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Can glycated hemoglobin be a biomarker for iatrogenic pancreatic iron deposition due to repeated blood transfusion in β thalassemia major? A pilot study in north indian population

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ABSTRACT

Introduction: To evaluate HbA1c, SOD, NO in children with beta thalassemia major. **Materials and Methods:** Case control study was performed in LHMC, New Delhi. The study comprised of 47 cases of thalassemia major and 47 age and sex matched healthy controls. Whole blood samples were collected under aseptic conditions. HbA1c and SOD were estimated by commercially available kits and NO was estimated by modified Griess method. **Results:** HbA1c, SOD and NO levels were very significantly high in cases as compared to controls. NO and SOD were very significantly correlated to HbA1c. AUC was > 0.7 for all the parameters which suggests that patients with beta thalassemia major can be evaluated for risk of oxidative stress by estimating SOD or NO. HbA1c levels can be used to evaluate risk of secondary diabetes. **Conclusions:** Positive correlation was present between HbA1c with NO and SOD which suggests interplay between the various pathways responsible for the clinical manifestations of the disease. Repeated blood transfusions lead to iron deposition in pancreas which leads to secondary diabetes. HbA1c may be used to evaluate risk of iron overload in these patients but further studies with large sample size and detailed iron profile are required to use HbA1c as a biomarker of pathological iron deposition in pancreas.

KEYWORDS

Thalassemia;
Oxidative stress;
Glycated hemoglobin;
Iron overload.

INTRODUCTION

Beta thalassemia is one of the most common hemoglobinopathies caused by mutations in the beta globin genes ranging from missense to frameshift mutations. It is of two types depending upon the globin chain which undergoes defective synthesis. In beta thalassemia, the imbalance between the alpha and beta chains makes the developing erythrocytes more fragile leading to early damage, ineffective erythropoeisis and anemia[1].

Chronic anemia is the reason behind recurrent blood transfusions in thalassemia major to tide over ineffective erythropoiesis. The consequent iron overload is causative of oxidative stress consequent to the production of reactive oxygen species such as superoxide and peroxide ions[2]. This oxidative stress is responsible for growth failure as well as hepatic, cardiovascular, endocrine, and neurological complications in beta thalassemia

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major. This oxidative stress is mitigated by the anti oxidant defense mechanisms of the body[3,4].

Nitric oxide (NO) is one of the most potent vasodilators known which is essential to vascular homeostasis. Besides its role on the endothelium, it also plays an important role in the maintenance of vasomotor tone, limits platelet aggregation and ischemia-reperfusion injury, modulates endothelial proliferation, and possesses anti-inflammatory properties[5,6]. However, in the wake of oxidative stress, it can generate peroxynitrite radical which is a highly reactive free radical. Abnormal NO metabolism is a hallmark of thalassemia. As hemoglobin is liberated into plasma during hemolysis, it reacts with and destroys NO, resulting in abnormally high NO consumption and the formation of reactive oxygen species. This hampers activation of guanylyl cyclase leading to impairment of vascular endothelial function[7,8].

Glycated hemoglobin (HbA1c) is a retrospective indicator of blood sugar homeostasis during the past 3 months. The impact of thalassemia syndromes on HbA1c test results depend on the pathologic processes involved and the assay method employed.

MATERIALS AND METHODS

The study was conducted by the department of biochemistry. The study comprised of 47 cases of thalassemia major and 47 age and sex matched healthy controls. Whole blood samples were collected under aseptic conditions in anti coagulated vacutainers. Glycated hemoglobin was estimated using commercially available kits on Daytons analyser (Randox Industries, Atrium, UK). Superoxide dismutase (SOD) was estimated by commercially available kit provided by Randox. Nitric oxide (NO) was estimated by modified Griess method.

STATISTICAL ANALYSIS

The data generated from this study was analyzed by using Statistical software SPSS version 13. The values are expressed as mean ± S.D with their 95 % confidence intervals. The continuous quantitative variables were initially tested for normality by using Kolmogorov-smirnov test and Shapiro-wilk test. Pearson correlation, student t test were used for statistical analysis.

RESULTS & DISCUSSION

Thalassemias are a group of disorders characterized by defective globin chain synthesis consequently leading to ineffective erythropoiesis and consequent hemolytic anemia. Thalassemia is most common in individuals whose ancestors originated from the Mediterranean region, Africa, southern China, Southeast Asia, and India. These disorders are also associated with metabolic abnormalities, oxidative stress and vascular dysfunctions[9]. Our study evaluated the levels of superoxide dismutase, nitric oxide and glycated hemoglobin in children with beta thalassemia major.

