

# Immunogenetic Study of Diabetes Mellitus in Relation to HLA DQ and DR

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## Abstract

**Introduction:** Type-1 diabetes mellitus (T1DM) which is also known as insulin-dependent diabetes is diagnosed mainly during childhood and accounts for approximately 5%–10% of all cases of DM. In India, early onset diabetes (<15 years age) constitutes about 1%–4% of the total diabetic population. The insulinitis as well as to a humoral (B cell) response with production of antibodies to IAA, GAD, and the protein tyrosine phosphatase IA2 (IA-2AA) is the main pathogenesis of T1DM. Human leukocyte antigen (HLA)-DR and DQ contributes approximately 40%–50% of the inherited susceptibility for T1DM and most frequently involved haplotypes are DRB1\*0301-DQB1\*0201, DRB1\*0301-DQA1\*0501-DQB1\*0201, and DRB1\*0401-DQB1\*0302. **Method and Material:** Total 70 cases of DM in age group of 10 years to 65 years and 25 healthy controls of same age group 30 cases of complicated diabetic mellitus were included in the study. 2 mL blood was taken in an EDTA vial for HLA typing and 5 mL blood was taken in a plain vial for anti-GAD antibody. HLA DQB1 and DRB1 were done by sequence specific priming polymerase chain reaction method. Indirect immunofluorescent test was used for anti-GAD antibody. Statistical analysis was performed using SPSS version-16. **Results:** Total 40.9% cases of type-I DM were found seropositive for anti-GAD antibody. None of the cases of type-II DM was anti-GAD antibody positive. HLA DRB1\*03010 were significantly more in diabetic patient ( $P < 0.011$ ) as compared to control. DRB1\*0403/6 shows that a relative risk of 1.08 was slightly more frequent in DM cases as compared to the control. DQB1\*0201 was significantly high ( $P < 0.004$ ) in DM patient as compared to control with a relative risk of 1.68. Correlation of DR, DQ antigen with types of DM showed that in type-I DM, DRB1\*03010 was significantly high ( $P = 0.009$ ) with a relative risk of 2.78 as compared type-II DM. In DQ typing, DQB1\*0201 was significantly high in type-I DM in comparison to type-II DM (65% vs. 30%,  $P = 0.026$ , RR = 2.05). Comparison of DQB1 in type-I DM with healthy control showed that DQB1\*0201 was significantly high in type-I DM as compared to healthy control ( $P = 0.0003$ , RR = 3.09). In type-I DM patient's homozygosity at DRB1\*03010, DRB1\*03010 was significantly high as compared to the control ( $P < 0.047$ , RR = 2.33). Correlation of anti-GAD antibody with DRB1 and DQB1 showed that 77.7% anti-GAD antibody positive cases were DRB1\*03010 positive. Similarly, in DQB1 typing, 66.6% anti-GAD positive cases have DQB1\*0201. **Conclusion:** Prevalence of anti-GAD antibody in Indian population was found up to 45%. HLA DRB1\*3010 and HLA DQB1\*0201 were the most susceptible haplotypes for type-I DM. HLA DRB1\*14 and HLA DRB1\*15 were the protective haplotypes for type-I DM. Susceptibility to type-I DM increases when the homozygosity for DRB1\*03010 was present. Diagnosis of type-I DM by anti-GAD antibody was possible in only 40.9% cases but if DRB1 and DQB1 typing is added in the diagnosis then diagnostic efficacy increases up to 83%.

**Keywords:** Diabetes mellitus, glutamic acid decarboxylase, human leukocyte antigen

## INTRODUCTION

Type-1 diabetes mellitus (T1DM) which is also known as early onset diabetes or insulin-dependent diabetes is diagnosed mainly during childhood and accounts for approximately 5%–10% of all cases of DM. The main pathogenesis of T1DM is a decrease in insulin production due to autoimmune destruction of pancreatic  $\beta$ -cells in islets of Langerhans.

