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**Title:** The frequency of celiac disease in Turkish children with cystic fibrosis

**Running Head:** Celiac disease in cystic fibrosis

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**Abstract**

**Objective:** We aimed to investigate the frequency of CD in children with cystic fibrosis.

**Patients and Methods:** This prospective study was carried out from October 2015 to March 2017. A total of 71 patients with CF and 73 age- and sex-matched healthy children were included. All groups were evaluated for CD in terms of clinical and laboratory findings. Firstly, total IgA and tissue transglutaminase IgA (tTG IgA) levels were measured. Anti-endomysium IgA antibodies (EMA) were analysed for those patients with positive tTG IgA. Gastroduodenoscopy was performed to patients with both positive tTG and EMA IgA antibodies.

**Results:** Only eight patients (11.2%) had tTG IgA positivity, while four of them (5.6%) had positive EMA antibodies. The pathological results were consistent with Marsh 2 classification score in two patients. In addition to that, HLA-DQ2 is present in those two patients. Those patients were accepted as potential CD. Two patients who were thought to have potential CD were reassessed after one year and celiac screening tests were detected as positive again.

**Conclusions:** Only two of 71 CF patients (2.8%) was diagnosed with potential CD. Our study results showed that there might be an association between CD and CF.

**Keywords:** Celiac disease, cystic fibrosis, small intestine biopsy.

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**Introduction**

Cystic fibrosis (CF) is the most common autosomal recessive disorder associated with a deficit in the Cystic Fibrosis Transmembrane Regulator (CFTR) gene localized on chromosome 7 and is life threatening among Caucasians (1). The global incidence of cystic fibrosis is 1 in 2500 newborns (2).

Celiac disease (CD) is an immune-mediated systemic disease characterized by intestinal villous damage at various levels, triggered by gluten intake in genetically susceptible individuals (3).

The prevalence of celiac disease is estimated to be 0.5-1% in different regions of the world (4). Celiac disease has two peaks which are between 1-2 years old and 30 years old (5).

Cystic fibrosis was first described in 1938 and has been considered a separate disease from CD since then. A patient with CD and CF was reported for the first time in 1969, after that date sporadic cases were reported (1).

Weight loss, steatorrhea, and diarrhea are associated with intestinal malabsorption in both diseases, so it is difficult to diagnose CD in patients with CF (6). It has been recommended that screening of CD should be performed in communities with a high prevalence of CD and those with CF particularly having malabsorption that does not respond to standard treatments, those with pausing in physical development, those with CF having comorbidity of autoimmune disease (1,6). There is no consensus on the screening of CD in children with CF.

There are very few studies investigating the prevalence of CD in patients with CF (6-9). To our knowledge, there are not any study that investigate the frequency of CD in children with CF in our country. For this reason, we aimed to investigate the frequency of CD in children with CF.

**Methods**

The study was conducted prospectively between October 2015 and March 2017 at the Outpatient Clinics of Pediatric Pulmonology and Gastroenterology. Seventy-one patients were included in the

study. Patients having incomplete information in files at the time of diagnosis, patients with coincidental disease and patients refused to participate in the study were excluded. Only three patients refused to participate the study. Patients followed up with the diagnosis of CF, received gluten, and wanted to participate the study voluntarily were included in the study. A total of 73 age- and -sex matched healthy children were included in the study as a control group.

The study protocol was approved by the local Ethics Committee (313608 / 06 October 2015). An informed written consent was obtained from the patients, the healthy controls and their parents before the study. Those who refused to participate in the study and those who did not receive gluten were not included in the study.

According to the generally accepted criteria, the diagnosis of CF was diagnosed (10). All groups were evaluated for clinical and laboratory findings in terms of CD. Venous blood samples were obtained from both groups. Each sample was divided into aliquots and samples were stored at -80°C until analysis.

