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Original article

Molecular Identification of HLA-DRB Exon 2 Genetic Variants in Young Adults with Diabetes Mellitus: A Pilot Sequencing Study

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Abstract

Background: *Genetic variation within the human leukocyte antigen (HLA)-DRB1 gene has been implicated in the pathogenesis of diabetes mellitus. This pilot study investigated nucleotide sequence variations in the HLA-DRB1 exon 2 region among young adults with diabetes mellitus.Methods: Ten young adults with diabetes mellitus were enrolled in this cross-sectional molecular study. Genomic DNA was isolated from peripheral blood samples, and DNA quality was assessed using NanoDrop spectrophotometry. The HLA-DRB1 exon 2 region was amplified by polymerase chain reaction (PCR) and analyzed by Sanger sequencing. Sequence alignment was performed using the reference HLA-DRB1 sequence.* ***Results:*** *Genomic DNA of adequate quality was obtained from all participants, and PCR amplification and sequencing were successfully completed in all samples. Sequence analysis showed that nine participants (90%) had complete similarity with the reference HLA-DRB1 exon 2 sequence, while one participant (10%) demonstrated a nucleotide sequence variation. All participants had HbA1c values above the diagnostic threshold for diabetes mellitus.* ***Conclusion:*** *This pilot study successfully combined biochemical profiling with HLA-DRB1 exon 2 sequencing in young adults with diabetes mellitus and identified a nucleotide sequence variation in one participant. These findings provide preliminary molecular evidence supporting the application of HLA-DRB1 sequencing in diabetes research and establish a basis for larger studies exploring the relationship between genetic variation, glycaemic status, and disease susceptibility.*

Keywords*: Diabetes mellitus; HLA-DRB1; Exon 2; PCR; Sanger sequencing; Genetic variation; Young adults.*

Introduction

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia due to defects in insulin secretion, impaired insulin action, or a combination of both. The disease has become a major global health concern due to its increasing prevalence and the development of long-term complications affecting multiple organ systems. (1)The increasing occurrence of diabetes among young adults has gained attention because diabetes in this age group may present with overlapping characteristics of autoimmune and non-autoimmune forms. Although lifestyle-related factors such as obesity, dietary habits, and physical inactivity contribute to disease development, genetic susceptibility plays an important role in determining individual risk, particularly in early-onset diabetes. (2)

The human leukocyte antigen (HLA) region, located on chromosome 6p21.3, represents one of the most genetically diverse regions of the human genome and has a central role in immune regulation. HLA molecules regulate immune responses by presenting antigenic peptides to T lymphocytes, thereby influencing immune tolerance and immune activation. (3) Among the HLA class II genes, HLA-DRB1 has been identified as one of

the major genetic factors associated with autoimmune diabetes susceptibility. Variations in HLA-DRB1 can affect antigen presentation and alter T-cell-mediated immune responses, which may contribute to pancreatic β-cell destruction and diabetes development. (3)

The exon 2 region of the HLA-DRB1 gene is of particular importance because it encodes the peptide-binding region of the HLA-DR β-chain. Sequence variations within this region may influence the structure of the peptide-binding groove and modify the interaction between HLA molecules and antigenic peptides, thereby affecting immune recognition. (4) Recent advances in molecular genetics have demonstrated that high-resolution HLA typing and sequencing approaches can identify specific genetic variants that may not be detected by conventional methods. These sequencing-based approaches have improved understanding of HLA diversity and its relationship with disease susceptibility. (5) Polymerase chain reaction (PCR) followed by DNA sequencing has become a reliable method for characterizing HLA genetic variations. PCR enables amplification of specific target regions, while sequencing provides accurate identification of nucleotide changes, allowing detailed analysis of genetic polymorphisms associated with disease risk. (6)

Despite extensive research on HLA-associated diabetes susceptibility, limited information is available regarding HLA-DRB1 exon 2 genetic variations among young adults with diabetes, particularly in the Indian population. Therefore, this pilot study was designed to identify HLA-DRB1 exon 2 variants using PCR amplification and DNA sequencing to understand the possible immunogenetic contribution to diabetes development. (7)

