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Original Article

Pathology Section

Study of Autoantibodies and DQ Antigens in Patient with Celiac Disease

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ABSTRACT

Introduction: Celiac Disease (CD) is a chronic autoimmune mediated disorder triggered by the ingestion of gluten. It is seen in genetically predisposed person and results in small intestine injury. Its aetiopathogenesis is not clear. Simple histopathology is not able to diagnose the disease many times.

Aim: Aim of present study was to assess the prevalence of HLA DQ alleles and autoantibodies in diagnosis of the disease and association of DQ antigens with Type 1 Diabetes mellitus (T1DM) and autoimmune hypothyroidism in CD patients.

Materials and Methods: Total 100 cases of CD and 31 healthy controls were studied, within a period of January 2015 to Febuary 2016. Autoantibodies like ANA, anti-tTg, anti-TPO and anti-scl 70 were done by ELISA kits. HLA DQ typing was done in 44 cases of CD, 20 cases of CD with T1DM, 22 cases of CD with autoimmune thyroid disease and 31 healthy controls. HLA DQ typing was done by SSO hybridisation method by Mr. SPOT machine.

Results: About 70% patients were children between 6 months to 20 years of age and female formed the maximum number of cases (60%). Anti-tTg ab was positive in all cases (100%), anti-Scl 70 Ab was positive in 25%, anti-TPO ab was found in 22% and ANA was positive in only 10% cases. Most frequent DQβ1 haplotype in CD were DQβ1*02:01 (45.5%, p<0.001) and DQβ1*02:02 (20.5%, p=0.007) while DQ*06:01 was significantly more common in controls suggesting its protective role. Among DQα1 typing DQα1*05:01 (45.5%, p<0.001) and DQα1*05:05 (40.9%, p<0.001) which were significantly more in CD than controls. Contrary to this DQα1*01:01, DQα1*01:03 and DQα1*01:04 were significantly reduced in CD patients. CD patients associated with T1DM and autoimmune thyroid disease had significantly more DQβ1 02:01, DQβ1*02:02, DQα1*05:01 and DQα1*05:05.

Conclusion: CD is an autoimmune disease, DQ typing should be kept in diagnostic criteria of CD. Association of autoimmune thyroid diseases and T1DM in CD is due to common sharing of these DQ antigens suggesting its role in predisposing autoimmune diseases.

Keywords: Anti nuclear antibody, Anti thyroid peroxidase antibody, Anti-scleroderma 70 antibody, Celiac disease, DQ allele, Hypothyroidism, Type 1 Diabetes mellitus

INTRODUCTION

The CD is a chronic immune mediated disorder triggered by the ingestion of wheat gluten and related proteins in barley and rye in genetically predisposed persons. It results in small intestinal injury [1-3]. CD is characterised by presence of Anti Endomysium Antibody (EMA) and Anti-tissue Transglutaminase antibody (anti-tTg) in serum or locally in the gut [4]. Prevalence rate of CD varies from 1-2% of the total population in the North America, South America, the middle-east and North Africa [4]. The disease incidence is also increased in the first degree relatives of CD patients and varies from 10-20% and higher incidence is also seen in person with other autoimmune diseases [5]. A significantly increased prevalence of some autoimmune diseases like Type 1 Diabetes Mellitus (T1DM) and autoimmune thyroid diseases have been found in CD patients and their first degree relatives [6]. CD is also common in Indian subcontinent. The incidence of CD in north Indian community is 1 in 96 or about 1%. More or less it is same as that of other part of world [7]. The aetiology of CD is influenced by both environmental and genetic factors. The Human Leukocyte Antigen (HLA) contributes to 40% of total genetic lineage [8]. The most of CD patient expresses the HLA DQ 2.5 heterodimer encoded by the HLA DQα1*05 (alpha chain) and HLA DQβ1*02 (beta chain) alleles. Both are carried either “in-cis” on the DR3-DQ2 haplotype or “in-trans” in individuals who are DR5-DQ7, and DR7-DQ2 heterozygotes.

