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BLOOD GROUP AND HUMAN LEUCOCYTE ANTIGEN SUB-TYPE AS DETERMINANTS TO KELOID FORMATION AND RECURRENCE IN KELOID PATIENTS
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BLOOD GROUP AND HUMAN LEUCOCYTE ANTIGEN SUB-TYPE AS DETERMINANTS TO KELOID FORMATION AND RECURRENCE IN KELOID PATIENTS

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ABSTRACT

Background: **The role of genetic factors in keloid is affirmed by the fact that keloids have been shown to occur among members of the same family.**

Objective: **To determine whether there is any association between patients' blood group and HLA sub-types to keloids and keloid recurrence.**

Design: **A prospective longitudinal study**

Setting: **The Kenyatta National Hospital between August 2018 and July 2020.**

Subjects/Participants: **Patients with keloids and a control of patients managed for other surgical conditions with no keloids. Blood was taken from each patient and analyzed for blood group and HLA sub-types using the sequence specific primer geno-typing. Data captured were summarized and analyzed using students T-test and Bonferroni correction. Probability values significance was at 0.05.**

Results: **A total of 90 patients with keloids and 59 in a control group were followed up in the study. The male to female ratio of the patients was 1:2. The most common blood group for both groups was blood group O at 51.3% and 49.2%, followed by blood group A and B respectively. Patients with keloids had a significantly higher positive alleles of HLADQA1*01 and HLADQB1*06. There was also an association between blood group A and keloid recurrence.**

Conclusion: **This study demonstrates that there is significant difference in some HLA sub-types and blood groups among patients who form keloids and non-keloid forming patients an indication of the possible role patient's genetics and immune could play in keloid pathogenesis and severity.**

Key words: **Blood group, Human Leucocyte Antigen, keloid, Recurrence**

INTRODUCTION

Pathogenesis of keloid disease is still not well established. Keloid occurrence has generally been classified as sporadic, in individuals with no known members of the family with keloids or familial where members of a given family have positive history of the disease. Familial keloids have been shown to contribute to about 50 per cent of keloids indicating a strong inheritance pattern (1).

A number of pedigree studies have demonstrated keloids to either have an autosomal dominant or recessive mode of inheritance (2-3). Other studies have suggested X linked mode of inheritance (4). HLA studies have demonstrated association between sub-types such as HLA DRB*16 and keloid formation (5). Majority of these studies have however been done in populations with low keloid prevalence with non in Africa, a keloid endemic zone.An association between ABO blood grouping and keloids have also been documented by a number of authors (6).

Keloids have high recurrence rates irrespective of the treatment modalities provided (7-8). Whether patients' genetic factors influence keloid recurrence has not been demonstrated. We undertook this study to demonstrate any significant differences between

various blood groups and HLA sub-types in patients with keloids and those without and to determine whether they could influence keloid recurrence.

MATERIALS AND METHODS

Study Design: A prospective longitudinal study

Study Setting: The Study was carried out at the Kenyatta National Hospital between August 2018 and July 2020.

Study Subject/Participants: Patients with keloids attending plastic surgical clinic were systematic randomly sampled into the study. A control group of healthy patients seeking aesthetic services with no keloids nor family history of keloids were also recruited for the study.

Data Collection and Sampling procedures: Patients with keloids in the study had history and physical examination taken. The mean surface area of keloids was measured by the grid iron technique. Pain and pruritus scores were determined using their respective visual analogue scores. Surgical excision of the keloids was done by a senior plastic surgeon followed by post excision superficial radiotherapy within 24 hours of surgery. Peri-operative management of the patients was similar for all patients. Recurrence was determined as either keloid regrowth or worsening pain and or pruritus at one year of follow up.