In India, it is estimated that over 97,700 children are suffering from T1DM.<sup>[1]</sup> In India, during 1990 various data were

collected from many hospital-based studies which suggest that early onset diabetes (<15 years age) constitutes about 1%–4% of the total diabetic population.<sup>[2]</sup>

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The importance of environmental risk factors for T1D was highlighted by various twin studies and seen that concordance rates for monozygous twins are higher than those for dizygous twins (approximately 30% vs. 10%, respectively).<sup>[3]</sup> It is not only the genetic susceptibility but also environmental triggers and epigenetic changes are also required to alter the immune system and initiating  $\beta$ -cell destruction.<sup>[4]</sup>

The inflammatory response within the islets cells (insulinitis) as well as to a humoral (B cell) response with production of antibodies to  $\beta$ -cell antigens which is caused by abnormal activation of the T-cell-mediated immune system in susceptible individuals is the most important pathogenesis of T1DM.

Antibodies against the islets cells were the first described, but have been supplanted by more specific autoantibodies to insulin (IAA), glutamic acid decarboxylase (GADA/GAA), and the protein tyrosine phosphatase IA2 (IA-2AA). The clinical onset of T1A can be preceded by the presence of one or more types of antibody over the years.<sup>[5,6]</sup>

The major risk factor for T1DM resides in the HLA region on chromosome 6p21 and this model has been supported by various linkage and association studies.<sup>[7,8]</sup> HLA-DR and DQ contributes approximately 40%–50% of the inherited susceptibility for T1DM.<sup>[9]</sup> Numerous studies have been done on the effect of the HLA-DR and DQ alleles, haplotypes, and genotypes on predisposition. Among the various HLA haplotypes, the most frequently involved in the susceptibility to T1D as risk factors are DRB1\*0301-DQB1\*0201, DRB1\*0301-DQA1\*0501-DQB1\*0201, and DRB1\*0401-DQB1\*0302(12). The alleles that are the most frequently reported risk factors are DRB1\*0301, DRB1\*0401,<sup>[10]</sup> DPB1\*0301(11), and DQA1\*0301(10). The DRB1\*1501-DQB1\*0602 haplotypes are the most common protective factor.<sup>[11,21]</sup>

## Aim

The present study was designed to assess the prevalence of anti-GAD antibody in different types of DM, to study the HLA-DR and DQ antigens in the young DM patient and healthy control, and to correlate the HLA-DR and DQ antigens with the age.

## METHOD AND MATERIAL

### Patient recruitment and plan of study

This study about the “**IMMUNOGENETIC STUDY OF DIABETES MELLITUS WITH SPECIAL REFERENCE TO DR, DQ ANTIGENS**,” was approved by the local Ethical Committee of Banaras Hindu University. Patients of young diabetes age group and complicated DM from the Department of Endocrinology and nephrology, SIR SUNDER LAL HOSPITAL, BHU, were consistently contacted. An informed consent was obtained during the consultation.

### Sample size

Total 70 cases of DM in the age group of 10 years to 65 years and 25 normal healthy controls were included in the study.

All DM cases which were diagnosed by biochemical test (according to the WHO criteria) included in the study. Clinical details are recorded in pretested performa in all cases.

### Collection of samples

2 mL blood was taken in an EDTA vial for HLA typing and 5 mL blood was taken in a plain vial for anti-GAD antibody.

### HLA DQ-B1 and DR-B1 typing

HLA DQB1 and DRB1 were done by sequence specific priming PCR method (SSP-PCR). The kit used for the typing of this gene was HLS-SSP kit of BAG Healthcare Germany.

### Principle of the test

DRB1\* alleles sequence-specific primer pairs were designed to selectively amplify target sequences that are specific to a single allele or group of alleles. This PCR SSP method was based on the principle that only Primer with completely matched sequence to the target sequence results in amplified products under control conditions to the target sequence and results in amplified products under control conditions. The result of amplified DNA fragments is a positive indication of the existence of allele-specific sequence within the genomic DNA. On the other hand, miss-match primer does not generate amplification. In addition to a sequence-specific primer, an internal control primer pair which amplified a conserved region of the housekeeping gene of cystic fibrosis is included in every PCR reaction mix and the PCR product of the internal control primer pair serves as an indicator of the integrity of PCR reaction.

