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Frequency of CCR5-delta32 Mutant Allele in Sudanese Patients with Sickle Cell Anemia

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Authors' contributions

This work was carried out in collaboration among all authors. Author TAMH designed the study, managed the literature search, wrote the protocol, wrote the Introduction and performed part of the statistical analysis. Author BOEH wrote the discussion. Author NAMG wrote the methodology of the study. Author RKAG wrote the abstract and conclusion, performed grammar, spelling, punctuation and language editing. Author SGE performed the statistical analysis, wrote the result and performed the final revision of the study. All authors read and approved the final manuscript.

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ABSTRACT

Background: Sickle cell anemia (CSA) is one of a group of hemoglobin disorders known as sickle cell disease in which the sickle β-globin gene is inherited. The pathophysiology of sickle cell disease (SCD) is based on the chronic hemolysis, vaso-occlusive episodes, infection and chronic inflammatory conditions. The CCR5 gene which encodes CCR5,Th5,cell associated chemokine receptor act as pro-inflammatory mediator, the presence of mutant allele known as CCR5 delta 32 makes it non- functional, and lower inflammatory picture. Thus it could confer a selective advantage on patient with sickle cell disease because it induce less efficientTh1 response (decrease inflammation and morbidity) its effect on the inflammatory response and morbidity in patients with sickled cell disease.
Objective: This study aimed at the detection of the frequency of CCR5delta 32 polymorphism among Sudanese patients with SCA.

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Materials and Methods: This is a case control study, conducted in Alneelain University –khartoum state during the period from August to December 2018. A total of study population, 60 participants (30 patients with SCA and 30 normal controls), were enrolled in this study. 2.5 milliliter of EDTA anticoagulated blood was collected from each subject. DNA was extracted by salting out method, and target DNA regions of the CCR5 delta 32 gene were amplified using allele specific polymerase chain reaction (AS-PCR).
Results: The frequency of CCR5 delta32 polymorphism in study populations was 0%.
Conclusion: There is no any CCR5 delta32 among Sudanese patients with sickle cell anemia.

Keywords: Sickle cell anemia; CCR5-delta32; mutant alleles; Sudan.

1. BACKGROUND

Sickle cell disease was first described by Herrick [1], a cardiologist, who observed sickle-shaped red cells in the blood of a medical student from west India, who suffered from chronic hemolytic anemia, was the first to suggest that sickle cell anemia was a homozygous state and sickle cell trait (the asymptomatic carrier state) was a heterozygous state of a genetic character that had not yet been defined. Linus Pauling [2] proposed that the sickling represented an abnormality of the hemoglobin molecule, based on the observation of the medical student that sickle cells, induced by deoxygenation, were birefringent. Birefringence indicated to Pauling that some type of molecular alignment or orientation existed inside these red cells, and since hemoglobin predominates overwhelmingly, it had to be this particular protein which was involved in the pathology. Electrophoretic studies confirmed this interpretation and the concept of molecular disease was born [3].

SCD is an autosomal recessive condition, It is a general term for abnormalities of hemoglobin structure, for example, hemoglobinopathies, in which the sickle gene is inherited from at least one parent. These genetic disorders are characterized by the production of HbS, anemia, and acute and chronic tissue damage secondary to the blockage of blood flow produced by abnormally shaped red blood cells.

Sickle cell anemia (HbSS), the most common form of hemoglobinopathy, is an expression of the inheritance of a sickle gene (HbS gene) from both parents as the most severe form of SCD. Individuals with this form also experience with worst symptoms at a higher rate. Other sickle cell disorders result from the coinheritance of the sickle gene. Common variants include HbSC disease occurs when you inherit the HbC gene from one parent and the HbS gene from the other. Individuals with HbSC have similar symptoms to individuals with HbSS. However the anemia is less severe and Beta -thalassemia affect beta globin gene production. If inherited with HbS gene you will have HbS beta thalassaema. Patients with this disease are living longer, new treatments are becoming available for adults as well as children, and early detection does matter [4].

The common sickling disorders consist of the homozygous state for the sickle cell gene which can be found on the short arm of chromosome, that is, sickle cell anaemia (HbSS), and the compound heterozygous state for the sickle cell gene (Sickle cell trait) and for another β chain variant (beta thalassemia). The sickle cell mutation results in a single amino acid substitution in the β globin chain (single nucleotide polymorphism) (GAG codon changing to GTG) of the B-globin gene which result in glutamic acid being substituted by valine at the sixth position of the polypeptide chain (3-5). Heterozygotes have one normal (βA) and one affected (βS) β chain gene and produce about 60% HbA and 40% HbS; homozygotes produce mainly HbS with small amounts of HbF. Compound heterozygotes for HbS and HbC produce almost equal amounts of each variant, where as those who inherit the sickle cell gene from one parent and β thalassaemia from the other make predominantly sickle hemoglobin [3].

