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ORIGINAL ARTICLE

Incidence and Prevalence of celiac disease in Hazara Division, KP Pakistan by anti-tissue transglutaminase antibody as diagnostic tool

ABDUL HASEEB[1], AZAM HAYAT[2], AAMER ALI KHATTAK[3], MUJADDADUR REHMAN[4], FAHEEM JAN[5], BILAL AHMED[6], USMAN AYUB[7], RIZWAN ULLAH[8], SHAFIQUER REHMAN[9], ANUM KHAN[10]
[1,2,4,5,6]Department of Medical Lab Technology, Abbottabad University of science and technology, Abbottabad Pakistan
[3,7]Department of Medical Lab Technology, The University of Haripur, Haripur Pakistan
[8,9,10]Department of Microbiology, Abbottabad University of science and technology, Abbottabad Pakistan
Correspondence to Abdul Haseeb, Email: abdul.haseeb576.ah@gmail.com

ABSTRACT

Aims: To check the prevalence of CD among different age groups and across gender in population of Hazara division and to determine the distribution of CD through ELISA technique.
Study design: A cross sectional study was designed at department of medical Lab Technology, University of Haripur, Haripur (KPK).
Place and duration: Samples were analyzed at Hamdard laboratory from November 2016 to May 2017.
Methods: we used ELISA technique in our study. According to the company instructions, the ELISA method was used to detect anti-tissue transglutaminase antibodies using this approach, which is sensitive, and specific. We target anti-tissue transglutaminase antibody by this technique which is an optimistic antibody assisting in the validation of the CD diagnostic test.
Results Total 8000 patients samples were collected from Gastro OPD of district head quarter (DHQ) hospital Haripur, Abbottabad and Mansehra of Hazara Division. Out of 8000 the 6880(86%) male and 1120(14%) females. About 568(7.1%) cases were highly positive while 120(1.5%) cases were weak positive and the rest of 7312 (91.4%) cases were negative. Out of 568(7.1%) highly positive cases 452(79.6%) were males and 116 (20.4 %) were females. Furthermore 120(1.5%) of weak positive cases 74 (61.7%) were males and around 46(38.3%) were female.
Conclusion: For centuries, CD has indeed been kept under wraps, with little to no known about what is a comparatively common health problem. This is only recently that we have improved our knowledge of its Prevalence, pervasiveness, prognosis, and pathophysiology that has assisted in the creation of new CD treatment strategies.
Key words: Celiac disease, ELISA, Anti-tissue transglutaminase, Gluten.

INTRODUCTION

Tissue transglutaminase is calcium dependent enzyme which plays role in post-translation modification of peptides and certain proteins with the formation of isopeptide bonds (Esposito et al., 2002). Tissue transglutaminase (tTG) is one of several blood tests that may be used to help diagnose Celiac disease (CD). People with CD often make antibodies that attack this enzyme (Ashtari S et al., 2001).

Celiac disease (CD/Gluten-sensitive enteropathy) is a small intestine inflammatory disease mostly occur by the ingestion of grains like gluten, wheat etc. (uddin Jamro et al., 2012). It is defined by global protein, nutrient, and vitamin impaired absorption. It is described histopathologically by a strong influence of T-cell infiltration in the epithelium as well as lamina propria of the intestinal wall, which could also result in complete damage of the villi, and also its reason was unidentified again until Dutch pediatrician Willem Dicke found a connection between both the usage of food ingredients and CD relapsing diahorrea. During food crisis during WWII, his clients' diseases continued to improve once bread has been replaced with non-cereal-containing foods. (Gatti S et al., 2020). CD might be differentiated from other malabsorptive disorders that result in villous atrophy, including such tropical (microbial) sprue as well as bacterial uncontrolled growth, with the advancement of serum tests including such immunoglobulin A (IgA) monoclonal antibody to gliadin (Wong et al., 2003).

It is widely accepted that wheat (gliadins), rye (secalins), and barley prolamins (hordeins) are toxic to the intestinal lumen of Celiac sufferers due to the high glutamine (>30%) but also proline (>15%) content, so although cereals and corn prolamins seem to be nontoxic due to the lower glutamine as well as proline subject matter (Lagana & Bhagat, 2019).

The high familial occurrence of celiac (about 10% of first relatives of individuals of celiac sufferers are affected by natural disasters) as well as its strict interconnection with certain human leukocyte antigen (HLA) class 2 alleles (up to 95% coeliac disease

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are HLA-DQ2 optimistic with traditional heterodimer, meanwhile the remainder 5% are HLA-DQ8 positive) confirm the disease's genetic susceptibility. Because roughly 20-30% of good health people in the western world have almost the same HLA pattern, the existence of HLA-DQ2 and/or HLA-DQ8 must be considered an important precondition for the illness but also can justify the advancement of CD by itself. As a direct consequence, non-HLA biological factors are likely to be necessary for disease. (Sharma L et al., 2022).