Our study demonstrated statistically significant increase in the levels of superoxide dismutase (SOD) in the cases as compared to the controls (TABLE 1). Our findings are in concordance with similar studies carried out by Afanes[10] and Abdalla et al[11]. A Low SOD level in thalassemics has been reported by Dhawan et al[12]. Iron overload due to repeated blood transfusions leads to peroxidative damage due to increased free radical production in beta thalassemia major and the antioxidant machinery attempts to reduce tissue damage by scavenging these free radicals.

TABLE 1: Biochemical parameters of the study population

Parameter	Cases	Controls	p value
HbA1c (%)	8.92 ± 2.1	5.02 ± 0.85	<0.001*
SOD	33.7 ± 16.1	15.9 ± 7.9	<0.001*
NO	29.7 ± 16.6	4.96 ± 3.3	<0.001*

***very significantly**

One of the most important antioxidant enzymes present in the human body is superoxide dismutase. It exists in two isoenzyme forms- erythrocytic and cytoplasmic. Superoxide is the main reactive oxygen species which reacts with nitric oxide radical and forms peroxynitrite thereby causing oxidative stress and cellular damage. SOD is the essential antioxidant that decreases the formation of reactive oxygen species and oxidative stress thus protecting the cells from damage. Erythrocyte superoxide dismutase protects the erythrocyte from being damaged during oxidative stress[13,14].

Besides oxidative stress, hemolytic anemias such as thalassemia are also associated with vascular abnormalities such as endothelial dysfunction. The underlying hemolysis initiates a deleterious impact on the delicate

arginine NO pathway[15]. The released hemoglobin destroys NO and initiates a myriad of other changes such as impairment of vascular function through the expression of adhesion molecules and potent vasoconstrictors such as endothelin 1[16]. The hemolytic process also releases arginase from the RBCs which limits the availability of arginine for polyamine synthesis essential for smooth muscle growth[17].

Our study demonstrated an increase in the levels of NO in the cases (TABLE 1). This may aggravate the oxidative process by forming peroxynitrite with superoxide radical. Our findings are in accordance with a study carried out by Naithani et al on 50 patients with thalassemia. SOD and NOx levels were significantly elevated in thalassemic children as compared to healthy controls[18]. Afanas demonstrated through his study in patients with thalassemia and fanconi's anemia that there is another pathway of free radical-mediated damaging processes in these pathologies, depending on the interplay between physiological free radicals superoxide and nitric oxide (NO)[19]. The central role of disordered NO metabolism in thalassemia is exemplified by the positive correlation of NO levels with the diseased status as well as an area under curve of 0.989 in ROC curve analysis (TABLE 2).

TABLE 2: ROC curve analysis

Parameter	AUC	95% CI	p value
HbA1c	0.966	0.91- 1.01	<0.001*
SOD	0.862	0.75- 0.97	<0.001*
NO	0.989	0.96 – 1.01	<0.001*

***very significantly**

Secondary diabetes is an established complication of transfusional iron overload[20]. Iron overload results in β-cell oxidant stress and decreased insulin secretory capacity secondary to β-cell apoptosis and desensitization of glucose induced secretion[21]. Elevated iron stores may also interfere with hepatic insulin extraction leading to peripheral Hyperinsulinemia[22,23]. Iron is a transition metal that can catalyze the conversion of poorly reactive free radicals into highly active free radicals. It has been suggested that formation of hydroxyl radicals catalyzed by iron may play a role in the development of diabetes because the highly active radicals can attack cell membrane, lipids, proteins, and DNA and cause tissue damage thus contributing to the development of insulin resistance[24-27].

Our study demonstrated a positive correlation between HbA1c with NO and SOD (TABLE 3). This implies interplay between the various pathways responsible for the clinical manifestations of the disease.

TABLE 3 : Pearson correlation analysis

Parameter	r value	p value
NO vs SOD	0.237	0.140
NO vs HbA1c	0.406	<0.001*
SOD vs HbA1c	0.673	<0.001*
Disease with SOD	0.519	0.001
Disease with NO	0.652	<0.001*
Disease with HbA1c	0.720	<0.001*

***very significantly**

Further research with larger sample size and detailed iron profile is recommended for correlation with glycated hemoglobin as a biomarker of pathological iron deposition in pancreas.

ABBREVIATIONS

Glycated hemoglobin (HbA1c),
Superoxide dismutase (SOD),
Nitric oxide (NO),
Area under receiver operating characteristic curve (AUC)

CONFLICT OF INTEREST

There is no potential conflict of interest among authors.

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