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Value of Gluten Patch Test in Diagnosis of Celiac Disease

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Abstract

Objective: Celiac disease is an intestinal disorder identified by mucus inflammation, villous atrophy and crypt hyperplasia. This disorder can be controlled by elimination of gluten from daily diet. Patients with celiac disease are at greater risk of gastrointestinal malignancy and non-Hodgkin lymphoma than are the general population. This study tries to present the value of gluten patch test for diagnosis of celiac disease.

Methods: In this investigation, the study population was divided into case and control groups. The case group consisted of patients with celiac disease. The control group were patients involved in celiac disease but suffering from other gastrointestinal disorders. Both gluten patch and placebo patch were attached to the skin between the scapulas. The results were read twice: 48 hours and 96 hours after the patch was applied. Patients who showed irritation reactions were withdrawn from this study. The results were analysed by SPSS software, Spearman's test, chi square, and Mann–Whitney tests.

Findings: The value obtained from the gluten patch test after 96 hours are as follows: specification at 95%, sensitivity at 8%, positive prediction value at 67%, and negative prediction value at 43%.

Conclusion: It can be concluded that the gluten patch test is not an efficient test for screening of celiac disease, however, it can be useful for diagnosis of celiac disease if employed and studied with clinical symptoms and serologic and biopsy tests. Furthermore, we should doubt our judgment if the result of gluten patch test for the patient with celiac disease is positive.

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Introduction

Celiac disease (CD) is a disorder of the small intestine known by mucosal inflammation, villous atrophy, and crypt hyperplasia, which appear upon exposure to dietary gluten and improve after withdrawal of gluten from the diet.

However, the serologic tests for celiac disease and the common use of upper endoscopy have complicated the definition. These tests have identified patients who may be involved in the disease but have variable degrees of histop-

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athologic symptoms and changes [1].

No definite single test can lead to a definite diagnosis of celiac disease for every individual. So, the most important step in diagnosis as a general rule is using serologic tests. The specific tests like IgA anti-tissue transglutaminase and IgA endomysial antibody can be recom-mended [2,3].

Patients with a positive IgA endomysial or transglutaminase antibody test should undergo a small bowel biopsy. Multiple biopsies should be taken from the second and third portions of the duodenum. The exact minimal number is uncertain; however, some experts believe that at least four should be obtained [4]. The duodenal mucosa may be atrophic with loss of folds, contain visible fissures, have nodular appearance or scalloped folds, but such findings are not universally acceptable and may be seen with some other disorders [5]. The diagnosis is usually established if there is a correlation between the serologic results and the biopsy findings. It is confirmed when symptoms resolve subsequently on a gluten-free diet.

Documentation of histological normalization is usually unnecessary. Tissue transglutaminase is a ubiquitous intracellular enzyme released by inflammatory and endothelial cells and fibroblasts in response to mechanical irritation or inflammation. Once it has been secreted, it cross-links glutamine-rich proteins like gluten proteins from wheat. However, it can also deamidate glutamine residues in gluten to glutamic acid. Deamidation causes a negative charge in gluten peptides, which increases their binding to HLA-DQ2 and DQ8, which potentiates their capacity to stimulate T-cells [6,7].

In addition to activation of pathogenic T cells, innate responses to gliadin are also involved in the immune response, and perhaps even necessary to trigger the gliadin-specific (adaptive) T-cell response in genetically potential individuals [8]. The innate immune system applies "pattern recognition" to provide an early response to stimuli such as RNA, DNA, lipopolysaccharide, or viral proteins, in contrast to the adaptive immune system, which depends on HLA-presentation and T-cell recognition and expansion. In celiac disease, certain cereal peptides can apparently initiate innate immune responses in macrophages, monocytes, dendritic cells, and intestinal epithelia via yet unknown receptors and mechanisms [8,9] in the immune system. When T-cells are stimulated, two types of T-cells are produced. One type is T-helper and the other one is memory T-cell. Memory T-cells usually survive for a long time, about 20-30 years [10]. Therefore, every time gluten enters the body of celiac patients, memory T-cells will be activated and trigger innate immune response.

This study tries to answer this question whether the immune response starts if gluten enters the body of celiac patients through skin and whether gluten skin patch test can be used for celiac disease diagnosis.

A patch test is based on the principle of a type IV (delayed) hypersensitivity reaction. This is where a substance is recognized by immune cells in the skin known as antigen-presenting cells (APCs). The APC moves down the lymphatic system to a lymph node where it presents antigen to T-cells. If the T-cell identifies the substance as a threat, it sends out all types of immune cells including more of its own type to where the antigen has entered the skin. This is what causes skin immune response in contact dermatitis. Patch test is the gold standard of food allergy diagnosis[11], and celiac disease is one of the non-IgE mediated types of food allergy [12].

