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Gamma Globin Gene "Switch On" by Dietary Source of Short Chain Fatty Acids as a Therapy for the Management of Sickle Cell Anaemia in Patients Attending Federal Medical Centre Gusau, Zamfara State

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Abstract
The effect of feeding Cow's Milk butter as a nutritional source of short chain fatty acids on the level of foetal haemoglobin (Hb F) in patients with Sickle Cell Disease (SCD) was studied. A significant increase ($P<0.05$) was observed in the level of Hb F (1.0% to 2.5%) in all the patients treated with Cow's Milk butter for a period of 6 months (180 days). An increase in the level of Hb F in the patients is an indication of molecular "switch on" of the gamma globin gene responsible for the production of gamma globin chain found in Hb F. Increase in production of Hb F can serve as a therapy for the management of sickle cell anaemia (SCA).
Key words: Sickle Cell Anaemia, Foetal Haemoglobin, Short Chain Fatty Acids, Nutritional Therapy and Gamma Globin chain.

INTRODUCTION

Sickle cell anaemia (SCA) was first reported by Herrick (1910) as a peculiar elongation and sickling of Red Blood Cell (RBC) occurring during severe anemia. The disease is a chronic haemolytic molecular disease (Pauling *et al,* 1949) that is genetically transmitted as an autosomal recessive disorder (Neel, 1949). The disease is one of the most severe genetic disorders in the world and it follows a more severe clinical course in Africa than the rest of the world. The disease being genetic, has no definitive cure, and an alarmingly high rate has been reported in Nigeria, especially among children from rural areas (Attah and Ekere,1975). Nigeria has the largest population of people with Sickle Cell Disease in the world, with over 150,000 babies born with the condition every year (Alex, 2015). Homozygous for the abnormal gene suffer from SCA while the heterozygous state is asymptomatic (Warsy *et al,* 1985). The molecular abnormality occurs in the beta globin chain of haemoglobin (Hb) in which glutamic acid, an acidic amino acid at position six of the chain is replaced by valine owing to mutation in the beta globin gene (Ingram, 1957).

The main pathophysiology of SCA is Hb polymerization that leads to erythrocyte rigidity and Vasoocclusion. The polymerization of deoxygenated Hb S is the primary event in the molecular pathogenesis of SCA resulting in a distortion of the shape of the RBC and a marked decrease in its deformability (Bunn, 1997).These rigid cells are responsible for the Vasoocclusive phenomina that are the hallmarks of the disease (Bunn, 1997). Recurrent episodes of Vasoocclusion and inflammation may results in damage of vital organs in the body.

The therapy for SCA is usually limited to supportive care during painful crisis, treatment of infections and care of the affected organs and maintenance of the steady state by the removal of the environmental factors known to precipitate a crisis (WHO, 1972). In severe case, the treatment include blood transfusion, hydration, use of antibiotics as well as oxygen therapy. Antipyretics and analgesics are commonly use (Pearson,1983). However, based on the understanding of the molecular pathogenesis of SCD, a number of independent approches to therapy have been proposed and developed (Bunn,1997). Therotically, one can change the DNA, transplant bone marrow, increase foetal haemoglobin (Hb F) synthesis and provide conditions or compounds which can increase erythrocyte oxygen affinity, decrease deoxyhaemoglobin S polymerization and decrease sickle cell membrane damage (Brewer *et al*, 1977 and Brewer and Bereza, 1982). However, interest in therapy of SCA by treatment of the molecular defect of the disease resulted in an observation that an unusually high level of Hb F plays a significant role in rendering the clinical course of the disease benign, suggesting the possibility of finding drugs or antisickling agents that would decrease the production of adult Hb in favour of Hb F (Pembrey *et al,* 1978). Hb F has a higher oxygen affinity under physiological conditions than does Hb A (White *et al*, 1978). Platt *et al* (1991) reports that the levels of Hb F in patients with SCA correlate inversely with the severity of the disease. Patients with the highest level of Hb F live longer and experience fewer painful crisis (Platt *et al*, 1991).