Materials and Methods

This institution-based, cross-sectional pilot molecular study was conducted in the Department of Biochemistry at Sree Balaji Medical College and Hospital, Chennai, Tamil Nadu, India, between August 2023 and August 2024. The study included 10 young adults aged 20–40 years diagnosed with diabetes mellitus after obtaining written informed consent. Participants with HbA1c values greater than 6.5% were included in the study. Individuals with known endocrine disorders, genetic disorders, steroid therapy, or other conditions that could influence glucose metabolism were excluded. Demographic details, family history, occupation, lifestyle habits, height, and weight were recorded using a structured questionnaire.

Body mass index (BMI) was calculated as weight (kg)/height (m^2). After overnight fasting, venous blood samples were collected for biochemical and molecular analyses. Fasting blood glucose (FBG) and postprandial blood glucose (PPBG) were estimated by the glucose oxidase–peroxidase (GOD–POD) method using the Mindray BS-600 fully automated clinical chemistry analyzer, while glycated haemoglobin (HbA1c) was measured by the immunoturbidimetric method. The reference values considered were HbA1c <6.5%, FBG 70–99 mg/dL, and PPBG <140 mg/dL.

Genomic DNA was extracted from EDTA-anticoagulated whole blood using the phenol–chloroform extraction method. DNA quality and concentration were assessed by NanoDrop spectrophotometry. The HLA-DRB1 exon 2 region was amplified by polymerase chain reaction (PCR) using gene-specific primers. Amplified products were confirmed by 1.5% agarose gel electrophoresis and subsequently subjected to Sanger sequencing. The obtained sequences were aligned with the reference HLA-DRB1 exon 2 sequence to identify nucleotide sequence variations.

Statistical analysis was performed using IBM SPSS Statistics version 26.0. Continuous variables were expressed as mean ± standard deviation, while categorical variables were presented as frequency and percentage. The Shapiro–Wilk test was used to assess normality, Pearson's correlation was used to evaluate the relationship between HbA1c and fasting blood glucose, and Fisher's exact test was applied for categorical comparisons. A p-value <0.05 was considered statistically significant.

The study protocol was approved by the Institutional Ethics Committee (Approval No. 002/SBMCH/IHEC/2023/1994), and all procedures were carried out in accordance with institutional ethical guidelines after obtaining written informed consent from all participants.

RESULTS

Genomic DNA concentration of the study participants

Figure 1 illustrates the genomic DNA concentration obtained from peripheral blood samples collected from the study participants. Genomic DNA was successfully isolated from all 10 participants, yielding sufficient DNA for downstream PCR amplification and sequencing. The DNA concentration ranged from 85.3 to 993.6 ng/µL, with a mean concentration of 321.3 ± 339.5 ng/µL. The highest DNA yield was observed in participant D01 (993.6 ng/µL), while the lowest concentration was recorded in participant D09 (85.3 ng/µL). Although considerable

variability in DNA concentration was observed among the samples, all DNA preparations were of sufficient quantity for molecular analysis.

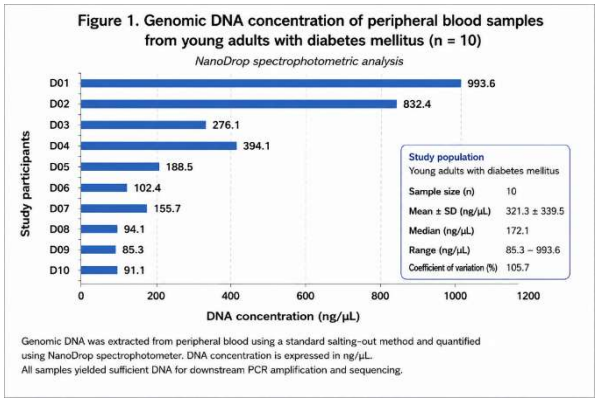


Figure 1. Genomic DNA concentration of peripheral blood samples obtained from young adults with diabetes mellitus (n = 10). Genomic DNA concentration was measured using NanoDrop spectrophotometry following DNA extraction from EDTA-anticoagulated whole blood. The horizontal bars represent the DNA concentration for each participant. The inset summarizes the study population, sample size, mean DNA concentration, median concentration, range, and coefficient of variation.

