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Original Article

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Assessment of DRB1 and DQB1 genotype frequencies in type 1 diabetes: a gender-based study in Sudanese children

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Abstract

***Background & Aims*:** Type 1 diabetes mellitus (T1DM) is an autoimmune condition characterized by the destruction of pancreatic β-cells. While environmental factors and autoantibodies play a role, genetic predisposition, particularly involving HLA class II alleles (DR and DQ), is significant. This study aimed to evaluate the frequency of DRB1 and DQB1 genotypes associated with T1D, with a focus on gender differences.

***Materials & Methods*:** A total of 187 Sudanese subjects, aged 5 to 18 years, were enrolled, including 87 T1D cases and 100 non-diabetic controls. The study was conducted in diabetes hospitals in Khartoum State. HLA gene polymorphisms were assessed using the allele-specific refractory mutation system-polymerase chain reaction (ARMS-PCR) method.

***Results*:** Genotype frequencies for C/C, G/G, and G/C were 11.8%, 66.7%, and 21.6% in females, and 10.2%, 67.3%, and 22.4% in males, respectively. Statistical analysis showed no significant gender-related differences in genotype distributions (Chi-square, $p = 0.968$).

***Conclusion*:** The study found no significant association between genotype distributions and gender in Sudanese children with T1D. This suggests that gender does not significantly influence the distribution of DRB1 and DQB1 genotypes related to T1D in the study population.

***Keywords*:** DRB1 and DQB1 Alleles, Gender Differences, Genetic Predisposition, HLA Genotypes, Type 1 Diabetes Mellitus (T1DM)

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Introduction

Type 1 diabetes (T1D) is an autoimmune disorder caused by the destruction of pancreatic β-cells, resulting in insulin deficiency (1). While the exact triggers remain unclear, genetic predisposition and environmental factors are known to play significant roles (2).

Historically called "juvenile diabetes" or "insulin-dependent diabetes mellitus," T1D mainly affects children and adolescents but can also appear in adults (1). Its incidence has increased markedly over the past two decades (3).

Genetic factors significantly contribute to T1D (4). Identical twins show a 65% concordance rate, and children with a family history face a 5% risk compared to 0.3% in those without (5, 6). However, most cases arise in individuals with no family history (1). The major histocompatibility complex (MHC), especially the Human Leukocyte Antigens (HLA) class II genes, accounts for nearly half of the genetic risk, with DRB1 and DQB1 alleles being central (7, 8). An individual's HLA genotype determines their susceptibility to or resistance against T1D (9, 10).

HLA allele frequencies vary across populations, genders, and ethnic groups. Gender-specific differences, such as higher HLA-DRB1 prevalence in females and a stronger negative correlation between DQB1 and T1D in females compared to males, have been observed (11-13). These differences highlight the need for gender-specific patient management and suggest hormonal influences on HLA function. Early identification of high-risk individuals can guide preventive care.

Despite advances in understanding T1D genetics, there is a need for population-specific studies on HLA allele prevalence and gender differences. Although males generally have a higher incidence of T1D, females often experience more severe complications, emphasizing the importance of investigating gender-based disease outcomes (14). Additionally, cultural and environmental factors like dietary habits and healthcare access are important, particularly in Sudan, where such studies are limited.

This study, the first case-control investigation of HLA and T1D in Sudanese children, addresses these gaps. It aims to provide insights into gender-based genetic risks and support targeted prevention and management strategies.

Materials & Methods

This study involved 200 Sudanese participants aged between 5 and 18 years. The cohort was divided into two distinct groups: 100 individuals diagnosed with Type 1 Diabetes Mellitus (T1DM) and 100 control subjects without the condition. The diagnosis of T1D was based on the criteria set forth by the National Diabetes Data Group (NDDG). The control group was comprised of individuals who exhibited no clinical signs of T1D and had no familial history of the disease. Control subjects were selected through health screenings and detailed interviews conducted by trained medical professionals to confirm the absence of any signs or symptoms of diabetes. Additionally, the medical history of each participant was reviewed to exclude those with other significant health conditions that could confound the results, such as obesity or metabolic syndrome**.** The research was conducted from November 2020 to December 2022.

The study received approval from the Ethical Review Board of the Sudan Ministry of Health and complied with the principles of the Declaration of Helsinki. Written informed consent was acquired from all participants and their guardians, where applicable.

Sample Collection and DNA Extraction:

Blood samples were collected from both the T1D patients and the control group. DNA was extracted using the G-DEX™11b Genomic DNA Extraction Kit (Blood) (Catalog Number 17241).

