

Effects of Hydroxyurea Hemoglobin F Level in Pediatric Patients with Sickle Cell Disease Attaining Jafaar Ibnouf Hospital - Khartoum

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ABSTRACT

Fetal hemoglobin (HbF) is the main hemoglobin throughout the fetal life and at birth, accounting for approximately 80% of total hemoglobin in newborn. HbF, when elevated during sickle cell disease (SCD), cause reduction of different crises associated with SCD. Elevation of HbF is achieved by hydroxyurea (HU) therapy as a tool to control SCD. This study was conducted in the period between May 2009 to December 2009 to determine the levels of HbF in SCD pediatric patients treated with hydroxyurea (HU) therapy and evaluate its effects on elevation of HbF which lead to the reduction of SCD crises. Ninety one Sudanese SCD pediatric patients with mean age of (6.0 years \pm 2.9), who were referred to Jafaar Ibnouf hospital in Khartoum city, were included in this study. Sixty one of the patients, designated group T, had been treated with 22 mg /Kg/ day of HU for seven months; and thirty patients had been treated with conventional treatment were used as control designated group C. Two and half ml blood sample was collected from each participant in Ethylene Diamine Tetra Acetic Acid (EDTA) container. HbF level was determined by using the denaturation method. The results showed that the mean of HbF levels in group T was 8.00 ± 0.259 while the mean of HbF level in the control group (C) which was 0.8 ± 0.2 .

المستخلص

الهيموقلوبين من النوع (F) هو الهيموقلوبين الرئيس في المرحلة الجنينية وحتى الولادة حيث يمثل 80% من الهيموقلوبين عند الأطفال حديثي الولادة. يرتفع تركيز هيموقلوبين (F) في حالة الإصابة بمرض الأنيميا المنجلية مسبباً المضاعفات المرتبطة بمرض الأنيميا المنجلية. يستعمل علاج يسمى هيدروكسي يوريا للتحكم في مرض الأنيميا المنجلية. أجريت هذه الدراسة في الفترة ما بين مايو 2009م حتى ديسمبر 2009 لتحديد تركيز هيموقلوبين (F) عند الأطفال المصابين بمرض الأنيميا المنجلية تحت العلاج بالهيدروكسي يوريا ولتقييم إرتفاع هيموقلوبين (F) والذي يقلل من المضاعفات الناتجة من هذا المرض. واحد وتسعون مصاباً بالأنيميا المنجلية متوسط أعمارهم $= 6 \pm 2.9$ عاماً والمترددون على مستشفى جعفر بن عوف بولاية الخرطوم تم إختيارهم لهذه الدراسة. واحد وستون مريضاً تم علاجهم بالهيدروكسي يوريا بتركيز 22mg/kg يومياً ولمدة 7 أشهر. هذه المجموعة سميت المجموعة (T)، ثلاثون مريضاً تم علاجهم بالطريقة التقليدية أخذوا كمجموعة ضابطة وسميت المجموعة (C). جمع 2.5 مل من دم كل مشارك في إناء يحتوي على مادة مانعة للتجلط (EDTA) لتحديد مستوى هيموقلوبين (F) بطريقة التفسير. أظهرت نتائج الدراسة أن متوسط هيموقلوبين (F) في المجموعة (T) $= 8.00 \pm 2.59$ جرام/ديسلتر، حيث إرتفع مقارنة بمتوسط هيموقلوبين (F) في المجموعة (C) $= 0.8 \pm 0.2$ جرام/ديسلتر.

KEYWORDS: Fetal hemoglobin (HbF); sickle cell disease; hydroxyurea therapy.

INTRODUCTION

Sickle cell anemia (SCA) is a common genetic disorder that causes considerable morbidity and mortality throughout the world. SCA results from an amino acid substitution of valine for glutamic acid at position 6 of the β -globin chain, which results in the polymerization of hemoglobin upon deoxygenation, leading to deformed dense red blood cells the predominant pathophysiological feature of SCA is vaso-occlusion, which leads to acute and chronic complications such as painful crises, acute chest syndrome and strokes. Patients with SCA have a markedly decreased life expectancy and their quality of life is greatly compromised by their disease⁽¹⁾. In 1910, Dr James Herrick working in Chicago, USA, reported 'Peculiar elongated and sickle shaped red blood corpuscles in a case of severe anemia'⁽²⁾. The inherited disease was subsequently called SCA, and has continued to attract the attention of medical scientists to the present day. SCA includes homozygous (Hb SS) sickle cell disease and compound heterozygous states such as sickle cell haemoglobin C (Hb SC) disease, sickle cell thalassaemia (Hb S thal), HbS/Hb D Punjab (Los Angeles), HbS/HbO-Arab, HbS/HbE, and HbS/Hb⁽³⁾. Hemoglobin S becomes polymerized and becomes poorly soluble when the oxygen tension is lowered and red cells that contain this hemoglobin become distorted and rigid. SCD occurs when an individual is homozygous for the sickle cell mutation or is a compound heterozygote for sickle haemoglobin and β -thalassemia, haemoglobin C, and some less common β -globin mutations. Diagnosis depends upon demonstrating the presence of the abnormal haemoglobin (S) in the red cells. The disease is characterized by haemolytic and by three types of crises, painful (vasoocclusive), sequestration, and aplastic crisis. Complications include splenic infarction and autosplenectomy, stroke, bone infarcts and aseptic necrosis of the