The incidence of individual CD in the United States population has been determined to be 0.95%, with the frequency of child CD widely expected to be 0.31%, for a high incidence tend to range from 0.69 % to 0.7 5%. (Kim et al., 2016). This rises to 1.01% between many non-Hispanic whites, while blacks and Hispanics in the U. S. have much reduced costs of CD, at 0.3% and 0.2%, in both (Ditah et al., 2015). These statistics are in line with the results of several epidemiologic studies research studies in Italy, which exhibit similar intervals of 0.2% to 0.74% (Tommasini et al., 2004).

The illness is unusual in East Asians and Pacific Peoples that has been credited to a lack of the specific HLA haplotype considered necessary for CD to happen or indeed a low gluten usage in the inhabitants (Abadie et al., 2011).

Presently, the best method for increasing the diagnosing percentage is specific instance findings meets certain prerequisites for vulnerable communities, a method that reduces costs whilst also trying to remain ethical. The quantity and quality of ingredients available for consumption, the form as well as wheat duration dough digestion, the spectral response of intestinal micro - organisms and how those who alter over time, infections of the intestine, and overall sources of stress are all possible future transitions in the compassion response rebalancing (Zanoni et al., 2006).

The objectives of the study were to check prevalence of CD among different age groups and across gender in population of hazara division and to determine the distribution of CD through ELISA technique.

METHODS AND MATERIALS

A cross sectional study was designed at department of medical Lab Technology, University of Haripur, Haripur (KPK). Samples were collected at Gastro OPD of district head quarter (DHQ) hospital Haripur, Abbottabad and Mansehra of Hazara division from November 2016 to May 2017 and were analyzed at Hamdard Laboratory Abbottabad.

Inclusion criteria: In this study, patients having minimum of 3 clinical features out of 6 (chronic diarrhea, malnutrition, short stature, anemia, abdominal distension, clubbing) were included.

Exclusion criteria: All the patients are suffering from diseases other than the above-included ones.

Data and Samples collection: Approximately 8000 test results with client information were gathered from CD suspects with symptoms of long-term diarrhoea, malnourishment, small stature, anemia, and abdominal discomfort (at least 3 out of 5).For the collection of data a standard design questionnaire was used including sex, age etc. The patients were interviewed orally. Whole blood samples were collected aseptically by venipuncture technique in Red cap clot activator gel tube. Clotted blood was centrifuge at 3000 rpm for 5 minutes for serum collection and was store at -20°C before further analysis. Anti-tissue transglutaminase antibody ELISA test was performed on semi-automatic plate reader (Urit China).

Ethical Consideration: Every person being evaluated provided written consent, as well as the institutional review board authorized the research.

Preparation of Sample: Patient's serum from clotted blood was separated and was used for analysis. Hemolysed and lipemic samples were avoided from analysis. Specimens were kept in the fridge at 2-8° C for up to 5 days or stored in a freezer at -20° C for up to 6 months analysis. Repeated freeze and thawing of samples was avoided in order to get quality result. Testing of heat-inactivated sample was not recommended.

IgA anti-tissue transglutaminase antibody assay: A human recombinant enzyme-linked immunosorbant assay (ELISA) model was used to calculate IgA anti-tTG antibody using an available commercially Kit NovaTec (Catalog No 3773-17). It was developed to assess IgA autoantibodies directed against tTG in a quantitative way. Microplates coated with purification methods tTG are used for the test. The plates can be divided into 12 components of 8 well and one per used or out of for 96 measurement techniques. Anti-tTG ELISA is a simple, non-invasive method for detection that offers an effective way to the immunoflurescent technique and therefore is perfect for testing suspected cases CD children and young adults. The products supplied are a divided sure to assist with 12 modules of 8 wells coated with recombinant human tTG. The sample material is moisturiser or erythrocytes, and the sample size needed is 10l of survey to be diluted 1:100 with buffer was added and 100l of prediluted specimen for every single dedication. At room temperature (20-28oC), the total long incubation period is 60 minutes. Human overexpressing tTG is encased on the solid matrix. As a result, the anti-tTG test kit only works by detecting tTG-specific autoimmune reactions. The anti-tTG permanent foundation a cut-off of 10 U/ml in a range of normal study with samples from good health blood donations. We evaluated serum IgA levels throughout all patients to make a ruling out a condition known specific serum immunoglobulin A defect

Statistical Analysis: All the data collected from the above mentioned assays was calculated by percentages and ratio of variables.

