Govaresh/ Vol. 21, No.4, Winter 2017; 266-271.

Original Article

Pitfalls in Estimation of Celiac Disease Prevalence Using Serology: A Cross-Sectional Study

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ABSTRACT

Background:
Celiac disease (CD) is an auto-immune disorder. The prevalence of CD has been estimated mainly based on serological tests. The aim of this study was to evaluate the seroprevalence of celiac disease in the adult general population of Mashhad, northeast of Iran and pitfall of serology in epidemiological studies considering the importance of serology titer.

Materials and Methods:
1558 subjects aged 35 to 65 years and 1025 individuals aged between 15 to 35 years were selected randomly from multistage cluster sampling papulation for this cross sectional study. Anti-tissue transglutaminase (tTG)-IgA assay was performed by ELISA(Enzyme-Linked Immunosorbent Assay). The manufacture's cut-off point of anti tTG was 20 IU/mL and the prevalence of positive serology was estimated based on being just above the upper limit of normal (20 IU/mL), twice or three times above the normal value at 40 and 60 IU/mL, respectively.

Results:
In both age group 35-65 year-old and 15 to 35 years adults, the prevalence of positive serology was 1.2% for anti-tTG level more than 60 IU/mL, which was three times of the kit references (95% CI: 0.7- 1.9) and (95% CI: 0.7-2.1), and based on our previous study in Mashhad if we consider the cut-off point as 76 IU/mL anti–tTG for mucosal atrophy, the prevalence of CD would be 0.69.

Conclusion:
Epidemiological data of CD is mainly based on serology and as these tests are to some extent non-specific at lower levels, the accuracy of the previous reported prevalence of CD in some studies are questionable and level of anti-tTG is important.

Keywords: Celiac disease, Tissue transglutaminase, Prevalence, Serology

please cite this paper as:
Shakeri MT, Ganji A, Ghayuor Mobarhan M, Ghavami Ghanbarabadi V, Rahimi L. Pitfalls in Estimation of Celiac Disease Prevalence Using Serology: A Cross-Sectional Study. *Govaresh* 2017;21:266-271.

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Received: 31 Jul. 2016
Edited: 14 Dec. 2106
Accepted: 15 Dec. 2016

INTRODUCTION

Celiac disease (CD) is an auto-immune disorder triggered by the ingestion of gluten-containing grains in genetically susceptible individuals(1). The prevalence of CD has been increasing over the past 20 years (1-3). It seems that improvement in diagnostic techniques, availability of multiple commercial kits and increased awareness of CD have led to an increase in the detection of CD.

In the United States and the European countries, the prevalence of CD was about 1%; although it varies in different countries (1-4). The CD in Sweden and Finland has been estimated to be approximately 2-3%, and 0.2% in Germany

(2-4). In healthy blood donors in Brasilia, the prevalence of CD was 1/67 to 1/681(5). Previous epidemiological studies showed a high prevalence of CD in some African and Asia-Pacific countries (6-7). Among 2500 Tunisian healthy blood donors, the prevalence of anti-endomysium antibodies in the general population was 1:355(8). Recent studies showed that gluten intolerance was also common among Indian population (9-11).

During last decade, many studies have been done in Middle Eastern countries for estimating prevalence of celiac disease base on serological screening and lead to reporting a wide spectrum in prevalence of CD from more than 1:80 to 1:166 (12-17) and in one study in Turkey seroprevalence of celiac disease was 1:115, but biopsy proven celiac disease was 1:158 (12,17). Not only genetic and environment are different worldwide, but also the screening policies are widely variable among different countries; so they may affect the accuracy of these reports.

Currently, two different categories of serological tests have been provided for initial evaluation of patients suspected of having CD: autoantibodies such as antiendomysial (EMA) and anti-tissue transglutaminase (tTG) tests, and antibodies against synthetic deaminated gliadin peptides (DGPs) (18-22). Anti tTG (IgA) by enzyme-linked immunosorbent assay (ELISA) method is now proved to be the best screening measurement(23).

IgA anti-tTG antibodies (ELISA) are now widely available in most Middle Eastern countries and Iran, which can help for rapid determination of CD prevalence(18). The specificity of serological tests especially tTG is variable, but they are still widely used to evaluate the prevalence of CD (24). It seems that tests with lower specificity are performed in this area because gold standard diagnostic procedures for CD such as biopsy sampling or HLA typing are either invasive or costs a lot of money.

Cut-off limit of anti-tTG is also important and low levels can be false positive due to other diseases. Leja and colleagues showed that considering the cut-off point of 30 for tTG led to 100% DQ2 or DQ8 positive results (25). Anderson showed that testing for HLA-DQ genes and confirmatory serology could reduce the numbers of unnecessary gastroscopies and help to estimate the prevalence of CD more precisely (26). In our unpublished data in celiac center of Mashhad, 96.4% of the patients with biopsy-proven CD had tTG level of 2-10 times above the upper normal limit (Ttg >40) and only 3.6% of the patients with CD had tTG level less than 40, including the patients with IgA deficiency. It is very clear that serology becomes more specific when it is significantly above the cut-off point and this is why some studies suggest that endoscopy and biopsy can be eliminated in patients with higher titers of antibodies (19-21).