A number of substances and drugs are known to "Switch on" the gamma globin chain genes and "turn off" some of the beta genes resulting in the production of Hb F. Among such drugs are 5 - azacytidine that can increase Hb F levels in baboons (Desimone *et al,*1982) and human subjects (Ley *et al,* 1982). Hydroxyurea has been shown to increase the production of Hb F in non – human primates as well as in patients with SCD (Platt *et al, 1984* and Charache et *al*, 1987). Short chain fatty acids and their derivatives also appear to induce Hb F production in sheep fetuses (Perrine *et al,* 1989), in normal baboon (Constantoulakis *et al* 1989) and in patients with SCD (Perrine *et al*, 1993 and Sher *et al* (1995). Stimulating Hb F production by increasing gamma globin stynthesis in patients with SCD would, if the production of sickle cell beta globin decreases, have a " sparing" effect on the formation of intracellular polymers. Increased levels of Hb F decrease the tendecy towards intra cellular polymerization of sickle Hb that charecteized SCA(Rodgers *et al*, 1990). Butyric acid and other short chain fatty acids(C_3-C_8) are mostly found in animal fat, with milk fats having substantial amount (Matazu *et al*, 2004). . Cow Milk butter (Manshanu – Hausa) is commonly consumed when fried into liquid in Northern Nigeria. There is no known toxic effect reported on consumption of Cow Milk butter. This study investigate the effect of Cow milk butter as a dietary source of short chain fatty acids in the production of Hb F that can serve as a therapy for the management of SCA.

MATERIALS AND METHODS

Chemicals

All chemicals used in this study are of analytical grade. They were purchased from British Drug House (BDH) chemicals, Poole, England.

Informed Consent

Parental consent and that of the authorities of Federal Medical Centre (FMC) Gusau, Zamfara State were obtained before the patients were recruited for the study. Ethical clearance was also obtained and all the patients and their parents were briefed on the nature of the study before commencement of the feeding protocol. Compliance was ensured and no drug and diet was allowed during the study period that may influence the parameter to be analyzed.

Sickle Cell Anaemia Patients

Ten (10) sickle cell anaemic patients (6 males and 4 females) aged 5 – 12 years attending the SCA clinic of FMC Gusau were recruited for this study. The genotype of each patient was confirmed using Hb electrophoresis.

Feeding Protocol

The patients were given a daily dose of 3 spoon full (15ml) of fried Cow milk butter(1.25mg/ml) as a dietary source of short chain fatty acids for a period of 6 months (180 days).

Blood Sample collection

Four (4) millitres of Venopuncture blood were collected from each of the patients using a plain plastic syringe by the Medical officer in charge of SCA clinic of FMC Gusau. The blood was placed in a labeled stoppered vial containing EDTA. The blood was collected for base line and serial analysis after every 20 days throughout the six month of the feeding protocol. All blood samples collected were immediately analyzed.

Hb Electrophoresis

Hb Electrophoresis of each patient blood was done according to the modified method of Jain and Dar (1981). The Hb electrophoresis was done to confirmed the genotype of each patient recruited for the study.

Determination of Hb F

The Hb F content of each patient blood sample was determined using the method of Betke *et al,* (1959). The method is based on the resistance of Hb F to alkali denaturation. Hb F shows a decrease rate of alkali denaturation, while other Hbs will be denatured within one (1) minute of exposure to an alkali solution (Dacie and Lewis, 1984). To 2.8ml of cyanmethaemoglobin (HiCN) solution, 0.2ml of 1.2mol/litre Sodium Hydroxide was added and mixed thoroughly. The mixture was allowed to stand for exactly 2 minutes at room temperature. 2ml of saturated Ammonium Sulphate were added and then mixed thoroughly. The mixture was allowed to stand for 10 minutes and then filtered through a whatman No 42 filter paper. The absorbance of the filtrate was taken at 540nm. A 25% standard was made by adding 0.7ml of the HiCN solution to 4.3ml of Drabkins solution. The percentage Hb F was calculated as follows :Hb F(%) = [A_{540} test x 25]/[A_{540} standard]

RESULTS AND DISCUSSION

Table 1 shows the Hb F content of the ten (10) sickle cell anaemic patients when treated with fried Cow Milk butter as a dietary source of short chain fatty acids for a period of 6 months (180 days). There was a significant increase ($P<0.05$) in the mean Hb F content of the patients from 1.0% to 2.5%. Treatment of SCA patients with short chain fatty acids could results in some haematological response which Sher *et al* (1995) defined as an increase in the Hb concentration of at least 2g per deciliter in patients with thalessemia and as a two fold increase in Hb F in patients with SCD.