### DNA isolation

The BAG EXTRA-GENE kit is most suitable for the DNA isolation since pure DNA can be obtained from whole blood in a short time without the use of toxic chemicals or solvents. The presence of heparin potentially inhibits PCR. Therefore, EDTA or Citrate Blood is recommended for typing. DNA should have the following purity indexes:

- $OD_{260}/OD_{280}$  = contamination with RNA: >1.5 and <2.0
- $OD_{260}/OD_{230}$  = contamination with salt, carbohydrate, or organic solvents: >1.8

### Amplification

All prealiquoted and dried reaction mixtures already contain allele and control-specific primers and nucleotides. These are supplied dried down in the reaction vial. Amplification parameters are optimized to a final volume of 10  $\mu$ l.

1. Remove the required number of HISTO TYPE HLA-SSP plates or strips and the 10  $\times$  PCR buffer from the kit.
2. Pipette the Master-Mix consisting of 10  $\times$  PCR-buffer, DNA solution, Taq-polymerase, and Aqua dest and mix well. The different HISTO TYPE SSP Kits do all work with the same master-mix and can, therefore, be combined. The composition of the master-mix depending on the number of reaction mixes is given in Table 1.

If a contamination control should be performed, produce the master-mix without the DNA solution first and pipette 10  $\mu$ l of this mix in the contamination control (colored

**Table 1: Composition of the master-mix depending on the number of reaction mixes**

No. of mixes	Aqua dest.	10 × PCR buffer	DNA-solution (25-40 ng/μl)	Taq-polymerase (5 U/μl)	Whole volume	Micro liter
1	7	1	2	0.08	10	μl
4	55	8	16	0.6	80	μl
8	69	10	20	0.8	100	μl
24	194	28	56	2.2	280	μl
30	235	34	68	2.7	340	μl
32	249	36	72	2.9	360	μl
48	360	52	104	4.2	520	μl
54	401	58	116	4.6	580	μl
56	415	60	120	4.8	600	μl
72	540	78	156	6.2	780	μl
80	595	86	172	6.9	860	μl
96	406	102	204	8.2	1020	μl

red). Afterwards, add the DNA solution and distribute the master-mix on the predropped reaction mixes.

The quantity should be 50–80 ng per mix. According to DNA concentration, the amount of DNA and water must be varied (e.g., for 24 mixes: 28 μl DNA solution (50 ng/μl) and 206 μl Aqua dest).

- After vortexing add 10 μl of this solution of this mixture immediately to the predropped and dried reaction mixtures. Change the tip after each pipetting step. Tightly close the tubes with the respective caps or foil.
- Slightly shake the plate/stripe downwards to dissolve the blue pellet at the bottom of the plate. All PCR solution should settle on the bottom.

Place the reaction tubes into the thermal cycler and tighten lid so that the reaction vessels do not warp in heating. Start the PCR program.

Amplification parameters:

Program Step	Temp.	Time	No. of Cycles
First Denaturation	96°C	5 min	1 cycle
Denaturation	96°C	20 s	5 cycles
Annealing + Extension	68°C	1 min	
Denaturation	96°C	20 s	10 cycles
Annealing	64°C	50 s	
Extension	72°C	45 s	
Denaturation	96°C	20 s	15 cycles
Annealing	61°C	50 s	
Extension	72°C	45 s	
Final Extension	72°C	5 min	1 cycle

By using thermal cyclers with a very fast heat and cooling rate, it is recommended to use a slower heat and cooling rate (~2.5°C/s).

### Gel electrophoresis

Separation of the amplification products is done by electrophoresis via a (horizontal) agarose gel. As electrophoresis buffer, 0.5 × TBE (45 mM of tris, 45 mM of boric acid, 0.5 mM of EDTA) buffer is recommended. The gel concentration should be 2.0%–2.5% of agarose. Allow the gel to polymerize

at least 30 min before sample loading. After amplification has been finished, take the sample out of the thermal cycler and load the complete reaction mixtures carefully in each slot of the gel. In addition, apply 10 μl of the DNA length standard for size comparison. Electrophoretic separation is done at 10–12 V/cm (with 20 cm distance between the electrodes approx. 200–240 V) for 20–40 min. After the run has been completed, the complete gel is stained in an ethidium bromide (EtBr) solution (approx. 0.5 μg/mL of EtBr in H<sub>2</sub>O or TBE buffer) for 30–40 min. As an alternative, EtBr (0.5 μg/mL) can also be added to the electrophoresis buffer or the agarose gel. If required, excess of EtBr can be removed by soaking the gel in H<sub>2</sub>O of o. 5 × TBE buffer for 20–30 min.

