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A Cross Sectional Study of Association of HLA typing with Haemoglobin Level in Sickle Cell Anaemia

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: Sickle-cell Disease (SCD) is the most common blood cell disorder affecting millions of people. In severe cases, regular blood transfusion is an essential practice to relieve clinical symptoms. However, since regular blood transfusion can lead to alloimmunization to foreign human leukocyte antigens (HLA), this may result in severe anemia due to red blood cell destruction. Therefore, this study aimed to determine the association between the hemoglobin level and the presence of HLA genotypes among Sickle Cell Anemia patients.
Methodology: A total of 64 SCD patients and 21 healthy donors seen at King Abdulaziz hospital between November 2019 and February 2021 were recruited for this study. Demographic data including ABO/Rhesus blood groups, hemoglobin concentration, were among the clinical information obtained. HLA genotyping was performed using Polymerase Chain Reaction-Sequence Specific Oligonucleotide (PCR-SSO). The data were cleaned using the Microsoft Excel and analysed using the statistical packages for Social Sciences (SPSS) version 24.
Results: The incidence of SCD is not strictly gender-related because of its transmission as an

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autosomal recessive disorder. Sixty-four individuals (33 females; 31 males) having SCD were analyzed. O blood group recorded the highest prevalence compared to other ABO blood groups in SCD patients. After analysing allelic association, HLA-A*02 was more frequent in SCD patients compared to control. After further allelic combination analysis of patients and compared with the control group, HLA-DQB1*02 was majorly involved in overexpression and decreasing hemoglobin level and significantly different among control and experimental groups.

Conclusion: Rhesus-positive blood types were more associated with the SCA. HLA- type II alleles could influence the clinical course of sickle cell disease and HLA-DQB1*02 was significantly different among SCD group and control individuals, which signifies the concept that the allele was overexpressed among patients resulting in low Hb level.

Keywords: Sickle cell anemia; HLA typing; anemia.

1. INTRODUCTION

Sickle cell disease (SCD) is an autosomal recessive disorder characterized by the formation of sickle hemoglobin (HbS) instead of normal hemoglobin (HbA). It was first identified in 1910 when Dr. Herrick reported an unusual blood finding and gave the term "sickle-shaped" to describe the appearance of Red Blood Cells (RBCs) [1]. It is caused by a point mutation in the β-globin gene resulting in replacing the 6^{th} amino acid glutamic acid to valine and thus formation of HbS [2]. Every year, more than 50 million people are affected by this disease, and they regularly need blood transfusions as they lack normal blood for the transport of oxygen. SCD is prevalent in North America (USA), Africa, South America (Brazil), West Indies, Algeria, Tunisia, Morocco, Germany, and France and affects millions of people each year [3]. The vaso-occlusive crisis, or sickle cell crisis, is a frequent severe complication of SCD [4, 5] It is characterized by periods of stability, and patients receive episodes of severe pain, especially in the back, chest, joints, and abdomen, with a requirement of regular blood transfusion [6]. Alloimmunization to RBCs is a common phenomenon that occurs as a result of repeated blood transfusion. However, this alloimmunization can be mediated by the Human Leukocyte Antigen (HLA), and particular HLA alleles may influence the development of alloimmunization in such patients [7].

Anemia is a common symptom in SCA patients. Identifying HLA typing can give us a close picture of the relationship between hemoglobin decrease level and genes involved and their allelic parts responsible for this decrease [8]. HLA and its three classes play an imperative role in the immune system encoding glycoproteins in differentiation from self to non-self within the body. HLA molecules are mainly involved in detecting and binding peptide molecules from non-self-antigens and display them on the surface of MHC (major histocompatibility complex) for easy detection by T cells [8]. Moreover, T cell is also specific in recognition of MHC class molecules. It has receptors on its surface called T cell receptors, which only recognize the peptide molecules bounds to its appropriate molecule encoded by HLA. All these HLA molecules are encoded by genes present on the short arm of chromosome 6. In this way, HLA typing can easily help determine genetic defects in SCD patients for their decreased hemoglobin level and alleles involved in most patients [9]. There are three different types of HLA genes: class I with HLA-A, B and C genes, HLA-E, F and G genes and many other genes which functions are still unknown, - class II with HLA-DR, DP and DQ notably, - Class III. In addition, these genes encode other proteins related to the immune response [8]. In this study, we have studied 64 patients with SCA symptoms and 21 control individuals and determine their susceptibility to disease and find out the association between hemoglobin level and HLA typing.