A number of studies have reported an increase in Hb F level when patients with SCA or other beta globin disorders were treated with short chain fatty acids which are regarded as antisickling agents. Perrine *et al,* (1993) reported that in patients with beta haemoglobinopathies, butyrate, a natural fatty acid, can significantly and rapidly increase foetal globin production to levels that can ameliorate beta globin disorder. An *in vivo* and *in vitro* studies with sodium Butyrate (NaB), an analogue of butyric acid, resulted in a stimulation of Hb F production in adult baboon (Constantoulakis *et al*, 1989). Similarly, Perrine *et al* (1988) have also shown that butyrate can inhibit gamma to beta globin gene switching in Vitro. Short chain fatty acids can directly induce the expression of gamma globin gene, resulting in the production of Hb F (Bunn, 1997). There was an evidence for selective stimulation of gamma globin gene by butyrate, with a relatively safe and fairly specific effect in maintaining foetal globin gene expression (Perrine *et al* 1993). This result in an increase in the synthesis of Hb F, which inhibit polymerization of Hb S.

In patients with SCD, when the Hb S is deoxygenated, the amino acid replacement result in a hydrophobic interaction with another Hb molecule, triggering an aggregation into large polymers. The polymerization of deoxygenated Hb S is the primary event in the molecular pathogenesis of SCD (Bunn, 1997), resulting in a distortation of the shape of the red cell and a marked decrease in its deformability. These rigid cells are responsible for the Vaso – occlusive phenomina that are the hallmark of SCD (Bunn, 1997). "Switching on" the gamma chain genes or "turning off" some of Hb F had been considered a treatment of SCA (Huntsman and Lehmann, 1974). Vanderploeg and Flavell (1980) reported that the gamma globin gene expression was found to be dependent on the extent of methylation of DNA sequence near the gamma genes. Perrine *et al* (1993) suggested that a further trial of the butyrate treatment is warranted to dtermine long term tolerance and efficacy in patients with SCA or beta thalessemia. Miller *et al* (1987) reported that butyrate has low toxicity

Table 1: Percentage(%) Hb F Value of SCA Patients treated with Dietary Source of Short Chain Fatty Acids.

	Treatment days									
Patients No.	**0**	**20**	**40**	**60**	**80**	**100**	**120**	**140**	**160**	**180**
1	1.2	ND	1.2	1.4	1.5	1.7	1.9	2.2	2.4	2.7
2	0.8	0.9	1.0	1.2	1.3	1.4	1.6	1.9	2.1	ND
3	0.9	0.8	0.9	ND	ND	1.7	1.9	2.0	2.2	2.5
4	1.6	1.6	1.7	1.9	2.0	ND	2.5	2.6	2.8	30
5	1.0	1.1	1.2	1.5	1.8	1.9	2.0	2.1	2.4	2.6
6	1.1	1.2	1.3	1.4	1.7	1.8	1.9	2.2	2.5	2.7
7	0.7	0.7	0.7	0.9	1.1	1.3	1.3	1.5	1.7	1.9
8	0.8	0.9	1.0	1.1	1.3	1.4	1.6	1.8	1.9	2.2
9	0.9	0.9	0.9	1.1	1.2	1.4	1.7	1.8	2.2	2.5
10	1.0	1.1	1.2	1.4	1.6	1.7	1.9	2.1	2.3	2.5
Mean	1.0 ± 0.20	1.0 ± 0.09	1.1 ± 0.21	1.3 ± 0.22	1.5 ± 0.21	1.6 ± 0.24	1.8 ± 0.26	2.2 ± 0.29	2.3 ± 0.22	2.5 ± 0.21

