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Frequency of Cameroon Haplotype in a Cohort of Sudanese Patients with Sickle Cell Disease

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ABSTRACT:

Background: Haplotypes provide useful population data. The beta-globin gene cluster was the first to be described and is still one of the most studied nuclear DNA segments used for investigation of evolutionary relationships of human populations.

Objective: The aim of this study was to determine the frequency of the Cameroon haplotype in a cohort of Sudanese patients with sickle cell disease.

Materials and Methods : This was a prospective, cross-sectional hospital-based cohort study involving 80 sickle cell disease patients from western Sudan (38 males and 42 female) who were 3-38 years old (mean age 9.5 ± 6.1), and presented to the sickle cell clinic at the Children Emergency Hospital Khartoum between March -April 2008. Following informed consent and using a non invasive technique (buccal wash) samples were collected. DNA was extracted using the chloroform method. β^S-globin gene Cameroon haplotype was determined by amplification refractory mutation system (ARMS) polymerase chain reaction technique. Primers were designed based on the published human beta globin chain sequence for known haplotype Cameroon.

Results: In the 160 alleles studied β^S gene was found to be linked to the Cameroon haplotype in 17 alleles 10.6% (95% confidence interval 6.31-16.47%). Eight patients were homozygous and one patient was heterozygous.

Conclusion: The Cameroon haplotye has a frequency of 10.6% in this cohort of Sudanese patients with sickle cell anaemia from western Sudan. We recommend further larger study to evaluate the frequency of the different known haplotypes and a longitudinal study to correlate the haplotypes to the clinical course of the disease.

Key words: Cameroon haplotype; sickle cell anaemia; Sudan

Introduction:

Patients with sickle cell disease share the same gene mutation, where thymine replaces adenine in the DNA encoding the ß-globin chain. Consequently, the amino acid valine replaces glutamic acid at the sixth position in the ß-globin protein product. [(1)] Although everyone with sickle cell disease shares a specific, invariant genotypic mutation, the clinical variability in the pattern and severity of disease manifestations is astounding. [(2)] ,the most likely causes of this inconstancy are disease modifying factors. A potential modulator of sickle cell disease now known is haplotype.

Haplotypes of sickle cell disease can be described as polymorphic restriction endonuclease sites in and around the mutant ß-globin gene.[(2)] Although the haplotypes have numeric identifiers, they

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are most commonly designated by the geographic areas in which they were first identified: Senegal, Benin, Central African Republic (or Bantu), Cameroon and Indian-Arab (or Asian). [3] In addition to its anthropologic interest, the majority of the chromosomes with the β^S gene have one of the five common haplotypes, although in every large series of sickle cell patients there is a minority of chromosomes (about 5%) associated with less common haplotypes, usually referred to as atypical haplotypes. The β^S gene is in linkage disequilibrium with the five main haplotypes. [4, 5]

The independent origin of the sickle mutation opens the possibility that haplotypes could differ in associated sickle cell disease severity. The three most common haplotypes in the Americas are Senegal, Benin, and Bantu/CAR. [6, 7] In African populations, each is associated with different degrees of disease severity. People with the Senegal haplotype, on average, have the least severe clinical course, while those with the CAR/Bantu haplotype, on average, have the most severe disease. People with the Benin haplotype usually have disease of intermediate severity. [7] Understanding the parameters that modify sickle cell disease severity would be enormously useful in treatment decisions. [8]

Haplotypes provide useful population data. The beta-globin gene cluster was the first to be described and is still one of the most studied nuclear DNA segments used for investigation of evolutionary relationships of human populations. [9, 10] Sickle cell anaemia is the most common inherited hemolytic anemia in the world and hence in Sudan. A pilot study in Sudan based on limited number of samples of sickle cell patients has indicated that the haplotypes of the S gene in the study group are more related to those of West Africa (Cameroon) haplotype. [10]

The objective of this study was to identify the Cameroon haplotype associated with sickle cell anemia mutation among Sudanese patients.

Materials and Methods:

The study was conducted in the sickle cell clinic. It is a prospective, cross-sectional and hospital-based study. Following informed consent eighty patients with sickle cell anaemia (38 males and 42 females) presented to the sickle cell clinic for follow up were enrolled in the study. The age range: 3-38 years (mean age 9.5 ± 6.1). Those under three years of age were excluded because they can not cooperate for the buccal brush and wash.

