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Evaluation of Tissue Transglutaminase IgA in Thalassemia Minor Patients

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ABSTRACT: Minor beta thalassemia is a common condition in children. The aim of the present study was the investigation of serum tissue transglutaminase IgA level as potential celiac disease in patients with thalassemia minor. This case control study was conducted on patients with beta thalassemia minor and healthy children in the years of 2014 to 2015. A total of 300 children were enrolled and IgA and tissue transglutaminase IgA levels were measured. The normal limit of tissue transglutaminase IgA was considered to be 20 IU/mL. Data analysis was performed using SPSS20 with 95% confidence of interval. Two groups of participants were matched regarding sex (Chi-Square=0.436 and P=0.509). Mean age of participantswas8.46 ± 4.54 and 7.74 ± 2.99 in minor and control respectively. Means of age, body mass index and serum tissue transglutaminase values were different between case and control while only serum tissue transglutaminase was significant (P=0.024). The status of tissue transglutaminase had a significant correlation with case and control (P<0.001). It is concluded that a positive association exists between β- thalassemia minor and celiac disease. Based on this result we could suggest a routine screening test for celiac disease in all children with β- thalassemia minor.

Keywords: Celiac, Children, Thalassemia minor, TTG IgA

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INTRODUCTION

Celiac Disease (CD) is an immune-mediated disorder which is generated by dietary gluten habits in genetically susceptible persons (Noori et al., 2016). The major staple diet for Iranian population (95% of the rural and 85% of the urban people) is wheat and its production especially in the southeastern areas like Sistan and Baluchistan province (S&B). S&B has wheat consumption higher than 160 kg per capita in a year (Nejad et al., 2011). The prevalence of CD is reported as 1-2% among western people and it affects 1.0% of people around the globe and it is about 1.5 to 2 folds higher among females than males (Noori et al., 2016). There are several non-gastrointestinal diseases or symptoms by which patients with CD may present. The prevalence of CD in patients with various diseases ranges from 1% for mental retardation to 14.6% in patients with iron deficiency anemia of unknown origin (Nejad et al., 2011). CD may have even a higher increase among individuals who have a first-degree relative with the disease (10 to 15%), type I diabetes (3 to 16%), Down's syndrome (0% to 19%), Turner's syndrome (3%) and IgA deficiency (9%) (Noori et al., 2016). In S&B province of Iran it has been reported that the prevalence of CD in patients with thalassemia major is 11.5% versus controls with 3.5%, and the prevalence in patients with congenital heart defects is 18.41% versus controls with 7.35% (Shahramian et al., 2015a and Shahramian et al., 2015b). Amongst different serologic tests (Anti Gliadin Antibodies (AGA), Anti Endomysial Antibodies (anti-EMA) and Anti Tissue Transglutaminase (anti-tTG) to evaluate and detect CD, anti-TTG is more common in application because of high sensitivity and specificity (Shahramian et al., 2015b). Thalassemia is characterized by decrease or absence of production of α- or β-globin chains, which results in two main types of α- or β -thalassemia. β -thalassemia major is a common genetic disorder that causes severe anemia from early childhood (Noori et al., 2014) and it has three mild (minor or carrier), medium (intermedia) and severe (major) forms. Every year, 100,000 neonates are born with hemoglobinopathies around the world (Noori et al., 2012). Thalassemia has a high prevalence in Mediterranean, Indian, North Chinese, and Pacific populations. Recently,

multiple blood transfusions and iron chelating agents have caused a significant improvement in the quality of life and life expectancy of these patients (Noori et al., 2012; Noori et al., 2014; Noori et al., 2015). In Iran, β-thalassemia has a high prevalence (around 10%) in north and in south. The prevalence of thalassemia alleles in most parts of the country has been estimated to be 4–8% (Rahimi, 2013). About 20000 patients with thalassemia major and 2 - 3 million carriers of thalassemia are living in this country (Jaafari et al., 2006). A high frequency of β-thalassemia in the S&B province with multiethnic population could be due to malaria and high rates of consanguineous marriage specially among Baluch population (Miri-Moghaddam et al., 2011). β-thalassemia major and CD have similar association with autoimmune disorders. The association between CD and β- thalassemia has been established in few case reports. Thalassemia intermedia can range from transfusion-dependent anemia to anemia in presentation that is slightly more severe than that in patients with thalassemia minor. Valizadeh and Shateri (2009) and Ramraj et al. (2014) have reported two cases of thalassemia minor and intermedia with CD (Ramraj et al., 2014 and Valizadeh and Shateri, 2009). The known clinical presentations of CD are a few (Fasano et al., 2003) and there are multiple similar symptoms in thalassemia and CD as well (Noori et al., 2016). Since both CD and thalassemia are two of the most common genetically based diseases, recognition and treatment of CD may improve the survival of thalassemia patients. There are only few case reports about CD in patients with β-thalassemia and we aimed to carry out this study with the objective of discovering the prevalence of CD in patients with minor thalassemia and its comparison with controls.