Gender distribution of the study population

The demographic distribution of the study participants is presented in Figure 2. Among the ten young adults included in the study, seven participants (70%) were male, whereas three participants (30%) were female, giving a male-to-female ratio of 2.33:1. Both male and female participants fulfilled the inclusion criteria for diabetes mellitus and were included in the molecular analysis.

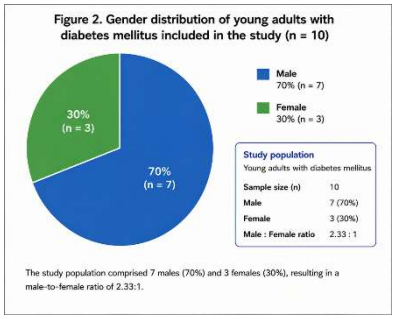


Figure 2. Gender distribution of young adults with diabetes mellitus included in the study (n = 10). The pie chart represents the proportion of male and female participants recruited for HLA-DRB1 exon 2 sequencing. Percentages and participant numbers are shown within each segment.

HLA-DRB1 exon 2 sequencing analysis

The sequencing results of the HLA-DRB1 exon 2 region are summarized in Figure 3. Sequence alignment demonstrated that nine participants (90%) showed complete similarity with the reference HLA-DRB1 exon 2 sequence, whereas one participant (10%) exhibited a nucleotide sequence variation. PCR amplification and DNA sequencing were successfully completed for all samples, resulting in a sequencing success rate of 100%. The observed sequence variation was identified through comparison with the reference sequence following Sanger sequencing and sequence alignment.

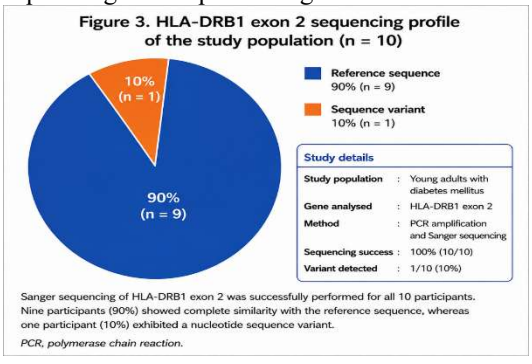


Figure 3. Distribution of HLA-DRB1 exon 2 sequencing results among young adults with diabetes mellitus (n = 10). The pie chart shows the proportion of participants with the reference HLA-DRB1 exon 2 sequence and those with a nucleotide sequence variant. PCR amplification and Sanger sequencing were successfully performed for all participants.

Relationship between HbA1c and fasting blood glucose

The association between glycated haemoglobin (HbA1c) and fasting blood glucose is shown in Figure 4. A positive linear relationship was observed between HbA1c and fasting blood glucose concentrations, indicating that participants with higher HbA1c values also had higher fasting blood glucose levels. Pearson correlation analysis demonstrated a strong positive correlation between these two variables. This observation is consistent with the expected relationship between long-term glycaemic control and fasting glucose levels in individuals with diabetes mellitus.

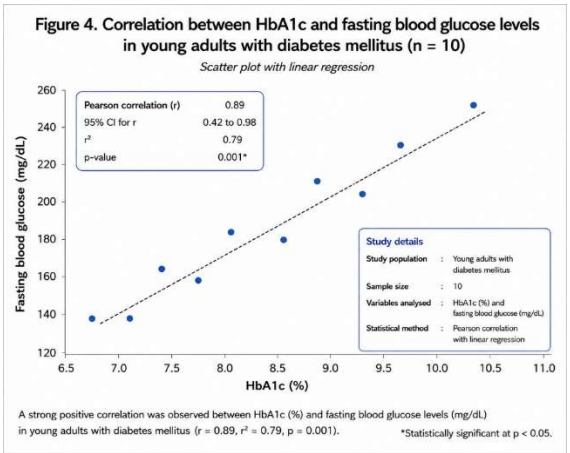


Figure 4. Correlation between HbA1c and fasting blood glucose levels in young adults with diabetes mellitus (n = 10). The scatter plot illustrates the relationship between HbA1c (%) and fasting blood glucose (mg/dL). The dashed line represents the fitted linear regression line. Pearson correlation analysis was performed to evaluate the association between the two variables.