femoral head, leg ulcers, priapism, pulmonary hypertension, and renal failure⁽⁴⁾. Hemoglobin S occurs with greatest prevalence in tropical Africa. HbF is the main hemoglobin component throughout fetal life and at birth, accounting for approximately 80% of total hemoglobin in newborns. HbF is produced from the sixth week of gestation and during the rest of fetal life, replacing the embryonic hemoglobin. After birth, HbF synthesis rapidly declines and HbF is gradually substituted by HbA in the peripheral blood, so that within the first two years of life, the characteristic hemoglobin phenotype of the adult with very low levels of HbF (less than 1%) is found^(1,2). Functionally, HbF differs mostly from HbA because it has a slightly higher oxygen affinity, explained by the low interaction with 2,3-DPG. This characteristic makes the delivery of oxygen through placenta easier, giving the fetus better access to oxygen from the mother's bloodstream⁽⁵⁾. Moreover, HbF is known to inhibit the polymerization of HbS and different agents have been introduced to increase HbF production for therapeutic aim⁽⁶⁾. The levels of HbF in erythrocytes account for a large part of the clinical heterogeneity observed in patients with SCD and β -thalassemia⁽⁶⁾. The Cooperative study of SCD identified HbF as a major prognostic factor for several clinical complications including painful events and acute chest syndrome. These clinical and epidemiological observations provided important clues about the beneficial role of HbF in ameliorating the clinical complications of SCD which is a major public health concern that has great impact on both individuals and society. HU treatment allows γ -globin genes to be more actively expressed. By killing cycling cells, HU changes the kinetics of erythroid proliferation, forcing more F cells to be produced from primitive progenitors. HU also produces nitric oxide and directly stimulates

fetal hemoglobin production. Because F cells are less likely in red cells with little Hb F to occlude vessels and cause membrane damage, HU treatment results in fewer symptoms, less severe hemolytic anemia, and lower mortality⁽⁷⁾. Some patients also display increases in their anaerobic muscular performance and aerobic cardiovascular fitness⁽⁸⁾. The hemoglobin S-containing erythrocytes became less dense, and hemolysis was reduced. These changes and the reduction in painful episodes preceded the increase in the hemoglobin F concentration⁽⁹⁾. HU should be reserved for patients with sickle cell anemia who have complications that are sufficiently severe to justify the burdens of treatment and who can comply with the treatment regimen.

This study aimed at determining the levels of HbF in SGD pediatric patients treated with hydroxyl urea therapy in Jafaar Ibnouf hospital in Khartoum state.

MATERIALS and METHODS

Ninety one SCD patients, who were referred to Jafaar Ibnouf hospital in Khartoum city, were informed about the study objectives and agreements for their participation were obtained. Sixty one of the patients had been treated with HU called T group, and thirty patients had been treated with conventional treatment called C group. Patient's samples were selected randomly to be matched in age, sex with control. Venous blood (2.5 ml) was collected from each patient into Ethylene Diamine Tetra Acetic acid (EDTA) containers.

Principle of denaturation method

This method based on the resistance to denaturation by alkali of HbF compared to HbA, the denaturation being activated by the ionization of buried, weakly acidic side chains (one tyrosine and two cysteines) present in HbA and not in HbF⁽¹⁰⁾. This is only a relative difference, and the conditions have been optimized over time in order that during the time of exposure to alkali most of the HbA is denaturated while the HbF is largely unaffected. Before the

exposure to alkali, all the hemoglobin forms are transformed in the more stable Cyanomehtemoglobin form by treatment with Drabkin's reagent. An optimized version of the method was proposed by Pembrey⁽¹¹⁾.

So HbF was estimated by denaturation method to measure the percentage of HbF in a mixture of hemoglobins⁽¹²⁾. Sodium hydroxide was added to a lysate and, after a set time, denaturation was stopped by adding saturated ammonium sulphate. The ammonium sulphate lowers the pH and precipitates the denatured hemoglobin. After filtration, the quantity of undenatured (unprecipitated) hemoglobin was measured. The proportion of alkali-resistant HbF was subsequently calculated as a percentage of the total amount of hemoglobin present⁽¹³⁾.

