

## Haptoglobin Phenotypes distribution among Sickle Cell anemia patients with different genotypes

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### Abstract:

**Introduction;** Sickle cell anemia (SCA) is a haemoglobinopathy due to single point mutation in the  $\beta$  chain of human haemoglobin. Sickle cell anemia is a form of haemolytic anemia, and it provides the most well documented example of acute and chronic haemolysis, which leads to acute crisis and may develop frequently of infections, and acute chest syndrome. Haptoglobin (Hp) is a unique acute phase protein that primarily scavenges haemoglobin released into circulation by haemolysis. **Materials and Methods;** Across sectional study was done to determine and compare the frequency of haptoglobin phenotypes among Sudanese patients with sickle cell anemia (SS), and sickle cell trait (AS). A 100 Sudanese patients were recruited in this study after informed consent; and diagnosed by SCA, based on their clinical evaluation, Sickling test, complete blood count (CBC), and Hb electrophoresis. Patient's age was range between 2 to 45 years. An EDTA venous blood was collected from each patient for haptoglobin phenotypes. Hp phenotypes have been performed by PAGE (Poly-acrylamide-gel-electrophoresis) protocol. **Results and discussion;** To our Knowledge, this study was the first among Sudanese with SCA patients. It appeared that Hp1-1 was taking the high percent (59%) in distribution between the patients. There was high frequency of haptoglobin phenotype 1-1 among

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*Sudanese sickle cell anemia (SS) patients, while Hp 2-2 was more frequent among Sudanese sickle cell anemia (AS) patients.*

**Key words:** Haptoglobin phenotypes(Hp),Sickle cell anemia (SS), Sickle cell trait (AS), Haemoglobin (Hb), PAGE

## **Introduction**

Sickle Cell Anemia (SCA) is a haemoglobinopathy due to a single point mutation in the  $\beta$ -chain of human haemoglobin [1].The amino acid valine replaces glutamic acid in the sixth position of  $\beta$  chain and form (Haemoglobin S)<sup>[2]</sup>. There is a form of sickle cell anemia which the mutation is homozygous (SS), in heterozygous individual there is only one sickle gene and one normal adult haemoglobin gene, or sickle cell trait (AS).Sickle cell anemia has provided the most well documented example of acute and chronic haemolysis and clinical vasculopathy, and it can lead to severe complication, and high mortality rate, under un usual circumstances <sup>[3, 4]</sup>. During intravascular haemolysis haemoglobin (Hb) escapes into plasma rapidly bound by the haptoglobin which is the protein that in humans is encoded by haptoglobin gene, and it produced mostly by hepatocyte but also by other tissues; e.g. skin, lung and kidney. Haptoglobin, in its simplest form; consists of two  $\alpha$  and two  $\beta$  chain connected by disulfide bridges. Polymorphism arises from the variant in  $\alpha$  chain<sup>[5]</sup>.Haptoglobin exists in two allelic form, hp1 and hp2, and it has three major phenotypes,Hp1-1,Hp2-2 and Hp2-1.Haptoglobin 1-1 phenotype is more likely to resist cellular oxidative stress than the hp2-2 phenotype<sup>[6]</sup>. Haptoglobin phenotypes have clinical significant marker, and association with various human pathologies, and development of sever complication<sup>[7,8]</sup>,for example haptoglobin 1-1 phenotype was associated with susceptibility to falciparum malaria ,also it associated with acute myeloid Leukemia, chronic myeloid Leukemia, chronic Lymphoid Leukemia and acute Lymphoid

Leukemia<sup>[9,10]</sup>. Haptoglobin phenotype 2-2is also found to be associated with atherosclerosis, cardiovascular disease, myocardial infraction as independent predictor of severity and extent of myocardial damage in patients with different risk factor and diabetes mellitus<sup>[11, 12]</sup>.The present study was therefore designed to determine the frequencies of Hp phenotypes distribution among Sudanese patients with sickle cell anemia; SS and AS genotypes.