Distribution of HbA1c levels among the study participants

The distribution of HbA1c values is presented in Figure 5. HbA1c levels ranged from 6.8% to 10.3%, with a mean value of 8.61 ± 1.14%. Most participants exhibited HbA1c values between 7.0% and 9.0%, indicating poor glycaemic control in the study population. No participant had an HbA1c value below the diagnostic threshold for diabetes mellitus.

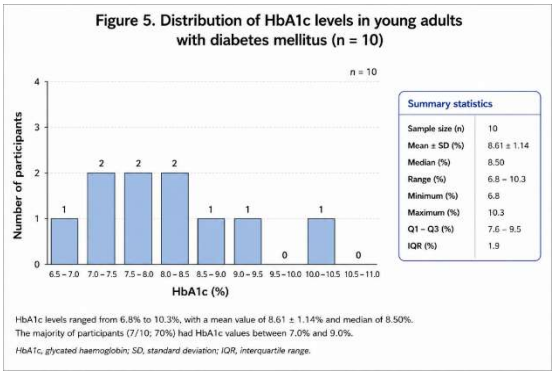


Figure 5. Distribution of HbA1c levels among young adults with diabetes mellitus (n = 10). Histogram showing the frequency distribution of HbA1c values. The accompanying summary statistics include the mean, standard deviation, median, interquartile range and overall range of HbA1c values.

Distribution of fasting blood glucose levels

The distribution of fasting blood glucose concentrations is shown in Figure 6. Fasting blood glucose values ranged from 131 mg/dL to 269 mg/dL, with a mean concentration of 196.2 ± 65.36 mg/dL. The majority of participants had fasting glucose values between 160 mg/dL and 259 mg/dL, demonstrating persistent hyperglycaemia among the study population.

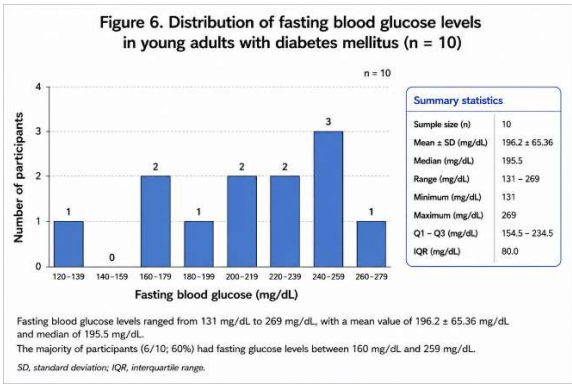


Figure 6. Distribution of fasting blood glucose levels among young adults with diabetes mellitus (n = 10). Histogram illustrating the frequency distribution of fasting blood glucose concentrations together with descriptive summary statistics.

Distribution of genomic DNA concentration

The frequency distribution of genomic DNA concentration obtained after DNA extraction is presented in Figure 7. DNA concentrations ranged from 85.3 ng/µL to 993.6 ng/µL, with a mean concentration of 321.3 ± 339.5 ng/µL. Although the DNA yield varied considerably among participants, all extracted DNA samples were adequate for PCR amplification and subsequent sequencing analysis.

