FREQUENCY OF CELIAC DISEASE IN PATIENTS WITH β-THALASSEMIA MAJOR REPORTED AT CHILDREN HOSPITAL LAHORE

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ABSTRACT

Background and Objective: β-thalassemia is the most common blood disorder with an autosomal recessive pattern caused due to reduced synthesis of β globin chains. The objective of the study is to determine the frequency of celiac disease in patients with β-thalassemia major.

Methods: The study was conducted at The Children's Hospital and The Institute of Child Health, Lahore from July 2015 to December 2015. In this cross sectional study, the prevalence of celiac disease in children with β-thalassemia major was conducted in a period of 6 months. Patients were screened for celiac disease through ELISA technique by the detection of anti-tissue transglutaminase (anti-tTG) IgA and IgG antibodies.

Results: A total of 83 patients with mean age ± SD 7.87 ± 3.82 were included in this study which showed 66.3% male and 33.7% females. A total of 9.6% (n = 8) patients showed positive results for (anti-tTG) IgA and IgG antibodies for celiac disease. Borderline cases were 6.0% (n = 5) and 84.3% (n = 70) patients showed negative results. Variables were quantified by descriptive analysis.

Conclusion: Patients have multiple symptoms well foundedwith celiac disease. As the prevalence of celiac disease is high in patients with β-thalassemia major, so it would be justifiable to screen them for celiac disease.

Keywords: β -thalassemia major, Celiac disease, HLA, ELISA (IgA & IgG antibodies).

INTRODUCTION

β-thalassemia major is a genetically determined blood disorder caused by the syntheticdefect of β globin chains. It results in the reduction of hemoglobin involving the affected chain, where each globin chain has separate genetic control.[1] As hemoglobin is red cell protein which works to carry oxygen to all parts of the body and its disorder results in red cell destruction, leading to anemia.[2]

β-thalassemiais prevalent in the Middle East, Mediterranean countries, India and Central Asia, Far East, Southern China and countries along north coast of South America and Africa. Carrier frequency shown in Sardinia (10.3%), Cyprus (14%), and Southeast Asia.[3] 3% area of the world's population carry genes for β-thalassemia and 60,000 thalassemic babies are estimated to have born all around the world.[4] Carrier rate in Pakistan ranges between 5 – 8% and around 5000 children each year are diagnosed with β-thalassaemia major.[5] As reported by Thalassaemia International Federation, only 200,000 patients with thalassaemia major are registered receiving regular treatment all over the world.[6]

Four protein subunits collectively form a haemoglobin molecule, two beta-globin chains and two alpha globin chains. So for 200 mutations reported as yet, of which point mutations were most important in functional regions of β globin gene.[7] βglobin gene deletions are uncommon. Haemoglobin β gene regulate the production of β globin chains. Haemoglobin β gene located on chromosome.[11] undergo mutations that cause thalassaemia with inadequate production of β-gobin chains of haemoglobin, with excessive alpha globin chains[8].

β-thalassaemia major patients clinically diagnosed upto 24 months of age. Infants are mostly presented with growth failure, diarrhoea, recurrent abdominal pain, hepatospleenomegaly and sometimes constipation.[9] Patients remain untreated in developing countries due to insufficient resources and also poorly transfused patients show clinical picture of growth failure, jaundice and skeletal changes.[10]

Celiac disease is a unique genetically determined immune mediated disorder persuaded by gluten, a storage protein of wheat and multiple similar proteins of barley and rye cosequencing in small intestinal mucosal damage and malabsorbption.[11] Celiac disease is categorised by immunological activation in lamina pro-

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pria of small bowel, and gluten free diet ensued full recovery.[12] Regular usage of gluten diet in sensitized individuals accelerate chronic inflammation of the small intestinal mucosa with variable outcomes ranging from villous atrophy, crypt hyperplasia and lymphocyte infiltration along the whole length of mucosa.[13]

Celiac disease occurs in all ages with different rates approaching 1% of general population[14].The disease is also manifested in Europe andEuropeanancestry populated countries. Celiac disease is also prevalent in Asia[15], Middle East,[16] North Africa,[17] and South America.[18] Celiac disease is variably prevalent in different groups, ranging from 0.5 to 1%.[19] 90% symptomatic individuals on estimation, remain undiagnosed.[20]

Celiac disease is activated by gliadin peptides part of gluten inducing sequential damage to mucosal epithelium through stimulating the innate and adaptive immune responses.[21] Damaged epithelium initiates the expression of interleukin-15 resulting in intraepithelial lymphocytes work up, as they become cytotoxic and kill enterocytes. A stress protein on their surface is presented, permeability changes results in the entry of gliadin in laminapropria, where its deamidation by an enzyme tissue transglutaminase, allowing its interaction with HLA-DQ2 (or HLA-DQ8) on antigen presenting cell's surface.[11] Presentation of gliadin to reactive CD4+T cell via T-cell receptor, causes the cytokine release with resulting tissue damage and expansionof B cells that produce antibodies.[22]