ND = Not Determined

CONCLUSION AND RECOMMENDATION

SCA being a genetic disorder has no definite cure. The disease can only be managed. A normal therapy for the disease include the use of drugs (antpyretics and analgesics) and blood transfusion in severe case. The use of nutritional source of short chain fatty acids could serve as a molecular therapy for the management of SCA. Short chain fatty acids can induce the expression of gamma globin gene resulting in the production of gamma globin chain found in Hb F. A high level of Hb F is known to inhibit polymerization of Hb S. Hb F also has a higher affinity for Oxygen than Hb A. From the result of this study, it could be concluded that consumption of Cow milk butter can serve as a therapy for the management of SCA, a molecular disease. Consumption of Cow milk butter over a long period of time may possibly result in the accumulation of short chain fatty acids in the body. A further study on the method of frying the Cow milk butter into liquid is recommended to be carried out to reduce the possible destruction of the fatty acids during heating.

A study using large number of patients and extending the feeding period to one year is also recommended in order to come up with a better conclusion on the role of Cow milk butter as a nutritional source of short chain fatty acids and their use as a molecular therapy for the management of SCA.

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Short-chain fatty acids, e.g. propionic acid (PA), are one of the principal products of the dietary fiber fermentation by microbiota. This leads us to hypothesize that PA could constitute the elusive link between colonic-microbiota metabolism and the physiology of human adipose tissue. To test this hypothesis we formulated the following research aims, after reviewing the recent knowledge about metabolic, immunological and potential adverse effects of PA: 1. To investigate the influence of PA on the production of various markers of physiologically relevant functions by human adipose tissue ex v... Short Chain Fatty Acids and Inflammation. Mediterranean Style Diets can Target Immune and Metabolic Outcomes Associated with Schizophrenia. Conclusions.Â Publications implicating premature mortality as a characteristic of schizophrenia have been ongoing in the literature for the past few decades. Some of these past reports have suggested that attenuated lifespan may be independent of schizophrenia symptom chronicity. During the 1960's the primary causes of mortality in schizophrenia were thought to be due to the following diseases or illnesses: infection, cardiovascular-renal, neoplasm, endocrine and metabolic, suicide/accident or other external causes, and other disease and unspecified causes (Niswander et al., 1963). Several short-chain fatty acids (SCFAs) specifically stimulate transcription in the Î³-globin gene promoter through histone deacetylase HDAC inhibition, resulting in global histone hyperacetylation [5, 47]. In contrast, some studies argue that globin histone hyperacetylation induced is not the primary mechanism of SCFA [5]; yet, HDAC inhibitors are often potent Î³-globin inducers [47, 48].Â In erythroid precursor cells, rapamycin preferentially induce Î³-globin mRNA accumulation, while being only minor for Î²-globin and none for Î±-globin mRNAs [49]. As its HbF-inducing effect is not related to cytotoxicity and cell growth inhibition, scientists are very interested in further studying if the enhancement of Î³-globin mRNA medicated by rapamycin is associated with XmnI polymorphism [49]. anemia. â€¢ Impaired globin synthesis. Thalassemia, severe protein deficiency. â€¢ Due to quantitive deficiency of Aplastic anemia, bone marrow replace-. hematopoietic progenitor cells ment by neoplastic cells (myelophthisis). â€¢ Impaired response to erythropoi-etin or decreased erythropoietin Anemia complicated chronic renal failure. production. â€¢ Caused by defective DNA synÂ Patients with acute gastroin-testinal blood loss sometimes have an elevation of blood urea nitrogen owing to impaired renal blood flow and perhaps to the absorption of digested blood protein. Patients with severe acute blood loss require resuscitation emergently. Chronic blood loss leads to the iron deficiency anemia. Induction of gamma globin expression to certain target levels reduces clinical severity in sickle cell disease, globin imbalance, and anemia in the beta thalassemias. However, achieving high level expression in diverse patients with variable baseline HbF levels is clinically challenging. To determine whether different therapeutics have complimentary molecular actions that could be combined for higher efficacy, we evaluated a panel of oral fetal globin-inducing therapeutics in clinical use or trials for effects on transcriptional suppressors of HBG, including components of the NuRD complex, LSD1 and HDACs 1-3, and the downstream repressor BCL11A, in erythroid progenitors cultured from sickle.