MATERIALS AND METHODS

This case-control study was conducted on 100 patients with minor thalassemia compared with 200 healthy participants to evaluate and compare the level of TTG from March 2015 to March 2016. Patients and control participants were collected randomly from those who have referred to Ali Asghar pediatric clinic of Zahedan University of Medical Sciences, Zahedan, Iran. The participants 'age was 1 to 18 years.

Inclusion and exclusion criteria

Healthy children were selected randomly among those who referred to the center for annual check-up and patients were selected to take part in the study after the confirmation of hemoglobin electrophoresis for thalassemia minor. According to the results of hemoglobin electrophoresis, the patients who had an HbA_2 higher than 3.5 % were considered as the case group. Exclusion criteria included IgA deficiency, a history of digestive, endocrine and metabolic disorders, iron deficiency, kidney disease, fever and chronic diseases.

Measurements and Tools

Height was measured using a scaled table in sleeping position for the children below 2 years old and by a scale in standing position for others. Besides, weight was calculated using a special Mika scale (made in Japan) for infants and by Rasa scale (made in Iran) for other children. Body Mass Index (BMI) was calculated by dividing a person's weight in kilograms to the square of height in meters and categorized in three main groups of underweight (<18.5), healthy (18.5-25) and Overweight (>25). Three milliliters of blood was drawn from these fasting children at 8:00 am. Samples were centrifuged and the separated serum was kept in a -70°C fridge till TTG IgA and total IgA was measured. Finally, they were transferred to the biochemistry laboratory, University of Medical Sciences, Zahedan, Iran under the cold chain compliance. Then, 250 microns of the isolated serum of these samples were used for serologic tests with recombinant ELISA. Normal limit of TTG IgA was considered to be 20 U/mL (Shahramian et al., 2015a; Shahramian et al., 2015b; Noori et al., 2016).

Ethical Approval

The study was approved by the ethics committee of Zahedan University of Medical Sciences, Zahedan, Iran. , The written informed consent was obtained from all patients' parents. A form was prepared for each patient which contained information about age, height, weight, sex, TTG IgA and total IgA.

Statistical Analysis

Data analysis was performed using SPSS version 20 (IBM Corp. Released 2011, IBM SPSS Statistics for Windows, Version 20.0, Armonk, NY: IBM Corp) with applying non parametric Mann-Whitney U, cross tabs contingency coefficient. Significance level of 0.05 was considered.

RESULTS

This study was conducted on 300 participants with the distribution of 200 and 100 for the control group and minor thalassemia patients respectively. The normality test

for the major variables of the study revealed that all the variables in the study namely age, BMI and TTG had non-normal distribution (Table 1). For the reason, all the statistical methods for the analysis were non-parametric tests. Table 2 indicates the sex distribution in categorized participants. Accordance to Pearson's chi-square test, the results revealed that the two groups of participants are matched in regarding gender (Pearson chi-Square=0.436 and P value= 0.509). Table 3 demonstrates the results of Mann-Whitney U test for age, BMI and TTG variables. Among these variables, only TTG had significant differences in groups of case and control (Mann-Whitney U=0.8404, P=0.024). Mean age of participants were 8.46 ± 4.54 and 7.74 ±2.99 for minor and control populations respectively (P=0.077). Correlation between TTG status (normal or ≤ 20 and abnormal or >20) and the main variables in the study are presented in the table 4. According to various study variables (Case-Control, BMI and Sex), the distribution percent of participants in different status of TTG was correlated to case control (Contingency Coefficient=0.291, P<0.001).

Table1. Kolmogorov-Smirnov (K.S) Normality test for Age, BMI and TTG Data

Variable	Mean	SD	K.S Value	P value
Age	7.9800	3.59062	.092	<0.001
BMI	21.4317	12.73186	.192	<0.001
TTG	19.1690	47.85579	.373	<0.001

BMI=Body Mass Index, TTG=Tissue TransGlutaminase

Table 2. Participants Sex distribution amongst case and control group in Zahedan, Iran

Participants	Statistics	Sex		Pearson Chi-Square value	P. value
		Males	Females		
Minor	Number	60	40	0.436	0.509
	Percent	60.00	40.00		
Control	Number	112	88		
	Percent	56.00	44.00		
Total	Number	172	128		
	Percent	57.30	42.70%		

Table 3. Results of Mann-Whitney U test for three main variables of the study among participants with minor thalasemia and control group in Zahedan, Iran

Variables	Groups	Mean	SD	Mean Rank	Sum of Ranks	Mann-Whitney U	P
Age	Minor	8.4600	4.53810	162.96	16296.00	8754.00	0.077
	Control	7.7400	2.99202	144.27	28854.00		
BMI	Minor	24.6450	18.02472	154.15	15414.50	9635.5	0.606
	Control	19.8251	8.61079	148.68	29735.50		
TTG	Minor	33.6839	69.48523	166.46	16646.00	8404	0.024
	Control	11.9116	29.66895	142.52	28504.00		

BMI=Body Mass Index, TTG=Tissue TransGlutaminase

Table 4. Results of correlation between TTG status and main variables in the study