More than 90% CD patients possess 1 or 2 copies of HLA DQ2.5 [9] encoded by allele DQα1*05/DQβ1*02 and 5% carries DQ8 encoded by DQα1*03/DQβ1*03:02 allele. Genome wide association studies have shown that 57 non HLA loci are also associated with CD and accounts for 18 % of the genetic variance [10].

This study will help in knowing the role of HLA DQ antigen and autoantibodies in CD and its clinical features. This study will also throw light whether these markers described in foreign country are applicable to our India or not.

The main aim of study was to find out prevalence of HLA DQ antigen and autoantibodies in CD patients especially of ANA, Anti-tissue Transglutaminase antibody (anti-tTg) Anti-Thyroid Peroxidase Antibody (anti-TPO), Anti-Scleroderma-70 Antibody (anti-Scl 70) and association of DQ antigens with T1DM and autoimmune hypothyroidism in CD patients.

MATERIALS AND METHODS

This case control study included 100 patients of CD and 31 healthy controls from January 2015 to August 2016. Cases were taken from Department of Paediatrics and Endocrinology of SS Hospital BHU. The power of the study is 80%. Samples were collected on the basis of feasibility and availability. All patients and controls were informed the purpose of the study, and their consent was obtained before collecting the blood samples. All agreed samples was taken during the given time period. This study was approved by Institute of Medical Sciences, Human Research Ethics Committee, Banaras Hindu University, Varanasi, India (No.Dean/2014-15/EC/1047).

The present study took only symptomatic patients diagnosed as CD based on elevated anti-tTg antibody, history, HLA and histopathology as defined by diagnostic criteria of European Society of Paediatric

Gastroenterology, Hepatology and Nutrition (ESPGHAN) described by Husby S et al., [11].

In all patients and controls 3 mL blood was taken in EDTA vial for HLA DQ study by PCR and 4 mL blood was taken in plain vial for autoantibodies analysis.

Autoantibodies analysis: The autoantibodies like Anti-Tissue Transglutaminase (Anti-tTg), Antinuclear Antibody (ANA), Anti-TPO and Anti-scleroderma 70 Ab were performed by Enzyme-Linked Immunosorbent Assay (ELISA) method. The autoantibodies were done by using kits of following company:

1. anti-tTg Ab were done by ELISA kit of AESKU.DIAGNOSTICS, Germany supplied by Immunoconcept, New Delhi. The normal value of the assay was <12 U/mL.
2. ANA Ab: were done by ELISA kit Eurodiagnostica Sweden supplied by M/S OSB Agencies Geetha colony New Delhi. The normal value of the assay was <40 U/mL.
3. Anti-TPO Ab was done by ELISA kit Eurodiagnostica Sweden supplied by M/S OSB Agencies Geetha colony New Delhi. The normal value of the assay was <87 IU/mL.
4. Anti-scleroderma 70 Ab were done by ELISA kit Eurodiagnostica Sweden supplied by M/S OSB Agencies Geetha colony New Delhi. The normal value of the assay was <3.2 U/mL.

The principle of the ELISA kit is based on indirect non competitive enzyme immunoassay for the quantitative and semi-quantitative determination of antibodies in human serum. The well of the solid phase were coated with a balanced mixture of autoantigen of nuclear antigens. The wells were Washed with 300 µL wash buffer and dispensed 100 µL of calibrators, negative control and diluted patient sample with diluents buffer (in ratio 1:101) into appropriate wells. Incubated for 30 minutes, fluids were aspirated from wells and wells were washed 3 times with washed buffer. 100 µL of Horse-Radish Peroxidase (HRP) conjugate was dispensed into all wells and incubated for 30 minutes. Fluid was aspirated from wells and wells were washed. 100 µL of TMB substrate was dispensed into all wells and incubated for 10 minutes in the dark. 50 µL stop solution was dispensed into all wells and absorbance (OD) was read at 450 nm and reference wavelength at 620 nm.