2. METHODS

2.1 Patient Selection

A cross-sectional observational study of HLA alloimmunization was undertaken in paediatric SCD patients between November 2019 and February 2021. The current study were conducted at King Abduaziz university hospital, Jeddah Saudi Arabia. Patients with SCD who had received three or more lifetime RBC transfusions were considered eligible for enrollment getting written informed consent. A total of 64 SCA samples were enrolled in this study. The Control group was composed of 21 samples obtained from normal healthy individuals with no history of transfusion.

2.2 Hematological and Molecular Analyses

Complete Blood Counts were carried out on EDTA (Beckton and Dickinson) tubes using Sysmex XE-2100 analyzer (Sysmex Corporation, Kobe, Japan). In addition, plasma collection was performed following hematological indices measurement, and DNA extraction was carried out using a Wizard® Genomic DNA Purification Kit (Promega) according to the manufacturer's protocol.

All samples were successfully genotyped for HLA-I and II alleles. By utilizing Hardy- Weinberg formulation, allele frequencies were determined to investigate their effect on the hemoglobin level. HLA class I and II genotyping was performed using sequence-specific oligonucleotides (PCR-SSOs) as performed previously by Satapornpong P *et. al.* [10]. The HLA class I alleles included HLA-A, HLA-B, and HLA-C, while HLA class II alleles included HLA-DRB1, HLA-DQA1, and HLA-DQB1.

2.3 Data Analysis

Data cleaning was performed using the Microsoft Excel and analysed using the statistical packages for Social Sciences (SPSS) version 24 (IBM Corp, Armonk, NY, USA). Descriptive statistics was performed for categorical and continues data and presented as frequency, percentage, mean and standard deviation. The chi-square and t-test tests were used to examine differences in the prevalence of different categorical and continuous variables. A *p*-value < 0.05 was considered statistically significant.

3. RESULTS

3.1 Patient Demographics

Among 64 SCD patients, 51.6% (n=33) were female while 48.4% (n=31) were male the age varied from 6 – 51 years. The control group comprises 28.5% (n=6) female and 71.5% (n=15) male. Characteristics of case and control groups are compared in Table 1. Age and gender were the only variable not equally distributed, with patients in the control group tending to be older (42 ± 16 years) than those with SCD (24 ± 10 years) (p = 0.0001). Rhesus positive blood group (62 out of 64 patients) was more prevalent. The O^+ blood group was predominant in 51% of the population compared to the control group with 57.1% of the O blood group phenotype. Whereas 28% of patients were phenotypically A^+. It exhibits the phenomenon that ABO/Rhesus blood group correlated with sickle cell disease and genome expression in the following order O > A > B > AB.

Moreover, the study inferred that 37 out of 64 (approx.57.8%) patients who have undergone transfusions in a range of 3-84 times carry HLA Class-I Antibodies in their serum, and 27 out of 64 (approx. 42.1%) patients were carrying HLA-II antibodies. This exhibits 37 alloantibodies positive and 27 alloantibodies negative patients. In the control group, out of 21 individuals, 6 carried HLA-I antibodies, and 2 carried HLA-II antibodies without any history of prior transfusion. Thus, it verifies the notion that patients with SCD who have undergone (> 3 times) blood transfusion process has exhibited 15 and 10 times more HLA -I and HLA-II alloantibodies than those of the control individuals and who have not experienced any kind of blood transfusion process. The frequency of occurrence of blood transfusion and Panel Reactive antibody testing is being shown in Fig. 1.