Variables	Options	Statistics	TTG		Contingency Coefficient	P. Value
			<=20 (Normal)	>20 (Abnormal)		
Groups of participants	Case	Number	74	26	0.291	P<0.001
		Percent	74.0%	26.0%		
	Control	Number	190	10		
		Percent	95.0%	5.0%		
	Total	Number	264	36		
		Percent	88.0%	12.0%		
Sex	Male	Number	150	22	0.028	0.625
		Percent	87.2%	12.8%		
	Female	Number	114	14		
		Percent	89.1%	10.9%		
	Total	Number	264	36		
		Percent	88.0%	12.0%		
BMI	Underweight (<18.5)	Number	135	20	0.050	0.684
		Percent	87.1%	12.9%		
	Healthy (18.5-25)	Number	77	8		
		Percent	90.6%	9.4%		
	Overweight (>25)	Number	52	8		
		Percent	86.7%	13.3%		
	Total	Number	264	36		
		Percent	88.0%	12.0%		

BMI=Body Mass Index, TTG=Tissue TransGlutaminase

DISCUSSION

The results of the present study showed that participants' mean age was matched between case and control as well as gender. Among age, BMI and TTG variables, only TTG was significantly different between these two groups. It was found that 26 patients had positive TTG IgA (> 20) when it was 10 for control group. In many studies, TTG IgA was a valuable diagnostic test for CD so that Makharia et al. (2011) with using this test found that the prevalence of CD among thalassemia patients was 11.5 percent. Approximately, Shahramian (2015b) found identical prevalence for CD in thalassemia patients that were significantly higher than controls (Shahramian et al., 2015b). In comparison, these two studies confirmed our results.

Honar et al. (2014) conducted a population based study to evaluate the prevalence of CD in patients with thalassemia major compared with controls. This study concluded a prevalence of CD as 0.6% in controls but none of the patients had positive TTG IgA so the results of the study was completely dissimilar with our findings. In patients who do not have a response to oral iron, several factors may contribute to the lack of response. Stomach pain and constipation can reduce oral iron absorption. Ongoing bleeding which is a cause of iron loss and iron deficiency are highlighted causes of anemia and iron deficiency anemia along with thalassemia trait are the most common forms of microcytic anemia (DeLoughery, 2014). The present study confirmed this fact that the prevalence of CD was significantly higher in patients with thalassemia compared with control children. Parakh et al. (2008), reported a ten-year-old boy that had thalassemia major with early onset of growth failure with finally diagnosed CD. Two other studies also reported an adolescent with weight gain arrest and short stature with final diagnostic of CD (Mangiagli and Campisi, 1996; Parakh et al., 2008). According to these case reports, not only in patients with thalassemia but also in patients with weight gain arrest and short stature, CD can be a concurrent disease. Valizadeh and Shateri (2009) reported a final diagnosis of CD for a 28-years-old-male with thalassemia intermedia, iron

deficiency anemia and history of pemphigus. Sacco et al. (2004) described a patient with concurrent thalassemia minor, porphyria and CD in Pakistan. Ramraj et al. (2014) reported a 53-year-old-female with coexistence of type II diabetes mellitus, hypertension, thalassemia minor and CD who underwent sleeve gastrectomy. All the above case reports establish the truth of high correlation between celiac and thalassemia that is identical with the results of the present study. Based on some common features between β-thalassemia and CD such as growth failure, iron deficiency and also based on some available case reports, probably should be said that there is a correlation between these two diseases. In both CD and β- thalassemia, short stature is a common complication and this disorder in CD could appear even without typical gastrointestinal symptoms. CD bears a close resemblance to β- thalassemia in growth hormone deficiency (Hashemi et al., 2008; Nijhawan et al., 2013; Wu et al., 2003) and this matter can strongly contribute to the correlation between CD and autoimmune diseases (Ch'ng et al., 2007; Fasano et al., 2003). The other reason for supporting the association between CD and β- thalassemia is the genetic similarities in HLA system; especially HLA DQB1 alleles which represent major genetic predisposition in CD and β-thalassemia (Bao et al., 2002; Mangia et al., 2011; Megiorni and Pizzuti, 2012). Sanseviero et al. (2016) conducted a study on celiac patients to assess iron deficiency anemia condition in a large cohort of pediatric patients with newly diagnosed CD. Their subjects included 518 patients of whom 156 had anemia including iron deficiency anemia associated with α- or β-thalassemia trait, thalassemia trait without iron deficiency and the remaining suffered from other forms of anemia. In our opinion their analysis confirmed that iron depletion and iron deficiency anemia area frequent findings at the diagnosis of.

CONCLUSION

It is concluded that there is a positive association between β- thalassemia minor and CD. We could suggest routine screening test for CD in all children with β-thalassemia minor.

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Competing interests

The authors declare that there is no conflict of interest.

Authors' contribution

NMN created the study conception and design,
IS, MH and SMD performed the acquisition of data,
ES analyzed and interpreted the data,
AT drafted the manuscript and performed the critical revision.

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