In this study we screened general population in northeast Iran using tTG test, aiming to assess its reliability for the diagnosis of CD. Considering the discrepancies related to specificities of tTG, we highlight the potential pitfalls in screening for CD when tTG is used for this purpose.

MATERIALS AND METHODS

Study group selection

The current study was a sub-analysis of two larger cross-sectional projects. The two studies were done by multistage cluster sampling method on about 12000 individuals in Mashhad with projects codes of 85134 and 88290 in two groups, 35-65 year-old and 15-35 year-old subjects. In the first stage, three classes were defined in Mashhad and in the next step, nine clusters within each center were defined by probability proportional to size (PPS) method. Individuals were selected randomly according to the demographic information available from health centers of Mashhad.

1558 subjects aged 35-65 years from the general population in the first group and 1025 subjects aged 15-35 years from the second group were selected for our study.

Serology and diagnosis

10 mL of venous blood sample was taken from the brachial vein and the sera were collected. Tissue transglutaminase (tTG) assay was performed by ELISA. Anti-tTG Kit (euro immune, Germany) was used in a research laboratory and results >20 IU/mL were considered positive based on references.

We contacted the patients who had positive serology and arranged a phone call interview and asked about all classical and non-classical symptoms of CD based on our check list. We could not contact two third of the patients because their cell phone numbers had changed or they had moved to another home or city since 5 years ago that the blood samples were collected. The patients whom we could contact were invited to come for endoscopy and anti-endomysial antibody test. The patients with titer more than 60, had high probability of CD by symptom. EMA was negative in all patients with anti-tTG level less than 60. Few patients accepted to do endoscopy, and their pathology had no mucosal atrophy in titer less than 3 times of normal limit of kit references. Unfortunately, most of our patients, whom we could contact did not accept to come for

Table 1: Characteristics of participants aged 35-65 years in each group with anti-tTG >20

Variable		Negative anti- tTG n=1496	Positive anti-tTG n=62	*p* value
Age (year), mean±SD		50.5±3.8	48.15±2.1	0.065
Sex, n (%)	Male	620 (41.5)	24 (38.7)	0.378
	Female	874 (58.5)	38 (61.3)	
BMI (kg/m^2), mean±SD		28.7±4.71	28.34 ± 4.62	0.372
ALT(mg/dL), mean±SD		18.02±15.84	16.59±12.28	0.642
AST(mg/dL), mean±SD		24.85±17.94	20.36±6.57	0.187

Data are shown as number (percent), or mean±SD, or median (IQR)
BMI (Body Mass Index), ALT(Alanine aminotransferase), AST(Aspartate aminotransferase)

Table 1: Characteristics of participants aged 35-65 years

Variable		Negative anti- tTG n=1496	Positive anti-tTG n=62	*p* value
Age (year), mean±SD		50.5±3.8	27.6±6.2	0.067
Sex, n (%)	Male	620 (41.5)	12(37)	0.36
	Female	874 (58.5)	20(63)	

Table 3: Seroprevalence of positive tTG in general population

Seroprevalence	tTG >20 IU/mL	tTG>40 IU/mL	tTG>60 IU/mL
First study (35-65)	(62) 4%	(37) 2.4%	(18) 1.2%
	(%95 CI: 3.1-5.1)	(95% CI 1.8-3.3)	(CI 95% :0.7-1.9)
Second study (15-35)	(32) 3.1%	(21) 2%	(12) 1.2%
	(%95 CI: 2.2-4.4)	(95% CI :1.4-3.2)	(CI 95% :0.7-2.1)

*t*TG: tissue transglutaminase

endoscopy to confirm the diagnosis of their CD.

Ethical considerations

Informed consent was obtained from the participants, and we conducted our study on stored sera. Ethics Committee of Mashhad University of Medical Sciences approved this study.

Statistical analysis

SPSS software version 16 was used for statistical analysis. Standard deviation and mean values were measured. P value less than 0.05 was considered as statistically significant. We evaluated the prevalence of anti-tTG in three groups, more than upper normal limit (more than 20), two times more than normal limit (tTG >40), and three times above normal limit (tTG > 60).

RESULT

1558 individuals were recruited. The age range of the subjects was 35-65 years. Of all the subjects, 59% were female and 41% were male. Mean age was 48 years in anti-tTG Ab-positive patients (more than 20 IU/mL) and 50 years in anti tTG Ab-negative individuals (table 1). In the group with positive serology, 39% of the subjects were male and 61% were female. Neither sex nor age was significantly different between the two groups. In the second group, 1025 individuals aged from 15 to 35 years were recruited Their mean age was 26 years (table 2). Of the subjects with positive anti-tTG, 37% were male and 63% were female. Neither age nor sex was significantly different between anti–tTG positive and negative subjects in this group (P>0.05, table 2).

Among 35-65-year-old subjects, the estimation of CD prevalence based on anti-tTG level more than 20 IU/mL was 4% (%95 CI: 3.1-5.1), 2.4% for anti-tTG more than 40 IU/mL (95% CI:1.8-3.3), and 1.2% for anti-tTG level more

than 60 IU/mL (95% CI: 0.7-1.9).