Total IgA tests by immunoturbidometric method (Roche Diagnostics GmbH, Mannheim, Germany) and tTG IgA tests (Catalog No.3503, Aesku Diagnostics Gmbh, Wendelsheim, Germany) by ELISA method were measured at the Central Biochemistry Laboratory of XXX. tTG IgG test was planned for patients with IgA deficiency. The cut-off value of tissue transglutaminase antibody was 12 U/mL. Anti-endomysium IgA antibody (EMA IgA) test (Inova Diagnostics, Inc. Lübeck, Germany) by IFA method was analysed in patients with positive tTG IgA in the ‘’XXX Laboratories Group’’. Gastroduodenoscopy was performed to the patients with both positive tTG and EMA IgA antibodies for definitive diagnosis.

**Biopsy procedures**

Gastroduodenoscopy was performed to the patients with both tTG and EMA antibodies positivity. One biopsy from duodenal bulb one and four biopsies from the duodenum were obtained. Biopsies were evaluated by the same experienced pathologist according to Marsh classification score (11).

**Statistical Analysis**

Statistical Package for Social Sciences for Windows, version 17.0 software (SPSS Inc, Chicago IL, USA) was used for statistical analysis. Frequency, percentage, and mean ± standard deviation (SD) and median (interquartile range) were used as descriptive statistics. Independent-Samples t-test was used for the nominal data with normal distribution. The Mann-Whitney U test was used for patients with not normally distributed variables, and the chi-square test was used to compare the relationship between categorical variables. P value was considered statistically significant as a <0.05.

**Results**

Seventy-one patients were included in the study. The mean age and weight were 9.94±5.55 years and 29.32±15.03 kg, respectively. 36 (50.7%) of the patients were female and 35 (49.3%) were male. There were 37 (50.7%) girls and 36 (49.3%) boys in the control group. The mean age of the control group was 9.67±5.36 years. When the patient and control groups were compared in terms of age, sex, height and weight, there was no significant difference between the groups (p>0.05) (Table 1).

56 of our patients (78.9%) have gastrointestinal symptoms such as chronic diarrhea, steatorrhea, and weight loss. Also, 15 of them (21.1%) have anemia for extra-intestinal symptoms.

tTG IgA positivity was detected in only eight patients (11.2%), then EMA antibodies was measured, and four (5.6%) patients had positive results (Table 2). Gastroduodenoscopy was performed to those patients. One from duodenal bulb and four biopsies from the duodenum were taken. The endoscopic appearance of two patients has normal mucosal appearance of the bulbus and scalloping of the duodenum. The pathologic results were consistent with Marsh 2 classification score in those two patients and HLA-DQ2 typing were also positive. Those patients were accepted as potential CD. Also, they have typical gastrointestinal symptoms such as chronic diarrhea and steatorrhea, so they are considered as typical celiac disease.

The other two patients have normal mucosal appearance of the bulbus and duodenum, the pathologic results were compatible with the Marsh 0 classification score. Two patients who were thought to have potential CD were reassessed after one year, and the celiac screening tests were detected positive again. Gastroduodenoscopy could not be performed to one patient due to chronic pulmonary infection. In the other patient, pathologic result was consistent with Marsh 1 classification score (Table 3).

In the control group, three (4.1%) children had tTG IgA positivity, but EMA antibody positivity was not detected in any of them.

**Discussion**

The CFTR gene associated with CF disease was identified in 1989. The most common CFTR defect is delta F 508 mutation, which is present in approximately 70% of patients with CF (2). Today, more than 1500 mutations have been identified (12). Different CFTR mutations result in different disease phenotypes. Some mutations may have little or no effect on the CFTR function or may cause mild forms of the disease (13).

The prevalence of CD has increased dramatically in the last 20 years due to the use of serological tests. Studies reported that its prevalence increased four times in the United States, while two times increase was detected in Finland (14,15). Because only 10% of patients are symptomatic, a majority of asymptomatic patients remains undiagnosed despite screening of the high risk populations (5,16).

Celiac disease is a life-long disorder and is associated with increased morbidity and mortality if left untreated (17). Celiac disease complications are predominantly in adults and include refractory celiac disease, decreased fertility in women, cancers and other autoimmune diseases. Compared with the

general population, the risk of developing cancer is twice as high (18). With a gluten-free diet, the risk of developing complications is likely reduced (19). Therefore, it is very important to diagnose CD early and to start treatment.