## **Material and Methods**

The study was designed as cross sectional study, conducted and approved by Alneelain University, Faculty of medical laboratory sciences and Khartoum hospitals, Khartoum, Sudan. A 100 Sudanese patients were recruited in this study after informed consent; and diagnosed by SCA, based on their clinical evaluation, Sickling test, complete blood count (CBC), and Hb electrophoresis. Patient's age was range between 2 to 45 years. An EDTA venous blood was collected from each patient for haptoglobin phenotypes. Hp phenotypes have been performed by PAGE (Poly-acrylamide-gel-electrophoresis) protocol according to Davis and Ornestein method and modified by Linkeusing the Mini-V8.10 (BRL, Life Technologies Inc, Gaithersburg, USA)<sup>[13,14]</sup>.

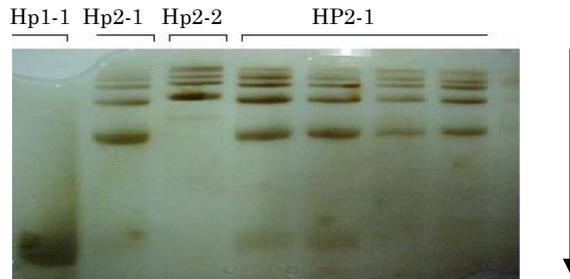
Data were analyzed using Statistical Package for Social Sciences (SPSS 16). The descriptive data was given as means  $\pm$  standard deviation .Chi square test and Anova test were used for assessment the differences between groups and significant obtained with P.value  $\leq 0.05$ .

## **Results**

### ***Haptoglobin phenotypes bands appearance***

Hp1-1 phenotype was appeared one thick band. Hp2-1 phenotype was appeared as multiple fines bands and had the

same thickness in addition to a band corresponds to the Hp1-1 band. Hp2-2 phenotype was showed a series of multiple bands, which were fine and close to each other. However, the fastest migrating band appears fainter than its preceding band.



**Plate (1):** Haptoglobin phenotypes on PAGE

### ***Haptoglobin phenotypes in all subjects***

The Hp phenotypes distribution percent in all subjects were 59, 28, and 13 for Hp1-1, Hp2-1 and Hp2-2 respectively. There was a significant difference between those percent (P.value=0.00).

### ***Hp-phenotypes and Hb electrophoresis***

An eighty of patients (80%) had homozygous (SS) hemoglobin, while twenty (20%) were heterozygous (AS). Out of the eighty HB (SS) patients, there were 54(67.5%) had Hp1-1 phenotype, 21(26.5%) had Hp2-1-phenotype and 5 patients (6.3%) had Hp2-2 phenotype. On the other 20 patients (AS) there were 5(25%) had Hp1-1, 7 (35%) had Hp2-1-phenotype and 8(40%) had Hp-2-2 phenotype. Hp1-1 phenotype was most prevalent in SS patients while Hp2-2 was common in AS patients.

### ***Hp-phenotypes and patient's gender***

***In SS patients***, there were 41 (51.2%) females and 39 males (48.8%). Twenty five (60.9%) of the females had Hp1-1 phenotype, 12(29.2%) had Hp2-1, and 4 (9.7%) had Hp2-2 phenotype; while in males 29 (74.3%) had Hp-1-1, 9(23%) had Hp-2-1, and one (2.5%) had Hp2-2 phenotype. According to gender, Hp 1-1 was the most distributed. ***In AS patients***, there

were 10 females (50%) and 10 males (50 %). Three of the females (15%) had Hp1-1, 3(15%) had Hp2-1, and 4 (20%) had Hp2-2, while there were 2 males (10%) had Hp1-1 phenotype, 4(20%) had Hp2-1 and 4 (20%) had Hp2-2 phenotype. Hp2-2 appeared with a high frequency in both gender.

### ***Hp-phenotypes and complete blood count***

#### ***Hb concentration and Hp distribution***

The means of Hb concentration in SS and AS patients were 6.3g/dl and 12.2g /dl respectively. There was highly significant difference between the two groups (P.value=000) .***In AS patients***, the means of Hb in patients with Hp 1-1, Hp2-1 and Hp2-2 phenotypes were 11.9g/dl, 12.2g/dl, and 12.5g /dl respectively. While in ***SS patients***, the means of Hb for Hp1-1, Hp2-1and Hp2-2 phenotypes were 6 g/dl, 5.9g/dl and 7.6g/dl respectively. Although there was a significant different between the two groups in haemoglobin level, but the highest concentration of Hb was appeared related to Hp2-2.