RESULTS

Total 8000 patients samples were collected from Gastro OPD of district head quarter (DHQ) hospital Haripur, Abbottabad and Mansehra of Hazara Division. Out of 8000 the 6880(86%) male and 1120(14%) females. About 568(7.1%) cases were highly positive while 120(1.5%) cases were weak positive and the rest of 7312(91.4%) cases were negative. Out of 568(7.1%) highly positive cases 452(79.6%) were males and 116(20.4%) were females. Furthermore 120(1.5%) of weak positive cases 74(61.7%) were males and around 46(38.3%) were female as shown in figure 1, 2.

In District Abbottabad total 2960 numbers of patients were examined (2480 male and 480 female). Out of these, 232(7.90%) cases were highly positive while 91(3.07%) cases were weak positive rest of 2637(89.03%) were negative. Out of 232(7.90%) highly positive cases, 201(86.63%) were males and 31(13.36%) were females. Furthermore 66(82.5%) male and 14(17.5%) females were weak positive as shown in table1.

In District Haripur total 3440 numbers of patients were examined (2960 males and 480 females). Out of these, 256 (7.4%) cases were highly positive while 76(2.20%) cases were weak positive rest of 3108 (90.34%) were negative. Out of 256(7.4%) highly **positive cases,** 221(86.32%) were males and 35(13.68%) were females. Furthermore 63(82.89%) were male and 13(17.10%) female were weak Positive as shown in table 2

In district Mansehra total 1600 numbers of patients were examined (1440 males and 160 females). Out of these, 80(5%) cases were highly positive while 29(1.81%) were weak positive rests of the 1491(93.18%) cases were negative. Of the highly positive cases 73(91.25%) were male while 7(8.75%) were females while in weak positive cases 21(72.42%) were male and 8(27.58%) were females as shown in table 3, 4.

The overall results shows that the increase in percentage of highly positive Anti-tissue transglutaminase IgA cases is in males 495 out of 568 cases and then females 73 out of 568. And increase in percentage of weak positive cases also shown in males 150 out of 196 and then in females 46 out of 196. Beside these overall negative cases were 7236 out of total 8000 from which 6270 out of 7236 were males and 966 out of 7236 were females as shown in table 5, 6.

Fig 1: District wise Prevalence of CD in male and female population of Hazara Division

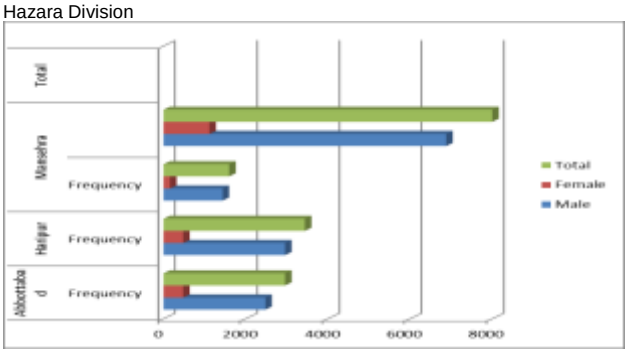

Fig 2: District wise Prevalence of CD disease

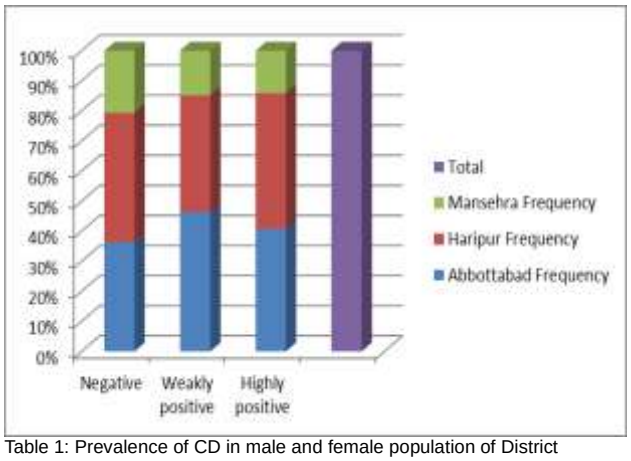

Table 1: Prevalence of CD in male and female population of District Abbottabad

	Frequency	Percent%	Cumulative%
Male	2480	83.7	83.7
Female	480	16.3	16.3
Total	2960	100.0	100.0

Table 2: Prevalence of CD in male and female population of District Haripur

	Frequency	Percent%	Cumulative%
Male		86.05	86.05
Female	480	13.95	13.95
Total	3440	100.0	100.0

Table 3: Prevalence of CD in male and female population of District Mansehra

	Frequency	Percent%	Cumulative%
Male	1440	90.0	90.0
Female	160	10.0	10.0
Total	1600	100.0	100.0

