Original Article

Retrospective Study of Tissue Transglutaminase Antibody Levels in Celiac Disease-Suspected Patients at Tertiary Care Hospital in Uttarakhand

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Abstract

Introduction: Celiac disease (CD) is a chronic gastrointestinal disorder arising due to gluten sensitivity in susceptible individuals. In India, one person per 100 is suffering from this disease. CD has been reported more in high wheat-consuming areas like northern India. However, the incidence of CD in patients attending tertiary care hospital of Uttarakhand has not yet been reported. Therefore, this study was planned. **Materials and Methods:** A retrospective study with 603 patients was done at AIIMS Rishikesh in Biochemistry Department. These patients were screened for CD by tissue transglutaminase antibodies (ttgA) levels using enzyme-linked immunosorbent assay method. Percentage of levels of ttgA <4 U/ml (normal value) and >4U/ml in male, female adults, and children was calculated. Chi-square test was applied to compare results. **Results:** Out of 603 patients, 23 (3.81%) had abnormally raised ttgA levels (>4 U/ml). Percentage (15.18%) of ttgA levels in male children was significantly ($P = 0.01$) higher than 2.56% in female children. Mean + standard deviation (SD) of normal and abnormal ttgA levels in patients was 1.17 + 0.45 versus 66.81 + 34.80 U/ml, respectively. Mean + SD of abnormal ttgA levels in children and adults was 72.84 + 41.91 versus 32.38 + 24.75 U/ml, respectively. There was a statistically significant difference in abnormal ttgA levels among children and adults with children predominantly being higher ($P = 0.0235$). **Conclusion:** This study shows that levels of ttgA suggestive of CD in children are higher as compared to adults and more in males than females attending tertiary care hospital of Uttarakhand.

Keywords: Adults, celiac disease, children, retrospective, tissue transglutaminase, Uttarakhand

Introduction

Celiac disease (CD) is one of the most communal, chronic, immune-mediated, and multifactorial gastrointestinal (GI) disorders. It has common genetic and environmental factors involved in addition to immunological factors.[1] The series of events in this disease is activated due to gluten sensitivity in susceptible individuals leading to an inflammatory reaction in the small intestine. Gluten is present in various cereals including wheat, rye, and barley.[2] Worldwide, the prevalence of CD is higher in children (0.1%–5.7%) as compared to adults (0%–1.9%) and this rise has been intensifying with passing years.[3]

On the basis of serological tests with biopsy results, if we compare the universal prevalence of CD, the former constitutes 1.4% and the latter is found to be 0.7%. It has been reported to be 4% in South America, 0.8% in Europe and Oceania, 0.6% in Asia, and 0.5% in Africa and North America. It has been also reported that as compared to males, the prevalence is higher in females (0.6% vs. 0.4%; $P < 0.001$).[4] As far as the worldwide incidence is concerned, 1 in 133 Americans, or about 1% of the population, has CD. Undiagnosed or misdiagnosed cases of CD have been reported to be as high as 83% in Americans.[4] In India, approximately 60–80 million people are estimated to be suffering from this disease. According to the latest data collected in North India,

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one person per 100 is suffering from this disease.[5] Reports of CD are also present from Maharashtra.[6] In India as well a wide breach has been reported between the prevalence of CD in the general population (1%) and diagnosis-based prevalence (0.02%–0.27%).[7] Thus, in India also, 90%–95% of the cases remain undiagnosed and unnoticed. The geographical distribution of this disease in India varies. The highest prevalence of CD in adults has been reported in northern India (1.23%), followed by northeastern India which shows an intermediate prevalence (0.87%) and southern India has the least prevalence (0.10%). It was also reported that mean daily wheat intake among these three areas was statistically significantly different. Northern India's average daily wheat intake was found to be 455 g, while northeastern India's average daily wheat intake was found to be 37 g and southern India 25 g. This statistically significant difference in wheat consumption among different regions implied that diet is likely to be the basis for much of the difference.[8] In comparison to the west, in India, the demographic profile of CD in children is different. It has been testified that in India, the proportion of male-to-female CD is 3:2. Patients of CD also show some unusual presentations. It has been reported that 20% of CD cases were short stature, 14% were anemic, 5% with confirmed constipation along with the family history, and 1.5% of subjects had rickets. Immunoglobulin A (IgA) deficiency (6% cases), asthma (2% cases), type 1 diabetes mellitus (T1DM) (1.5% cases), autoimmune hepatitis and seizures (1.5% cases), juvenile rheumatoid arthritis, Down syndrome, and nephrotic syndrome (0.7% cases) are other common association observed in Indian children.[9]

