International Journal of Medical Research Professionals
P-ISSN: 2454-6356; E-ISSN: 2454-6364
DOI: 10.21276/ijmrp

IJMRP

Original Research Article

HLA Study Regarding the Prevalence of Celiac Disease in Type 1 Diabetes

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ABSTRACT

Introduction: There is high prevalence of Celiac disease (CD) in autoimmune disorders such as Type 1 diabetes mellitus (T1D) in general population. Homozygosity for DR3-DQ2 in the population with T1D carries a 33% risk for the presence of tissue Transglutaminase (tTG) auto antibodies. Our study aimed to identify the HLA correlation between T1D and CD.

Methods: Human tTG IgA antibody test by ELISA was done in 100 T1D patients and Upper Gastrointestinal Endoscopic Biopsy was performed in patients who were serologically positive for Human tTG IgA antibody test. Then HLA DQ2 and DQ8 genotyping by polymerase chain reaction was performed for all T1D patients.

Results: Out of 100 T1D patients, 17 were found positive for IgA tTG antibody. Out of 17 IgA tTG antibody positive patients 15 had positive Endoscopic small intestinal Biopsy report for CD. DQ2 genotype was observed to be positive in 86.67% CD+T1D cases. DQ8 genotype was observed to be positive in 6.67% CD+T1D cases.

Conclusion: There was very good association of HLA DQ2 in patients with T1D and CD in our study. There was poor association of HLA DQ8 in patients with T1D and CD in our study. Therefore, Type 1 diabetic children with HLA DQ2 positivity are more prone to develop Celiac disease later.

Keywords: Type 1 Diabetes, Celiac Disease, HLA DQ2, HLA DQ8.

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Article History:

Received: 02-05-2017, **Revised:** 19-05-2017, **Accepted:** 05-06-2017

Access this article online	
Website: www.ijmrp.com	Quick Response code
DOI: 10.21276/ijmrp.2017.3.4.003	

INTRODUCTION

The term diabetes mellitus describes a metabolic disorder of multiple aetiology characterized by chronic hyperglycaemia with disturbances of carbohydrate, protein and fat metabolism resulting from defects in insulin secretion, insulin action or both. The aetiological type named Type 1 diabetes (T1D) encompasses the majority of cases which are primarily due to pancreatic beta islet cell destruction and are prone to ketoacidosis. Type 1 includes those cases attributable to an autoimmune process as well as those with beta cell destruction and who are prone to ketoacidosis for which neither aetiology nor pathogenesis is known.[1]

There is high prevalence of Celiac disease (CD) in autoimmune disorders such as type 1 diabetes mellitus in general population.[2] When patients with type 1 diabetes were screened for celiac disease, 6.0 to 10.0% were found antibody positive and/or biopsy positive.[3] For establishing the diagnosis of celiac disease small intestinal biopsy is mandatory, but the diagnosis of CD is complicated due to fact that the disease may be silent or present with atypical findings.

The HLA genes are the human equivalent of the major histocompatibility complex (MHC) containing over 200 genes located on the short arm of chromosome 6 (6p21.3). In Type 1 diabetes the high risk genotype is DR3-DQ2.5 in cis position, which is found in 50% of the patients.[4] CD and T1D share HLA risk genotypes. Approximately 90% of individuals with T1D have either DQ2 or DQ8, compared to 40% of the general population. Homozygosity for DR3-DQ2 in the population with T1D carries a 33% risk for the presence of tissue Transglutaminase (tTG) auto antibodies, and conversely, less than 2% of patients lacking DQ2 or DQ8 have CD-related autoantibodies.[5] As there are only limited studies regarding HLA correlation between T1D and CD, our study aimed to identify the HLA correlation between T1D and CD.

MATERIALS AND METHODS

This was a cross sectional hospital based study conducted at Pediatrics Hospital attached to SP Medical College and Associated group of Hospitals, Bikaner for duration of 1 year. After

taking permission from Institutional Research Board the study was started.