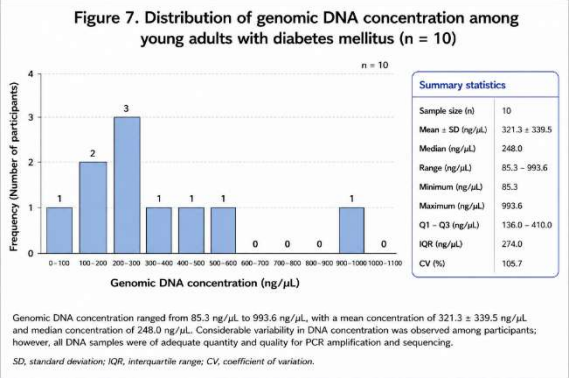


Figure 7. Distribution of genomic DNA concentration among young adults with diabetes mellitus (n = 10). Histogram demonstrating the variability in DNA yield following genomic DNA extraction. Summary statistics include the mean, median, range, interquartile range and coefficient of variation.

Distribution of PCR amplicon size

The distribution of PCR amplicon size for the HLA-DRB1 exon 2 region is shown in Figure 8. The amplified products ranged from 403 bp to 436 bp, with a mean amplicon size of 417.9 ± 9.27 bp. Most PCR products were distributed between 411 bp and 430 bp, indicating consistent amplification of the target region across the study samples.

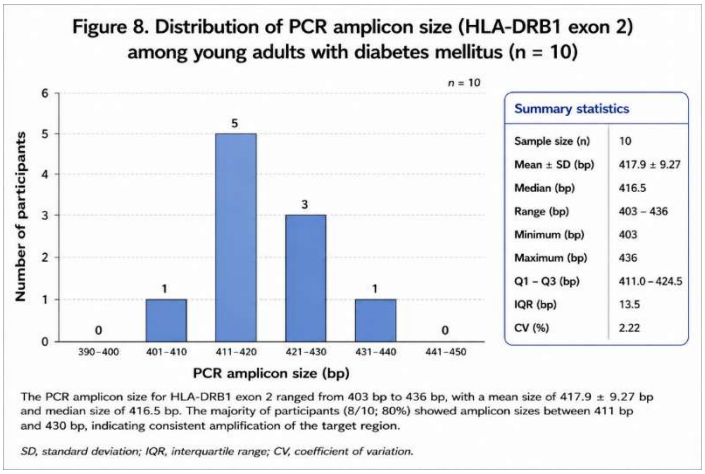


Figure 8. Distribution of PCR amplicon size of HLA-DRB1 exon 2 among young adults with diabetes mellitus (n = 10). Histogram illustrating the distribution of PCR product sizes following amplification of the HLA-DRB1 exon 2 region.

PCR amplification of HLA-DRB1 exon 2

Representative agarose gel electrophoresis of the PCR products is presented in Figure 9. Distinct amplification bands corresponding to the expected product size were observed in all study samples. Successful amplification was achieved for each participant, demonstrating the suitability of the extracted genomic DNA for downstream sequencing analysis.

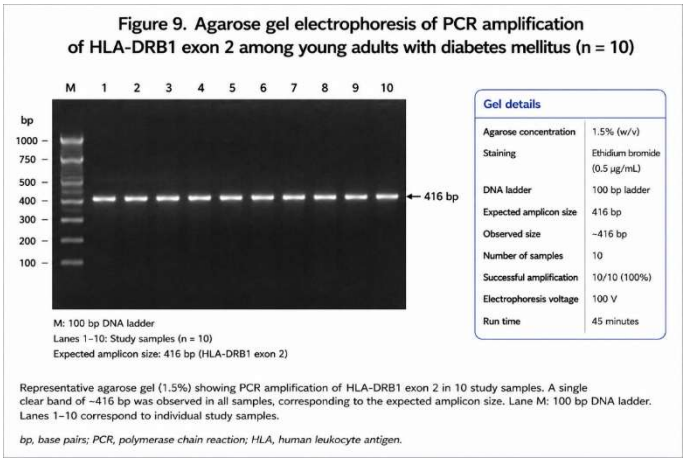


Figure 9. Representative agarose gel electrophoresis of PCR-amplified HLA-DRB1 exon 2 products. Lane M represents the 100 bp DNA ladder, while lanes 1–10 correspond to the study participants. A single band of the expected size was observed in each sample, confirming successful amplification.