Most of the symptoms of CD can be seen as gastrointestinal system findings of CF. Furthermore, it is difficult to distinguish between these two conditions in patients with CF since clinical findings of iron deficiency anemia and lack of fat-soluble vitamins are equally seen among people with and without CD (6,7). Mucosal changes in the small intestine and its associated malabsorption may worsen the nutritional status and affect the survival duration. Thus, diagnosing CD in co-existing CF patients may play an important role in the treatment efficacy (7).

Some hypotheses have been proposed to explain the coexistence of these two diseases. In patients with cystic fibrosis, small bowel mucosa may have greater contact with the gluten protein due to incomplete digestion and pancreatic insufficiency. This can play an important role in comorbidity. However, malnutrition can also cause some additional mucosal damage. In patients with malabsorption, feeding with a high-energy diet results in more antigen burden and gluten peptides may pass more easily to the epidermis and lead to the development of CD (7,20).

Venuta et al. (21) reported a case report with an association of CD and CF, and they had opted for 15 cases of coexistence of CD and CF reported in the literature until 1999.

Studies have reported that the incidence of CD among children with CF is between 0.4-2.6% (6-9).

In a multi-center study, the prevalence of CD was found to be 1.2% (1/83) in 790 Scandinavian patients with CF (6). In this study, six patients had already diagnosed with CD, whereas four were just diagnosed with CD. In addition, serological tests were positive in two patients, but normal histology of duodenal biopsy was detected. These patients were scheduled for follow-up because of suspected potential CD. Based on the prevalence studies conducted earlier, it has been estimated that the prevalence of CD among Scandinavian patients with CF was 2-3 times higher than the general population. CD was diagnosed in three patients before the diagnosis of CF, while it was synchronously diagnosed with CF in two patients. According to clinical findings, it was difficult to

distinguish between these two conditions, iron deficiency and deficiency of fat-soluble vitamins were found to have equal prevalence in these patients. In this study, it has been recommended that screening should be performed for older than nine month old children with CF or those receiving gluten for more than three months in populations with high-prevalence of CF, such as Sweden (6).

In a study including 230 CF patients in Poland, the prevalence of CD was found 2.6%. tTG IgA positivity was detected in eleven patients, EMA IgA positivity was detected in six of those patients. Gastroduodenoscopy was performed to five of them, four of them diagnosed with CD at that moment, whereas two of them were previously diagnosed. The incidence of CD was five times higher in patients with CF than in healthy populations. In addition, tTG positivity and EMA negativity were found in four patients and gastroduodenoscopy was performed to those four patients and pathologic results were detected as normal. Moreover, those patients were planned to be followed up for a long time, as they might have latent CD (7). It has been suggested that CF as a risk factor should be examined as a consequence of this study, and it is thought that CF has a tendency to cause CD.

Abdominal pain was detected in 11% of the 500 CF patients, and two of those patients (0.4%) were diagnosed with CD (8). It has been stated that the most common cause of admission to the hospital in older children with CF might be abdominal pain.

In a study conducted by Valleta et al. (9), the incidence of CD was found to be 0.4% in 1100 CF patients and reported to have a higher incidence of CD in population with CF than in healthy population.

The current approach to CD has changed with the development of highly sensitive and specific serological tests. Both EMA IgA and tTG IgA tests have been shown to be highly sensitive for CD (22). European Association of Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) recommends total IgA and tTG IgA tests for the initial screening of CD. It has been suggested that EMA IgA test should be performed in patients with tTG IgA positivity. If this test is found positive, small bowel biopsy should be performed (3). In patients with IgA deficiency, tTG IgG or EMA IgG may

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help in decision for the biopsy (16). EMA and tTG IgA tests have more than 95% sensitivity and specificity when used together (23).