3.2 Analysis of HLA Typing

3.2.1 Class I allele frequencies

Results indicated that the seven most frequent alleles of HLA-A locus within HLA Class -I observed in SCD were A*02 (35.5%), A*30 (3.1%), A*01 (2.3%), A*03 (2.3%), A*68 (2.3%). A*32 (2.1%) and A*24 (1.6%) compared to A*02 (2.1%), A*03 (4.0%), A*01 (2.4%), A*30 (2.4%), A*68 (2.4%), A*23 (1.6%) and A*26 (1.6%) in control group. Among patient's HLA-I, HLA-A*02 was predominant (Table 2). On HLA-B locus, the most frequent alleles in SCD were B*51 (6.8%), B*58 (3.1%), B*07 (2.6%), B*41 (2.6%), B*08 (2.1%), B*50 (2.1%) and B*39 (1.8%) compared to B*51 (7.9%), B*35 (4.8%), B*50 (4.8%), B*58 (3.2%), B*13 (2.4%), B*07 (1.6%), and B*15 (1.6%) in control. HLA-B*513 was playing a significant role in reducing Hemoglobin levels in SCD compared to control group (6.00±1.91, and 13.8±1.39 respectively). Finally, with regards to HLA-C locus, HLA-C*04 has the lowest Hb concentration in SCD (7.00±0.51) when compared to the control (14.06±0.92). Moreover, results indicated that sickle individual expressed some HLA-I alleles (HLA-1*11, *36, *47, *66,

*74) that were not expressed in normal individuals (Table 2).

3.2.2 Class II allelic frequencies

Within HLA Class-II typing, the three most frequent alleles were observed on the HLA-DQ locus (DQB1*02 (13.8%), DQA1*01 (11.5%), DQA1*05 (10.4%) in SCD group. Among HLA-II, DRB 1*01 haplotypes were playing a significant role in decreasing hemoglobin levels (6.9±0.0). Whereas, on HLA-DQA locus and HLA-DQB locus, four alleles were observed notably from which DQA 1*01 and DQB 1*02 were of prime importance. Similar to HLA-I, the SCD individual expressed alleles (DQA1*01, DQA1*03 and DQA1*05) that were not expressed in normal individuals (Table 3).

Table 1. Demographic analysis of SCD and Control groups

Parameters	SCD patients	Control	*p*-value
Number of samples (n)	64	21	
Gender			0.0001
Females	33	6	
Males	31	15	
Age Mean ± S.D (Range) (years)	24±10, (6–51)	42±16, (11–73)	0.0001
Rhesus D blood group Frequency (%)			0.0001
Positive	62 (96.9)	20 (95.2)	
Negative	2 (3.1)	1 (4.6)	
ABO Blood Group Frequency (%)			0.0001
O	33 (51.6)	13 (61.9)	
B	11 (17.2)	1 (4.8)	
AB	1 (1.6)	1 (4.8)	
A	19 (29.7)	6 (28.6)	
Hb concentration (g/dl) Mean ± S.D	7.92±1.53	14.12±0.97	0.0001
RBCs count (x10^6 cells/µL)	2.73±0.58	4.95±0.44	0.0001
>3 prior blood transfusions	64/64 (100%)	0/21 (0%)	0.0001
Mean of transfusions ± S.D	18±10	0	

Age in years, hemoglobin concentration (Hb) and number of blood transfusions are expressed as the mean ± standard deviation (range). The P-value is calculated by t-test or chi-square comparison, as appropriate.