HLA DQ typing: HLA DQ typing was done in 44 cases of CD, 20 cases of CD with T1DM, 22 cases of CD with autoimmune thyroid disease and 31 healthy controls. HLA DQ typing was done by Sequence Specific Oligonucleotide (SSO) probe hybridisation method on instrument called as Mr. SPOT, BAG health Care, Germany and supplied by Shiva Scientific, New Delhi. DNA sample was extracted by salt-out and phenol chloroform method. A final reaction volume of 20 µL for the Polymerase Chain Reaction (PCR) was constituted, which contained 10 µL of master mix (biotynylated primers, dNTPs, taq polymerase, reaction buffer, 0.05% sodium azide), 5 µL of $MgCl_2$ (6 mm containing 0.001% Proclin R 300) and 5 µL of DNA (15-30 ng/µL). The Sequence specific amplification with biotinylated primers was carried out on an Biorad Thermal cycler under the following conditions: 96°C for 2 min, 10 cycles of 96°C for 15 s, 65°C for 60 s, followed by 20 cycles of 96°C for 10 s, 61°C for 50 s and 72°C for 30 s. Whole amplified product was inserted into heating bar of Mr. SPOT machine for SSO hybridization. Process as indicated on the screen was followed.

Interpretation

The resulting coloured dots in the bottom of each well are photographed by Mr. SPOT and the image is transferred into the Histo Match software installed on the PC user. Image analysis programme of the Histo Match software determines the intensity of each spot in the array and compares the intensity of background and displays the results.

STATISTICAL ANALYSIS

All data were analysed using Statistical Package for Social Sciences (SPSS, Chicago, Illinos, USA), version 16. Pearson's Chi-square (χ^2) and Relative Risk (RR) test were used to compare differences between the frequencies as per the requirement. A p-value <0.05 was considered significant for all analysis.

RESULTS

CD was more common in between 6 months to 10 years of age (52%). As a whole 70% patients were upto 20 years of age and 25% were young adults between 21 to 40 years of age and only 5% patients were above 41 years of age [Table/Fig-1].

Age in years	CD patients total N=100	
	Number of CD patient	Percentage (%)
0-10	52	52.0
11-20	18	18.0
21-30	17	17.0
31-40	08	08.0
41-50	03	03.0
>60	02	02.0

[Table/Fig-1]: Showing age wise distribution of patients with CD.

Sex wise distribution of patients showed that majority patients were female (60 cases) and 40 were males. Female to male ratio was 1.5:1.

Among the clinical manifestations analysed in 100 cases of CD showed that failure to thrive and weight loss were the most common manifestations seen in 79% cases followed by chronic recurrent diarrhea (71%), anaemia (66%), short stature (62%) and pain in abdomen (56%).

Autoantibodies in CD Patients and Controls

Anti-tTg was positive in all cases (100%). Anti-scleroderma 70 antibody was significantly positive in cases (p=0.026) as compared to control [Table/Fig-2].

Autoantibody	CD positive cases total N=100		Controls N=31		χ^2	p-value
	Positive cases	Percentage (%)	Positive cases	Percentage (%)		
Anti-tTg Ab	100	100.0	0	0	131.0	<0.001
Anti-TPO Ab	22	22.0	3	9.7	2.327	0.127
Anti-scleroderma Ab	25	25.0	2	6.5	4.976	0.026
ANA	10	10.0	1	3.2	1.412	0.235

[Table/Fig-2]: Showing autoantibody positivity in CD patients and controls.

HLA DQ Typing in CD Patients and Controls

DQβ1 typing in CD and control showed that DQβ1*02 was found in significantly higher percentage (65.9%) than controls (0%). High resolution typing showed that 45.5% had DQβ1*02:01 (p<0.001) and 20.5% had DQβ1*02:02 (p<0.007). Contrary to this, DQβ1*06:01 was significantly reduced in CD patients (p<0.001) suggesting that DQβ1*06:01 has protective role. DQβ1*03:03 was also reduced in CD patients but it was not significant (p=0.053) [Table/Fig-3].