### Documentation and interpretation

For documentation, visualize the PCR amplification using a UV transilluminator (220–310 nm) and photograph it with a suitable camera, film, and filters (e.g., Polaroid, film type 667 or video system, thermal paper KP65HM-CE). Choose exposure time and aperture such that the bands are drawn sharp and stand out against the dark background (approximates aperture 11, exposure time 1 s).

For interpretation, use the specificity table and evaluation diagram. Only bands that have the correct size compared to the DNA length standard should be considered positive.

### Estimation of anti-GAD antibody

Indirect immunofluorescent test was used for anti-GAD antibody. The kit used for anti-GAD antibody was Medizyme Anti GAD-96 by MEDIPAN Germany.

### Principle

It is a double antibody technique. The unlabeled antibodies which have bound to the antigens are visualized by a fluorescent antiglobulin reagent directed at the unlabeled antibodies.

### Procedure

Patient sample is diluted in 1:5 with PBS. 50 mL of this is poured in wells containing cell section of monkey pancreatic islet cells.

It is incubated for 30 min at 4°C.

Section are washed with phosphate buffer saline.

FITC conjugated IgG is added to each well.

Sections are incubated in dark for 30 min.

Wash in PBS.

Mount in glycerol; PBS mixture (9:1).

It is seen in a fluorescent microscope with filter.

Fluorescent emission appeared green at 529 nm wavelength.

In positive cases, green fluorescence was seen in cytoplasm of the cell of islet.

### Presentation of result and statistics

Statistical analysis was performed using SPSS version-16. The various parameters studied during observation period were compared using the Chi-square test for noncontinuous variable. For continuous variables, Mann–Whitney U test and ANOVAs test were used. The results were presented in absolute frequencies, percentages, averages, standard deviation, and relative risk. The critical value of “*P*” indicating the probability of significant difference was taken as <0.05 for comparison.

## RESULTS

Total 22 cases of type-1 DM, 48 cases of type-2 DM, and 25 healthy controls were included in the study. Nine cases of type-1 DM were detected between 10 years to 20 years. Ten cases were seen in third decade (14.3%) and maximum cases were of type-II DM occurring after 40 years.

Among total type-1 DM cases, most of the cases were male patients (68.18%) in comparison to the female patients (31.8%). Majority of type-1 DM cases (86.36%) were below 30 years of age. Only 3 cases were found >30 years of age.

Total 09 cases were found seropositive for anti-GAD 65 antibody, which were 12.8% of total DM cases, while in control, no case was seropositive for anti-GAD 65 antibody. Among 09 cases, all were type-1 DM cases and none of the cases of type-II DM was found seropositive for anti-GAD 65.

Among total 09 cases of type-1 DM, 6 cases (66.6%) were male and 33.3% cases were female. Seven cases (77.7% of total GAD 65 positive cases) were below 20 years of age and 22.2% cases were between 21 and 30 years of age.

HLA DR typing in relation to healthy control as a whole showed that HLA DRB1\*03010 were significantly more in diabetic patient ( $P < 0.01$ ) as compared to control. Relative risk was high for DRB1\*03010 (1.615) followed by DRB1\*1310 (1.513), DRB1\*0701 (1.38), and DRB1\*0401 (1.13). DRB1\*0403/6 shows a relative risk of 1.08 and was slightly more frequent in DM cases as compared to the control [Table 2].

Correlation of DQB1 in DM with control showed that DQB1\*0201 was significantly high ( $P < 0.004$ ) in DM patient as compared to control with a relative risk of 1.68. DQB1\*0301

**Table 2: HLA-DR in diabetic patients and control**

HLA allele	DM (n=40)		Control (n=20)		P	RR
	No.	%	No.	%		
DRB <sub>1</sub> *03010	14	35	1	5	0.011	1.615
DRB <sub>1</sub> *0401	3	8	1	5	0.714	1.135
DRB <sub>1</sub> *0403/6	5	12.5	2	10	0.776	1.08
DRB <sub>1</sub> *0701	11	27	4	20	0.5271	1.138
DRB <sub>1</sub> *0901	1	2.5	1	5	-	-
DRB <sub>1</sub> *1101	5	12.5	1	5	0.3613	1.286
DRB <sub>1</sub> *1201	1	2.5	1	5	0.7412	0.8919
DRB <sub>1</sub> *1310	3	8	2	10	<0.9999	1.513
DRB <sub>1</sub> *1401	1	2.5	0	0	-	-
DRB <sub>1</sub> *1404/5	6	15	9	45	0.0114	0.5297
DRB <sub>1</sub> *1501/1502	17	43	11	55	0.3602	0.8447