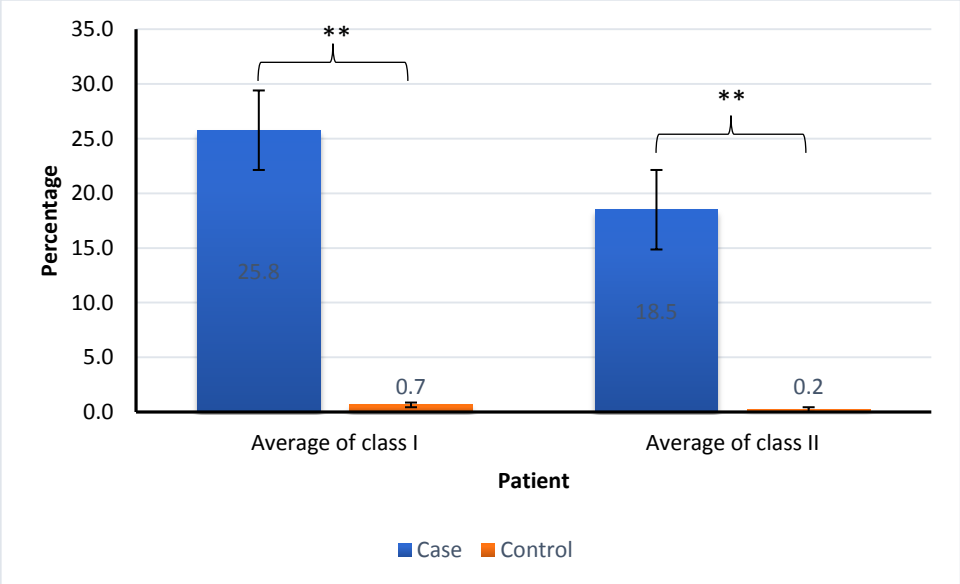

Fig. 1. Graphical representation of Alloimmunization following blood transfusion for SCA patients and no transfusion for the control group. ** indicate *p*-value = 0.001

Table 2. HLA -A, B and C allele frequency and Hb level

allele	Group									
	Control					Patient				
	Allele Frequency	RBC count ($x10^6$ cells/µL)		Hb (g/dl)		Allele Frequency	RBC count ($x10^6$ cells/µL)		Hb (g/dl)	
		Mean	S.D	Mean	S.D		Mean	S.D	Mean	S.D
A*01	2.4% [a]	5.00	0.20	13.93	0.90	2.3%[a]	2.54	0.56	7.42	1.73
A*02	27.5%[b]	4.96	0.46	14.19	1.03	35.5%[a]	2.79	0.57	8.03	1.47
A*03	4.0%[a]	4.80	0.47	13.80	0.98	2.3%[a]	2.74	0.54	8.07	1.72
A*11	0.0%[b]					1.3%[a]	3.01	0.67	7.74	1.74
A*23	1.6%[a]	4.80	0.28	13.75	0.35	1.0%[a]	2.49	0.41	7.15	1.08
A*24	0.8%[a]	4.00	0.00	12.80	0.00	1.6%[a]	3.05	0.43	8.55	1.55
A*26	1.6%[a]	4.50	0.00	13.25	0.07	0.8%[a]	2.37	0.31	7.73	1.50
A*29	0.0%[b]					0.3%[a]	1.97	0.00	7.22	0.00
A*30	2.4%[a]	5.33	0.35	15.07	0.83	3.1%[a]	2.72	0.77	7.64	2.13
A*31	0.8%[a]	4.80	0.00	13.00	0.00	0.8%[a]	2.98	0.68	8.83	1.10
A*32	0.8%[a]	5.30	0.00	15.00	0.00	2.1%[a]	2.84	0.43	7.54	0.87
A*33	1.6%[a]	5.50	0.00	15.20	0.00	0.8%[a]	2.54	0.30	8.87	1.48
A*36	0.0%[b]					0.3%[a]	1.83	0.00	6.30	0.00
A*47	0.0%[b]					0.3%[a]	2.04	0.00	6.70	0.00
A*66	0.0%[b]					0.5%[a]	2.30	0.17	6.50	0.28
A*68	2.4%[a]	5.03	0.25	14.03	0.95	2.3%[a]	2.67	0.64	8.43	1.62
A*74	0.0%[b]					1.3%[a]	2.66	0.70	8.28	1.70
B*06	0.0%[b]					0.3%[a]	3.24	0.00	10.50	0.00
B*07	1.6% [a]	4.85	0.21	13.75	0.35	2.6%[a]	2.77	0.98	7.44	2.26
B*08	0.8%[a]	4.00	0.00	12.80	0.00	2.1%[a]	2.89	0.66	8.72	2.07
B*13	2.4%[a]	4.93	0.61	13.80	1.39	0.8%[a]	2.19	0.55	6.00	1.91
B*14	0.0%[b]					0.5%[a]	2.47	0.38	7.10	0.28
B*15	1.6%[a]	5.30	0.00	14.80	0.00	1.3%[a]	2.68	0.61	6.54	0.53
B*18	0.8%[a]	4.50	0.00	13.50	0.00	1.0%[a]	3.07	0.52	8.35	2.33
B*27	0.0%[b]					0.3%[a]	3.27	0.00	7.10	0.00
B*35	4.8%[a]	4.83	0.28	13.58	0.47	0.5%[a]	2.32	0.13	7.35	0.21
B*37	0.0%[b]					0.3%[a]	2.20	0.00	6.80	0.00
B*38	0.8%[a]	5.50	0.00	16.00	0.00	0.3%[a]	3.09	0.00	8.90	0.00
B*39	0.8%[a]	5.00	0.00	14.40	0.00	1.8%[a]	2.78	0.54	7.83	0.85
B*40	0.8%[a]	5.00	0.00	14.00	0.00	0.8%[a]	3.20	0.53	8.23	2.44
B*41	0.8%[a]	4.60	0.00	13.50	0.00	2.6%[a]	2.75	0.40	8.20	1.27
B*42	0.0%[b]					0.3%[a]	2.54	0.00	7.10	0.00
B*44	0.8%[a]	4.80	0.00	13.10	0.00	0.3%[a]	2.01	0.00	6.60	0.00
B*45	0.0%[b]					0.3%[a]	1.83	0.00	6.30	0.00
B*47	0.0%[b]					0.8%[a]	2.59	0.16	7.03	0.42
B*48	0.0%[b]					0.3%[a]	3.76	0.00	12.40	0.00
B*49	0.8%[a]	5.50	0.00	16.00	0.00	0.5%[a]	2.87	0.81	9.11	0.84
B*50	4.8%[a]	5.07	0.59	14.38	1.17	2.1%[a]	2.92	0.41	8.42	0.62
B*51	7.9%[a]	4.95	0.45	14.11	0.98	6.8%[a]	2.83	0.53	8.54	1.39
B*52	0.0%[b]					0.3%[a]	2.73	0.00	8.80	0.00
B*53	0.8%[a]	4.50	0.00	13.50	0.00	1.8%[a]	2.41	0.34	7.51	0.87
B*57	0.0%[b]					0.5%[a]	2.19	0.01	6.55	0.35

