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ORIGINAL ARTICLE

Hypocholesterolemia and Increased Plasma 7-ketocholesterol Levels in Pediatric Sickle Cell Patients

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ABSTRACT

Introduction: Hypocholesterolemia is the most documented lipid abnormality in sickle cell disease which is also characterized by increased oxidative stress. We investigated plasma levels of oxysterols, oxidized cholesterol derivatives, in the plasma of sickle cell disease patients and compared with controls.

Material and Methods: Twenty steady state sickle cell disease patients and 8 healthy controls were enrolled in the study. 7-ketocholesterol and cholestane-3β,5α,6β-triol levels, were measured by an LC-MS/MS method. Total cholesterol levels were determined by enzymatic colorimetric method.

Results: Mean plasma total cholesterol levels were significantly lower (109.0±17.4 mg/dl versus 149.6±28.8 mg/dl) (p=0.002) and 7-ketocholesterol levels were higher (10.6±1.9 mg/dl versus 9.0 ±1.2 mg/dl) (p=0.033) in the sickle cell disease group versus control group. When patients were grouped according to their genotype, Sβ patients (N=10) had higher cholestane-3β,5α,6β-triol levels (p=0.033).

Discussion: To our knowledge this is the first study investigating plasma oxysterols, particularly 7-ketocholesterol and cholestane-3β,5α,6β-triol in sickle cell disease . We found a significant increase in mean 7-ketocholesterol levels in sickle cell disease patients compared to controls. Patients also had hypocholesterolemia that is typical in many sickle cell disease patients. Apart from being an oxidative stress marker, 7-ketocholesterol may be a modifier of plasma cholesterol concentration. The metabolic roles of cholesterol oxidation products in diseases warrant further research to explain the tremendous nuclear effects of these oxysterols.

Key words: Oxysterol, 7-ketocholesterol, cholesterol, hypocholesterolemia, oxidative stress

INTRODUCTION

Sickle cell disease (SCD) is a hemoglobinopathy caused by a point mutation in the beta globin gene. It is a chronic condition characterized by anemia, vaso-occlusion, inflammation and increased oxidative stress [1-3]. Patients with sickle cell disease have increased morbidity and mortality due to involvement of organs such as liver, spleen, kidney, lungs and brain [4]. Mutated hemoglobin (Hb) is unstable and decomposes easily to heme and globulin (9). This free heme further releases its iron that both heme and iron attacks membrane lipids producing oxidized lipids in various forms (10). Ischemia reperfusion injury developed as a result of vasoocclusive crisis also aggravates free radical production. There is an increasing number of reports on the lipid abnormalities in serum and erythrocytes of patients with SCD such as hypocholesterolemia [5-6], relative hypertriglyceridemia decreased HDL [6], decreased apolipoprotein AI and apolipoprotein B [7] compared to controls. Anemia, inflammation and oxidative stress are three pathological processes that may result with lipid abnormalities in sickle cell disease [8]. Various abnormalities concerning lipid metabolism were reported in patients with SCD in which hypocholesterolemia is the most documented lipid abnormality [5-6]. The chronic anemia results with overstimulation of erythropoiesis that consumes plasma cholesterol pool for new membrane synthesis [9-10]. There is a previous report which suggested a possible link between oxidative stress and

hypocholesterolemia in SCD patients whose plasma cholesterol levels were negatively correlated to hemolysate MDA levels [11]. Cholesterol is converted to more polar compounds by the addition of oxygen containing groups such as epoxy, hydroxy or ketone and these oxidative decomposition products of cholesterol are named oxysterols [12]. Various oxysterols are produced in body at lower quantities during cholesterol metabolism to perform recently understood metabolic roles. However, oxysterol concentrations increase in pathologic conditions. 7-ketocholesterol is the major, oxysterol assumed to be produced in vivo mainly by free-radical attack on biomembranes and on low density lipoprotein particle (LDL) [13]. Cholestane-3β,5α,6β-triol is another oxysterol produced during increased oxidative stress. Cholesterol oxidation products were previously investigated in the erythrocytes from SCD patients by two consecutive studies. In the first study, various oxysterols were inserted into normal and sickle RBC membranes to observe the effect on the membrane fluidity and it is concluded that oxidized cholesterol derivatives perturbate membrane dynamics and that this might contribute to membrane pathology in SCD [14]. In the next study 7-ketocholesterol levels were measured in the membrane of sickle erythrocytes and it was found that membranes from sickle erythrocytes contained higher 7-ketocholesterol levels than normal erythrocytes [15]. 7-position derivatives of cholesterol were also shown to induce cytotoxic effects on macrophages in vitro [16]. To our knowledge there is no report in the literature investigating oxysterol levels in the plasma of SCD patients.