**Table 3: HLA-DQ in diabetic patient and control**

HLA allele	DM (n=40)		Control (n=20)		P	RR
	No.	%	No.	%		
DQB <sub>1</sub> *0201	19	48	2	10	0.004	1.68
DQB <sub>1</sub> *0301	5	12.5	1	1	0.3613	1.286
DQB <sub>1</sub> *0302	8	20	7	35	0.2063	0.75
DQB <sub>1</sub> *0303	3	8	2	10	0.7412	0.8919
DQB <sub>1</sub> *0501	30	75	14	70	0.6797	1.091
DQB <sub>1</sub> *0601	14	35	8	40	0.7048	0.9301
DQB <sub>1</sub> *0602	3	8	2	10	0.7412	0.8919

was not significantly high in DM patient with a relative risk of 1.28 [Table 3].

Correlation of DR antigen with types of DM showed that in type-I DM, DRB1\*03010 was significantly high ( $P < 0.009$ ) with a relative risk of 2.78 as compared to type-II DM. Next was DRB1\*0401 and DRB1\*0403/6 which showed a relative risk of 1.37 and 1.75, respectively, although it was not found statistically significant due to small number of cases. In type-II DM, DRB1\*1501/1502 and DRB1\*0701 were high (55% and 30%, respectively) as compared to the type-I DM [Table 4].

In DQ typing, DQB1\*0201 was significantly high ( $P < 0.026$ , RR = 2.05) in type-I DM as compared to the type-II DM. DQB1\*0302 was not significantly high in type-1 DM in comparison to type-II DM patient (25% vs. 15%) with a relative risk of 1.33. Contrary to this, DQB1\*0301, DQB1\*0601, and DQB1\*0602 were more frequent in type-II DM as compared to type-1 DM, although that was not found statistically significant [Table 5].

Comparison of type-I DM with healthy control showed that DRB1\*03010 was significantly high in type-I DM patients as compared to control with a relative risk of 3.11 and  $P < 0.0002$ , while the frequency of DRB1\*1404/5 and DRB1\*1501/1502 was more in control as compared to type-1 DM patient. After the DRB1\*0301, the relative risk was high for DRB1\*0403/6 (1.41) followed by DRB1\*0401 (1.37) [Table 6].

**Table 4: Correlation of DR antigen with types of diabetes mellitus**

DR antigen	Type-I DM (n=20)		Type-II DM (n=20)		P	RR
	No.	%	No.	%		
DRB <sub>1</sub> *03010	12	60	2	10	0.00091	2.78
DRB <sub>1</sub> *0401	2	10	1	5	0.5483	1.37
DRB <sub>1</sub> *0403/6	4	20	1	5	0.1516	1.75
DRB <sub>1</sub> *0701	5	25	6	30	0.7233	0.878
DRB <sub>1</sub> *0901	0	0	1	5	<0.0001	-
DRB <sub>1</sub> *1101	2	10	3	15	0.6326	0.777
DRB <sub>1</sub> *1201	0	0	1	5	-	-
DRB <sub>1</sub> *1310	1	5	2	10	-	-
DRB <sub>1</sub> *1401	0	0	1	5	-	-
DRB <sub>1</sub> *1404/5	4	20	2	10	0.3758	1.41
DRB <sub>1</sub> *1501/1502	6	30	11	55	0.1098	0.579

**Table 5: Correlation of DQ antigen with type of diabetes mellitus**

DR antigen	Type-I DM (n=20)		Type-II DM (n=20)		P	RR
	No.	%	No.	%		
DQB <sub>1</sub> *0201	13	65	6	30	0.0266	2.05
DQB <sub>1</sub> *0301	1	5	4	20	0.1516	0.368
DQB <sub>1</sub> *0302	5	25	3	15	0.4292	1.333
DQB <sub>1</sub> *0303	1	5	2	10	0.5483	0.649
DQB <sub>1</sub> *0501	15	75	15	75	1	1
DQB <sub>1</sub> *0601	5	25	9	45	0.1851	0.619
DQB <sub>1</sub> *0602	1	5	2	10	0.5483	0.649