allele	Group									
	Control Allele Frequency	RBC count (x10^6 cells/µL)		Hb (g/dl)		Patient Allele Frequency	RBC count (x10^6 cells/µL)		Hb (g/dl)	
		Mean	S.D	Mean	S.D		Mean	S.D	Mean	S.D
B*58	3.2%[a]	5.15	0.42	14.70	0.95	3.1%[a]	2.61	0.53	7.55	1.24
B*73	0.0%[a]					1.3%[a]	2.39	0.76	7.22	0.65
C*02	0.0%[b]					0.3%[a]	3.27	0.00	7.10	0.00
C*03	0.8%[a]	4.70	0.00	14.00	0.00	2.1%[a]	2.83	0.57	7.79	1.45
C*04	7.9%[a]	4.96	0.37	14.06	0.92	2.3%[a]	2.45	0.54	7.00	0.51
C*05	0.0%[a]					0.5%[a]	3.07	0.29	6.40	0.99
C*06	7.9%[a]	5.02	0.53	14.17	1.13	4.9%[a]	2.69	0.39	7.97	0.98
C*07	5.6%[a]	4.94	0.59	14.37	1.27	8.1%[a]	2.71	0.68	7.97	1.87
C*08	0.0%[a]					1.0%[a]	2.89	0.65	8.08	2.96
C*12	1.6%[a]	5.00	0.00	14.20	0.28	2.1%[a]	2.94	0.64	7.79	1.65
C*15	2.4%[a]	4.97	0.47	13.93	1.12	4.9%[a]	2.80	0.70	8.52	1.59
C*16	6.3%[a]	4.93	0.45	14.05	1.00	3.9%[a]	2.58	0.53	7.76	1.50
C*17	0.8%[a]	4.60	0.00	13.50	0.00	2.9%[a]	2.76	0.32	8.26	1.16
C*18	0.0%[b]					0.3%[a]	2.01	0.00	6.60	0.00

