Effects of hyperbilirubinaemia on fructosamine assay in sickle cell anaemia

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Abstract

Background: *Studies have shown that high levels of bilirubin interfere with the colorimetric estimation of fructosamine both in-vivo and in-vitro. None of the previous studies was conducted in a defined clinical disorder.*

Aim: *The aim of this study was to demonstrate the effects of hyperbilirubinaemia on the measurement of fructosamine in patients with sickle cell anaemia (Hb SS), a highly prevalent genetic disorder in our society.*

Methodology: *Serum fructosamine, glucose, albumin and total bilirubin were measured in 150 Hb SS patients and 100 healthy volunteers as controls.*

Results: *The mean fructosamine concentration was statistically similar in Hb SS patients and controls. No statistical difference was found between the mean fructosamine concentration at different levels of serum total bilirubin in Hb SS patients and controls*

Conclusion: *These results show that high levels of bilirubin in patients with sickle cell anaemia, in the steady state, do not interfere with the colorimetric estimation of fructosamine.*

Keywords: Hyperbilirubinaemia, Fructosamine, Sickle cell anaemia

Introduction

Hyperbilirubinaemia punctuates the course of sickle cell disease due to chronic haemolysis and defective bilirubin metabolism resulting in increased levels of conjugated and unconjugated bilirubin in the blood.[1,2] Also, a variety of clinical conditions in sickle cell disease result in acute, marked elevations of both total and conjugated bilirubin together with hepatic tenderness and enlargement. These conditions include viral hepatitis (often transfusion related), intrahepatic sickling (sickle cell hepatopathy) and intrahepatic cholestasis (both benign and progressive).[3,4,5,6]

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No conflict of interest have been declared by the author

The interfering effects of high serum levels of bilirubin on the estimation of serum fructosamine (an intermediate- term glycaemic index) have been reported in literature. Some of the studies observed that high serum levels of bilirubin positively interfered with fructosamine assay[7,8] while others observed no effect.[9,10] None of the quoted studies was conducted in patients with defined clinical condition.

The aim of this study therefore, is to evaluate the effect of hyperbilirubinaemia on fructosamine estimation in patients with sickle cell anaemia, a highly prevalent clinical condition in our environment.

Materials and Methods

A total of two hundred and fifty subjects were studied. They were made up of:

(I) One hundred and fifty patients with sickle cell anaemia (Hb SS), aged between 15 and 40 years. All the patients had Hb SS phenotype and were in steady state, attending the sickle cell disease clinic of the Ahmadu Bello Teaching Hospital (ABUTH), Zaria.

(II) One hundred healthy volunteers aged between 15 and 60 years served as controls. They all had haemoglobin AA phenotype who presented at the blood donor bay of ABUTH, Zaria.

The study was approved by the Ethical Committee of the hospital and informed consent was duly obtained from each subject.

About five milliliters of venous blood was collected from each subject into plain tube from which serum was obtained into clean bijou bottle after centrifugation. Total bilirubin and albumin were measured on the same day of blood collection while the remaining sera were stored frozen at -20^{o} C and batched tested for fructosamine estimations fourthnightly.

Albumin and total bilirubin were measured by standard operating procedures,[11,12] while fructosamine was estimated using the nitroblue tetrazolium method.[13]

Table 1: Serum fructosamine, random glucose, albumin & total bilirubin (mean ± SD and Range) in Hb SS patients and controls

	Hb SS (n=150)	**Controls (n=100)**	**P value**
Fructosamine (mmol/L)	1.3 ± 0.36 (0.7 – 1.9)	1.4 ± 0.3 (0.6 – 2.2)	0.05
Glucose (mmol/L)	4.3 ± 1.1 (2.8 – 5.6)	4.6 ± 0.9 (3.0 – 5.8)	>0.05
Albumin (g/L)	40 ± 4.7 (28 – 52)	38 ± 2.8 (30 – 45)	0.05
Total Bilirubin (µmol/L)	43 ± 8.8 (11 – 160)	7.4 ± 1.4 (4 – 18)	0.05

SD - Standard Deviation

Statistical analysis was performed using descriptive and inferential statistical test on the Statistical Package for the Social Sciences (SPSS) version 20. A p-value less than or equal to 0.05 was used to define statistical significance.

Results

The mean values of serum fructosamine, glucose and albumin were statistically similar in both Hb SS patients and controls (P > 0.05, Table 1). However, the mean total bilirubin concentration was significantly higher (P Â 0.05) in Hb SS patients than the controls. No correlation was found between fructosamine and total bilirubin in the Hb SS patients (r= -0.16, P > 0.05). Similarly, no correlation was observed between fructosamine and bilirubin fractions (conjugated and unconjugated) among the Hb SS patients.

Table 2 shows no statistical difference between the mean fructosamine values at different concentrations of serum total bilirubin in Hb SS patients and the controls. Also, no significant statistical difference was found between fructosamine levels at different concentrations of both conjugated and unconjugated bilirubin in Hb SS patients and controls.