From each patient and the control group blood was taken and analyzed for blood groups and HLA sub-types. HLA typing was done using sequence specific primer geno-typing (SSP). DNA extraction was done using Qiapen extraction kitTM. PCR master mix preparation was done by adding commercial kit from OlerrupTM. PCR master Mix was aliquoted into 96 well plates containing Primer Mixes. PCR amplification was done using QiaxelTM automated machine which uses a commercial cartridge. Interpretation was done by SSP-typing HLA allele software.

Data Analysis: Data captured were summarized and analysed using students T-test and Bonferrini correction to compare means. Probability values significance was at 0.05.

ETHICAL CONSIDERATION

This study was approved by the local ethics and research committee; KNH/UON/ERC (P611/07/2018). Informed consent was sort from all patients to participate in the study. For the minors informed consent was taken from the parents or legally acceptable representatives.

RESULTS

A total of 90 patients with 104 keloids and a control group of 59 patients were followed up during the study. The male to female ratio was 1:2 with 60 females and 30 male patients.For the control group the male to female ratio was 1:2 with 39 female and 20 male patients.The age range was 15 to 65 years with a mean age of 29.5 and a median age of 20 -25 years. The age range for control patients was 15.5 to 64 years with mean age of 29.7 years and a median age of 20-25 years. There was no statistical significance difference between the keloids group and the control group (Table 1). The anatomical location of the keloids were as follows; ears (n=47), abdomen (n=10), cheek (n=15), upper limb (n=5), back (n=13), chest and neck (n=13) and scalp (n=1).

Table 1*: Age of patients with keloids and the control group*

Age of presentation (years)	Keloid group	Control group
10-15	3	2
15-20	19	10
20-25	18	14
25-30	15	12
30-35	12	8
35-40	10	6
40-45	7	4
45-50	4	3
> 50	2	2
Total	90	59

All 90 patients and 59 controls had their blood taken for blood groups while 80 of the 90 patients had their blood taken for HLA studies. Eighteen patients of the 80 had keloid recurrence (KR) while 62 did not have any form of recurrence (NKR).

The most common blood group for patients with Keloids and the control group was blood group O at 51.3% and 49.2% (*P*-value 0.552), followed by blood group A and B respectively. There was no statistical significance difference in the blood groups of patients who formed keloids and the control group (Table2).

Table 2*: Blood group types for patients with keloids and those without*

Blood group	Total Count	Keloids N = 90	Control N = 59	
		%	%	P-Value
O	75	51.3	49.2	0.552
A	45	31.3	28.8	0.480
B	23	13.7	18.6	0.175
AB	6	3.7	3.4	0.932

Comparison between blood groups in patients with KR and NKR patients demonstrated that keloid patients with blood group A were more prone to recurrence compared to the other blood groups (p-value <0.011) while patient with blood group B were least likely to have keloid recurrence (p-value <0.001) (Table 3).

Table 3: *Comparison of blood groups in patients with keloids recurrence (KR) and those without (NKR).*

Blood group	Total Count	Non Recurrent N = 71	Recurrent N = 19	P-Value
		%	%	
O	45	50.0	55.6	0.124
A	28	29.0	38.9	0.011
B	13	17.7	0	<0.001
AB	4	3.3	5.50	0.516

Out of 90 patients with keloids who were followed up in the study 80 patients had their blood analysed for HLA. Of the 80 patients 18 had keloid recurrence (KR)and 62 had no recurrence (NKR).Comparison of the HLA sub-types between the control group and patients who had keloids showed significance differences in the alleles of HLA DQA1*01, DQB1*05, DQB1*06, and DRB1*15 all in favour of patients who had keloids (P Value <0.05)(Table 4).