Table 4: Prevalence of total cases of CD in District Mansehra

	Frequency	Percent%	Cumulative%
Negative	1491	93.18	93.18
Weakly positive	29	1.81	1.81
Highly positive	80	5.0	5.0
Total	1600	100.0	100.0

Table 5: Prevalence of CD in total male and female population of Hazara Division

	Frequency	Percent%	Cumulative%
Male	6880	86.0	86.0
Female	1120	14.0	14.0
Total	8000	100.0	100.0

Table 6: Overall results of CD in Hazara Division

	Frequency	Percent%	Cumulative%
Negative	7236	90.45	90.45
Weakly positive	196	2.45	2.45
Highly positive	568	7.1	7.1
Total	8000	100.0	100.0

DISCUSSION

CD is a very common condition with a prevalence of biopsy proven disease as 1% worldwide (Greco et al., 2011). The number of subclinical cases of CD is very high due to subtle manifestations closely mimicking other common conditions, such as primary malnutrition and inflammatory bowel syndrome. Frequency of CD in study population of present study, which includes high-risk population, was 9%. This result closely correlates with the results of Aziz *et al* who studied similar high risk population in Karachi (Aziz et al., 2007).

In this study if we categorize the population in two main etiological groups the male and female. In this study, total of 8000 patients suffering with gastrointestinal diseases (6880 male, 1120 female) were examined for anti-tissue transglutaminase antibody from Hazara division. Male to female ratio was 1:6.14 in present study. Out of which, 568(7.1%) were highly positive (495 males and 73 females), 196(2.45%) were weak positive (150 males and 46 female) and 7236 were negative. High prevalence rate of CD is seen in district Abbottabad with 7.9% followed by district Haripur with 7.4% and 5% in district Mansehra.

Study conducted by waqar rabbani *et al*, 2013 reported 13% highly positive cases of the disease using biopsy while our study shows 7.1 % highly positive cases. This difference is due to technique used for test. Biopsy is more specific than serological Tissue transglutaminase test. (Rabbani et al., 2013)

Prevalence of CD in Hazara Division is 7.1% in the present study while study conducted by Neetu Beniwal *et al*, 2017 shows prevalence of 14.42% which was little higher and dissimilar with our present study. One reason for little raise of the disease is non-hygienic environment of India and overpopulation Beniwal (Beniwal et al., 2017).

We also compare our study with diabetic patients suffering from CD having 14.3% of prevalence rate. The results were slightly higher than result reported in our present study. It might be because some studies shows that diabetic patients are at high risk for CD (Castellaneta & Francavilla, 2015).

The preceding discussion shows that tTG is an outstanding tool for screening for CD, with very great specificity, clarity, predictive value, and negative predictive value. A highly positive tTG value, i.e. tTG>50 IU/ml, has a 100% predictive value, suggesting adequate diagnostic performance to avoid endoscopic duodenal histology and clinic pathologic investigation of intestinal mucosa. As a consequence, a highly positive tTG is an outstanding CD screening tool. Histopathology is the moment gold method for determining CD. However, it requires specialist skills and costly hardware that are not readily available in many areas of the country. Endoscopy as well as duodenal mucosal histopathological examination should be reserved for situations with tTG positivity higher than 50 IU/ml, as well as tTG negative instances with such a high clinical suspicion of CD. A biopsy inspection should be managed to perform after six weeks of GFD to prove mucosal recovery after gluten withdrawal in questionable instances. The conversation makes it obvious that diagnosis has been the most essential step in CD planning. This is largely attributable to the varied clinical characteristics of CD, which can be quite diverse as well as subtle at points of time. The situation is complex by large set of sub cases that lack characteristic symptoms. Correct detection is essential for determining the treatment strategy.

CONCLUSION

For centuries, very little known regarding Celiac disease, despite the reality that it is a comparatively common condition. It is only recent times that we have improved our knowledge of its Prevalence, pervasiveness, diagnostic test, and pathogenesis that has helped in the creation of new CD treatment interventions. There are various future methods to trying to treat CD that, if successful, will complement or even replace the currently only successful therapy, the use of GFD. A improved comprehension of CD pathogenesis allows for future CD treatments that catalyze the hydrolysis toxic gliadin peptide, help stop toxic gliadin peptide uptake, block specific participants of specific glutathione byproducts by parenchyma, reestablish self - tolerance to gluten, ' transforming autoimmune disease to diet and lifestyle gliadin, as well as restore immunity.

Conflict of interest: Nil

Authors' Contribution: A. Haseeb, B. Ahmed, R. Ullah and F. Jan conducted the research; while A.Hayyat, M. Rehman,

S.Rehman, A.Khan, proceed the data analysis. U.Ayub and A. khattak wrote the manuscript.

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