#### ***Packed Cell Volume and Hp distribution***

The means of PCV in SS and AS patients were 20.6% and 39.7% respectively. There was significant difference between the two groups (P.value =0.03).In AS patients, the means of PCV in patients with Hp 1-1, Hp2-1 and Hp2-2 phenotypes were 39%, 39.7%, and 40.2% respectively. While in SS patients, the means of PCV for Hp1-1, Hp2-1 and Hp2-2 phenotypes were 20.6%, 19.4% and 25.1% respectively. The distribution of Hp2-2 was related to the highest result of PCV in both groups.

#### ***Red blood cell count (RBCs) and Hp distribution***

The means of RBCs count in SS and AS patients were  $2.5 \times 10^6/\mu\text{l}$ , and  $4.8 \times 10^6/\mu\text{l}$  respectively. There was significant difference between the two groups (P.value=0.00).

***In AS patients***, the means of RBCs count according to Hp 1-1, Hp2-1 and Hp2-2 phenotypes were  $4.4 \times 10^6/\mu\text{l}$ ,  $5.0 \times 10^6/\mu\text{l}$  and  $4.8 \times 10^6/\mu\text{l}$  respectively. While in ***SS patients***, the means of RBCs count for Hp1-1, Hp2-1 and Hp2-2 phenotypes were  $2.2 \times 10^6/\mu\text{l}$ ,  $2.1 \times 10^6/\mu\text{l}$  and  $2.6 \times 10^6/\mu\text{l}$  respectively.

#### ***Red blood cells indices (MCV, MCH, MCHC)***

***In SS patients***, the means of MCV, MCH and MCHC were 89fl, 27.9pg and 30.5 g/dl respectively. While in AS patients, the means were 94fl, 33.3pg and 32.2gm/dl respectively. There was significant difference between those means (P.value=0.00).

#### ***Total white blood cell count (TWBC) and Hp distribution***

The mean total white blood cells count of SS and AS patients was  $14.2 \times 10^3/\mu\text{l}$ , and  $5.1 \times 10^3/\mu\text{l}$  respectively. There was significant difference between the two groups (P.value=0.00). ***In AS patients***, the means of total white blood cells count in patients with Hp1-1, Hp2-1 and Hp2-2 phenotypes were  $5.2 \times 10^3/\mu\text{l}$ ,  $5.0 \times 10^3/\mu\text{l}$  and  $5.3 \times 10^3/\mu\text{l}$  respectively. While in ***SS patients***, the means of TWBCs count for Hp1-1, Hp2-1 and Hp2-2 phenotypes were  $13.6 \times 10^3/\mu\text{l}$ ,  $14.9 \times 10^3/\mu\text{l}$  and  $18.0 \times 10^3/\mu\text{l}$  respectively. The highest count in TWBC was appeared with Hp2-2, either the count within normal level or above.

#### ***Platelets count and Hp distribution***

The mean of platelets count in SS and AS patients was  $394.4 \times 10^3/\mu\text{l}$  and  $245.4 \times 10^3/\mu\text{l}$  respectively. There was significant difference between the two groups (P.value=0.001).

***In AS patients***, the means of platelets count in patients with Hp 1-1, Hp2-1 and Hp2-2 phenotypes were  $225.8 \times 10^3/\mu\text{l}$ ,  $225.7 \times 10^3/\mu\text{l}$  and  $275.0 \times 10^3/\mu\text{l}$  respectively. While in ***SS patients***, the means of platelets count for Hp1-1, Hp2-1 and Hp2-2 phenotypes were  $400.0 \times 10^3/\mu\text{l}$ ,  $378 \times 10^3/\mu\text{l}$  and  $389.8 \times 10^3/\mu\text{l}$  respectively.

### ***Hp phenotypes and Clinical features***

The present study also afforded an opportunity to examine the association of the Hp phenotypes with the clinical features.

### ***Hand/Foot syndrome and Hp distribution***

***In SS patients*** there were 64 patients suffered from hand/foot syndrome. Hp1-1 was the more distributed 44. ***In AS*** group no patients suffered from hand/foot syndrome.

### ***Leg ulcer and Hp distribution***

***In SS patients*** there were 5 patients with leg ulcer. Three of them had Hp1-1, one had 2-2 and the last one had 2-1 phenotype. ***In AS*** group no patients suffered from leg ulcer.