MATERIALS and METHODS

Patients

We enrolled SCD patients who were under routine follow-up, free of vaso-occlusive crisis and transfusion for the last three months in the study. Two patients recovering from crisis were also analyzed for the below parameters. The diagnosis of SCD was established according to hemoglobin (Hb) electrophoresis and β globin gene mutation analysis. The control group was consisted of healthy children were of similar age without any acute or chronic disease with normal Hb electrophoresis. The study was approved by the Ethics Committee of Mersin University (2014-115).

Sampling

Blood was drawn into EDTA containing tubes kept on ice at least for 5 min, centrifuged at 2500 rpm for 10 minutes (Hettich, Germany). Plasma samples were separated, aliquoted and frozen immediately at –80°C.

Cholesterol and oxysterol measurements

We measured plasma total cholesterol levels with the enzymatic colorimetric method (Beckman-Coulter, Pasadena, CA, USA) and 7-ketocholesterol and cholestane-3β,5α,6β-triol levels by the LC-MS/MS (Schimadzu, Japan) with method from Jiang et al. [17].Statistics: The results were expressed in terms of arithmetic means ±standard deviation (SD). Nonparametric statistics were used. Difference between the two groups was determined by Mann–Whitney U-test. Correlation between the parameters was calculated by Spearman correlation coefficient, $p < 0.05$ was considered statistically significant. SPSS (SPSS 19.0 for Windows, Chicago, IL, USA) was used for statistical analysis.

RESULTS

Demographic characteristics and laboratory values of the patients are given on Table 1. Mean plasma total cholesterol levels were significantly lower and 7-ketocholesterol levels were significantly higher in the steady state SCD group when compared to the healthy control group as shown in Table 1. There was no significant difference between groups

Table 1. Age, sex, white blood cell (WBC) count, cholesterol and oxysterol levels in SCD patients and healthy children.

	Sickle cell disease N=20	Healthy N=8
Age	14.4 ±3.7	14.2 ±3.6
Sex (M/F)	13/7	6/2
WBC (x103cells/µl)	10.970 ±5.61 *	8.00 ±0.89
Total cholesterol (mg/dl)	109.0 ±17.4**	149.6±28.8
7-ketocholesterol (ng/ml)	10.6±1.9***	9.0 ±1.2
cholestane-3β,5α,6β-triol (ng/ml)	6.5 ±2.4	5.3±2.6

*p=0.001
**p=0.002
***p=0.033

Table 2. Total cholesterol and oxysterol levels in SCD patients grouped according to their genotype.

	SS N=10	Sβ N=10
Total cholesterol (mg/dl)	103 ±18.4	115.1 ±14.7
7-ketocholesterol (ng/ml)	10.2 ±1.5	10.9 ±2.2
cholestane-3β,5α,6β-triol (ng/ml)	5.0 ±2.4	8.0±1.2*

*p=0.02

for cholestane-3β, 5α, 6β-triol. However, when the patients were grouped according to genotype, either being SS or Sβ, cholestane-3β, 5α, 6β-triol levels were found to be significantly higher in Sβ group (Table 2).