Note: Values in the same row and subtable not sharing the same subscript are significantly different at p< .05 in the two-sided test of equality for column proportions. Cells with no subscript are not included in the test. Tests assume equal variances.

a. This category is not used in comparisons because its column proportion is equal to zero or one.

b. This category is not used in comparisons because there are no other valid categories to compare

Table 3. HLA-DQA1, DQB1 and HLA-DRB1 allele and haplotype frequencies in the studied cohort

allele	Group									
	Control Allele Frequency	RBC count (x10^6 cells/µL)		Hb (g/dl)		Patient Allele Frequency	RBC count (x10^6 cells/µL)		Hb (g/dl)	
		Mean	S.D	Mean	S.D		Mean	S.D	Mean	S.D
DQA1*01	0.0%[a]					11.5%[b]	2.75	0.66	8.07	1.75
DQA1*02	0.0%[a]					3.1%[b]	2.94	0.53	8.26	1.04
DQA1*03	0.0%[a]					7.8%[b]	2.63	0.50	7.88	1.34
DQA1*04	0.0%[a]					0.3%[b]	2.74	0.00	6.90	0.00
DQA1*05	0.0%[a]					10.4%[b]	2.70	0.56	7.77	1.56
DQA1*06	0.0%[a]					0.3%[b]	2.86	0.00	5.70	0.00
DQB1*02	4.3%[a]	4.80	0.49	13.78	0.97	13.8%[b]	2.75	0.57	7.89	1.47
DQB1*03	11.9%[b]	4.96	0.51	14.19	1.10	7.6%[b]	2.65	0.46	7.81	1.37
DQB1*04	1.2%[b]	5.00	0.00	14.00	0.00	0.3%[b]	2.74	0.00	6.90	0.00
DQB1*05	16.7%[b]	5.01	0.40	14.24	0.97	7.3%[b]	2.67	0.58	8.21	1.75
DQB1*06	6.0%[b]	5.14	0.31	14.46	0.94	4.4%[b]	2.85	0.77	7.82	1.73
DRB1*01	2.4%[b]	5.00	0.85	14.20	1.70	1.8%[b]	2.28	0.68	6.99	1.97
DRB1*03	9.5%[b]	4.84	0.45	13.93	0.96	8.1%[b]	2.75	0.56	7.85	1.62
DRB1*04	11.9%[b]	4.96	0.51	14.19	1.10	8.1%[b]	2.64	0.49	7.88	1.34
DRB1*07	4.8%[b]	4.83	0.62	13.63	1.09	2.9%[b]	3.04	0.42	8.44	0.88
DRB1*08	0.0%[a]					1.0%[b]	2.30	0.22	6.53	0.43
DRB1*10	4.8%[b]	4.93	0.34	13.95	0.98	1.0%[b]	2.55	0.26	7.45	0.68
DRB1*11	3.6%[b]	5.07	0.40	14.37	0.75	1.6%[b]	2.78	0.56	8.03	1.40
DRB1*12	0.0%[a]					0.3%[b]	2.86	0.00	5.70	0.00

allele	Group									
	Control Allele Frequency	RBC count (x10^6 cells/µL)		Hb (g/dl)		Patient Allele Frequency	RBC count (x10^6 cells/µL)		Hb (g/dl)	
		Mean	S.D	Mean	S.D		Mean	S.D	Mean	S.D
DRB1*13	3.6% [b]	5.23	0.40	14.93	0.92	1.3% [b]	2.67	0.75	7.96	1.49
DRB1*14	1.2% [b]	5.00	0.00	14.00	0.00	0.8% [b]	2.94	0.71	9.80	2.27
DRB1*15	3.6% [b]	5.17	0.29	14.50	1.32	3.1% [b]	2.88	0.85	7.77	1.87
DRB1*16	4.8% [b]	4.83	0.39	13.90	0.80	3.4% [b]	2.84	0.57	8.69	1.51

Note: Values in the same row and subtable not sharing the same subscript are significantly different at p< .05 in the two-sided test of equality for column proportions. Cells with no subscript are not included in the test. Tests assume equal variances.

a. This category is not used in comparisons because its column proportion is equal to zero or one.

b. This category is not used in comparisons because there are no other valid categories to compare.