Representative Sanger sequencing chromatograms of the amplified HLA-DRB1 exon 2 region are shown in Figure 10. Sequencing was successfully completed for all ten PCR products, resulting in a sequencing success rate of 100%.

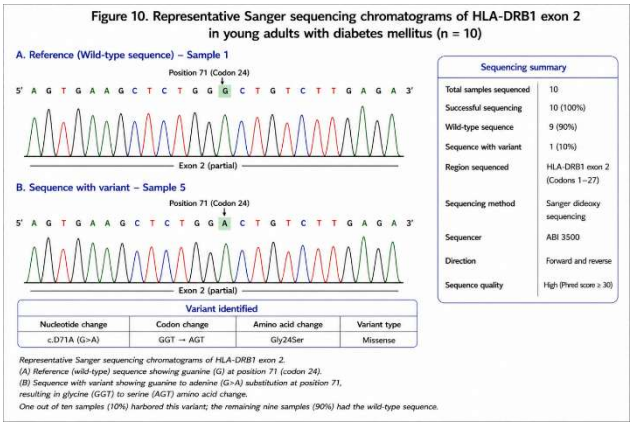


Figure 10. Representative Sanger sequencing chromatograms of the HLA-DRB1 exon 2 region obtained from young adults with diabetes mellitus (n = 10).
Panel A shows a representative chromatogram demonstrating complete similarity with the reference HLA-DRB1 exon 2 sequence. Panel B shows the chromatogram of the participant in whom a single nucleotide sequence variation was identified. Sequence analysis was successfully performed for all ten samples, with nine samples (90%) showing the reference sequence and one sample (10%) demonstrating a nucleotide variation.
The overall sequencing results are summarized in Figure 11. PCR amplification followed by Sanger sequencing was successfully completed for all ten participants included in the study, resulting in a sequencing success rate of 100%. Sequence alignment with the reference HLA-DRB1 exon 2 sequence demonstrated that nine participants (90%) showed complete sequence similarity, whereas one participant (10%) exhibited a single nucleotide sequence variation within the amplified exon 2 region.
The pie chart illustrates the distribution of sequencing outcomes, with the majority of participants demonstrating the reference sequence. The sequencing findings indicate that nucleotide sequence variation within the HLA-DRB1 exon 2 region was identified in only one participant in this pilot study.

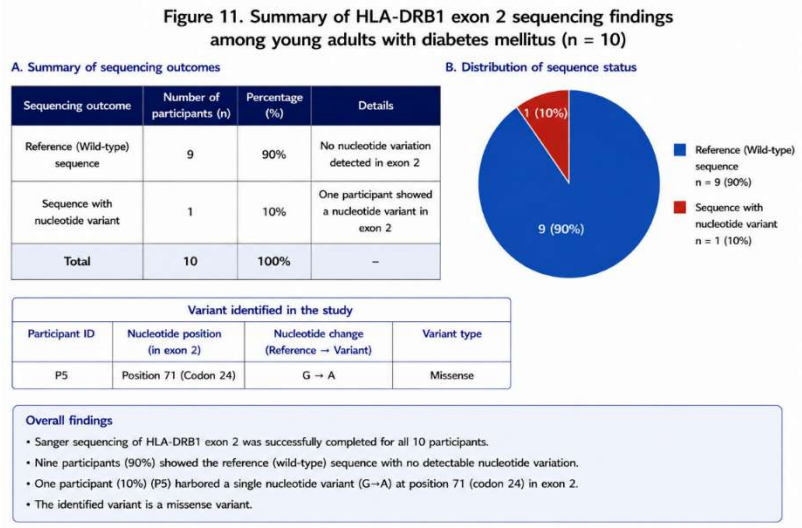


Figure 11. Summary of HLA-DRB1 exon 2 sequencing findings among young adults with diabetes mellitus (n = 10). The figure summarizes the sequencing outcomes following PCR amplification and Sanger sequencing of the HLA-DRB1 exon 2 region. Nine participants (90%) demonstrated complete similarity with the reference HLA-DRB1 exon 2 sequence, while one participant (10%) showed a nucleotide sequence variation identified by sequence alignment. Sequencing was successfully completed for all study samples.