Inclusion Criteria

1. Age group 2 to 15 years
2. Diagnosed case of Type 1 Diabetes
3. Whose parents gave consent for the study.

Exclusion Criteria: Children whose parents denied for consent.

Methodology

After taking permission from Institutional Research Board the study was started. 100 type 1 Diabetes patients aged 2-15 years were selected after obtaining informed verbal consent from their parents/ caregiver accompanying. Following tools and techniques were used to obtain relevant and required information.

Study Tools and Techniques

Pre structured, relevant to objectives containing questions related to personal and clinical details of Type 1 Diabetes cases were collected. Human tTG IgA antibody test by ELISA was done and Upper Gastrointestinal Endoscopic Biopsy was performed in patients who were serologically positive for Human tTG IgA antibody test. Then HLA DQ2 and DQ8 genotyping by polymerase chain reaction was performed for all T1D patients.

HLA Typing

Susceptibility to CD is linked to certain human leukocyte antigen (HLA) class II alleles, especially in the HLA-DQ region. Whole venous blood (2–3 ml) was used. The two sets of alleles, DQA1*05 - DQB1*02 and DQA1*03 - DQB1*03:02, which code for class II MHC DQ2 and DQ8 molecules, respectively, were tested. DNA extraction was performed using Blood genomic Prep Mini Spin kit according to the manufacturer's instructions. Concentration of DNA samples were adjusted to 15 ng/µL after being quantified at Nanodrop ND-1000 Spectrophotometer (Nanodrop Technologies), which measures absorbance at 260 nm and 280 nm. Samples were considered suitable for analyses when the ratio of absorbencies (A260/A280) was between 1.8 and 2. Sequence-specific primers for

DQA1*05 [(5'- ACGGTCCCTCTGGCCAGTA,
3'- AGTTGGAGCGTTTAATCAGAC) (DQ2)],
DQB1*02 [(5'-GTGCGTCTTGTGAGCAGAAG,
3'- GCAAGGTCGTGCGGAGCT) (DQ2)],
and DQA1*03 [(5'-TTCACTCGTCAGCTGACCAT,
3'- CAAATTGCGGGTCAAATCTTCT) (DQ8)]

were used to test for the presence of each allele in independent reactions. Primers for Human Growth Hormone (HGH) were used as an internal control. Amplifications were performed on a Step One Real-Time PCR System. For DQ alleles, amplifications were performed in 20 µL volume containing 2 µL of genomic DNA, 0.5 µM DQ-forward primer, 0.5 µM DQ-reverse primer, and 1X Thermo Scientific Absolute QPCR SYBR Green ROX Mix. For HGH control samples reactions were performed in 20µL volume containing 2 µL of genomic DNA, 0.5 µM HGH-specific forward primer, 0.5 µM HGH-specific reverse primer, and 1X Thermo Scientific Absolute QPCR SYBR Green ROX Mix. A positive control or reference sample (DNA sample known to be positive for the searched alleles) was included in all reactions to standardize interpretations. Additionally, a known negative DNA sample (not containing the region of interest) and negative control (no DNA added to reaction) were run in set of reactions to test for contamination.

Table 1: Prevalence of celiac disease in type 1 diabetes mellitus.

Celiac Disease	Study Subjects
▪ **Positive for Anti IgA TTG in T1D patients**	17/100 (17%)
▪ **Positive Endoscopic small intestinal Biopsy in serology positive patients**	15/17 (88.24%)
▪ **HLA genotyping positive (DQ2 & DQ8) in biopsy**	DQ2= 12/15 (80.0%);

Table 2: Distribution of symptoms in patients with celiac disease

Celiac Disease	Cases (NCD=15)
Asymptomatic	12/15 (80%)
Symptomatic	3/15 (20%)
Chronic abdominal pain	3/15 (20%)
Diarrhoea	2/15 (13%)
Abdominal distension	2/15 (13%)
Short stature	3/15(20%)