Among 15-35-year-old subjects, the prevalence of CD was estimated 3.1% (95% CI: 2.2-4.4) for anti-tTG> 20 IU/mL, 2% (95% CI: 1.4-3.2) for anti-tTG> 40 IU/mL, and 1.2% (%95% CI: 0.7-2.1) for anti-tTG>60 IU/mL.

The detailed prevalence of IgA anti-tTG level in groups 1 and 2 is presented (table 3).

We considered that the prevalence of CD in northeast Iran would probably be 1.2% based on serology more than 60 IU/mL or 3 times above the normal limit, clinical symptoms, and EMA positive results.

DISCUSSION

The aim of this study was to evaluate the sero prevalence of CD in a large general adult population in northeast Iran. Serological screening tests in general population and at risk groups can result in early identification of patients with CD (21). A comparison between recent studies in European and Middle Eastern countries has shown that the prevalence of CD is equal in both areas (27). The prevalence of CD is the proportion of people with positive serology at a specified time in a random sample of general population but many studies were performed in special groups like healthy blood donors (28).

CD was regarded as a rare entity in Iran until 10 years ago, however, by applying serological procedures, it is now estimated that CD is also a common disease in Iran similar to western countries. But it is not yet clear how far we can trust serological tests in estimation of CD prevalence. The specificity of serological tests such as anti-tTG is variable and it is still used widely for estimation of CD prevalence (24).

The first study among the Iranian population was conducted on healthy blood donors in Tehran, in 1999. Based on that study, 12 individuals were EMA positive. Histopathological changes according to Marsh in seropositive patients were: Marsh I in three patients, Marsh II in four patients and Marsh III lesions in five patients. The results of this study showed that the prevalence of CD in this group was 1/166. However if we consider only Marsh III as CD, the prevalence of CD would be 1/400 (13).

Another study in Mazandaran (Sari) conducted by using cluster sampling showed that CD was identified in 13 out of 1438 subjects. Small bowel biopsy was performed for nine patients. The results were as follows: Marsh 0 in one patient, Marsh I in eight, Marsh II in two, and Marsh three in two patients, respectively (30). The patients with Marsh I-II might not have CD. Another cluster sampling conducted in Kerman in 1361 individual from general population showed that 16 of 1361 subjects had positive serology for CD. Marsh 0 was seen in one case, Marsh I in eight cases, Marsh II in two cases, and Marsh III in two cases. In these two studies, the overall prevalence of CD was estimated as 1:120 and 1:91, respectively (Sari and Kerman) (30). The authors indicated that if they considered only March III and positive serology as CD, the prevalence of the condition would be 1/700.

In another study on 1440 individuals aged 20 to 83 years of general population in Shiraz (southern Iran), the prevalence of CD by tTG, EMA, and biopsy was 0.5%, which is less than other areas (31). It seems that the prevalence rate drops when intestinal biopsies are undertaken.

In most of the previous studies, anti tTG level has not been considered in estimating the prevalence of CD. However the specificity of serological kit, might be low when the manufacture's cut-off point is used. (24) This is reflected in a study by Marcis Leja and colleagues, who used HLA typing DQ2/DQ8 in addition to serological tests to assess the prevalence of CD in Latvia. They demonstrated that by using a cut-off >20 IU/mL for anti tTG as positive, only 41.86% of seropositive cases had a positive DQ2 or DQ8. Interestingly when they used a cut-off >30 U, the DQ2/DQ8 test was positive in 100% of seropositive cases (12). So it seems we need to consider a cut-off point more than the reference kit to have more specificity of tTG for diagnosis of CD (32) and based on our previous study in Mashhad if we consider the cut-off point as 76 IU/mL anti–tTG for mucosal atrophy,(32) the prevalence of CD would be 0.69.

Our findings by using different cut-offs for anti-tTG highlight the possible pitfalls in estimation of true prevalence of CD. Furthermore, the diagnosis of CD is usually made based on multiple parameters. Since early 1990, there have been numerous guidelines and algorithms to lead towards an acceptable diagnostic policy and yet there are some cases who do not fulfill these criteria. Our study aimed to highlight the pitfalls that may mislead us in epidemiological studies. Based on serology, the estimated prevalence of CD reported in general population might be inaccurate and overestimated.

One limitation of our study was that we did not use endoscopy and duodenal biopsy for all patients for accurate diagnosis. More studies with updated knowledge and based on current literature are needed for estimating the true prevalence of CD in Iran.

CONCLUSION

Epidemiological study of CD in some areas is mainly based on serology and as these tests are non-specific at low level, we highlight the pitfalls of these studies. By not considering the titer of tTG, the prevalence of CD in general papulation might be overestimated.

ACKNOWLEDGMENTS

Research Council of Mashhad University of Medical Sciences supported this research. This paper was the subject of a thesis by Azita Ganji MD, the MPH candidate of Mashhad University of Medical Sciences. We would like to thank Ms Tavalayee and Ms Mohamadi for their collaboration in laboratory.

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