Human leukocyte antigen (HLA), especially HLA-DQA1 and HLA-DQB1 are genetically predisposing alleles in celiac disease.[19] MHC class 2 alleles presented on short arm of HLA-DQ locus 22 for necessary for phenotypic expression of celiac disease.[13] Celiac disease share close resemblance with β-thalassemia major due to genetic predisposition and by endocrinal abnormalities. HLA-DQB1 allele is susceptible with pathogenesis to β-thalassaemia major.[19]

Rational of the Study

This study suggests that patients with β-thalassaemia major are at increased risk of developing the celiac disease due to genetic predisposition. Moreover, this study purposes the guidelines to evaluate common clinical features in β-thalassaemia major and celiac disease patients. So, it may be helpful to take therapeutic measures after screening celiac disease in β-thalassaemia major patients and in agreement that patients should be on follow up because the disease is progressive in nature.

METHODS

A cross sectional study was conducted from July 2015 to December 2015 on 83 patients with β-thalassaemia major aged 1 – 15 years who referred to OPD thalassaemia centre of The Children's Hospital and The Institute of Child Health Sciences affiliated with University of Health Sciences, Lahore. The study was approved by the Ethics Committee of The Children`s Hospital and The Institute of Child Health, Lahore. Patients were diagnosed on the basis of routine complete blood count and haemoglobin electrophoresis. Clinical findings were also evaluated. Patients were undergoing regular blood transfusion and enrolled in the study after the parent's informed consent. Exclusion criteria was designed as the patients not willing to participate in the study and patients suffering from chronic diarrhoea, weight loss and vomiting due to conditions like gastroenteritis, ulcerative colitis, crohn's disease etc. Also patients having no clinical features suggestive of any endocrinal disorder that might affect the growth were not included in the study. All demographic data of the patients which were studied includes age and gender, anthropometric data such as weight, height, and gastrointestinal symptoms in β-thalassemia major patients common with celiac disease such as diarrhoea, growth failure, recurrent abdominal pain, abdominal distention, finger clubbing and edema. Patients were tested for anti-tissue transglutaminase (anti-tTG) IgA&IgG antibodies. Serological tests were performed in the Department of Diagnostic Immunology, The Children's Hospital and The Institute of Child Health, Lahore with human recombinant enzyme linked immunosorbant assay (ELISA) method using acommercial available kit; (BL Diagnostika, Mainz, Germany). For anti-tTG antibody, normal range as determined by the manufacturer was 9 – 11 U/mL. The data was analysed by using SPSS 22.0. Qualitative variables were expressed by percentages and frequencies. Quantitative variables were analysed by descriptive measures.

RESULTS

Among 83 patients 55 (66.3%) were male and 28 (33.7%) were female. Patients with mean age ± SD 7.87 ± 3.82, mean height ± SD were 2.66 ± 2.84 and with mean weight ± SD were 18.51 ± 8.25 were enrolled in the study (Table 1). Out of 83 cases, recurrent abdominal pain was in 66 (79.5%) patients and 71 (85.5%) patients have abdominal distention. A total of 40 (48.2%) patients show the positive dirrhoeal history. Growth failure was seen in 44 (53.0%) patients. Finger clubbing was prominent in 24 (28.9%) patients. Edema was present in 34 (41.0%) patients while 49 (59.5%) patients were negative for this (Table 2). Celiac disease positive cases for IgA & IgG were 8 (9.6%) and 70 (84.3%) patients were negative and borderline cases were 5 (6.0%) (Table 3).

DISCUSSION

β-thalassaemia is catagorized by reduced β-globin chains with varied heterogeneity of molecular defects. Children with thalassaemia major have several symptoms suggestive of celiac disease. Based on some

common features between β-tha-lassaemia major and celiac disease and also on some available case reports, there is a relation between two diseases. In our study, we investigated the frequency of celiac disease in patients with β-thalassaemia major.

We took a total 83 patients between 1 to 15 years of age. Similarly another study was also carried out by Sharamian *et al.* on β-thalassaemia major patients in pediatric age group.[19] Honar in 2014 conducted an investigation for determining the frequency of celiac disease in children with β-thalassaemia major.[23] He took 215 β-thalassaemia major patients with mean age of 12.7 ± 4.4 years which favours the age limit of our study. Moreover, various studies and cases were reported to determine the frequency of celiac disease and compromised growth in β-thalassaemia major patients of pediatric age group.[19,23 -27]

In our study male to female ratio was 2:1 indi-cating more male thelassaemic patient. Similarly, Sharamian *et al.* in 2015 took a total of 620 children and more were males (52.6%) as compared to females (47.4%).[19] Honar conducted an investigation in 2014 for determining the frequency of celiac disease in children with β – thalassaemia major with 52.1% male and 47.9% females.[23] All these studies favours the gender distribution of our study. As it does not have any genetic basis, celiac disease equally affects both males and females. In a study conducted by Montuori in 2014 reported celiac disease in a 21 year old thalassaemia major female.[24]