2. MATERIALS AND METHODS

A total of 60 DNA samples were collected from Sudanese's 30 of them were SCD patients diagnosed by electrophoresis, and 30 as healthy control both of them ranging between 1 - 18 years of age.

Complete blood count was done using full automated hematological analyzer (SYSMX KXN-21 Japan.

DNA was extracted by salting out method, and target DNA regions of the CCR5 delta 32 gene were amplified using allele specific polymerase chain reaction (AS-PCR) (Rimiller et al.1988).

2.1 Analysis of the CCR5Δ32 Polymorphism

To analyze the *CCR5* polymorphism, genomic DNA was extracted from leukocytes using a salting out method [5].

The CCR5 polymorphism was detected by allele specific polymerase chain reaction (AS-PCR), using the following CCR5-specific primers:

CCR5Δ32 F5 CTTGGGTGGTGGCTGTGTTT3

and

CCR5Δ32 R-5 AGTTTTTAGGATTCCCGATAGC 3

The reaction mixture consisted of template DNA 5 µl, premix (i-Taq) 3 µl, each of primer 1 µl and distilled water10 µl. Thermocycling conditions consisted of initial denaturation at 95°C for 2 minutes, 14 cycles consisting of: 95°C for 30 second, 59.9°C decreased 0.5 per cycle for 30 second and 72°C for 30 second; 19 cycles more consisting of: 95°C for 30 second, 52.9°C for 30 second and 72°C for 30 second; and Final extension at 72°C for 5 minutes, hold at 4°C until further steps. The amplified fragments were separated on 3% agarose gel stained with ethidium bromide and demonstrated by gel documentation system.

2.2 Demonstration of PCR Product

5 µl of the PCR product (ready to load) was electrophoresed on 3% agarose gel, and was stained with ethidium bromide, 1X TBE buffer was used as a running buffer. The voltage applied to the gel was 60volt with time duration 40 minutes. 100 bp DNA ladder was used as molecular weight marker with each patch of samples. Finally, PCR product was demonstrated by gel documentation system SYNGENE.

2.3 Data Analysis

Data was analyzed using statistical package for social science (SPSS) version 25.

3. RESULTS

3.1 Demographic Data

This is a case control study done at Alneelain University, Faculty of Medical Laboratory Sciences. A total of 60 patients homozygous with SCA (Hb-SS), 26 (43%) males and 34 (57%) females; ranging between 1-18years as case group and 30 healthy appearing subjects as control group; 15(50%) males and 15 (50%) females.

3.2 Molecular Analysis

3.2.1 Detection of CCR5/CCR5delta32

Normally CCR5/CCR5delta32 genotypes were determined by amplification. The normal allele generates a137pb band while the CCR5delta32 allele generates a105pb band.

Our results showed the frequency of the CCR5/CCR5delta32 in population with sickle cell anemia (patients and controls) was 0.0 % (Fig. 1) and (Table1).

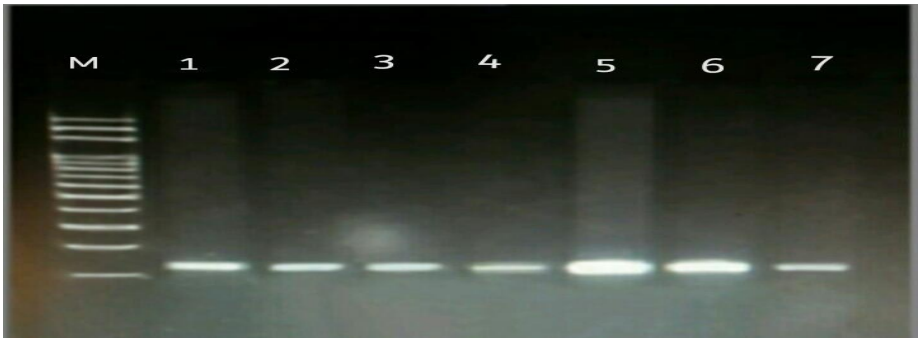

Fig. 1. A garose gel showing the CCR5 gene product in samples from a population of Sudanese SCA patients, M=100bp ladder, 1-7 patients with normal Alleles (137pb)

Table 1. Frequency of CCR5 delta 32 polymorphism among study group

CCR5 delta32	Case	Control
Positive	0.0 (0.0%)	0.0 (0.0%)
Negative	30 (100%)	30 (100%)
Total	30 (100%)	30 (100%)

The current study revealed that mean of Hb level was significantly lower in SCA patients compared with those in the normal control group (P. value = 0.00). The mean of leucocytes count was significantly higher among patients compared with control (P. value = 0.00), while mean of neutrophils was significantly lower in patient in comparison with control (P. value = 0.000). On the other hand, mean of lymphocytes and platelets counts were significantly higher in case group compared with normal control group (P. values = 0.00 and 0.005, respectively) (Table 2).