Our study was based on updated ESPGHAN guideline. tTG IgA positivity was detected in eight patients (11.2%), then the levels of EMA IgA antibody were measured for these patients and positive results were detected in four (5.6%) patients (Table 2). The gastroduodenoscopy was performed in those patients. The pathologic results were compatible with Marsh 2 classification score in two patients who had also positive HLA-DQ2 tests. Potential CD was thought in those two patients, it was planned that those patients were followed up serologically and clinically (24,25). In the other two patients, the pathologic results were detected as normal. Two patients who were thought to have potential CD were reassessed after one year, and the celiac serologic tests were found positive again. The first patient could not undergo endoscopy due to chronic infection. In the second patient who underwent endoscopy, the pathologic result was compatible with Marsh 1 classification score. In latter patient, the patient did not have a gluten-free diet, the pathologic result of second intestinal biopsy may be associated with patchy distribution of villous atrophy of CD. Also, first patient, who could not undergo endoscopy, did not have a gluten-free diet. If we had performed gastroduodenoscopy to first patient, we could detect Marsh 2 classification score. Therefore, in this study the frequency of potential CD was 2/71.

As a limitation, the number of cases may be small due to the fact that our study was single-centered, but only three follow-up patients did not accept to participate voluntarily. Therefore, the impact of our study may be weak, but we still consider that this study is important for our country, as it is the first study on this issue.

In a study conducted in healthy Turkish children aged between 6-17 years in our country, the prevalence of CD was detected as 0.47% (26). According to our study results, the frequency of potential CD was found as 2.8% in patients with CF. We detected that the CD was seen approximately six times more in the CF population than healthy children.

In conclusion, despite the small number of cases, we suggest that all children with CF should be screened for CD. Multicentric studies with more children with CF are needed to provide more precise evaluation.

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**Tables**

Table 1. Demographic characteristics and laboratory findings of the patient and control groups

| | Patient group (n=71) | Control group (n=73) | p |
|---|---|---|---|

| | | | |
|---|---|---|---|
| Age (years)* | 10.75 | 10.00 | 0.873 |
| Height (cm)* | 134.00 | 137.00 | 0.440 |
| Weight (kg)* | 27.40 | 37.00 | 0.090 |
| Hemoglobin (mg/dL)** | 12.61±1.07 | 12.78±1.48 | 0.434 |
| MCV** | 80.95±5.04 | 79.39±4.87 | 0.060 |
| Plt (/$mm^3$)* | 319.00 | 287.00 | 0.066 |
| tTG IgA (U/mL)* | 1.70 | 0.90 | 0.001 |
| Total IgA (mg/dL)* | 141.00 | 133.00 | 0.414 |
| Age at diagnosis (months)* | 6.00 | - | |
| Sweat chloride level (mEq/L)** | 97.11±21.8 | - | |

*Plt=thrombocytes, MCV= mean corpuscular volume, tTG=tissue transglutaminase.*

**Data are presented as median (interquartile range).*

***Data are presented as mean ± standard deviation.*

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Table 2. Data of patients with positive tissue transglutaminase antibody

| Patient number | tTG-IgA (U/mL) | Total IgA (mg/dL) | EMA-IgA | Marsh classification score |
|---|---|---|---|---|
| 1 | 300 | 84 | + | 2 |
| 2 | 47.1 | 147 | + | 2 |
| 3 | 72.9 | 241 | + | 0 |
| 4 | 15 | 69.3 | + | 0 |
| 5 | 31.4 | 184 | - | - |
| 6 | 32.6 | 92 | - | - |
| 7 | 26.5 | 187 | - | - |
| 8 | 33.8 | 210 | - | - |

*tTG=tissue transglutaminase, EMA= anti-endomysium antibody*

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Table 3. Laboratory data of patients with potential celiac disease

| Patient Number | tTG-IgA (U/mL) | Total IgA (mg/dL) | EMA-IgA | Marsh classification score |
|---|---|---|---|---|
| 1 | 300 | 84 | + | 1 |
| 2* | 32 | 147 | + | - |

*tTG=tissue transglutaminase, EMA= anti-endomysium antibody*

**Gastroduodenoscopy could not be performed due to chronic infection.*

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