In our study, abdominal distention and abdominal pain were the major clinically frequent features in patients. Growth failure and diarrhoea were also the renowned one. Sharamian *et al.* in 2015 studied 200 patients of thalasaemia with growth failure.[19] Similarly Ramraj in a case report in the yearof 2014 documented a female with history of recurrent diarrheal history[25]. Montuori diagnosed a thalassaemic girl in 2014 with history of diarrhoea and abdominal pain.[24] Ciccocioppo in year 2013 explained a boy and girl with complaints of abdominal discomfort and diarrhoea.[26] Ankit Parakh in 2008 reported a thalassaemic boy with history of growth failure.[27] All these researches were in favour of our study. In contrast to our study Honar *et al,* in 2014 studied β-thalassaemia major patients with clinical features of constipation and vomiting which shows that thalassaemic patients can present with constipation and vomiting[23] as well.

Approximately 9.6% children in our study were positive for screening of celiac disease and 6% shown the borderline results, and negative results were in 84.3% of cases. Most of the children presented with recurrent abdominal pain, abdominal distention, growth failure and diarrhea typical to β-thalassaemia major and also suggestive of celiac disease. Similarly Sharamian *et al.* in 2015 observed tTG IgA titers for celiac disease in β-thalassaemia major cases with prevalence of 11.5%.[19] Montouri in 2014 reported a β-thalassaemic major girl who, have tTG IgA titer more than 100.[24] A case report conducted by Ciccociopoin year 2013 diagnosed thalassaemia girl and a boy with clinical features suggestive of celiac disease. Results of all these studies favour the results of our study.[26] Diarrhoea, growth failure, abdominal pain was common in celiac disease

Table 1: *Demographic and anthropometric characteristics in patient with β-thalassaemia major.*

	Frequency	*Percent*		
Gender F/M	28/55	33.7/66.3		
	Mean	*Standard Deviation*	*Minimum*	*Maximum*
Age, year	7.87	3.82	1.00	15.00
Height, feet	2.66	2.84	1.00	5.00
Weight, kg	18.51	8.25	1.00	50.00

Table 2: *Frequency of symptoms in β-thalassaemia major patients common with celiac disease.*

	Positive Cases	**Negative Cases**
Diarrhea	40 (48.2%)	43 (51.8%)
Growth failure	44 (53.0%)	39 (47.0%)
Recurrent abdominal pain	66 (79.5%)	17 (20.5%)
Abdominal distention	71 (85.5%)	12 (14.5%)
Finger clubbing	24 (28.9%)	59 (71.5%)
Oedema	34 (41.0%)	49 (59.5%)

Table 3: *Frequency of celiac disease in patients with β-thalassaemia major.*

	< 9 (negative)	*9 – 11 (Border Line)*	*> 11 (Positive)*	*Total*
anti-tTG IgA	70 (84.3%)	5 (6.0%)	8 (9.6%)	83 (100%)
anti-tTGIgG	70 (84.3%)	5 (6.0%)	8 (9.6%)	83 (100%)

and β-thalassaemia major. Growth failure was most pronounced in patients due to defects in growth hormone secretion as the underlying mechanism. According to common findings, such as growth failure, genetic and autoimmune correlation between celiac disease and β-thalassaemia major is logical.

In contrast, Honar in 2014 observed that none of the β-thalassaemia major patients of the study were positive for anti-tTG IgA and IgG. Results of his study do not support our study. As eight cases in his study were of IgA deficient while none of the patients in our study was IgA deficient and he took patients with clinical features of constipation and vomiting while none of the patient shows the dirrhoeal history as in our study. In Honar study, there was nothing common between celiac disease and β-thalassaemia major and he did not in suggest any screening for celiac disease. We resulted that there was a high correlation between celiac disease and β-thalassaemia major which is dissimilar with the study by Honar. However, a review article about epidemiology of celiac disease in Iran showed a very low prevalence of celiac disease in Shiraz.[23]

According to our study the frequency of celiac disease should be concerned, as incidence of celiac disease is shown in patients with β-thalassaemia major. Patients with thalassaemia major should be screened for celiac disease, even without typical symptoms of celiac disease through detection of anti-tTG IgA and IgG antibodies. Intestinal biopsy should be done for patients showing positive serology.

Author's Contribution

- Hira Ali did the research, data collection, lab work and manuscript writing.
- Dr. Farhana Shehzad was the supervisor and corresponding author of data collection, lab work and manuscript writing.
- Mariam Zameer helped in result interpretation and manuscript writing.
- Saba Aziz designed and did the statistical analysis.

It is ***concluded*** that patients with β-thalassaemia major presenting with clinical features suggestive of celiac disease are at increased risk of developing the celiac disease due to genetic predisposition. So these patients should be on close follow-up for timely diagnosis of celiac disease.

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