4. DISCUSSION

Sickle cell disease is an inherited chronic haemolytic anaemia caused by homozygosity for the hemoglobin S (HbS) gene (Hoff brand, et al. 2005). HbS results from a single nucleotide substitution (GAG → GTG) at the sixth codon of the β-globin gene (HBB), which causes glutamic acid to be replaced by valine at the sixth position of the polypeptide chain [6].

The pathophysiology of SCD is based on the polymerization of deoxygenated HbS, leading to chronic hemolysis as sickling induces membrane fragmentation, complement mediated lysis and vasoocclusive episodes which initiated by adhesion of young deformable red cells to the vascular endothelium [7]. It has been suggested that these episodes are associated with a chronic inflammatory condition with abnormal endothelial function involving interactions between the endothelium and sickle reticulocytes and white blood cells and thrombocytes. SCD patients have elevated levels of inflammatory mediators [8]. A growing number of studies investigating the importance of the immune system in the pathophysiology of SCD have suggested that inflammation and morbidity are closely associated in this disease [9].

C-C chemokine receptor type 5, also known as CCR5 or CD195, is a protein on the surface of white blood cells that is involved in the immune system acts as a receptor for chemokines, in which T cells are attracted to specific tissue and organ targets. The CCR5 is an important receptor of a pro-inflammatory chemokine, acting as an inflammatory mediator [10]. The presence of CCR5Δ32 deletion makes it non- functional, conferring a lower inflammatory picture due to a less efficient response, this lead to decrease clinical severity and morbidity of sickles cell diseases, the presence of CCR5delta32 allele reduce the inflammatory response at low level leading to a less severe inflammatory state and a less severe vase-occlusive crisis [11].

This study showed that the frequency of the CCR5delta32 polymorphism among Sudanese patients with SCD and normal control group was 0%.

Our findings agreed with a study carried out by Mariana et al. [12], who demonstrated a total of 795 DNA samples from patients with SCA from Northeastern Brazil and concluded that study participants had null genotype on the other hand CCR5delta32 among both the case study and control group reflecting the history of immigration from very varied ethnic backgrounds.

The present findings were incongruent with Chies and Hutz [9] who demonstrated the genotype CCR5 delta32 among 79 sickle cell patients and reported that high frequency (5.1%)

Table 2. Comparison of hematological parameters among study population

Parameters	Case (Mean±SD)	Control (Mean±SD)	*P-value*
White blood cells	13.65 ± 6.24	5.96 ± 1.5	0.000
Lymphocytes	50.3 ± 11.79	35.20 ± 9.5	0.000
Monocytes	15.05 ± 6.82	8.70 ± 2.9	0.000
Neutrophil	34.77 ± 13.24	56.87 ± 9.9	0.000
Hemoglobin	7.94 ± 1.47	13.77 ± 1.0	0.000
Platelets	414.5 ± 170.5	321.9 ± 62.4	0.005

P. value considered significant if less than 0.05

of the CCR5delta32 variant among individuals from an admixed Brazilian population with sickle cell anemia, compared with healthy control from the same ethnic group (1.3%) also were inconsistent with study of Vargas et al. [13] who studied 52 sickle cell patients and found the frequency of CCR5 delta32 was 5.0% in SCA patients from the Southern Brazil compared with 2% in normal control. The frequency of CCR5 delta32 allele varies widely among the world but it is much lower in population of American, African and East Asian origin [12]. The disagreement of studies mentioned emphasis that CCR5 delta32 was very rare in Africa, on the other hand might be attributed to different ethnic backgrounds [9,13].

Hematological features and clinical severity of SCD are influenced by gender, genetic, and environmental factors. It was observed that WBC and PLT count are generally higher in SCD patients compared with healthy counterparts [14].

Therefore, WBC and PLT counts are expected to increase in all patients who may present with any form of complication associated with SCD as we observed in the current study. Higher PLT count seen in SCD could be attributed to a possible splenic sequestration as well as chronic inflammation.

The lower level of Hb among SCD patients in the current study agreed with the work of [15-17] as chronic hemolysis shortened RBC survival and reduces Hb level.

5. CONCLUSION

This study concluded that:

- The frequency of CCR5delta32 in study population was 0%.
- The Hb level and mean of neutrophils count were significantly lower among patients than controls.
- The mean of lymphocytes and platelets counts were significantly higher among patients when compared with the control group.

CONSENT AND ETHICAL APPROVAL

Data were collected with verbal informed consent from all participants or those legally responsible for them and the questionnaires were filled. The ethical clearance of this study was accepted by department of hematology college of medical laboratory science Alneelain University (no 017/07).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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