DISCUSSION

To our knowledge this is the first study investigating plasma oxysterols, particularly 7-ketocholesterol and cholestane-3β, 5α, 6β-triol in the plasma of patients with SCD. We found a significant increase in mean 7-ketocholesterol levels in SCD patients compared to controls. Patients also had hypocholesterolemia that is typical in many SCD patients. Cholestane-3β, 5α, 6β-triol levels were higher in the patients with Sβ genotype than SS genotype..Oxysterols mediate various roles in cholesterol metabolism in addition to being cholesterol metabolites themselves. 7-Ketocholesterol is suggested to have a role in the inhibition of cellular cholesterol synthesis by binding to oxysterol-related-binding-protein related protein-2, depletion of which reduces cholesterol biosynthesis [18]. Such an inhibition may contribute to other factors causing low blood cholesterol such as anemia in SCD patients.7-ketocholesterol was shown to reduce nitric oxide synthesis and release by vascular endothelial cells [19]. Increased 7-ketocholesterol levels in the plasma of SCD patients should be investigated further for any role in the vascular pathology such as chronic inflammation and vaso-occlusions observed in these patients. Oxysterols also effect sterol synthesis by regulating sterol element-binding protein (SREBP) function [20] and this may be an important contribution to hypocholesterolemia in SCD patients. SREBPs are transcription factors which are bound to the nuclear membrane and endoplasmic reticulum membrane when inactive. When activated they are cleaved in the Golgi apparatus and subsequently move into the nucleus to bind specific DNA sequences (Sterol Response Elements, SREs), initiating sterol synthesis by upregulating the synthesis of required enzymes [21]. SREBP activation relies on the level of cholesterol in the cell, which is when the SREBP cleavage-activating protein (SCAP) comes to effect. SCAP is a sensor for cholesterol, when cholesterol is low, SCAP escorts SREBP to the Golgi apparatus. In the Golgi, SREBP is cleaved and the amino terminal domain (referred to as nSREBP) moves to the nucleus and performs its aforementioned function [20]. Cholesterol is a natural inhibitor of this path as it is the end product. However, 7α-Hydroxycholesterol, 7β-Hydroxycholesterol, and 7-Ketocholesterol have been shown to inhibit SCAP escort of SREBP with high potency [22], by inducing SCAP binding with insig-1 and insig-2 which are ER membrane anchor proteins; thus blocking the SCAP-SREBP complex's movement to the Golgi [23-24]. In light of these studies, the elevated 7-ketocholesterol levels that we found in the SCD group may play an important role in the hypocholesterolemia of SCD. It is worth noting that while the mechanism of SCAP inhibition by cholesterol is known; it is as of yet unclear how oxysterols induce the SCAP/insig binding [25]. Besides relevance of all the above literature in explaining a possible association between 7-ketocholesterol and SCD pathology such as hypocholesterolemia, the source of plasma 7-ketocholesterol is also an important concern. Increased plasma 7-ketocholesterol in SCD may presumably be a result of increased LDL oxidation in SCD as reported previously [26]. Another study reported a positive correlation between plasma and erythrocyte 7-ketocholesterol levels in diabetic patients [27]. Therefore, 7-ketocholesterols from erythrocyte membrane may also contribute to measured plasma concentration of 7-ketocholesterol. We also investigated cholestane-3β, 5α, 6β-triol levels which were suggested to cause endothelial damage in animal studies [28]. However, we did not find a significant difference for cholestane-3β, 5α, 6β-triol levels between patients and controls. SB patients who had a better disease course than SS patients had a higher concentration of cholestane-3β, 5α, 6β-triol. There is a positive correlation between cholestane-3β, 5α, 6β-triol and total cholesterol levels in the patient group (r=0.0434, p=0.039). This may explain the increased cholestane-3β, 5α, 6β-triol levels

in Sβ patients. This is a preliminary report with a limited number of patients and controls as part of a larger project investigating lipid metabolism in SCD. Patients also had hypocholesterolemia that is typical in many SCD patients. Apart from being an oxidative stress marker, 7-ketocholesterol may be a modifier of plasma cholesterol concentration. The metabolic roles of cholesterol oxidation products in diseases warrant further research to explain the tremendous nuclear effects of these oxysterols.

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