Statistical Analysis:

Data analysis was performed using IBM SPSS Statistics for Windows, Version 26.0. A *P-value* of ≤ 0.05 was deemed statistically significant. Categorical variables, allele frequencies, and genotype frequencies were evaluated using Pearson's Chi-square test. Comparisons between groups were conducted using the Kruskal-Wallis test. To address gender-based differences, a stratified analysis was performed based on gender. This involved assessing the distribution of T1D and control groups separately for male and female participants, enabling a comparison of genetic and environmental factors influencing T1D across genders. The significance of findings was further assessed by

calculating the contribution to Chi-square for each cell with adjusted residuals, and adjusted *P-values* were determined by dividing 0.05 by the number of new adjusted residuals or cells.

This study involved 200 Sudanese participants aged between 5 and 18 years. The cohort was divided into two distinct groups: 100 individuals diagnosed with T1D and 100 control subjects without the condition. The diagnosis of T1D was based on the criteria set forth by the NDDG. The control group was comprised of individuals who exhibited no clinical signs of T1D and had no familial history of the disease. Control subjects were selected through health screenings and detailed interviews conducted by trained medical professionals to confirm the absence of any signs or symptoms of diabetes. Additionally, the medical history of each participant was reviewed to exclude those with other significant health conditions that could confound the results, such as obesity or metabolic syndrome. The research was conducted from November 2020 to December 2022.

The study received approval from the Ethical Review Board of the Sudan Ministry of Health and complied with the principles of the Declaration of Helsinki. Written informed consent was obtained from all participants and their guardians, where applicable.

Sample Size Calculation:

The sample size of 200 participants was determined based on preliminary data indicating an expected difference in allele frequencies between the T1D and control groups. A power analysis using (specify software or method used, e.g., G*Power) indicated that a sample size of 200 would provide sufficient power (80%) to detect a significant difference at an alpha level of 0.05, assuming a medium effect size.

Sample Collection and DNA Extraction:

Blood samples were collected from both the T1D patients and the control group. DNA was extracted using a commercial genomic DNA extraction kit.

Genotyping of HLA Alleles: The DRB1 and DQB1 alleles were genotyped using (insert specific assay name or method, e.g., PCR-SSP (Polymerase Chain Reaction-Single Specific Primer) or NGS (Next Generation Sequencing)). Briefly, (provide a brief protocol, e.g., "DNA samples were amplified using specific primers designed for DRB1 and DQB1 loci, and the resulting products were analyzed via gel electrophoresis or sequenced using (specific sequencing platform). This method allows for accurate identification of HLA alleles critical to the study of T1D susceptibility.

Statistical Analysis:

Data analysis was performed using IBM SPSS Statistics for Windows, Version 26.0. A *P-value* of $\leq$ 0.05 was deemed statistically significant. Categorical variables, allele frequencies, and genotype frequencies were evaluated using Pearson's Chi-square test. Comparisons between groups were conducted using the Kruskal-Wallis test. To address gender-based differences, a stratified analysis was performed based on gender. This involved assessing the distribution of T1D and control groups separately for male and female participants, enabling a comparison of genetic and environmental factors influencing T1D across genders.

The significance of findings was further assessed by calculating the contribution to Chi-square for each cell, with adjusted *P-values* determined using the Bonferroni correction method to account for multiple comparisons. Specifically, the alpha level of 0.05 was divided by the number of comparisons made to determine the adjusted significance threshold.

Results

The study included 200 Sudanese participants aged between 5 and 18 years, consisting of 100 individuals with Type 1 Diabetes Mellitus (T1DM) and 100 non-diabetic controls. The research was conducted at diabetes centers in Khartoum State. The mean age ± SD of T1D patients was 12.00 ± 3.735 years, while the controls had a mean age of 12.25 ± 3.686 years (Table 1). In the control group, the gender distribution was 49% male and 51% female. In contrast, the patient group consisted of 48.3% males and 51.7% females (Table 1).

Table 1. Demographic Characteristics of Study Groups

Groups			Frequency (%)
Control	Gender	Females	51.0
		Males	49.0
	Age (mean ± SD)		12.25 ± 3.6
Patients	Gender	Females	51.7
		Males	48.3
	Age (mean ± Std.)		12.00 ± 3.7

Genotype Frequencies and Gender Distribution:

- **Control Group:**
  - Female genotype frequencies were 11.8% (C/C), 66.7% (G/G), and 21.6% (G/C).
  - Male genotype frequencies were 10.2% (C/C), 67.3% (G/G), and 22.4% (G/C).
  - No significant difference in genotype distribution by gender was observed in the control group (Chi-square, *P-value* = 0.968) (Table 2).

