

Alterations of Homocysteine in Sickle Cell Anaemia

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ABSTRACT

The serum homocysteine levels of sickle cell patients attending General Hospital Owerri, Nigeria were evaluated to determine whether or not the serum levels of these homocysteine were normal. One hundred confirmed sickle cell patients (HbSS), and thirty sickle cell patients in crisis age 5 to 30 years were selected. One hundred normal subjects (HbAA) age 5 to 30 years were used as control. The mean level of homocysteine was significantly increased in sickle cell anaemia ($p < 0.05$), when compared with the control (HbAA). However, the mean level of homocysteine was significantly more increased in sickle cell crisis compared with sickle cell anaemia. The result suggests, that sickle cell anemia is linked with elevated homocysteine level, which could lead to increased risk of hypercoagulability and thromboembolic complications as well as cardiovascular risk in sickle cell anaemia.

Key words: Homocysteine, Sickle cell anaemia, General Hospital, Owerri.

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INTRODUCTION

Homocysteine is an amino acid and breakdown product of protein metabolism. It is biosynthesized from methionine by the removal of its terminal C methyl group (Meekoo et al. 2004). It can be recycled into methionine or converted into cysteine with the help of certain B-vitamins. It has been linked to an increased risk of heart attacks and strokes. Elevated homocysteine levels are thought to contribute to plaque formation by damaging arterial walls. High levels may also act on blood platelets and increase the risks of clot formation; however, whether high levels of homocysteine actually cause cardiovascular disease has yet to be agreed upon (Selhub, 1999). Homocysteine may be requested as part of a screen for people at high risk for cardiac attack or stroke. It may be necessary in individuals who have a family history of coronary artery disease without other known risk factors, for instance smoking, high blood pressure, or obesity. Indeed, the actual role that

homocysteine plays in the progression of cardiovascular disease has not been established, so the utility of the screening test continues to be questioned (Sibrian-Vazquez et al., 2010). Low serum homocysteine may be due to genetic insufficiencies of the enzymes needed for its metabolism, as well as nutritional deficits in vitamin cofactors, or to cardiovascular disease. Similarly, low intake and plasma concentrations of folate and vitamins B₆ and B₁₂ have been associated with increased serum homocysteine. Urine and blood homocysteine may be used to help diagnose homocystinuria if a doctor suspects that an infant or child may have this inherited disorder (pandey et al., 2012). Amino acid cysteine is biosynthesized through homocysteine. Cystathionine β -synthase catalyses the condensation of homocysteine and serine to give cystathionine. This reaction uses vitamin B₆ as a cofactor. Cystathionine γ -lyase then converts this double amino acid to cysteine, ammonia,

Table 1. Homocysteine levels in sickle cell anaemia and control.

Parameters	Homocysteine ($\mu\text{mol/L}$)	t-value	P-value
HbAA	13.64 \pm 4.8	-----	-----
HbSS (n=100)	24.22 \pm 6.2 *	3.255	0.006
HbSS-crisis (n=30)	27.9 \pm 6.58*	4.054	0.001

*Significantly different from control at $P < 0.05$.

and α -ketobutyrate (Lutfi et al., 2014). Homocysteine alterations have been associated with sickle cell disease (SCD). SCD is a genetic disorder of red blood cell synthesis that can affect the skeletal system due to accelerated hematopoiesis and bone infarction (Nnodim et al., 2014). People with SCD possess high level of sickle cell haemoglobin. Hence, sickle cell haemoglobin (Hbs) is a form of abnormal haemoglobin occurring in the red cells of sickle cell disease patients. Really, it is an important cause of morbidity and mortality among black individuals (Nnodim et al., 2014). The impact of this disease on society is very high. Many may have been dying of SCD, yet not much research has been done in Nigeria towards better understanding and diagnosis (Nnodim, 2014). It is on the light of the above that this study was embarked upon to evaluate status of homocysteine in SCD patients. This study was equally undertaken so that the knowledge gained from the research work may suggest a better understanding of SCD.

MATERIALS AND METHODS

One hundred sickle cell patients (HbSS) diagnosed by haemoglobin electrophoresis, aged 5 to 30 years were selected for the study. One hundred HbAA normal subjects were used as control. Also, thirty sickle cell patients in crisis were also involved. Both male and female were equal. Blood sample: In all subjects, 5 ml of venous blood was collected into a non anticoagulated tubes. The samples were spun in a Wisterfuge (model 684), centrifuge at 1000 g for 10 min and the serum collected into a clean dry bijoux bottle. Serum homocysteine was estimated. Informed consent of the participants was obtained and was conducted in line with the ethical approval of the hospital.

Biochemical assay

Serum homocysteine was determined by enzymatic colorimetric method for the quantitative determination of homocysteine, using Globe diagnostics kit, Italy. It is based on a series of enzymatic reactions causing a decrease in absorbance value due to reduced Nicotinamide adenine dinucleotide (NADH) to oxidized Nicotinamide adenine (NAD⁺). Homocysteine concentration in the sample is directly proportional to the quantity of NADH converted to NAD⁺ (\AA 340 nm).

Statistical analysis

The results were expressed as mean \pm standard deviation. The statistical evaluation of data was performed by using student's t- test. The level of significance was calculated at $P < 0.05$.

RESULT AND DISCUSSION

The mean level of homocysteine was significantly increased in HbSS and HbSS- crisis when compared with the control ($P < 0.05$) (Table 1). SCD is a genetic disorder characterized by hypercoagulable state and increased risk of thromboembolic events, a rare but significant complication of SCD (Nnodim et al., 2014). Ischemic complications are a major cause of morbidity and mortality in patients with SCD (Lubinska, 2006). The results of this study indicated a significant increase in the level of homocysteine in sickle cell anaemia compared to control. This implies that high homocysteine levels are associated with sickle cell anaemia. Homocysteine (Hcy) is a sulphur-containing amino acid. It is formed from the conversion of methionine into cysteine, which metabolized through 1 of 2 pathways where vitamin B₁₂- and folate-dependent re-methylation pathway that regenerates methionine, or a vitamin B₆-dependent trans-sulphuration pathway that converts Hcy to cysteine (Jager et al., 2001; Drzewoski et al., 2000). Hence, low levels of these vitamins/co-factors are associated with hyper-homocysteinemia, which can be classified as mild, moderate, or severe. The increases in serum Hcy concentration can be linked to genetic defects in the enzymes involved in homocysteine metabolism. Also, the increased homocysteine could be associated with nutritional deficiencies in vitamin co-factors, as well as other factors such as chronic diseases (Houston et al., 1997; Ebesunun and Obajobi 2012). Patients with sickle cell anemia have significantly higher mean homocysteine level compared to control. This observation is consistent with the results of other studies (Martí-Carvajal et al., 2009; Mei et al., 2010).

Conclusion

Sickle cell anemia therefore is linked with increased homocysteine level, which. promote the elevated risk of hypercoagulability and thromboembolic complications.

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