minitab using version SPSS statistical package. T test was used to find the P value of significant differences ,the level of significance was 0.05 (or less) in all statistical testing, (p value less than 0.05).

RESULTS AND DISCUSSION

Expression of AGA-IgA level in celiac patients and control group

Table 1. Showed the mean and standard deviation ofAgA-IgA level in the studied groups (patients and control group). The statistical analyses revealed that the mean level of AGA-IgA was 39.96 U / ml in patients with significantly increase ($P \leq 0.001$) than control group (1.32 U / ml). Specificity and sensitivity of AGA-IgAis approximately 90%, and around 85%-90% respectively (8) .However ,our results showed highly significantly increase ($P \leq 0.001$) between patients and control group regarding AGA-IgA expression by ELISA .

Dahele*et al*., (2001) showed that AGA-IgA level was elevated in 61% of all untreated patients(9). Other study done by Baudon etal., (2004) reported that 18 of 30 had high level of AGA IgA antibodies(10). While Brain etal.,(2013) was showed the positive predictive value ofAGA IgA serum and small bowl biopsy of CD patients(11) .The AGA-IgA is the oldest marker determined by the ELISA method, it has specificity about 90%, and the sensitivity is around 85%-90% in celiac disease patients (12).

Expression of AGA-IgA level in male patients and control group

Table 2. referred to the expression of AGA-IgA in celiac patients in comparison with control group. There was highly significant ($P \leq 0.003$) difference between male patients of celiac disease and male control group in relation to AGA-IgA expression

Expression of AGA-IgA in female celiac patients and control group

As shown in table 3. the expression of female patients with celiac disease had mean value 44.21 U / ml of AGA-IgA, while the mean value of AGA-IgA in femalescontrol group was 1.36 U / ml. Statistical analysis of data by using t-test showed a significant increase in value of AGA-IgA in male celiac patients compared to femalescontrol group, ($P \leq 0.05$).

Expression of AGA-IgG levels in celiac patients and control group

As shown in table 4. there is a significant increase ($P \leq 0.02$) of the AGA-IgG means in CD patients (40.56 U / ml) when compared with control group (6.40 U / ml). It was well now known , the ingestion of gluten in celiac disease people leads to damage in the small intestine , which lead to immunoresponse, and this subsequent lead to production anti-gliadin antibodies. Study of Tonutti etal.,(2009) was found high specificity of the AGAs IgG test (72.2%) in CD patients (13) .However ,in recent study achieved in Iraq the researchers was found that 83 (20.15%) of CD patients were seropositive for AGA-IgG(14). Our result was coming in agreement with following studies: Kocna et al., (2002) who observed that the highest sensitivity (94%) was obtained for AGG in CD patients(15).Al-Mayouf etal.,(2003) reported the levels of AGA -IgG were high in 14 patients (77.8%) (16). Moreover, Baudon et al., (2004) showed that 28 of 30 patients had high level of AGA IgG(10).

Expression of AGA-IgG level in male patients and control group

As shown in table 5 the expression of 27 male patients had mean value of AGA-IgG for male celiac patients group was 16.58 U / ml, while the mean value of AGA-IgG in male control group was 5.57 U / ml. Statistical analysis of data by using t-test showed the mean level of AGA-IgG was significantly increase in male celiac patients when compared with male celiac control group ($P \leq 0.003$).

Expression of AGA-IgG in female celiac patients and female control group

As shown in table 6 female patients had mean value 62.15 of AGA-IgA U / ml, while the mean value of AGA-IgA in female control group was 7.06 U / ml. Statistical analysis of data showed a significant increase in values of AGA-IgA in female celiac patients compared to female control group, ($p < 0.01$).

Expression of tTG-IgA level in celiac patients and control group

Table 7 revealed the mean of tTG-IgA levels was highly increased in CD patients (81.08) when compared with control group (3.51) with significantly difference ($P \leq 0.01$.)

It is well documented that the presence and levels of IgA-tTG used as predictors and monitor of untreated celiac disease (17),so for this reason, the study of this enzyme is of great importance. Dahele *et al.,* (2001) showed the tTGIgA concentration was elevated in untreated CD patients (81%), compared with treated CD patients (1%) and non-celiac (3%) (9). The important role of tTGIgA antibodies as a marker of untreated CD patients is well reported in several studies (18,10,19).As well as, other researcher showed positive predictive value of IgA anti-tTG for biopsy of coeliac disease such as Lock *et al.,* (2004)(20). The study of Bonamico*et al.,* (2008) on anti-tTG-IgA reported it is highly sensitive for CD patient's diagnosis and for the follow up of patients under gluten-free diet (21). Tissue transglutaminase considerable in many studies as a major antigen in the autoimmune response as coeliac disease (22). tTG - IgA is suitable marker for screening and diagnosis of CD patients among first degree relatives (23). Finally Hasan*et al.* (2016) in their study demonstrated that twenty eight of 168 CD patients had high percentage of anti-tissue transglutaminase antibody (17.2%)(24).

Expression of tTG-IgA level in male patients and male control group

Table 8. Showed the expression of 27 male patients mean value of tTG-IgA was 60.69 U / ml, while the mean value of tTG -IgA in male control group was 3.53 U / ml. Analysis of data by using t-test to compare between the two groups showed significant increase in value of tTG-IgA in celiac male patients compared to male control group, ($P \leq 0.05$).