Buccal cell sample collection was done by a non invasive method using a soft toothbrush. Then 10 ml of 70% isopropanol was added to the sample. A cell pellet was collected by centrifugation at 3.000 for 15 minutes, the supernatant was discarded and the cells resuspended in lysis buffer (STE+SDS) and stored at -20°C until extraction later. [11]

DNA extraction from buccal brush samples was performed using chloroform method. [12]

Amplification refractory mutation system was used to amplify the haplotype defining mutation Cameroon. Polymerase chain reaction was carried out for DNA amplification. PCR reagents were added in 0.5 ml sterile eppendorf. A final total volume of 25ul containing 1µl genomic DNA, 100µM of each of the four deoxynucleotides, 1.0µm of each primer and 0.5µg of taq DNA

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polymerase in buffer composed of 10mM Tris PH 8.3 and 1.5 mM $Mgcl_2$. PCR reaction was carried out for 40 cycles. Each cycle consisted of denaturation at 94°C for 35 seconds, annealing at 59°C for 1 minute and extension at 72°C for 2 minute. There was a final cycle for 5min at 72°C to allow the completion of PCR products.

Detection of the PCR Product

Agarose Gel Electrophoresis

Sixty seven mls of distilled water and eight mls of 10X Tris Boric EDTA (TBE) were added to 1.2 gms of agarose. 1.5 mls ethidium bromide (10mg/ml) were added to the gel and mixed. The mixture was poured into horizontal electrophoresis gel tank with a suitable size comb and the gel was left for 30 minutes to polymerize. TBE buffer was used as a running buffer. 5ul of the PCR product was loaded into the agrose gel. The electrophoresis running was in 100 volts and for 30 minutes. The PCR products were visualized under UV light and photographed. The DNA size was determined using standard DNA markers (Fig 1).

Sequence of the primers

The primers were designed based on the published human beta globin chain sequence for known haplotype Cameroon [3]. The primer was shown in table 1 (N for normal and M for mismatch)

Table 1: The primer

Haplotype	Primer Sequence	Size of PCR Product
Cameroon	**R(C1)** AGGTGCTTTATGGCATCTCTCCCA**N** **R(C2)**AGGTGCTTTGTGGAACCGTTTCA **M** **(AF)**AAGAGACTAAGATTTGTCC **N**	**360bp**

Results:

A total of 80 individuals with confirmed sickle cell anemia by Hb electrophoresis were evaluated. Their age ranged between 3-38 years with a mean of 9.5 years and the standard deviation between the age groups was 6.1years.They were 38 males and 42 females.

Most of the patients in the study group were originally from Western Sudan, and they were 58 patients constituted (72.5%). While those from Central, Eastern, and Southern Sudan were less represented and constituted 22 patients (27.5%). No one was from Northern Sudan.

The majority of the patients were from the Messeria tribe and they were 24 patients (33.8%). It also represented a considerable percentage in the group with Cameroon haplotypes as it constituted (44.4%) (Table2).

Forty eight of patients' parents (60%) had consanguineous marriage of which 30 parents (37.5%) were first degree cousins.

The present residence of the majority (67.2%) is periurban areas and most (60.9%) were from low

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socioeconomic status.

Mutation detection method

Haplotype analysis was done for the 80 patiets. In the 160 chromosomes studied β^s gene was found to be linked to the Cameroon haplotype in 17 alleles (10.6%; 95% confidence interval 6.31-16.47%), eight patients were homozygous and one patient was heterozygous.

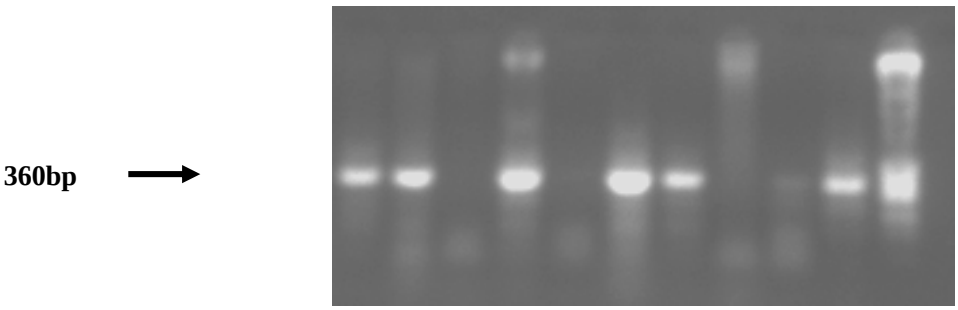

Figure 1: Agarose gel electrophoresis shows the results of ARMS-PCR for amplification of β cluster Cameroon haplotype. Lanes 1, 2, 4, 6, 7, 10, 11, PCR products of the samples targeting Haplotype 17 (Cameroon, 360bp).