**Table 6: Correlation of DR antigen between type-I diabetes mellitus patient and control**

HLA allele	Type-I DM (n=20)		Control (n=20)		P	RR
	No.	%	No.	%		
DRB <sub>1</sub> *03010	12	60	1	5	0.0002	3.11
DRB <sub>1</sub> *0401	2	10	1	5	0.548	1.37
DRB <sub>1</sub> *0403/6	4	20	2	10	0.375	1.41
DRB <sub>1</sub> *0701	5	25	4	20	0.704	1.14
DRB <sub>1</sub> *0901	0	0	1	5	-	-
DRB <sub>1</sub> *1101	2	10	1	5	0.548	1.37
DRB <sub>1</sub> *1201	0	0	1	5	-	-
DRB <sub>1</sub> *1310	1	5	2	10	0.548	0.649
DRB <sub>1</sub> *1401	0	0	0	0	-	-
DRB <sub>1</sub> *1404/5	4	20	9	45	0.09	0.461
DRB <sub>1</sub> *1501/1502	6	30	11	55	0.109	0.579

Comparison of DQB1 in type-I DM with healthy control showed that DQB1\*0201 was significantly high in type-I DM as compared to healthy control ( $P < 0.0003$ , RR = 3.09), while DRB1\*0601 was more frequent in control as compared to

the type-1 DM patient (40% vs. 25%) but that was not found statically significant [Table 7].

In type-I DM patient's homozygosity at DRB1\*03010, DRB1\*03010 was significantly high ( $P < 0.047$ , RR = 2.33), whereas no significant homozygosity of any DRB1 allele was found in type-II DM [Tables 8 and 9].

Correlation of anti-GAD antibody with DRB1 and DQB1 showed that 77.7% anti-GAD positive cases were DRB1\*03010 positive and 22.2% cases were either DRB1\*0401 or DRB1\*0403/6 positive. Similarly, in DQB1 typing, 66.6% anti-GAD positive cases have DQB1\*0201 and 55.5% have DQB1\*0501. In rest of the cases, associated haplotypes were DQB1\*0301, DQB1\*0302, and DQB1\*0601 [Table 10].

## DISCUSSION

In the present study, the prevalence of type-1 DM was more in male patient (68.18%) in comparison to the female (31.8%). Majority of type-1 DM cases (86.36%) were below 30 years of age. However, there was no major difference in the mean age of diagnosis between male and female patients (20.6 years vs. 21.42 years). Only three cases were found >30 years of age which could be due to late diagnosis of disease in these patients. The youngest patient was a 10-year-old female.

### Anti-GAD 65 antibody in diabetes mellitus present study of diabetic

Auto-immunity plays a major role in pathogenesis of type-1 DM. The risk of progression to clinical diabetes increases with the number of autoantibodies detected.<sup>[12]</sup> Mehra *et al.*, 2007,<sup>[13]</sup> showed that GAD 65 antibody was present in approximately 20%–26% cases of type-1 DM in India.

In the present study, the prevalence of anti-GAD antibody in our patient with type-1 DM was 40.9%, which was slightly higher than that reported for the Indian population. There was a significantly higher prevalence of anti-GAD in male patient in comparison to the female (66.6% vs. 33.3%). With regard to the age, there was significantly higher prevalence of anti-GAD in IDDM patient below 20 years of age (77.7%) with a mean age of 16.14 years. In western country, a study done in Spain by Serrano Rios *et al.*<sup>[14]</sup> found anti-GAD antibody positivity of 64.3%, which was more common in male (75%) as compared to female and 62.5% cases were below 10 years of age. When total DM cases were compared, only 12.8% of cases found to be seropositive. None of the patients of type-2 DM found to be seropositive for anti-GAD 65 antibody. These data indicate that autoimmunity plays a major role in the pathogenesis of type-1 DM.

