ORIGINAL ARTICLE

New diagnostic biomarkers for celiac disease in Egyptian children: Cyclophilin A and Netrin-1

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ABSTRACT

Background: Celiac disease is a chronic inflammatory disease of the small intestine. Cyclophilin A (CYPA) is a highly abundant protein in the cytoplasm of most mammalian cells. Netrin-1 is a laminin-related secreted protein that is broadly expressed in numerous tissues. Our aim was to determine the efficiency of both markers in the diagnosis of celiac disease and their relations to clinical findings.

Methods: This study was conducted on 50 children (mean age: 8±3.2 years) with celiac disease and 48 age and sex matched healthy controls. Circulating serum CYPA, Netrin-1, and anti-tissue transglutaminase antibodies were measured using ELISA kits.

Results: Both markers were significantly higher in celiac disease patients compared to controls. Patients presented with low birthweight in 5%, 15% of the patients were the offspring of consanguineous families, delayed milestones in 10%, abdominal pain in 35%, diabetes Type 1 was found in 10%, all patients had increased anti-tissue glutaminase levels, and upper endoscopy lesions in 25%. Significant positive correlations were noted between anti-tissue transglutaminase antibodies and both markers.

Conclusion: Both markers had good diagnostic performance for celiac disease among Egyptian children.

Keywords: Celiac Disease; Diagnosis; Cyclophilin A; Netrin-1.

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INTRODUCTION

Celiac disease is an autoimmune enteropathy described through changes in the intestinal mucosa due to gluten ingestion in genetically predisposed individuals. The three pillars used for its diagnosis are serum antibody detection, duodenal biopsy, and genetic study. Removing gluten from the diet is effective for most individuals, achieving clinical improvement, a progressive decrease in antibody titers, and recovery of the duodenal mucosa (1). The occurrence of celiac disease is growing, partially because of testing for, and improved recognition of, the disease (2). The criteria for celiac disease diagnosis are fluctuating, however, diagnosis in adults still count on the incidence of atrophied duodenal villous whereas the patient is on a diet including gluten, together with outcomes from examinations in serum. If proven scalable and accurate, analyses that identify tetramer complexes of gluten-HLA could be utilized in diagnosis done in the conditions of a gluten-free diet in absence of intestinal biopsy. Moreover, serum anti-tissue transglutaminase represents the most common diagnostic marker (3). Celiac disease patients also demonstrate the presence of alleles of HLA- DQ2 or HLA- DQ8, class II major histocompatibility complex as a genetic risk factor (4,5).

Recent improvements in the comprehension of celiac disease pathogenesis still emerging that may entangle the gluten exposure role. Numerous studies have revealed that the gluten quantity utilised by the child might impact on the age of onset of celiac disease in hereditarily predisposed persons. Novel recommendations from the European Society of Paediatric Nutrition, Gastroenterology, and Hepatology permit celiac diagnosis on serology, ignoring endoscopic biopsies for infants. Updated guidelines and latest information in adults do not favour biopsies for patients who are genetically vulnerable for celiac disease who have been identified via clinical signs of celiac disease with serology (6).

Cyclophilin A (CYPA) is a universally dispersed protein belongs to the family of immunophilin. CYPA has the activity of peptidyl prolyl cis-trans isomerase (PPIase), that controls protein trafficking and folding. CYPA largely activates pro-inflammatory pathways, and it is a direct chemoattractant for inflammatory cells. In addition, it enhances the production of reactive oxygen species and shares in increasing the proliferation of macrophages. High expression of CYPA associates with deprived outcome of patients with inflammatory diseases (7).

Netrin-1 is a neuronal regulator signal which controls cytoskeleton rearrangement, cellular migration, and activation in several cell kinds. It is a chemotropic protein expressed in tissues and stimulates both repulsive and attractive responses of migration (8). Netrin-1 regulates the immune response via the suppression of macrophage and neutrophil migration (9). Its expression is on vascular endothelium where it is regulated by inflammatory cytokines and infection. Netrin-1 acts as a powerful suppressor of migration to altered chemotactic stimulants in vitro and in vivo (10). These facts propose that expression of Netrin-1 endothelially might prohibit migration of basal cell to the tissues and that its down-regulation with the onset of inflammation/sepsis might help employment of leukocyte (11).

The development of new diagnostic biomarkers can provide a better noninvasive approach in the diagnosis of celiac disease. Further elucidations of the role played by CYPA and Netrin-1 on celiac disease will help in designing novel pharmacological therapies for this disease.

MATERIAL AND METHODS

This study comprised 50 children (mean age: 8±3.2 years) with celiac disease and 48 age and sex matched healthy controls. All celiac disease cases were on a gluten-free diet for more than six months. They were recruited from the outpatient clinics of the Clinical Genetics Department, National Research Centre, Egypt. The diagnosis was established based on clinical features, biochemical investigations, and upper endoscopy assessment. All samples were collected after obtaining the parents' informed consent using a form approved by the Ethical Committee of the National Research Centre (6).

