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FETAL HEMOGLOBIN LEVELS IN SICKLE CELL DISEASE PEDIATRIC PATIENTS TREATED WITH HYDROXYUREA DRUG

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ABSTRACT

Background. Fetal hemoglobin (HbF) is the main hemoglobin throughout the fetal life and at birth, accounting for approximately 80% of total hemoglobin in newborn. Hb F when is elevated during sickle cell disease (SCD) cause the reduction of different crises associated with SCD. This mechanism is achieved by hydroxyurea (HU) therapy as a tool to control SCD. Objectives. This study was conducted to determine the levels of HbF in SCD pediatric patients treated with hydroxyurea therapy . Subjects and methods. Ninety one Sudanese SCD pediatric patients who referred to JafaarIbnouf hospital in Khartoum city, Sudan included in this study. Sixty one of patients had been treated with 22 mg /Kg/ day of HU for seven months, those patients known as group T , and thirty patients had been treated with conventional treatment used as control known as group C. 2.5 ml blood sample was collected from each participant in Ethylene Diamine Tetra Acetic Acid (EDTA) container. Hb F level was determined by using denaturation method. Results. The mean ± Standard deviation (Mean±SD) for participators age was (6.0±2.9) mod=3.0 years. The results showed that the mean of HbF Level in group T was 08.87±02.15 that found to be raised when compared with the mean of Hb level in control group (C) which was (0.8±0.2).

Keywords. Sickle cell disease, Fetal hemoglobin, Hydroxyurea, Sudanese patients.

Introduction

Sickle cell anemia (SCA) is a common genetic disorder that cause considerable morbidity and mortality throughout the world, and 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 included homozygous (Hb SS) sickle cell disease or sickle cell 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 hemoglobins Gower I, Gower II and Portland. 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 easer, giving fetus better access to oxygen from the mother's bloodstream.[6] Moreover, HbF is known to inhibit the polymerization of HbS and different agents able to increase HbF production have been introduced 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, 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)Somepatients also had increases in their anaerobic muscular performance and aerobic cardiovascular fitness.(8) The hemoglobin S–containingerythrocytes became less dense, and hemolysis was reduced. Thesechanges and the reduction in painful episodes preceded the increasein 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.

Materials and Methods

Ninety one SCD patients who referred to JafaarIbnouf hospital in Khartoum city, Sudan informed about the study objectives and agreements for their participation were obtained. Sixty one of patients had been treated with HU called T group , and thirty patients had been treated with conventional treatment called C group .Patients samples selected randomly to be matched in age, sex with control in Khartoum state. 2.5 ml of venous blood 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 there optimized over time in order that during the time of exposure to alkali most of the HbA is denaturized while the HbF is largely unaffected. Before the exposure to alkali, all the hemoglobin forms are transformed in the more stable Cyanomehtemoglobin form by means of treatment with Drabkin's reagent. An optimized version of the preliminary method has been proposed by Pembrey(11). So Hb F estimated by denaturation method to measure the percentage of Hb F in a mixture of hemoglobins(12).Sodium hydroxide is added to a lysate and, after a set time, denaturation is 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 is measured. The proportion of alkali-resistant HbF is then calculated as a percentage of the total amount of hemoglobin present (15).

Procedure.

0.25 ml of lysate was added to 4.75 ml of cyanide to make a hemoglobin cyanide (HiCN), after that 2.8 ml of HiCN transferred into a new glass tube and allowed to be equilibrated 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 using the Whatman paper No.42 into a clean test tube. This filtrate contained alkaline- resistant hemoglobin (HbF). Total Hb was measured by transferred of 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 at 420nm against water blank. The percentage of alkali- resistant was calculated as followed :-

Hb F % = a 420 alkai-resisitant hemoglobin × 100

A 420 total Hb× 100

The Results

The mean of participators age in this study was (6.09±2.98) mod=3.00 years. Sixtey one of patients were treated with HU and remaining are treated with other drugs.

Level of Hb F in group T is (8.00±2.59) which was found to be raised significantly when compared with groups C (0.81±0.21) (0.47±0.28) respectively(P=0.000). Table3.

Table.1.Frequency of patients treated and untreated with hydroxyurea

	Number
Patients treated with hydroxyurea	61
Patients not treated with hydroxyurea	30

Table:2Hb F Levels and P values in study groups

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

Discussion

Sickle cell anemia is a well known haemoglobinopathies was 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 (Hb S 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 Hb F levels (Mean ± SD) when groupT compared with group C, (P=0.00), as shown in table 2. This finding agreed with several studies such as Multicenter Study of Hydroxyurea (MSH) and Griffin P. Rodgers"*et al*,1999 [(14)] , Past, Adragna NC "*et al*" in1994 [(15)] , Steinberg MH and his colleagues in 1997, and recent researches by Mary Catherine Beach and others [(16)] .Also this results similar to the results of study done in India by HraminderSingh,*etal*, 2011 reported that the level of HbF was increased in SCD patients treated with HU [(17)]. Also this study proved that the gene of SCA is quite prevalent in the West of Sudan..

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