### HLA DQ and DR in diabetes mellitus

Genetic susceptibility is important in the development of type-1 DM. Genome wide association studies have identified multiple genetic susceptibility loci for type-1 DM as well as type-2 DM. The most important is HLA-locus on chromosome

6p 21. The HLA locus contributes as much as 30%–50% of genetic susceptibility in type-1 DM (Nobel *et al*, 1996).<sup>[22]</sup> Many previous studies have shown the association of type-I DM with HLA in different ethnic groups. There is evidence showing that 40%–50% of the inherited susceptibility for the disease is contributed by HLA-DR–DQ.<sup>[9]</sup>

The HLA haplotypes that are the most frequently reported as involved in the susceptibility to T1D as risk factors are DRB1\*0301-DQB1\*0201, DRB1\*0301-DQA1\*0501-DQB1\*0201, and DRB1\*0401-DQB1\*0302.<sup>[11]</sup>

Most of the previous studies in India have shown an increase in the association of DRB1\*0301 and DQB1\*0201 allele in type-1 DM in comparison to the control.<sup>[13,15]</sup> Present study also shows significant increase of DRB1\*0301 ( $P$ -value 0.0002, RR = 3.11) and DQB1\*0201 ( $P$ -value 0.0003, RR = 3.09) allele in type-1 DM in comparison to the control.

**Table 7: Correlation of DQ antigen between type-I diabetes mellitus patient and control**

HLA allele	Type-I DM (n=20)		Control (n=20)		P	RR
	No.	%	No.	%		
DQB <sub>1</sub> *0201	13	65	2	10	0.0003	3.09
DQB <sub>1</sub> *0301	1	5	1	5	1	1
DQB <sub>1</sub> *0302	5	25	7	35	0.490	0.777
DQB <sub>1</sub> *0303	1	5	2	10	0.548	0.649
DQB <sub>1</sub> *0501	15	75	14	70	0.723	1.13
DQB <sub>1</sub> *0601	5	25	8	40	0.311	0.692
DQB <sub>1</sub> *0602	1	5	2	10	0.548	0.649

Most of the previous studies showed an increase association of DRB1\*0401 in IDDM patient.<sup>[16,17]</sup> But, in Indian population, Rani *et al.*, in 1996,<sup>[15]</sup> did not find any significant association between DRB1\*0401 and IDDM. Present study showed a slight increase frequency of DRB1\*0401 allele in IDDM patient (10%) in comparison to the control (5%) but this association was not found statistically significant ( $P$  = 0.548, RR = 1.37). In many studies, DRB1\*0403/6 was negatively associated with type-1 DM and have a protective effect.<sup>[18,19]</sup>

Mehra *et al.*, in 2007,<sup>[13]</sup> also showed that DRB1\*0403/6 have a negative association with type-I DM and have a protective effect in Indian population but we did not find any negative association of DRB1\*0403/6 with type-1 DM patient.

Present study showed a slight increase frequency of DRB1\*0403/6 in type-1 DM patient as compared to control but statistically, it was not significant (20% vs. 10%,  $P$  = 0.375).

In many studies, DQB1\*0601 have been shown to be negatively associated with type-1 DM.<sup>[19,20]</sup> Present study also showed that DQB1\*0601 was more frequent in control in comparison to the IDDM patient (45% vs. 25%,  $P$  = 0.311, RR = 0.692) but statistically it was not found to be significant.

DRB1\*14 and DRB1\*15 were negatively associated with type-1 DM.<sup>[19]</sup> DRB1\*1401 and DRB1\*1501 were the protective haplotypes for IDDM.<sup>[20]</sup> Our study also showed a negative association of type-1 DM with DRB1\*14 and DRB1\*15. In the present study, DRB1\*1401/5 was decreased significantly in total DM patient (15%) in comparison to the control (45%) with a  $P$  value of 0.0114. When type-I DM patients were correlated with control, there was a significant increase in DRB1\*1404/5 in control (45%)

**Table 8: Homozygosity and heterozygosity of various HLA-DR alleles in type-I DM**

DR alleles	Diabetic cases (n=20)		Control (n=20)		P	RR
	No.	%	No.	%		
*03010, *03010	5	25	0	0	0.047	2.33
*03010, *1501	3	15	0	0	0.2304	-
*1501, *1501	3	15	0	0	0.2304	-
*1501, *0701	2	10	1	5	0.8483	-
*03010, *0401/0403	2	10	0	0	0.4872	-
*1501, with alleles other than *03010	0	0	6	30	0.2020	-
*3010, *0701	1	5	0	0	<0.9999	-