Discussion

Random/fasting glucose measurement is a reflection of the glycaemic state at the time a blood sample is taken. Over the years other tests have been developed to give a better clinical picture of how the glycaemic control has been in the diabetic patients over a period of time and are thus more advantageous than measurement that give the value of blood glucose concentration at a point in time. These tests are glycated haemoglobin (HbA1c) and fructosamine. Thus, fructosamine and glycated haemoglobin (HbA1c) are used as medium-term and long-term indicators respectively, to monitor glycaemic control in diabetics. Fructosamine is a generic name given to a compound known as plasma protein ketoamines.

It is formed by a spontaneous non-enzymatic reaction between a carbonyl group of a glucose molecule and an amino group of plasma proteins, mainly albumin.[14] It provides the clinician with an index of glycaemia over a short period of time (2-3 weeks) due to the high turnover of human serum albumin in blood and the degree of glycation in serum proteins.[15,16] HbA1c, on the other hand, describes a chemically stable conjugate of haemoglobin with glucose. It is formed slowly,

Table 2: Fructosamine concentrations (mean and SD) at different levels of serum total bilirubin in Hb SS patients

Total Bilirubin (µmol/L)	Number of patients (n)	Fructosamine (mmol/L) mean (SD)	t	p
11 – 40	78	1.29 (0.33)	1.93	Ã0.05*
41 – 70	64	1.33 (0.35)	1.13	Ã0.05*
71 – 100	2	1.6 (0.28)	0.69	Ã0.05*
101 – 130	4	1.7 (0.17)	1.25	Ã0.05*
131 – 160	2	1.5 (0.21)	0.17	Ã0.05*

SD - Standard Deviation
**p > 0.05: Hb SS Vs Controls – no significant difference*

non-enzymatically, and irreversibly at a rate that is proportional to the concentration of glucose in the blood. The level of glycated haemoglobin in a blood sample provides a glycaemic history of haemoglobin glycation over the life span of the erythrocyte, the cell that contains the haemoglobin. Thus, HbA1c indicates the average glucose concentration over the preceding 6-8 weeks, as the average life span of a red blood cell is about 120 days.[17]

However, this test is inaccurate in patients with haemoglobinopathies, such as sickle-cell disease, recent change in diet over a 6-week period, blood loss accompanied afterward by blood transfusions and haemolytic anaemia.[18,19,20] In such cases, serum fructosamine plays an important role in glycaemic control and diabetes monitoring.

Patients with sickle cell disease have a wide range of high serum levels of bilirubin, an analyte that has been reported to interfere with the colorimetric estimation of fructosamine in the laboratory.[1,21,22] Various reasons have been suggested as being responsible for the interference observed in those studies.[7,8,9,23] These include the type of instruments used for the measurement of fructosamine, the colour of the jaundiced sample and presence of high levels of bilirubin in the sample. Anaja and colleagues (2003) observed that significant influence of bilirubin on fructosamine estimation was mainly seen in individuals with serum bilirubin concentrations above 100 µmol/L.[8] This is in contrast to the findings of this present study in which no significant statistical difference was observed between the mean fructosamine values obtained at different concentrations of serum total bilirubin (up to a maximum of 160 µmol/L) in the Hb SS patients studied and the controls. In a study of patients with mild hyperbilirubinaemia (serum bilirubin concentrations between 5 and 83 µmol/L), Dominiczak *et al* (1989) did not demonstrate any significant difference between the mean serum fructosamine concentration in normobilirubinaemic subjects and patients with mild hyperbilirubinaemia.[4] They concluded that mild increase in serum bilirubin did not cause significant colorimetric interference in the assay of fructosamine.

Studies have also shown that raised serum levels of bilirubin is a common feature in patients with sickle cell anaemia due to chronic haemolysis and abnormal bilirubin metabolism.[1,2,3] Johnson *et al* (1982) observed in their study that 72 out of 100 patients with sickle cell anaemia had isolated elevation of serum bilirubin, with no other clinical or laboratory evidence of liver disease.[3] Total bilirubin concentration seen in these steady state conditions were usually less than 100 µmol/L[1,3] thus suggesting that Hb SS patients in the steady state are not likely to have high serum levels that can interfere with fructosamine estimations as confirmed in this study.

Severe hyperbilirubinaemia (levels up to 1000 µmol/L) that is seen in Hb SS patients with some forms of complications like intrahepatic cholestasis, sickle cell hepatopathy, viral hepatitis, are usually not persistent following the resolution of such complications.[2,3,4,24,25] Buchanan *et al* (1977) described high levels of bilirubin (up to 974.7 µmol/L) in six children with sickle cell hepatopathy but the hyperbilirubinaemia resolved spontaneously within 2 to 8 weeks with no subsequent recurrence.[4]

Unlike the previous studies on this subject matter, this study was conducted in a defined clinical disorder (sickle cell anaemia) that has high prevalence among the black African population, including Nigeria.[26,27] With the increasing incidence rate of non-communicable diseases both globally and locally, this study is imperative as more cases of diabetes mellitus is expected among diverse groups of individuals in our society.[28,29,30]

Conclusion/Recommendation

This study has clearly shown that the high serum levels of bilirubin commonly

encountered in steady state sickle cell anaemia do not interfere with the laboratory estimation of fructosamine. It is therefore recommended that fructosamine test can be carried out on sickle cell anaemia patients preferably during the steady state, as indicator of medium-term glycaemic control.

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