This literature shows that both intestinal and extraintestinal clinical presentations are manifested in this disease which vary largely. The remarkable variability of the disease makes the diagnosis challenging. Classical CD includes signs and symptoms of malabsorption which include diarrhea, vomiting, abdominal pain, malnutrition, and growth failure. Aphthous ulcers, neuropathy, iron deficiency anemia, and dermatitis herpetiformis are the common extraintestinal manifestations.[10] Osteopenia, dyspepsia, anemia, and osteoporosis are more common in adults, whereas in children, predominant features include growth failure, fatigue, failure to thrive, and GI manifestations such as abnormal bowel changes, vomiting, and abdominal pain.[11] It has been reported that concurrence of CD and T1DM is 5–7 times more prevalent as compared to CD alone. Various studies have estimated that the occurrence of CD in TIDM varies from 3% to 16%, with a mean prevalence of 8%.[12,13]

Tissue transglutaminase (tTG or TG2) is an enzyme with a molecular weight of 78 kDa. Anti-transglutaminase antibodies are formed in response to gluten that are cross-linked to tTG. Transglutaminase-specific B-cell response is stimulated by this gluten that eventually results in the production of IgA and IgG anti-transglutaminase antibodies.[14] Glutamine residues are deaminated by tTG, as a result of which epitopes are created. These epitopes are responsible for increase in the binding affinity of the gluten peptide to the antigen presenting T-cells. Finally, an adaptive immune response is activated which leads to the inflammation of the small intestine and subsequent villous atrophy.[15]

Geographical differences in CD are also contributed by different dietary patterns. In India also, it has been reported more in high wheat-consuming areas like northern India. It has been previously revealed in a dynamic case study that the prevalence of CD in northern India schoolchildren[16] was ~1 in 300 and among adults, it was reported to be 1 in 96.[17] However, the incidence of CD in Uttarakhand area has not yet been reported. Therefore, a retrospective analysis of tissue transglutaminase antibodies (ttgAs) levels to diagnose CD in adults and children attending a tertiary care hospital of Uttarakhand has been done.

Materials and Methods

Study design

This was a retrospective study.

Sample size

The sample size of the study was 603 patients.

Study setting

The study was conducted at Gastro-Biochemistry Laboratory of Department of Biochemistry in AIIMS Rishikesh, Uttarakhand.

Age range of all these patients was 3–70 years. These patients were screened for CD by ttgA levels during June 2019 and February 2020. Waiver for informed consent of all participants who were enrolled in this retrospective study and the ethical clearance was received from the Institute Ethical Committee. Clinical symptoms in these patients were diarrhea, abdominal pain, short stature, and anemia. Three milliliter blood was taken, centrifuged and ttgA levels were measured using Quanta Lite enzyme-linked immunosorbent assay kit from Inova Diagnostics.[18]

Statistical analysis

Percentage of levels of ttgA <4U/ml and >4U/ml in male and female adults and male and female children was calculated and to compare the results, Chi-square test was used.

Mean ± standard deviation (SD) of ttgA levels in all groups was also calculated and the results were analyzed by using unpaired *t*-test.

Results

Out of the total of 603 patients, 446 were adults and 157 were children. The age range of adults was 25 years to 70 years with mean ± SD (45.5 ± 17.31 years), while the age range of children was 3 years to 17 years with mean ± SD (12.21 ± 3.91 years). When distribution in terms of sex was categorized in adults, 278 were males and 168 were females. Out of 157 children, the total number of males was 79 and the total number of females was 78.

Of the total 603 patients, 580 patients had ttgA levels <4U/mL (normal) and 23 patients had ttgA levels >4U/mL (abnormal). These results indicate that 23 patients out of 603 (3.81%) had abnormally raised ttgA levels. Out of these abnormal 23 patients, 14 were children (12 males and 2 females) and 9 were adults (8 males and 1 female). Out of the 157 children, 14 (8.9%) had ttgA levels suggestive of CD. On the other hand, out of 446 adults, 9 (2.01%) had raised ttgA levels suggestive of CD. Out of these 14 children with raised ttgA levels, 4 had T1DM mellitus (26.6%), 3 had diarrhea, 2 had anemia, 2 had tuberculosis, and 3 were of short stature. Among 9 adults, 4 had anemia, 3 had loss of appetite, and 2 had bloating.

The incidence of high ttgA levels suggestive of CD in female and male children was 2.56% and 15.18%, respectively. The incidence of high ttgA levels suggestive of CD in male and female adults was 2.87% and 0.59%, respectively. It has been observed that the percentage of high levels of ttgA is more (P = 0.01) in male children as compared to female [Table 1].