DQα1 typing revealed DQα1*05:01 and 05:05 was significantly increased in CD patients (p<0.001 and p<0.001). Contrary to this DQα1*01:01, DQα1*01:03 and DQα1*01:04 were significantly reduced in CD patients as compared to controls suggesting its protective role [Table/Fig-4].

It was noticed that DQβ1*02:01 genotype analysis revealed that DQβ1*02:01 homozygosity was very common (45.5%) in CD patients followed by DQβ1*02:02 (18.2%) DQα1*05:01 (36.4%) [Table/Fig-5].

Correlation of DQβ1 with T1DM in CD patients showed again increased frequency of DQβ1*02:01 (70%) and DQβ1*02:02 (65%)

DQβ1 typing	Group I CD (N=44)		Group II Control (N=31)		χ^2 I vs. II	p-value	RR
	N	%	N	%			
DQβ1*02:01	20	45.5	0	0.0	19.215	<0.001	1.833
DQβ1*02:02	9	20.5	0	0.0	7.206	0.007	1.257
DQβ1*03:01	6	13.6	5	16.1	0.090	0.764	0.821
DQβ1*03:02	8	18.2	3	9.7	1.051	0.305	2.074
DQβ1*03:03	6	13.6	10	32.3	3.758	0.053	0.332
DQβ1*05:01	4	9.1	1	3.2	1.005	0.316	3.000
DQβ1*05:02	5	11.4	5	16.1	0.357	0.550	0.667
DQβ1*05:03	4	9.1	2	6.5	0.172	0.678	1.450
DQβ1*06:01	3	6.8	17	54.8	21.446	<0.001	0.060
DQβ1*06:02	2	4.5	5	16.1	2.884	0.089	0.248
DQβ1*06:03	2	4.5	4	12.9	1.726	0.189	0.321
DQβ1*06:04	3	6.8	5	16.1	1.655	0.198	0.380

[Table/Fig-3]: Showing distribution of DQβ1 in CD and Control group.

DQα1 typing	Group I CD N=44		Group II Control N=31		χ^2 I vs. II	p-value	RR
	N	%	N	%			
DQα1*01:01	5	11.4	10	32.3	4.962	0.026	0.269
DQα1*01:02	13	29.5	10	32.3	0.063	0.802	0.881
DQα1*01:03	5	11.4	18	58.1	18.655	<0.001	0.093
DQα1*01:04	1	2.3	9	29.0	11.270	0.001	0.057
DQα1*02:01	12	27.3	6	19.4	0.625	0.429	1.562
DQα1*03:01	6	13.6	2	6.5	0.985	0.321	2.289
DQα1*05:01	20	45.5	0	0.0	19.215	<0.001	1.833
DQα1*05:05	18	40.9	1	3.2	13.653	<0.001	20.769

[Table/Fig-4]: Showing distribution of DQα1 in CD patients and control.

Homozygous alleles	CD (N=11)		Control (N=04)	
	N	%	N	%
DQβ1*02:01	5	45.5	0	0.0
DQβ1*02:02	2	18.2	0	0.0
DQα1*01:01	0	0.0	2	50.0
DQα1*02:01	0	0.0	2	50.0
DQα1*05:01	4	36.4	0	0.0

[Table/Fig-5]: Showing distributions of homozygous alleles in CD and Control groups.

which was statistically significant (p<0.001 and p<0.001) [Table/Fig-6].

DQβ1 alleles	Group I CD with T1DM (N=20)		Group II Control (N=31)		χ^2 I vs. II	p-value	RR
	N	%	N	%			
DQβ1*02:01	14	70.0	0	0.0	29.911	<0.001	3.333
DQβ1*02:02	13	65.0	0	0.0	27.043	<0.001	2.857
DQβ1*03:01	4	20.0	5	16.1	0.125	0.723	1.300
DQβ1*03:02	2	10.0	3	9.7	0.001	0.970	1.037
DQβ1*03:03	2	10.0	10	32.3	3.347	0.067	0.233
DQβ1*05:01	2	10.0	1	3.2	1.008	0.315	3.333
DQβ1*05:02	4	20.0	5	16.1	0.125	0.723	1.300
DQβ1*05:03	2	10.0	2	6.5	0.212	0.645	1.611
DQβ1*06:01	1	5.0	17	54.8	13.222	<0.001	0.043
DQβ1*06:02	0	0.0	5	16.1	3.576	0.059	0.839
DQβ1*06:03	0	0.0	4	12.9	2.800	0.094	0.871
DQβ1*06:04	2	10	5	16.1	0.386	0.535	0.578