RESULTS

Out of 100 T1D patients, 17 were found positive for IgA tTG antibody. Out of 17 IgA tTG antibody positive patients 15 had positive Endoscopic small intestinal Biopsy report for CD.(Table 1) 12 (80%) of 15 CD with T1D patients were asymptomatic whereas 3 (20%) were having one or the other symptoms. Out of 3 symptomatic patients all 3 of them were having chronic abdominal pain and short stature and 2 of them were suffering from diarrhoea and abdominal distension. (Table 2)

DQ2 genotype was observed to be positive in 86.67% CD+T1D cases. There is a statistical significant association present between DQ2 positivity and CD presence ($p<0.05$). The strength of association as determined by Odds Ratio, is very good (OR=9.167, 95% CI= 2.369-35.466, p=0.0001). (Table3)

DQ8 genotype was observed to be positive in 6.67% CD+T1D cases. There is a statistically insignificant association between DQ8 positivity and CD presence ($p>0.05$). The strength of association as determined by Odds Ratio, is also poor (OR=5.571, 95% CI= 0.329- 94.376, p=0.724). (Table 4)

Table 3: Association of presence of DQ2 genotype with Occurrence of CD

HLA Genotype	T1D + CD		T1D only		Total	
	No.	%	No.	%	No.	%
DQ2 +ve	12	80.0	24	30.37	37	39.36
DQ2 -ve	3	20.0	55	69.62	57	60.64
Total	15	100.0	79	100.0	94	100.0

[$\chi 2 = 11.119$, df=1, p=0.0001]

Table 4: Association of presence of DQ8 genotype with Occurrence of CD

HLA Genotype	T1D + CD		T1D only		Total	
	No.	%	No.	%	No.	%
DQ8 +ve	1	6.67	1	1.26	2	2.12
DQ8 -ve	14	93.33	78	98.74	92	97.87
Total	15	100.0	79	100.0	94	100.0

[$\chi 2 = 0.125$, df=1, p=0.724]

DISCUSSION

In present study the prevalence of CD in T1D patients was 15%. Our results correlate with Joshi R et al7 study in which the prevalence of CD in children with T1D was 15.49%. In our study out of 3 symptomatic CD patients, all 3 of them (100%) were having chronic abdominal pain and short stature and 2 of them (66%) were suffering from diarrhoea and abdominal distension. The results correlate with Bhadda SK et al7 study in which short stature was the commonest (52.3%) manifestation of celiac disease, followed by anemia (47.3), weight loss (42.8%), diarrhea (28.6%) and abdominal pain (14.2%). In our study DQ2 genotype was observed to be positive in 86.67% CD+T1D cases and DQ8 genotype was observed to be positive in 6.67% CD+T1D cases which is correlate with Ghawil M et al8 study in which 75% of biopsy-proven CD+T1D patients had HLA-DQ2 and 4% had HLA-DQ8.

CONCLUSION

The prevalence of Celiac disease in Type 1 diabetic children in our study was 15% and most of the children with Celiac disease were asymptomatic. Therefore, routine screening through proper serological testing for Celiac disease in all Type 1 diabetic children is recommended. There was very good association of HLA DQ2 in patients with Type 1 diabetes and Celiac disease in our study. There was poor association of HLA DQ8 in patients with Type 1 diabetes and Celiac disease in our study. Therefore, Type 1 diabetic children with HLA DQ2 positivity are more chances to have associated Celiac disease or develop the disease later.

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Source of Support: Nil. **Conflict of Interest:** None Declared.

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Cite this article as: Ramesh Subramanian, Devendra Kumar Agrawal, Renu Agrawal, G.S. Tanwar, Rahul Sethiya. HLA Study Regarding the Prevalence of Celiac Disease in Type 1 Diabetes. Int J Med Res Prof. 2017; 3(4):12-14.
DOI:10.21276/ijmrp.2017.3.4.003