### ***Stroke and Hp distribution***

***In SS group*** the study found that, there were 23 patients suffered from stroke. Six of them had Hp2-1, one had 2-2 and 16 had Hp 1-1. ***In AS*** group no patients suffered from stroke.

### ***Splenomegaly and Hp distribution***

***In SS group*** there were 51 patients with splenomegaly. Four of them had Hp2-2, 44 had Hp 1-1 and 3 had 2-1 phenotype. ***In AS*** group no patients suffered from splenomegaly.

### ***Jaundice and Hp distribution***

***In SS group*** there were 3 patients with jaundice. Two of them had Hp2-2 and one had Hp1-1. ***In AS*** group no patients suffered from jaundice.

### ***Fever and Hp distribution***

***In SS group*** the study found that, there were 20 patients suffered from fever. Seven had Hp 2-1, 11 had Hp1-1 and 2 had Hp 2-2. ***In AS*** group no patients suffered from fever.

### ***Hydroxy urea and Hp distribution***

***In SS patients*** there were 52 patients treated by hydroxyurea. 39 patients with Hp1-1, 9 patients with Hp2-1 and 4 patients with Hp2-2. While AS patients were not.

### **Discussion**

Haptoglobin, beside the hemoglobin binding function, is an acute-phase reactant protein; it had antioxidant and immune modulatory properties that had been extensively investigated [15]. Recently has been demonstrated that different Hp phenotypes showed distinct antioxidant activities and that Hp 2-2 phenotype is a less effective protector than Hp1-1<sup>[16]</sup>.

To our Knowledge, this study is the first among Sudanese with SCA patients. It appeared that Hp1-1 was taking the high percent (59%) in distribution between the patients. It is therefore this interesting finding was agreed with a study done by *A.D. Adekile M.Z. Haider*, 2009, who were observed that Hp1-1 was most prevalent among Nigerian SCA patients (73%). This consequently raises confirms the possibility that Nigerian SCD patients and Sudanese SCA patients were shared the same ethnic and geographic distribution concern the African roots. The patients were classified according to Hb electrophoresis into SS and AS group. Hp1-1 was the most frequent (67.5%) in sickle cell anemia patients (SS), while Hp2-2 was the most frequent (40%) in sickle cell trait patients (AS). In SS group, the distribution of Hp1-1 was agreed with Sadrzadeh, S, who confirmed that, there was association between Hp1-1 and sickle cell anemia <sup>[17]</sup>. Moreover agreed with Ostrowski RS, and Moreira HW, who were reported that there was strong association between Hp 1-1 phenotype and sickler patients.<sup>[18][19]</sup> These interested findings were explained that the predominant association between Hp1-1 and Hb binding in the presence of high rate of hemolysis in SS patients. The most interesting finding also in this study, that the highest

count of total white blood cells was related to Hp2-2 either in SS group or AS group. However the sickle cell trait patients (AS) showed normal white blood cells count comparing with sickle cell anemia (SS) showed leukocytosis. Recent evidences indicate that haptoglobin is involved in the immune response as well. The strong genetic pressure favoring the 2-2 phenotype suggests an important role of haptoglobin in human pathology<sup>[24]</sup>.

Angiogenesis function for Hp plays an important role in a variety of physiological and pathological conditions, including tumor growth, wound healing, and chronic inflammatory disorders. Hp2-2 is more angiogenic than the other phenotypes.<sup>[21]</sup> We also explored between the Hp phenotypes and patients clinical features; Hand Foot syndrome, splenomegaly , stroke and fever were associated Hp1-1, while appearance of jaundice and leg ulcer were related to Hp2-2 even though the low frequency of Hp2-2 distribution in patients. In the future, further exploration of the role of Hp in the immune system may lead to better understanding of the effect of Hp polymorphism in disease and will eventually contribute to a better tailored treatment.

## **Conclusions**

High frequency of haptoglobin phenotype 1-1 among Sudanese patients with sickle cell anemia(SS) and haptoglobin 2-2 phenotype among Sudanese sickle cell trait(AS). Homozygous sickle cell anemia(SS) patients have lower values of red cell parameters, but higher values of white cell and platelets counts compared to heterozygous sickle cell trait(AS) patients.

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