Table (2): Tribes of Patients in the Study Group

Tribe	**Group with Cameroon Haplotypes** - No **(%)**	**Group with no Cameroon Haplotypes** - No **(%)**
Messeria	04(44.4)	24 (33.8)
Silihab	00(00)	04 (5.6)
Hawsa	00 (00)	06 (8.4)
Fallata	00(00)	03 (4.2)
Banihalba	00(00)	03 (4.2)
Barno	01 (11.1)	04 (5.6)
Taaisha	00 (00)	06 (8.4)
Rizigat	02 (22.2)	07 (9.8)
Massalleat	01 (11.1)	03 (4.2)
Kawahla	00(00)	04 (5.6)
Bidairia Tungur	00 (00) 01 (11.1)	03 (4.2) 04 (5.6)
Total	**9 (100.0)**	**71 (100.0)**

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Discussion:

A total of 80 individuals with confirmed sickle cell anemia by Hb electrophoresis were evaluated. Their age ranged between 3-38 years with a mean of 9.5±6.1 years. They were 38 males and 42 females. In agreement with previous studies in the Sudan, most of the patients in the study were originally from Western Sudan constituted (72.5%). [13, 14] The majority of patients were from the Messeria tribe consistent with previous studies. [15, 16] High percentage of consanguineous marriages was observed in (60%) of which (37.5%) were first degree cousins which was consistent with the finding of previous study in the Sudan. [14,16] The majority of the patients in the study were from periurban areas (67.2%) where there is lack of adequate health services and specialist doctors and most were from low socioeconomic class (60.9%).It was thought that poorer socioeconomic classes tend to manifest more severe clinical course. [17] In a study in Benin, it was found that intensive socio-medical intervention can provide sustained clinical improvement in SS children. [18]

The β^s mutation is identical in all population groups, the pattern of haplotypes is important in distinguishing one population from another. This characterization of haplotypes has allowed for cultural and ethnic mosaics of certain populations and cleared out, at least partially, the reasons for the clinical heterogeneity of sickle cell anemia benign or severe clinical set of symptoms of anemia depends on the haplotype. [6, 7]

In this study we looked for the Cameroon haplotype in Sudanese patients suffering from sickle cell anemia who reported to the sickle cell clinic. This was based on previous studies. [10, 19, 20] The study reported 10.6% Cameroon haplotype which is lower than what has been reported in a pervious study which reports that the haplotypes of the S gene in Sudan are more related to those of the West Africa haplotypes where the most prevalent is the Cameroon haplotype which constitutes 23.4% of the five studied haplotypes. [10] Another two studies in Sudan reported that Cameroon haplotype was very prevalent in the studied immigrant group of the Hawsa tribe. [19, 20] The patients with Cameroon haplotyes in this study are from western Sudan and they belong to the Afro-Asiatic-speaking groups. This reflects that those populations migrated to the Sudan from north and western Africa.

Conclusion:

Sickle-cell anemia is a genetic disease, of great importance to people and is a highly prevalent disease in western Sudan. This disease is a cause of morbidity and mortality in populations that bear Hb S in its homozygous form. All molecular and genetic characteristics of the disease are currently known. However, there is yet no treatment that can make red blood cells bearing hemoglobin S function normally, and thus allow the carrier to lead a normal life.

The Cameroon haplotye is prevalent among Sudanese patients with sickle cell anemia from western Sudan who belongs to the Afro-Asiatic-speaking groups.

It must be mentioned that in Sudan few studies acknowledging the genetics of sickle cell anemia have been reported. All The previous studies including this study were based on small samples. We recommend further larger study to evaluate the frequency of the different known haplotypes

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and a longitudinal study to correlate the haplotypes to the clinical course of the disease.

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