[Table/Fig-6]: Showing DQβ1 distribution in CD patients with T1DM.

Similarly, in DQα1, DQα1*05:01 (60% vs. 0%, p<0.001) and DQα1*05:05 (50% vs. 3.2%, p<0.001) were significantly more common in CD with T1DM patients as compared to controls. DQα1*01:03 and DQα1*01:04 were significantly reduced in CD patients with T1DM [Table/Fig-7].

DQα1 alleles	Group I CD with T1DM (N=20)		Group II Controls (n=31)		χ^2 I vs. II	p-value	RR
	N	%	N	%			
DQα1* 01:01	3	15.0	10	32.3	1.906	0.167	0.371
DQα1* 01:02	9	45.0	10	32.3	0.844	0.358	1.718
DQα1* 01:03	2	10.0	18	58.1	11.782	0.001	0.080
DQα1* 01:04	0	0.0	9	29.0	7.051	0.008	0.710
DQα1* 02:01	4	20.0	6	19.4	0.003	0.955	1.042
DQα1* 03:01	1	5.0	2	6.5	0.046	0.830	0.763
DQα1* 05:01	12	60.0	0	0.0	24.323	<0.001	2.500
DQα1* 05:05	10	50.0	1	3.2	15.723	<0.001	30.000

[Table/Fig-7]: Showing HLA DQ1 distribution in CD patients with T1DM.

Similarly, anti-TPO Ab positive cases also had significantly increased expression of DQβ1*02:01 (63.6% vs. 0% p<0.001) DQβ1*02:02 (36.4% vs. 0%, p<0.001) [Table/Fig-8].

DQβ1 alleles	Group I Anti-TPO positive CD patients (N=22)		Group II Controls (N=31)		χ^2 I vs. II	p-value	RR
	N	%	N	%			
DQβ1* 02:01	14	63.6	0	0.0	26.809	<0.001	2.750
DQβ1* 02:02	8	36.4	0	0.0	13.277	<0.001	1.571
DQβ1* 03:01	2	9.1	5	16.1	0.556	0.456	0.520
DQβ1* 03:02	3	13.6	3	9.7	0.201	0.654	1.474
DQβ1* 03:03	2	9.1	10	32.3	3.943	0.047	0.210
DQβ1* 05:01	1	4.5	1	3.2	0.062	0.804	1.429
DQβ1* 05:02	1	4.5	5	16.1	1.720	0.190	0.248
DQβ1* 05:03	0	0.0	2	6.5	1.475	0.225	0.935
DQβ1* 06:01	5	22.7	17	54.8	5.465	0.019	0.242
DQβ1* 06:02	2	9.1	5	16.1	0.556	0.456	0.520
DQβ1* 06:03	1	4.5	4	12.9	1.052	0.305	0.321
DQβ1* 06:04	0	0.0	5	16.1	3.918	0.048	0.839

[Table/Fig-8]: Showing HLA DQβ1 distribution in anti-TPO positive CD patients.

DQα1*05:01 (63.6% vs. 0%) and DQα1*05:05 (40.9% vs. 3.2%) were also significantly increased (p<0.001 and 0.001) in anti-TPO positive cases [Table/Fig-9] contrary to this DQα1*01:01, DQα1*01:03 and DQα1*01:04 were significantly reduced in anti-TPO positive cases.