Table 4: *HLA sub-types comparison between patients with keloids and normal controls*

Allele	Keloids N = 80		Control N = 59		P-Value	Bonferroni correction P-value
	Allele +ve (%)	Allele –ve N (%)	Allele +ve N (%)	Allele –ve N (%)		
DQA1*01	83.8	16.2	44.1	55.9	<0.0001	0.0004
DQA1*02	5.0	95.0	13.6	91.5	0.129	
DQA1*03	8.8	91.3	13.6	86.4	0.415	
DQA1*04	7.5	92.5	5.1	94.9	0.733	
DQA1*05	51.3	48.7	47.5	52.5	0.732	
DQA1*06	6.3	93.7	6.8	93.2	1	
DQB1*01	3.8	96.2	1.7	98.3	0.637	
DQB1*02	10.0	90.0	8.5	91.5	1	
DQB1*03	22.5	77.5	23.7	76.3	1	
DQB1*04	20.0	80.0	20.3	79.7	1	
DQB1*05	55.0	45.0	30.5	69.5	0.006	
DQB1*06	55	45	0	100	<0.0001	
DQB1*07	1.3	98.7	0	100	1	
DRB1*01	20.0	80.0	27.1	72.9	0.415	
DRB1*02	2.5	97.5	5.1	94.9	0.650	
DRB1*03	23.8	76.2	25.4	74.6	0.844	
DRB1*04	11.3	88.7	6.8	93.2	0.557	
DRB1*05	6.3	93.7	5.1	94.9	1	
DRB1*06	3.8	96.2	0	100	0.086	
DRB1*07	6.3	93.7	13.6	86.4	0.155	
DRB1*08	7.5	92.5	6.8	93.2	1	
DRB1*09	1.3	98.7	3.4	96.6	0.574	
DRB1*10	2.5	97.5	0	100	0.508	
DRB1*11	16.3	83.7	15.3	84.7	1	
DRB1*12	3.8	96.3	3.4	96.4	1	
DRB1*13	12.5	87.5	13.6	86.4	1	
DRB1*14	6.3	93.7	6.8	93.2	1	
DRB1*15	52.5	47.5	22.	78.0	0.004	
DRB1*16	1.3	98.7	3.4	96.6	0.574	

Bonferreni correction revealed HLA DQA1*01 and HLADQB1*06 to be significantly different from the control. Analysis of HLA sub-types between patients who had keloids recurrence (KR) and those without (NKR), revealed DQBI*06 to be elevated in the keloid recurrent group. (P value <0.05) Bonferreni correction however revealed that the difference was not of statistical significance. (Table 5).

Table5: *HLA sub-types analysis between patients with keloid recurrence (KR) and those without (NKR) revealed statistical significance difference with HLA allele DQB1*06*

	Non Recurrent Keloids (NKR) N = 62		Keloid recurrent (KR) N = 18		P-Value	Bonferroni correction P-value
	Allele +ve	Allele –ve	Allele +ve	Allele –ve		
Allele	N (%)	N (%)	N (%)	N (%)		
DQA1*01	80.6	19.4	94.4	5.6	0.278	0.0006
DQA1*02	4.8	95.2	5.6	94.4	1	
DQA1*03	11.3	88.7	0	100	0.340	
DQA1*04	8.1	91.9	5.6	94.4	1	
DQA1*05	48.4	51.6	61.1	38.9	0.426	
DQA1*06	4.8	95.2	11.1	88.9	0.313	
DQB1*01	4.8	95.2	0	100	1	
DQB1*02	8.1	91.9	16.7	83.3	0.370	
DQB1*03	22.6	77.4	22.2	77.8	1	
DQB1*04	17.7	82.3	27.8	72.2	0.338	
DQB1*05	58.1	41.9	44.4	55.6	0.421	
DQB1*06	48.4	51.6	77.8	22.2	0.033	
DQB1*07	0	100	5.6	94.4	0.225	
DRB1*01	21.0	79.0	16.7	83.3	1	
DRB1*02	1.6	98.4	5.6	94.4	0.402	
DRB1*03	22.6	77.4	27.8	72.2	0.754	
DRB1*04	9.7	90.3	16.7	83.3	0.413	
DRB1*05	6.5	93.5	5.6	94.4	1	
DRB1*06	4.8	95.2	0	100	1	
DRB1*07	6.5	93.5	5.6	94.4	1	
DRB1*08	6.5	93.5	11.1	88.9	0.612	
DRB1*09	0	100	5.6	94.4	0.225	
DRB1*10	1.6	98.4	5.6	94.4	0.402	
DRB1*11	14.5	85.5	22.2	77.8	0.475	
DRB1*12	4.8	95.2	0	100	1	
DRB1*13	14.5	85.5	5.6	94.4	0.442	
DRB1*14	6.5	93.5	5.6	94.4	1	
DRB1*15	58.8	45.2	44.4	55.6	0.593	
DRB1*16	1.6	98.4	0	100	1	