Expression of tTG-IgA in female celiac patients and female control group

Table 9 referred to the expression of tTG-IgA in female patients and control group, the result showed the mean value of tTG-IgA was 98.84 U / ml in patients, while the mean value of tTG -IgA in female control group was 3.50 U / ml. Statistical analysis of data by using t-test showed a significant increase in values of tTG-IgA in celiac male patients compared to female control group, ($P \leq 0.01$).

Expression of tTG-IgG level in celiac patients and control group

As shown in table 10 the finding demonstrated a significant differences between patients and control groups ($p < 0.02$) in relation to the level of tTG-IgG ,due to elevation of tTG-IgG concentration in patients (23.06) when compared with control group (4.60). Some study, showed no association of anti-TGc Abs and celiac disease could be shown (25) However,our result was compatible with several studies , Hansson etal. (2002) showed the level of tTG-IgG was increased in untreated celiac disease children younger than 5 y of age than older children (26) .However, Villanueva, (2017) reported although tTG-IgG was highly elevated in our study but it is not sufficient for diagnosis of CD patients (27), because tTG-IgG is detected in several autoimmune disorders, with variable frequency and isotypes depending on the condition, so , tTG-IgG is lower applied than tTG- IgA. Moreover, Temur, (2017) showed that higher rate of tTG-IgG antibodies in CD patients related with chronic hepatitis D virus (28).

Expression of tTG-IgG level in male patients and male control group

As shown in table 11 statistical analysis of data showed significant increasing in values of tTG-IgG in maleceliac patients when compared to male control group, ($P \leq 0.05$).The mean of tTG-IgG was 12.07 in patients while it was 4.09 in control group .

Expression of tTG-IgG in female celiac patients and female control group

Table 12 cleared the expression of tTG-IgG in female patientsand female control group.Analysis of data by using t-test showed significant increase in values of tTG-IgG in female celiac patients compared to control group, ($P \leq 0.05$).

CONCLUSION

Our results indicated that AGA and tTG play important role in initiation and development of CD.

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Table 1. Level of AGA-IgA in celiac patients and control group

AgA-IgA	N.	Mean	Std. Deviation	Std. Error Mean	P value
Patients	58	39.96	55.54	7.35	P≤0.001 (S)
Controlgroup	27	1.32	1.88	0.36	

Table 2. Level of AgA-IgA in male patients and control group

AGA-IgA	N.	Mean	Std. Deviation	Std. Error Mean	P value
patients	27	35.24	54.55	10.49	P≤0.003
Controlgroup	12	1.26	1.62	0.46	(S)

Table 3. Level of AGA-IgA in female celiac patients and control group

AGA-IgA	N.	Mean	Std. Deviation	Std. Error Mean	P value
Patients	31	44.21	57.00	10.40	P≤0.006
Controlgroup	15	1.36	2.12	0.54	(S)

Table 4. Level of AGA-IgG in celiac patients and control group

AgA-IgG	N.	Mean	Std. Deviation	Std. Error Mean	P value
Patients	58	40.56	78.61	10.41	P≤0.02 (S)
Controlgroup	27	6.40	4.63	0.89	

Table 5. Level of AGA-IgG in male patients and male control group

AGA-IgG	N.	Mean	Std. Deviation	Std. Error Mean	P value
patients	27	16.58	16.00	3.08	P≤0.003 (S)
controlgroup	12	5.57	4.92	1.42	

Table 6. Level of AGA-IgG in female celiac patients and female control group

AGA-IgG	N.	Mean	Std. Deviation	Std. Error Mean	P value
patients	31	62.15	103.38	18.87	P≤0.01 (S)
controlgroup	15	7.06	4.45	1.14	

Table 7. Level of tTG-IgA in celiac patients and control group

TtG-IgA	N.	Mean	Std. Deviation	Std. Error Mean	P value
patients	58	81.08	172.64	22.66	P≤0.01 (S)
Controlgroup	27	3.51	3.37	0.65	

Table 8. Level of tTG-IgA in male patients and male control group

tTG-IgA	N.	Mean	Std. Deviation	Std. Error Mean	P value
patients	27	60.69	151.30	29.11	P≤0.05
controlgroup	12	3.53	3.61	1.04	

Table 9. Level of tTG-IgA in female celiac patients and female control group

tTG-IgA	N.	Mean	Std. Deviation	Std. Error Mean	P value
patients	31	98.84	189.96	34.11	P≤0.01 (S)
controlgroup	15	3.50	3.30	0.85	

Table 10. Level of tTG-IgG in celiac patients and control group

TtG-IgG	N.	Mean	Std. Deviation	Std. Error Mean	P value
patients	58	23.06	59.57	7.82	P≤0.02 (S)
Controlgroup	27	4.60	6.06	1.16	

Table 11. Level of tTG-IgG in male patients and male control group

tTG-IgG	N.	Mean	Std. Deviation	Std. Error Mean	P value
patients	27	12.07	20.14	3.87	P≤ 0.05 (S)
controlgroup	12	4.09	3.81	1.10	

Table 12. Level of tTG-IgG in female celiac patients and female control group

tTG-IgG	N.	Mean	Std. Deviation	Std. Error Mean	P value
patients	31	32.62	78.66	14.12	P≤0.05
controlgroup	15	5.01	7.51	1.93	