DQα1 alleles	Group I Anti-TPO positive CD patients (N=20)		Group II Controls (N=31)		χ^2 I vs. II	p-value	RR
	N	%	N	%			
DQα1*01:01	1	4.5	10	32.3	6.009	0.014	0.100
DQα1*01:02	4	18.2	10	32.3	1.312	0.252	0.467
DQα1*01:03	5	22.7	18	58.1	6.541	0.011	0.212
DQα1*01:04	0	0.0	9	29.0	7.694	0.006	0.710
DQα1*02:01	1	4.5	6	19.4	2.462	0.117	0.198
DQα1*03:01	3	13.6	2	6.5	0.777	0.378	2.289
DQα1*05:01	14	63.6	0	0.0	26.809	<0.001	2.750
DQα1*05:05	9	40.9	1	3.2	11.937	0.001	20.769

[Table/Fig-9]: Showing HLA DQα1 distributions in anti-TPO positive CD patients.

DISCUSSION

In present study, we found that majority of patients (70%) were children between 6 months-20 years of age and 52% children were below 10 years of age only 30% patients were adults. Similar to our findings the US based study by Llorente-Alonso MJ et al., reported that 67% patients of CD were children and 14.3% were adults [12]. However, Freeman HJ et al., from USA found bimodal peak of CD, first peak in children (8-12 years) and second peak during third to fourth decades of life [13]. While one of the studies from India reported increasing prevalence of CD in adults [14].

In the present study, we found female predominance (60%) showing a female to male ratio of 1.5:1. Similar to our study Elli L et al., of Spain also reported the female predominance in CD with female to male ratio of 2.3:1 [15]. Megiorni F et al., from America also reported that CD is twice more frequent in females than males [16]. They proposed that female predominance was due to increased frequency of HLA DQ 2 haplotype in females.

Major clinical manifestation of CD patients were weight loss and failure to thrive (79%) followed by chronic diarrhea (71%), short stature (62%), anaemia (66%) and pain in abdomen. Our study is in accordance with other Indian study [17,18]. Some studies from India reported short stature in 58%, delayed puberty in 31%, rickets in 8%, carpopedal spasm in 6% and gastrointestinal symptoms in 86% [19].

There was strong association of T1DM with CD (40%) in present series. More or less similar observations were noted by Philip R et al., from Australia (40%) and Bakker SF et al., from Italy (42%) [19,20]. One study from India found prevalence of T1DM in 25% cases in CD [19]. As a whole prevalence rates of T1DM from both cross-sectional and longitudinal studies range from 1.6-16.4% worldwide [21].

Reports on association of autoimmune thyroid diseases in CD patients are variable. In the present study we found that 22% patients had autoimmune hypothyroid diseases while Hakanen M et al., found much higher incidence of 38% [22]. While Velluzzi F et al., and Casta MG et al., reported incidence of about 29.7% and 30.5% [23,24]. Contrary to this Ventura A et al. reported low frequency of autoimmune thyroid diseases (14.4%) [25].

We also studied the ANA in CD patients which was detected in 10% cases only. In a study carried out by da Rosa Utiyama SR et al., studied in 56 patients with CD and 118 first degree relative and 101 healthy controls, ANA was detected in 9% while Caglar E et al., reported ANA positivity in 12% patients with CD [26,27].

Anti-scleroderma 70 antibody was detected in 25% patients with CD. There are variable reports in literature. Gomez-Puerta JA et al., studied 6 cases of CD and found all had anti-scl 70 antibody [28], while one of the Indian study found anti-TPO antibodies positivity in 53% and clinical hypothyroidism in 28% CD cases [19]. In our study, all anti-TPO Ab positive cases had autoimmune hypothyroidism but anti-scl 70 Ab positive cases and ANA positive cases did not have any classical presentation of scleroderma or SLE.

Author found that most frequent HLA DQ genotype in CD patient is DQ2 heterodimer composed of DQβ1*02, DQα1*05. DQβ1*02:01 was seen in 45.5% ($p<0.001$) while DQβ1*02:02 in 20.5% ($p=0.007$) which collectively forming 66% in total CD patients. Risk ratio was 1.833 and 1.257 respectively for 02:01 and 02:02 which indicate that their presence increases the risk for CD.