DISCUSSION

The influence of genetic factors in keloid formation could be extrapolated from the facts that keloids seem to portray a distinct pattern of inheritance and a strong familial tendency (1-3). Though not unique to keloids, genetic factor has been shown to have an influence on various disease processes whether infective, benign or malignant in origin. Most studied and easily applied genetic variants have been the ABO and the HLA systems probably due to the fact that they are routinely used during blood transfusions as well as tissue typing and organ transplants. Our study noted some significant associations between these two genetic systems of the body and keloid patients.

The role and influence of ABO blood groups in the pathogenesis and outcome of keloid management has been established before. Ramma kishnan *et al* in India found blood group A to be significantly higher in patients with keloids than in the normal population (9).These findings were similar to those of Shaheen *et al*. in a Syrian study who found blood group A to be significantly more common in patients with keloids than in the normal cohort (6). However, Abbas Moure Toure in Togo, like in our study demonstrated no

statistical significance difference with blood groups in patents with keloids and a control population of patients who presented with other dermatological conditions (10). Our study on the other hand found keloid patients with blood group A to be more prone to keloid recurrence compared to the other blood group with blood group B being less prone to recurrence.

Human leucocyte Antigen (HLA), key genes responsible for the development of the cellular immune system, have been shown to play a critical role in not only autoimmune conditions but also in benign and malignant conditions. Mutations on some of the alleles are thought to be responsible for the development of a number of autoimmunedisorders such as celiac disease, ankylosing, spondylisthesis and rheumatoid arthritis (11-12). Even closer to keloids sclerotic skin conditions such as sarcoidosis and systemic sclerosis have also been shown to have associations with particular alleles (13).Though keloids disease are not classified as autoimmune disease several studies have shown an association between some alleles and the disease bringing to the fore possibilities that the immune system could play a critical role in keloid formation.Wen-Sheng Lu found DQB1*0501, B*07-DRB1*15, DQB1*0503-DRB1*15 ($P<0.05$) to be associated with keloid formation among Chinese Ham patients cohorts (14). Another study by Brown et al found an association between HLA DRB1*15 and keloid formation in a patient cohort of Caucasians (15). However, in a study in the Caribbean with Brown et al no association was demonstrated between HLA DRB*15 alleles and keloid patients of African descent (16). In our study we demonstrated HLA alleles DQA*01, DQB1*05, DQB1*06 and DRB1*15 to be significantly associated with keloid patients (P value <0.05).After Bonferroni corrections alleles DQA1*01 and DQB1*06 were still significantly associated with keloid formation. Even further Allele DQB1*06 were found to be significantly associated with keloid recurrence (P value <0.05). This was however found to be insignificant after Bonferroni correction.

CONCLUSION

Keloid patho-physiology from our study findings seem to be influenced by the genetic composition of the patients. Patients with blood group A are more prone to keloid recurrence a possible indication of the disease severity in this group of patients. While a number of HLA sub-types were identified to be significantly associated with keloid disease in our cohort, HLA DQA1*01 and DQB1*06 seem to be more associated with the disease. The association between several type 2 alleles and keloid formation in our study and other studies strongly suggest an immunological aspect in keloid formation akin to auto inflammatory diseases.

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