When gluten comes into contact with the skin of a celiac patient (through patch test), tissue transglutaminase in skin cells can transmute gluten to glutamic acid, and APCs move down to the lymphatic node (where there are gluten-specific memory T-cells) and present gluten as an antigen to T-cells, the immune system is thus activated and produces hypersensitivity reaction type IV. Therefore, it can be stated that skin patch test can be considered as a method for diagnosis of celiac disease.

In this study, we try to determine the effectiveness of patch test in diagnosis of celiac disease compared to that of biopsy.

Subjects and Methods

This study was carried out in Al-Zahra University Hospital affiliated with Isfahan University of

Medical Sciences, Isfahan, Iran. We collected comprehensive clinical and histological data of all patients suspected of celiac disease undergoing endoscopy from November 2005 until June 2009.

We called 300 patients and invited them to participate in the study.

Only 57 patients met the inclusion criteria and participated in our study. All the 57 patients had biopsy taken from small intestine. They were less than 31 years old. The patients were divided into two groups, the case group and the control group.

In the case group, there were 30 patients with typical celiac disease criteria; they had symptoms of celiac disease, positive serologic test results, villous atrophy in biopsy, and their symptom resolved subsequently on a gluten-free diet.

In the control group, there were 27 patients with no suspicion of celiac disease. We investigated whether these patients suffered from dyspepsia or had intestinal diseases other than celiac disease, such as GERD, H. pylori infection, or gastroenteritis. All patients in the control group had normal villous architecture.

In this study, we used Viaskin, which is a type of gluten patch test produced by DBV Technology, France. Viaskin has a polymer plate containing gluten, with positive and negative poles (the negative pole is placed on the skin of patient. When Viaskin is placed on the skin, the gluten in the polymer plate is solved by natural moisture of the skin and absorbed, as a result of which the immune system is activated.

In this study, we placed both gluten patch test and placebo patch test on the skin between scapulas of the patient. After 48 hours, we removed both patch tests and reported the responses twice; after 48 hours and 96 hours.

The result of patch tests had five grades: grade 0 meant no reaction, grade I: mild erythema, grade II: severe erythema, grade III: erythema and eventually papules or only papules, and grade IV meant erythema, vesicles, and pustules.

Four patients in the case group and eight patients in the control group who showed irritation reactions were withdrawn from this study.

In order to determine the diagnostic value of gluten patch test, once we chose grade 0 as negative response and once grade 0 and I as negative response.

We determined sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of gluten patch test both after 48 and 96 hours.

Statistical differences between the study groups were evaluated using Spearman's test, Mann–Whitney test, or chi square test, as appropriate. $P<0.05$ was considered statistically significant.

Table 1: Primary reason for endoscopy and small intestine biopsy in study and control groups

Descriptions	Control group (*n*=27) NO (%)	Case group (*n*=30) NO (%)
Normal esophagus	18 (66.7)	25 (83.3)
Esophageal reflux	8 (29.6)	4 (13.3)
Achalasia	1 (3,7)	0
Esophageal reflux with *Candida*	0	1 (1.8)
Normal gastric	12 (44.4)	13 (43.3)
Mild gastritis	3 (11,1)	0
Chronic gastritis	2 (7.4)	6 (20)
***H. pylori* infection with gastritis**	5 (18.5)	9 (30)
Mild chronic gastritis	5 (18.5)	2 (6.7)
Normal duodenum	11 (40.7)	0
Mild duodenitis	6 (22.2)	0
Chronic duodenitis	5 (18.5)	0
Mild chronic duodenitis	5 (18.5)	0
Partial villous atrophy	0	3 (10)
Marsh II	0	1 (3.3)
Marsh IIIA	0	6 (20)
Marsh IIIB	0	17 (56.7)
Marsh IIIC	0	3 (10)

Table 2: The value for gluten patch test in diagnosis of celiac disease in comparison with small intestine biopsy

GPT value	Sensitivity	Specificity	PPV	NPV
After 48h with grade 0 considered as negative response	38.5%	84.2%	76.9%	50%
After 48h with grades 0 and I considered as negative response	30.8%	84.2%	72.7%	47%
After 96h with grade 0 considered as negative response	8%	95%	67%	43%
After 96h with grades 0 and I considered as negative response	8%	95%	67%	43%

GPT: Gluten Patch Test; NPV: Negative Predictive Value; PPV: Positive Predictive Value

Sensitivity and specificity, positive predictive value (PPV), and negative predictive value (NPV) of gluten patch test in detecting celiac disease were determined. The analyses were carried out using SPSS software.