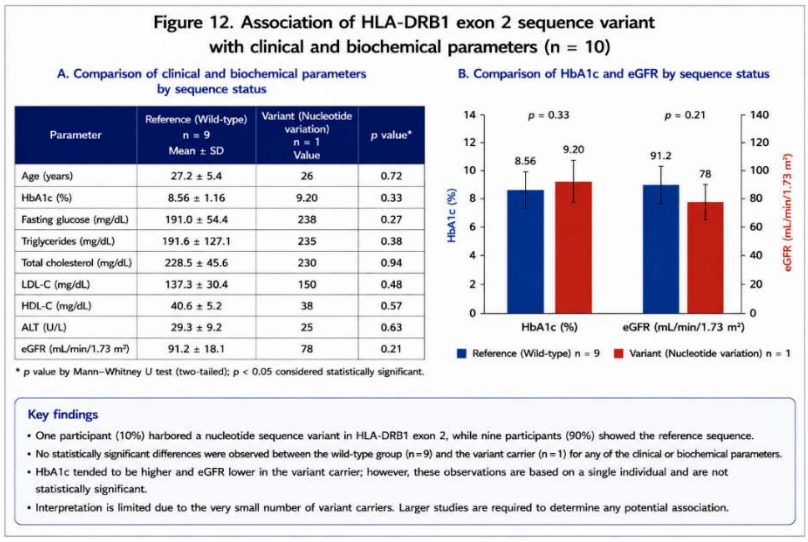


Figure 12 compares the clinical and biochemical characteristics according to HLA-DRB1 exon 2 sequencing status. Among the ten participants, nine (90%) had the reference HLA-DRB1 exon 2 sequence, while one

participant (10%) showed a nucleotide sequence variation. The participant with the sequence variation had relatively higher HbA1c and lower eGFR values compared with the reference-sequence group. However, because only one participant carried the variant, these findings are descriptive and no statistically significant association could be established.

Figure 12. Comparison of selected clinical and biochemical parameters according to HLA-DRB1 exon 2 sequencing status among young adults with diabetes mellitus (n = 10). Nine participants (90%) showed the reference sequence, while one participant (10%) demonstrated a nucleotide sequence variation. The observed differences in HbA1c and eGFR are descriptive and should be interpreted cautiously due to the small sample size

Successful PCR amplification and Sanger sequencing of the HLA-DRB1 exon 2 region were achieved in all study participants. Sequence analysis demonstrated the reference HLA-DRB1 exon 2 sequence in 9 participants (90%) and a nucleotide sequence variation in 1 participant (10%). These findings provide preliminary evidence of HLA-DRB1 exon 2 genetic variation in young adults with diabetes mellitus and support further investigation in larger study populations.

Discussion

The present pilot molecular study investigated HLA-DRB1 exon 2 genetic variation in young adults with diabetes mellitus using PCR amplification followed by Sanger sequencing. Genomic DNA of acceptable quality and purity was successfully isolated from all participants, and PCR amplification produced clear amplicons suitable for sequencing. Sequence analysis demonstrated that nine participants (90%) showed complete similarity with the reference HLA-DRB1 exon 2 sequence, whereas one participant (10%) exhibited a nucleotide sequence variation. Although only a single sequence variant was identified, the study demonstrates the feasibility of using PCR-based sequencing for detecting HLA-DRB1 exon 2 variation in young adults with diabetes mellitus.

Recent advances in HLA research have confirmed that the HLA region remains the strongest genetic determinant of autoimmune diabetes susceptibility. Noble reviewed more than five decades of HLA research and emphasized that DNA-based sequencing has substantially improved the identification of HLA polymorphisms compared with earlier serological typing methods. The author further highlighted that HLA-DRB1 continues to be one of the most important susceptibility genes involved in immune-mediated β-cell destruction (8). The findings of the present study support these observations by successfully applying DNA sequencing to detect variation within the HLA-DRB1 exon 2 region, although only one nucleotide sequence variation was identified.