Procedure

Lysate (0.25 ml) was added to 4.75 ml of cyanide to make a hemoglobin cyanide (HiCN), after that 2.8 ml of HiCN was transferred to a new glass tube and allowed to equilibrate at 20°C. To the same test tube 0.2 ml of 1.2 mol/l of NaOH was added and mixed well with HiCN on a vortex mixer for 2 to 3 seconds. After 2 minutes, 2 ml of saturated ammonium sulphate was added to the same test tube and also mixed well on a vortex mixer. Tube left to stand for 5 to 10 minutes at 20°C. Solution was filtered twice through a Whatman filter paper No.42. The filtrate containing alkaline-resistant hemoglobin (HbF). Total Hb was measured by transferring 0.4 ml of HiCN into another test tube and 13.9 ml of water was added to the same test tube. The absorbance of alkali-resistant and total Hb were detected using spectrophotometer set at 420nm against water blank. The percentage of alkali-resistant was calculated as follows:

$$HbF \% = \frac{a_{420} \text{ alkali - resistant Hb}}{a_{420} \text{ total Hb}} \times 100\%$$

RESULTS

The measured level of HbF in group T was (8.00 ± 2.59) which was found to be raised significantly compared with group C (0.81 ± 0.21), (P=0.000), (Table 1):

Table 1: HbF Levels and P values in the study groups

Group	(Mean ± SD)	P value
T	8.00 ± 2.59	0.000
C	0.81 ± 0.21	

It was noticed that most of the patients belonged to tribes living in the west of Sudan where the gene of sickle cell anemia is most prevalent. The

highest frequency of participators was found to be from Meseria tribe (n=51) 35%. The distribution of participant's tribe is shown in figure 1 below.

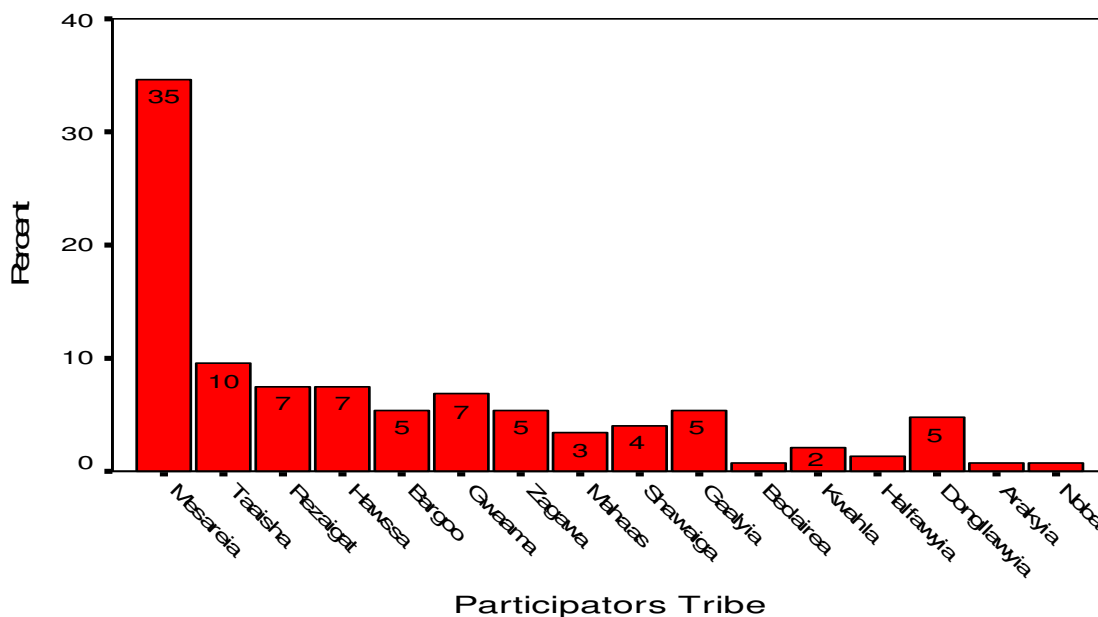


Figure 1: Frequency of participant's tribes in this study

DISCUSSION

SCA is a well-known haemoglobinopathies considered as endemic disease in certain areas of the world. It has been recognized now that it may have a wide geographic distribution, whose clinical manifestations arise from the tendency of the haemoglobin HbS (or sickle haemoglobin) to polymerize and deform red blood cells into the characteristic sickle shape leading to various types of crises. In Sudan sickle cell disease considered as serious problem either in Khartoum state or in rural areas. The results of this study showed significant differences in HbF levels (Mean \pm SD) when group T compared with group C, (P = 0.00), as shown in table 1. This finding agreed with several studies such as Multicenter Study of Hydroxyurea (MSH) and Griffin P. Rodgers *et al.*⁽¹⁴⁾, Past Adragna N. C. *et al.*⁽¹⁵⁾, Steinberg MH and his colleagues, and recent such researches by Mary Catherine Beach and others⁽¹⁶⁾. Also this result is similar to the results of study done in India by Hraminder Singh *et al.* which reported that the level of HbF was increased in SCD patients treated with HU⁽¹⁷⁾. HU is now used as drug of choice in reduction of SCD crises.

CONCLUSIONS

HbF levels increased significantly in SCD patients treated with HU compared to the control group and this study proved that HU still had high efficiency to reduce the suffering of SCD patients.

ACKNOWLEDGMENT

High gratitude to Jaffar Ibnouf hospital staff, for their full cooperation to complete this work. Also our appreciation goes to all patients and their families for their support to complete this work.

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