In DQα1 we found predominant allele was DQα1*05:01 (45.5%) and DQα1*05:05 (40.9%) in CD patients. Its association with CD was significant ($p<0.001$). RR for DQα1*05 was 1.833 and 20.769.

Vidales MC et al., studied 136 cases of CD found frequency of DQα1*05:01 in 59.2% and DQα1*02:01 in 26% while DQα1*01:04 and DQα1*03:02 were found in very low frequency (6.7%) [29]. In DQβ1 most frequent alleles were DQβ1*02:01 (47.1%) and DQβ1*02:02 (25.4%) and DQβ1*03:01 (12.5%) which is very much close to our observation.

Study conducted in western India found high prevalence of DQ2 (95%) and DQ8 (94%) among celiac patients as compared to controls (12%). DQ2.5 (DQα1*0501-DQβ1*0201) and DQ2 (DQβ1*02) haplotypes were common in CD patients (70%) [30]. However in this study, about 66% patient had DQ2 and 13.6% had DQ8 haplotypes.

Piccini B et al., also reported that 64% patients had DQ2 heterodimer and 13.5% had DQ8 haplotype that influences development of CD [31]. Megiorni F et al., also supported our observation and reported that 90-95% of CD patients carry DQ2.5 heterodimer encoded by DQβ1*03:02 in combination of DQα1*03 variant [32]. It was very interesting to note that 63% patients were homozygous for DQβ1*02 gene and 36.4% were homozygous for DQα1*05:01 suggesting a link between homozygosity for their alleles and increased risk for CD. Similar to our work some other reports also reported that DQ2.5 homozygotes are several times more likely to have CD than the DQ2.5 heterozygotes [33]. These homozygotes also have elevated risk for severe complication of the disease.

Major histocompatibility complex class II HLA DQ2 and DQ8 confers greatest disease susceptibility. The prevalence of autoimmune disease e.g., T1DM and autoimmune thyroid diseases are increased in CD patients. HLA DQ typing in CD with T1DM and without T1DM was analysed. It was found that DQβ1*02:01 (70%), DQβ1*02:02 (65%), DQα1*05:05 (50%) and DQα1*05:01 (60%) were significantly more common in CD associated with T1DM than CD alone. Barker JM et al., reported that DR3-DQ2 with T1DM carried a 33% risk for the presence of anti-tTg antibody and CD [34].

Camarca ME et al., reported that approximately 90% of the individuals with T1DM have either DQ2 or DQ8 compared to 40% of the general population [35]. Some of the studies from Northern Spain and USA also found increased frequency of DQβ1*02:01 in T1DM [36,37]. Some of the studies from India Singh S et al., also found increased frequency of DQβ1*02:01 in about 80% patients of T1DM [38]. HLA DQ genotype were also studied in anti-TPO positive CD patients and found that anti-TPO positive CD patients have genotype compatible with CD e.g., DQβ1*02:01 (63.6%), DQβ1*02:02 (36.4%), DQα1*05:01 (63.6%), DQα1*05:05 (40.9%).

The coexistence of CD and autoimmune thyroid diseases is thought to be partly due to common genetic predisposition. Lorini R et al., and Badenhoop K et al., proposed that DQ2 and DQ8 haplotypes are over represented in many autoimmune diseases including thyroid diseases and graves' disease [39,40].

Limitation(s)

Smaller sample size may limit the result validation. Due to logistic reason, number of controls could not be matched with the number of cases. Further the specificity and sensitivity of such gene typing needs to be carried out further.

CONCLUSION(S)

The present study suggests that CD is an autoimmune disease where the genetics have important role. HLA DQ antigen contributes more than 65% cases of CD hence it may be kept in diagnostic criteria of CD. HLA DQβ1*02 and HLA DQα1*05 alleles predisposes for development of other autoimmune diseases like T1DM and autoimmune thyroid disease. The involvement of genes other than HLA can be studied in the future to know the exact prevalence of these genes in CD.

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