Findings

Median age of control group was 9.08 (range: 3-16) and in control group was 11.27 (range: 4-31). Twelve patients (40) in case group and 15 patients (55.6%) in control group were females. The values for gluten patch test after 48 hours and 96 hours are shown in Table 2. The best results were obtained when we reported the responses of gluten patch test after 96 hours, in which sensitivity 95%, specificity 8%, PPV 67%, and NPV 43% were reported. Therefore, gluten patch test is not efficient in screening celiac disease.

In this study, we compared responses to gluten patch test with severity of villous atrophy in patients with celiac disease (partial atrophy of villus was considered as mild and Marsh IIIC was of the greatest severity), Spearman's test showed that there is a direct relationship between GPT response after 48 h and severity of villous atrophy (r=0.26 P=0.04); For example, we can say patients with Marsh IIIC will show a higher grade of GPT after 48 h than patients with partial atrophy, but there is no relationship between response to GPT after 96 and severity of villous atrophy.

There is an inverse relationship between patients' age and response to GPT after 96 h (confirmed by Spearman's test results: r=-0.25, P=0.04). This means that after 96 hours, older patients will show a lower grade of GPT response than younger patients. But there is no relationship between age and response to GPT after 48 h.

In 30 patients with celiac disease, six patients discontinued gluten-free diet. There was no relationship between response to GPT and continuing gluten-free diet (confirmed by chi-square test results, P=0.16).

In this study, there was a direct relationship between serology test results (Anti t-TG IgG, Anti Gliadin IgA) of CD patients and responses to GPT after 96 hours (by Mann–Whitney test, P=0.051 P=0.03). We can therefore say there is a relationship between negative Anti Gliadin IgA and negative Anti t-TG IgG on the one hand and grade 0 in gluten patch test after 96 h on the other hand.

But there is no relationship between results of these serology tests with results of GPT after 48 hours, and there is no relationship between the results of other serology tests (Anti Endomysial Ab IgA, IgG, Anti Gliadin Ab IgG, Anti t-TG IgA) and responses to GPT.

Discussion

For the first time, the diagnostic value of gluten patch test for celiac disease was investigated in this study. Different studies have shown that skin patch test is a reliable test for diagnosis of food allergy and delayed hypersensitivity reaction [13,14] and useful to diagnose both mediated and non-mediated IgE reactions [15].

Several studies have shown that skin patch test is sensitive in diagnosis of food allergies associated with atopic dermatitis, particularly in young children [16,17]. In a study, specificity of skin patch test in diagnosis of cow milk allergy was determined to be 95% and for hen eggs to be100% [18]. In 46 patients with allergic eosinophilic esophagitis, the sensitivity of both

skin patch test and skin prick test were 97% and specificity of both was 5% in diagnosis of foods that cause AEE [19]. In another study on 19 patients with food protein-induced entrocolitis syndrome (FPIES), sensitivity of skin patch test was shown to be 10% and its specificity to be 71% in diagnosis of foods associated with FPIES [20]. All the studies showed that skin patch test is useful for food allergy diagnosis and because celiac disease is a food allergy and is a type of non-IgE mediated reaction [12], it was expected that gluten patch test could be an effective test in diagnosis of celiac disease. However, this study showed that gluten patch test cannot be such an efficient test in screening of celiac disease. Nevertheless, the gluten patch test can be useful in diagnosis of CD when employed with clinical symptoms, serology test, and biopsy of small intestine. We should doubt our diagnosis if a patient with CD shows positive response to GPT after 96 h.

Regarding the value of this study, we should doubt the statement that CD is a kind of food allergy; Perhaps it is better to present celiac disease as an autoimmune disease. This hypothesis is reinforced by association of celiac disease with other autoimmune diseases such as diabetes type I [21,22], selective IgA deficiency [23,24], autoimmune thyroid disease [25,26], and auto-immune myocarditis [27,28]. In this hypothesis, in CD patients, gluten is probably similar to epithelium of small intestine and when patients use gluten, the autoimmune system will be activated. Nonetheless, proving the hypothesis requires further studies.

Conclusion

This study showed that there is a direct relationship between severity of villous atrophy in CD patients and the response to gluten patch test after 48 h.

Is the grade of villous atrophy predictable by the grade of gluten patch test result after 48 hours? This needs a study with a larger population. There is a direct relationship between gluten patch test results after 96 h and Anti Gliadin IgA and Anti t-TG IgG results.

Will gluten patch test after 96 h be able to be replaced with Anti Gliadin IgA and Anti t-TG IgG? This also needs further research.

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Conflict of Interest: None

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