Mean ± SD of normal and abnormal ttgA levels in patients was 1.17 ± 0.45 and 66.81 ± 34.80, respectively. Mean ± SD levels of abnormal ttgA levels in children and adults were 72.84 ± 41.91 and 32.38 ± 24.75, respectively. The statistical comparison showed a statistically significant difference (P = 0.0235) in abnormal ttgA levels among children and adults with children predominantly being higher [Table 2].

Discussion

In recent years, the number of CD cases has increased and continues to be on a rise across all affected regions. In our study, CD-suspected cases in children (8.9%) are significantly higher as compared to adults (2.01%), signifying that children are more prone to this disease in this part of northern India. A similar observation has been reported globally;[3] however, in our study, percentage of children suffering from CD is higher as compared to global distribution. To the best of our knowledge, there is no study from India in which the percentage incidence of CD has been compared between adults and children in the same study. Our study is a hospital-based study where both children and adults have been included in the same study and percentage of children suspected with this disease is much higher (8.9%). In contrast to this, the occurrence of CD in school children aged 3 years to 17 years has been reported to be 1 in 300 (0.3%).[16] In another community-based study where only adult males agreed to participate, the prevalence of the disease was approximately 1% (1 in 96 cases). However, the prevalence of disease in our study is comparatively higher (2.01%).[17] This difference again may be due to the hospital-based study of ours. One of the community-based studies in adults emphasizing the regional-based prevalence of CD showed that as compared to males (0.5%), CD was significantly more common in females (0.8%). In contrast to that study, in our study, males (2.87%) were predominantly affected as compared to females (0.59%).[8]

One of the studies emphasizing the regional association with CD using ttgA levels estimated the occurrence of this disease in Haryana, Assam, and Tamil Nadu in 23,331 healthy adults. The study concluded that the prevalence of celiac ttgA levels correlated through wheat intake with 1.23% in Haryana (North), 0.87% in Assam (northeastern), and 0.10% in south.[8] In contrast to this study where only healthy adults were included, in our study along with adults, children were also included in the same study. This study also proves that in northern India where the cultivation of wheat has been an ancestral culture, this disease is relatively common.[5] Hence, it also proves that both genetic and environmental factors explain the reason behind the relative rarity of CD in southern India. In our study, the overall percentage of CD was reported to be 3.81% (North) which is higher as compared to the above study (1.23%). Although both the studies are from North India, previous study is community based, while our study is hospital based. This may be the reason of higher percentage in our society.

Table 1: Percentage normal and abnormal levels of tissue transglutaminase antibody in adults and children (n=603)

Adults (n=446)				Children (n=157)			
Male (n=278), n (%)		Females (n=168), n (%)		Males (n=79), n (%)		Females (n=78), n (%)	
<4 U/ml	>4 U/ml	<4 U/ml	>4 U/ml	<4 U/ml	>4 U/ml	<4 U/ml	>4 U/ml
270 (97.13)	8 (2.57)	167 (99.41)	1 (0.59)	67 (84.82)	12 (15.18)*	76 (97.44)	2 (2.56)

*P=0.01 between TTGA levels >4 U/mL of male and female children. TTGA: Tissue transglutaminase antibody

Table 2: Mean±standard deviation of tissue transglutaminase antibody levels (U/ml) in adults and children

	Normal TTGA levels (n=580)	Abnormal TTGA levels (n=23)	P	Abnormal TTGA levels in children (n=14)	Abnormal TTGA levels in adults (n=9)	P
Mean	1.71	66.81	<0.0001	72.84*	41.91	0.0235
SD	0.45	34.80		32.28	24.75	
SE	0.018	7.26		8.63	8.25	

*P=0.0235 between mean±SD of abnormal TTGA levels in children and adults. TTGA: Tissue transglutaminase antibody, SD: Standard deviation, SE: Standard error

In our study, we observed the history of type I diabetes mellitus in 5 out of 14 (26.6%) affected children. This indicates that T1DM has an important association with CD; therefore, its screening is important for the timely and relevant treatment of the patient. In the literature also, it has been reported that in children suffering from CD, there is a greater risk of consequent T1DM. A study including 9243 children diagnosed with CD showed a hazard ratio of 2.4 with a 95% confidence interval indicating that in children, there is an association of CD with amplified risk of T1DM.[19]

Conclusion

This study shows that ttgA levels which suggest the presence of CD in children are higher as compared to adults attending the tertiary care hospital of Uttarakhand. This study also suggests that it is more in male children than female children. CD is a treatable condition and greater awareness and better understanding with regular follow-ups will help in the management and treatment of this disease.

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Conflict of interest

There are no conflicts of interest.

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