4. DISCUSSION

Sickle Cell Disease (SCD) is one of the most common inherited hemoglobinopathy worldwide [11]. However, the incidence of SCD is not strictly gender-related because of its transmission as an autosomal recessive disorder. In particular, the gender-related differences in paediatric SCD are not well characterized [12]. To address this matter, we analyzed the clinical records of 64 individuals (33 females; 31 males) having SCD. We found that male and female were equally affected with SCD, but the blood transfusion ratio (>3 times) was more among males than females. This confirm the hypothesis that males have more episodes of crises than females, and the reason could be attributed to the higher bioavailability of nitric oxides in females [13, 14].

Similarly, while carrying out the frequency of the blood group distribution among our cohort, it was revealed that O and A blood group individuals are mostly associated with genotype SS (SCD), followed by B, and the least prevalent is AB. Again, this agrees with the study performed by Alagwu *et al.* [15].

Red blood cell transfusion is the option of choice for SCA treatment. However, one of the drawbacks of this practice is the alloimmunization. Previous studies have indicated that patients with SCD represent a sub-population of transfused patients at much higher risk of alloimmunization than the general transfused individuals. In the SCD population, it has been determined that RBC antigens are known to contribute to the increased risk of alloimmunization in patients with SCD. The risk of alloimmunization in patients with SCA has been reported to range from 4-40% [16]. Our findings stated that some non-transfused control had HLA antibodies, consistent with other reports of natural or spontaneous HLA alloimmunization in unsensitized individuals. The aetiology of such antibodies is unknown, and it feels that their production may be triggered by non-allogenic stimulus [17]. Our observation was that 37 out of 64 patients were HLA Class-I alloimmunized after having blood transfusions in the range of 3-84 times. Whereas in the control group, 6 out of 21 individuals exhibited HLA-I antibodies without having any history of blood transfusion. Similarly, 27 out of 64 individuals had HLA-Class II antibodies with the same blood transfusions range. This denotes the concept that the role of HLA alloimmunization could become increasingly important in SCA as HLA antibody testing could help select donors avoid HLA mismatches that would otherwise act against a patient's specific HLA-antibodies [18].

The HLA on chromosome 6p21.3 carries more than 200 genes within this 3Mb segment, many involved in various immune functions and mechanisms [19]. The HLA class I (HLA-A, B, and C) and class II (DRB1, DQB1, DPB1, and DQA1) loci encode cell surface heterodimeric proteins that bind antigenic peptides and are the most polymorphic genes in the human genome [20]. The HLA system plays a key role in the immune system, and disease susceptibility, resistance, and progression are influenced greatly by HLA genes [21]. The consequences of sickle mutation and its downstream effects are variable. Complications due to chronic hemolytic anemia, episodic vaso-occlusion with painful episodes, and chronic organ damage lead to SCA phenotypes [22]. Tamaouza *et al.* worked on 80 patients with sickle cell disease and found significantly more patients without infections carrying HLA-DRB1*15 alleles than patients with infections.

Confirmation of these findings using a larger sample size considering age and sex matched groups sufficiently powered to decrease the probability of false-positive associations will be needed to elucidate the contribution of HLA haplotypes and genotypes to SCD.

5. CONCLUSION

Results indicated that SCD patients have different allele frequency compared to control group. Additionally, results proposed that certain HLA alleles were observed in only SCD not in the control group. Also, HLA type I and II alleles could influence the clinical course of sickle cell disease as HLA-A*02 and HLA-DQB1*02 were highly expressed among affected individuals, which signifies the concept that the allele was overexpressed among individuals that could resulting in low Hb level. Larger scale prospective studies are needed to endorse the current findings and elucidate the role HLA-A*02 and HLA-DQB1*02 in reducing Hb level.

CONSENT

As per international standard or university standard, patient's written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

Ethical approval was obtained from the ethics and research committee at the Faculty of Applied Medical Sciences, King Abdulaziz University (Ref.No. 2019-021).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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