**Table 9: Homozygosity and heterozygosity of various HLA-DR alleles in type-II DM**

DR alleles	Diabetic cases (n=20)		Control (n=20)		P	RR
	No.	%	No.	%		
*03010, *03010	0	0	0	0	-	-
*03010, *1501	1	5	0	0	-	-
*1501, *1501	2	10	0	0	-	-
*1501, *0701	2	10	1	5	0.5483	1.37
*1310, *1310	1	5	0	0	<0.9999	-
*1113, *1113	1	5	0	0	-	-
*1501, with alleles, other than *03010	5	25	6	30	0.7233	0.878

**Table 10: Correlation of DQ and DR antigen with anti-GAD antibody**

HLA allele	Type-I DM (n=20)		Control (n=20)		anti-GAD positive cases (n=9)	
	No.	%	No.	%	No.	%
DRB <sub>1</sub> *03010	12	60	1	5	7	77.7
DRB <sub>1</sub> *0401	2	10	1	5	1	11.1
DRB <sub>1</sub> *0403/6	4	20	2	10	1	11.1
DRB <sub>1</sub> *0701	5	25	4	20	0	0
DRB <sub>1</sub> *0901	0	0	1	5	0	0
DRB <sub>1</sub> *1101	2	10	1	5	0	0
DRB <sub>1</sub> *1201	0	0	1	5	0	0
DRB <sub>1</sub> *1310	1	5	2	10	0	0
DRB <sub>1</sub> *1401	0	0	0	0	0	0
DRB <sub>1</sub> *1404/5	4	20	9	45	1	11.1
DRB <sub>1</sub> *1501/1502	6	30	11	55	1	11.1
DQB <sub>1</sub> *0201	13	65	2	10	6	66.6
DQB <sub>1</sub> *0301	1	5	1	5	1	11.1
DQB <sub>1</sub> *0302	5	25	7	35	2	18.18
DQB <sub>1</sub> *0303	1	5	2	10	0	0
DQB <sub>1</sub> *0501	15	75	14	70	5	55.5
DQB <sub>1</sub> *0601	5	25	8	40	1	11.1
DQB <sub>1</sub> *0602	1	5	2	10	0	0

in comparison to the IDDM cases (45% vs. 20%,  $P = 0.09$ ,  $RR = 0.461$ ). Similarly, DRB1\*1501 was more frequent in control in comparison to the IDDM patient (55% vs. 30%,  $P = 0.109$ ,  $RR = 0.579$ ). Thus, our study suggests that DRB1\*1404/5 and DRB1\*1501 have a protective role for type-I DM.

Rani *et al.*, in 1999, showed that individuals who were homozygous for DRB1\*0301 allele were more susceptible for type-1 DM. In the present study, we also found that homozygosity for DRB1\*0301 was significantly more in type-I DM patient (25%) in comparison to the control (0%) with a  $P$  value of <0.05.

## CONCLUSION

The prevalence of anti-GAD antibody in Indian population was found up to 45%. Anti-GAD antibody positivity was more frequent in male patient as compared to female.

HLA DRB1\*3010 and HLA DQB1\*0201 were the most susceptible haplotypes for type-I DM.

HLA DRB1\*14 and HLA DRB1\*15 were the protective haplotypes for type-I DM but DRB1\*0401 and DRB1\*0403/6 did not show any significant association. Susceptibility to type-I DM increases when the homozygosity for DRB1\*03010 was present but no definite homo or heterozygosity was found for type-II DM. Diagnosis of type-I DM by anti-GAD antibody was possible in only 40.9% cases but if DRB1 and DQB1 typing is added in the diagnosis then diagnostic efficacy increases up to 83%. Hence, for diagnosis of type-I DM, both anti-GAD antibody and DR, DQ typing to be done.

Furthermore, studies are required to establish the role of DR, DQ typing and anti-GAD antibody in the diagnosis of type-I DM.

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## Conflicts of interest

There are no conflicts of interest.

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