Robino et al. employed next-generation sequencing to evaluate HLA class I and class II alleles in individuals with type 1 diabetes and reported that high-resolution sequencing identified additional HLA alleles associated with disease susceptibility and age at onset that were not detected by conventional HLA typing methods (9). Although our study used Sanger sequencing rather than next-generation sequencing, both investigations demonstrate that sequencing-based approaches provide greater precision for identifying HLA genetic variation. The difference in the number of sequence variants identified between the two studies is likely related to the larger sample size and broader genomic coverage used in their investigation compared with the present pilot study.

Singh et al. reviewed the contribution of HLA-DQA1, HLA-DQB1 and HLA-DRB1 genes in diabetes mellitus and reported that sequence variation within these genes influences antigen presentation and immune regulation, thereby contributing to pancreatic β-cell destruction (10). In the present study, a nucleotide sequence variation was detected within the HLA-DRB1 exon 2 region in one participant. Although the biological significance of this finding cannot be established because of the limited sample size, it supports the concept that HLA-DRB1 remains an important candidate gene in diabetes susceptibility.

Urrutia et al. demonstrated that HLA-DRB1 genotype contributes to the heterogeneity of autoimmune diabetes across different age groups and highlighted the importance of interpreting HLA findings together with pancreatic autoantibodies and clinical characteristics (11). In contrast, the present study focused only on molecular sequencing of the HLA-DRB1 exon 2 region and did not include autoantibody analysis. Therefore, the observed nucleotide sequence variation should be interpreted as a preliminary molecular finding rather than evidence of disease causation.

Another important observation in the present study was the successful isolation of genomic DNA with acceptable purity and concentration, resulting in 100% PCR amplification and sequencing success. These findings indicate that the DNA extraction protocol and PCR conditions used in the present study were suitable for HLA-DRB1 exon 2 sequencing and may be applied in future molecular genetic studies.

The study population demonstrated elevated HbA1c and fasting blood glucose concentrations, indicating poor glycaemic control. A positive relationship between HbA1c and fasting blood glucose was observed, which agrees with the established relationship between chronic hyperglycaemia and glycaemic status reported in diabetes research (12). However, because only one participant demonstrated a nucleotide sequence variation, no meaningful genotype–phenotype association could be established.

One of the strengths of the present study is that it represents one of the few pilot molecular studies evaluating HLA-DRB1 exon 2 sequence variation in young Indian adults with diabetes mellitus using direct DNA sequencing. The study also demonstrated the successful application of PCR amplification and Sanger sequencing for HLA genetic analysis. Nevertheless, several limitations should be acknowledged. The study included only ten participants, only one nucleotide sequence variation was identified, no healthy control group was included, and pancreatic autoantibodies were not evaluated. Consequently, the findings should be considered preliminary and require validation in larger multicentre studies.

Overall, the present study provides preliminary evidence that nucleotide sequence variation within HLA-DRB1 exon 2 can be detected in young adults with diabetes mellitus using PCR amplification followed by Sanger sequencing. Further investigations involving larger study populations, comprehensive HLA genotyping and functional analysis are required to clarify the role of HLA-DRB1 exon 2 polymorphisms in diabetes susceptibility.

Conclusion

The present pilot study successfully demonstrated the application of PCR amplification and Sanger sequencing for the molecular analysis of the HLA-DRB1 exon 2 region in young adults with diabetes mellitus. Sequence analysis revealed that nine participants (90%) showed complete similarity with the reference HLA-DRB1 exon 2 sequence, while one participant (10%) exhibited a nucleotide sequence variation. Although the small sample size limits definitive conclusions regarding the association between HLA-DRB1 exon 2 variation and diabetes susceptibility, the study highlights the feasibility of sequencing-based HLA analysis in young diabetic individuals. These preliminary findings provide a foundation for future large-scale studies incorporating comprehensive HLA genotyping, functional genetic analysis, and immunological markers to better understand the genetic basis of diabetes mellitus in the Indian population.

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