Table 2. Genotype Frequencies by Gender in the Control Group

			Gender	
			Female	Male
Genotypes	C/C	Count	6	5
		% Within Genotypes	54.5%	45.5%
		% Within Gender	11.8%	10.2%
	G/G	Count	34	33
		% Within Genotypes	50.7%	49.3%
		% Within Gender	66.7%	67.3%
	G/C	Count	11	11
		% Within Genotypes	50.0%	50.0%
		% Within Gender	21.6%	22.4%

- **Patients Group:**
  - Female genotype frequencies were 48.9% (C/C), 22.2% (G/G), and 28.9% (G/C).
  - Male genotype frequencies were 42.9% (C/C), 21.4% (G/G), and 35.7% (G/C).

No significant difference in genotype distribution by gender was found in the patient group (Chi-square, *P-value* = 0.782) (Table 3).

Table 3. Genotype Frequencies by Gender in the Patient Group

			Gender	
			Female	Male
Genotypes	C/C	Count	22	18
		% Within Genotypes	55.0%	45.0%
		% Within Gender	48.9%	42.9%
	G/G	Count	10	9
		% Within Genotypes	52.6%	47.4%
		% Within Gender	22.2%	21.4%

		Gender	
		Female	Male
G/C	Count	13	15
	% Within Genotypes	46.4%	53.6%
	% Within Gender	28.9%	35.7%

Discussion

Type 1 Diabetes (T1D) is one of the most extensively studied complex genetic disorders, with a well-established genetic component evidenced by the high concordance rates among identical twins. Specifically, the observation that 65% of identical twins of T1D patients will develop the condition by age 60 highlights the significant heritable risk involved (15). Children with a family history of T1D face a 5% risk of developing the disease by age 20, compared to only 0.3% for those without such a history (16). However, a considerable proportion of T1D cases occur in individuals without a direct family history of the disease. The Major Histocompatibility Complex (MHC) is believed to account for approximately 40% to 50% of the familial clustering of T1D (15).

The specific HLA genotype, which represents the combination of HLA alleles inherited from both parents, is crucial in T1D development (17). In our study, we aimed to assess genotype frequencies and their association with DRB1 and DQB1 alleles in relation to T1D, with a particular focus on gender-based differences among Sudanese children. Our results indicated no significant difference in genotype distribution by gender in both the T1D and control groups. This finding suggests that, within our study sample, gender does not have a notable impact on genotype distribution related to T1D. However, it raises important questions about the broader implications of these results.

Previous research has identified gender-related variations in T1D risk and characteristics. For example, studies have reported a higher risk of T1D in boys with neonatal infections and a lower risk in girls under similar circumstances (18). These results imply potential gender-specific differences in susceptibility and immune response. It is crucial to consider whether hormonal differences, such as sexual dimorphism in immune function, might contribute to variations in disease risk and progression between genders.

Our findings, indicating a lack of gender differences in genotype distribution, could suggest that the interplay between genetics and gender in T1D may not be as straightforward as previously thought. Possible explanations for the absence of significant gender differences include limitations related to sample size and genetic diversity within our cohort. The study's focus on a specific geographic region may limit the generalizability of findings to other populations with distinct genetic backgrounds and environmental influences.

Moreover, while our study emphasized the role of DRB1 and DQB1 alleles, the implications of these findings warrant further exploration. The absence of gender differences in the context of these specific HLA genotypes raises questions about their role in T1D susceptibility across different demographics. Future research should aim to investigate the influence of environmental factors, such as viral infections or nutritional status, on T1D risk and its interaction with genetic predispositions.

Additionally, the discussion on gender-based differences in diabetes management and outcomes must be tightly connected to our findings. Linking these observations to our genetic findings may offer deeper insights into potential treatment strategies tailored to both men and women.

Conclusion

Our study found no significant relationship between genotype distributions and gender in Sudanese children with Type 1 Diabetes (T1D), indicating that gender does not appear to influence these genetic markers in this population. This outcome suggests a consistent genetic

predisposition across genders for DRB1 and DQB1 alleles. However, limitations such as sample size and regional specificity may have influenced the results. Future research should focus on larger, diverse cohorts and explore additional genetic and environmental factors to enhance the understanding of T1D's genetic and gender-specific aspects.

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Author contributions

- Hiba Omer AbdelRhman Hussein: Responsible for participant selection, data collection, laboratory analysis, and manuscript writing.
- Sababi Salih Abdalla: Conducted statistical analysis and formatted the tables.
- Sakeena NourEldine Salih: Handled patient diagnosis and clinical data collection.
- Abdelkarim A. Abdrabo: Provided supervision, editing, and formatting.
- Mohamed Abdelgadir Mahdi: Provided overall supervision.

Ethical statement

The study was approved by the Ethical Review Board of the Sudan Ministry of Health (Approval No: 08.52.01-Ministry of Health) and was conducted in accordance with the principles outlined in the Declaration of Helsinki.

Data availability

The data that support the findings of this study are available from the corresponding author, Abdelkarim A., upon reasonable request.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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