Serum CYPA, Netrin-1 and anti-tissue transglutaminase antibodies levels were determined by ELISA (R&D Systems) according to the manufacturer's instructions.

Data are reported as mean ± standard deviation. For the comparison of normally distributed variables between groups, Student's t-test was used. Pearson's correlation coefficient was used to test the strength of any association between different variables. SPSS for Windows (SPSS Inc., Chicago, IL) was used. All tests were 2-tailed and statistical significance was set at the p0.05 level.

RESULTS

This study included 50 patients, 28 were females and 25 were males (1.12:1), Table 1 shows the characteristics of these patients. The mean levels of CYPA and Netrin-1 were increased in celiac disease patients compared to controls as shown in Table 2. Significant positive correlations were detected between anti-tissue transglutaminase antibodies and both CYPA and Netrin-1 levels in celiac disease patients (Table 3).

Table 1. The clinical characteristics of children with celiac disease.

Characteristics and variables	N=50
Female/male ratio	1.12:1
Consanguinity	15%
Low birthweight	5%
Delayed milestone	10%
Abdominal pain	35%
Diabetes mellitus type 1	10%
Anti-tissue glutaminase	100%
Upper endoscopy lesions	25%
Mean of anti-tissue glutaminase (U/ml)	36±12.55

Table 2. Levels of CYPA and Netrin-1 in CD patients and controls.

Biochemical markers	Group	Mean± Std. Deviation	P
CYPA (ng/mL)	Controls	6.26 ± 0.37	0.001
	Celiac disease	7.68 ± 0.72	
Netrin-1 (ng/mL)	Controls	145.49 ± 15.81	0.001
	Celiac disease	181.38 ± 19.13	

Table 3. Correlation between anti-tissue transglutaminase antibodies, CyPA, and Netrin-1 levels in celiac disease patients.

Biochemical markers	Pearson correlation	Anti-tissue transglutaminase antibodies
CYPA	r	0.919
	p	0.000
Nertin-1	r	0.611
	p	0.020

DISCUSSION

Celiac disease is a mediator for immune systemic syndrome initiated in hereditarily vulnerable persons via eating of gluten found in wheat and associated cereal grains. In adulthood, the intestinal biopsy sampled by endoscopy is the gold standard for celiac disease diagnosis (12). Generally, celiac disease prevalence varies from 4.5% among high-risk persons to 0.75% in subjects not-at-risk (13). High-risk persons comprise the relatives of celiac disease patients, adults or children with celiac disease related signs (i.e., constipation, abdominal pain and diarrhea), and adult or children individuals with celiac disease-related conditions (i.e., Down syndrome, infertility, osteoporosis, anaemia, Diabetes Mellitus Type 1) (14). Our findings found low birthweight in 5%, 15% of the patients were the offspring of consanguineous families, delayed milestones in 10%, abdominal pain in 35%, diabetes Type 1 was found in 10%, all patients had increased anti-tissue glutaminase and upper endoscopy lesions in 25%.

The syndrome is described by a variable grouping of clinical manifestations contingent on exposure to gluten in diet, the incidence of CD-specific antibodies in serum (anti-endomysium antibodies and anti-tissue transglutaminase (anti-TG2)), and diverse grade of enteropathy. The alleles of class II major histocompatibility complex was shown in celiac disease patients (15). Several studies have examined anti-TG2 autoantibodies production in patients' intestines with explicit celiac disease at diagnosis. Anti-TG2 autoantibodies deposited in the intestine were noticed in 100% of cases in adults with untreated celiac disease (16) . More changeability was described in pediatrics. Mucosal precipitates were recognized in 96 to 100% of celiac patients who were untreated (17). In children younger than 2 years of age this percentage is decreased (73%). Generally, the disease happens between 6 and 18 months of age, after the taking of weaning foods comprising prolamins. Cyclophilin B (CYPB) and Cyclophilin A (CYPA) are the best investigated members of the family targeting it to the endoplasmic reticulum(18). CYPA, a multifunctional protein, is known to be an inflammatory mediator that is released from different kinds of cells in response to inflammatory stimuli. Several studies have revealed that CYPA levels are increased in disorders associated with inflammatory conditions (19-21). Netrin-1 is a laminin-related released protein that is broadly synthesised in various tissues, comprising renal tissues. In previous studies Netrin-1 was shown to have a role in and acceleration of angiogenesis (22), growth and regulation of inflammation, and the migration of vascular endothelial cells tumor progression (23,24). In the present study results provided evidence for the role of Netrin-1 in celiac disease by showing an increase of its levels in patients compared to controls. A previous study in Egyptian patients with celiac disease and Type I diabetes reported genetic linkage of HLA genotypes and Egyptian celiac disease patients (25).

In conclusion, both CYPA and Netrin-1 markers have potential clinical efficacy in the diagnosis and follow-up of celiac disease among Egyptian children. Further studies are required to introduce these biomarkers into the traditional management of the celiac disease to avoid endoscopy, especially in pediatric patients.

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