academicJournals

Vol. 8(2), pp. 39-42, February 2014
DOI: 10.5897/AJBR12.095
ISSN 1996-0778 ©2014 AcademicJournals
http://www.academicjournals.org/AJBR

African Journal of Biochemistry Research

Full Length Research Paper

Serum lipids and oxidized low density lipoprotein levels in sickle cell disease: Assessment and pathobiological significance

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Accepted 24 January, 2014

One hundred and eighteen (118) subjects aged 15 to 36 years divided into control subjects (AA n = 42), heterozygous sickle cell patients (AS n = 33) and homozygous sickle cell patients (SS n = 43) were investigated for a lipid profile including the measurement of oxidized low density lipoprotein (LDL) to assess the risk of early atherosclerosis in sickle cell disease. The results show that total, high density lipoprotein (HDL) and LDL plasma cholesterol levels are significantly lower in the sickle cell patients than in control group ($p < 0.05$). In contrast, the triglyceride levels, the ratio of triglycerides to HDL-cholesterol and the oxidized LDL fraction are higher in patients ($p < 0.05$). These lipid abnormalities could represent a cardiovascular risk for sickle cell disease patients.

Key words: Atherosclerosis, dyslipidemia, oxidized low density lipoprotein **(**LDL), sickle cell disease.

INTRODUCTION

Sickle cell disease (SCD) is a genetic disorder caused by a single substitution (GTG for GAG) at the beta globin gene on chromosome 11. This gene defect codes for the sickle beta-hemoglobin characterized by the substitution of valine for glutamic acid at the sixth position of the beta-chain (Pauling et al., 1949; Ingram, 1957). This inherited affection also called Sickle cell anemia (SCA) is a condition characterized by defect in plasma and erythrocyte lipids associated with a chronic oxidative stress (Oztaz et al., 2011). These two morbid processes disturb lipid homeostasis (Rice et al., 1986; Diatta et al., 2002) and induce lipidoperoxydation which promotes the accumulation of malondialdehyde, lysophosphatides and oxidizing agents (Diatta et al., 1999; Hebbel et al., 1982). These products, which are found at higher levels in the erythrocytes and plasma of SCA patients, act as powerful catalysts for LDL oxidation. The generated hydroperoxides and oxidized low density lipoproteins (oxLDL) induce an atherogenic process and serial deleterious effects (Peluso et al., 2012; Itabe, 2009). They promote a defect in lipid metabolism and abnormalities in lipids homeostasis by compromising the Lecithin cholesterol acyltransferase and the paraoxonase activity (Bielicki and Forte, 1999; Aviram et al., 1999). These two enzymes have been shown to play a key role in esterification of cholesterol on high density lipoprotein (Santamarina-Fojo et al., 2000) and in the lipid oxidation (Hine et al., 2012).

Consistent with these data involved in atherogenesis, the aim of this study is to evaluate plasma lipids and oxidized LDL concentrations in SCA patients in order to verify the presence of an atherogenic lipid profile.

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Table 1. Lipid profiles in homozygous sickle disease (SS) and sickle cell traits (AS) compared to healthy controls (AA).

Biological parameter	AA (n=42)	AS (n=33)	SS (n=43)
Chol (mg/ml)	1.63 ± 0.42	1.57 ± 0.38	1.18 ± 0.21*
HDL-c (mg/ml)	0.47 ± 0.14	0.43 ± 0.13	0.32 ± 0.07*
LDL-c (mg/ml)	0.98 ± 0.33	1.00 ± 0.32	0.69 ± 0.18*
Triglyceride (mg/ml)	0.79 ± 0.22	0.84 ± 0.27	0.94 ± 0.39*
Oxidized LDL (U/L)	59.79 ± 12.84	42.75 ± 13.7	70.05 ± 0.82*
LDL-c /HDL-c	2.16 ± 1.06	2.47 ± 0.99	2.21 ± 0.82
Triglyceride/HDL-c	1.67 ± 0.66	2.22 ± 1.51	3.08 ± 1.99 *

The values are expressed as mean ± standard deviation. *significantly different from the control group (AA) ($p < 0.05$).

MATERIALS AND METHODS

The subjects of this study were randomly selected from Aristide Le Dantec hospital and from two monitoring centers for SCA patients, namely the CNTS (Centre National de Transfusion Sanguine) and Albert Royer Children's Hospital of Fann.

These health facilities are located in Dakar in Senegal. Eligibility criteria of this study population included absence of traditional cardiovascular risk factors (obesity, diabetes mellitus, hypertension, tabagism, alcoholism) and absence of any therapy or affection underlying dyslipidemia. Informed consent from each subject was obtained for participation in the study. Then, 5 ml-blood was collected, by venipuncture in the antecubital fossa, in a tube containing lithium heparin and 5 ml in a dry tube. The serum obtained from the dry tubes was divided into aliquots and frozen at -80°C for the lipid profile and quantification of oxidized LDL. The heparin tubes were used for Emmel test and hemoglobin electrophoresis.

Plasma cholesterol and triglycerides were determined by enzymatic methods according to the manufacturer instructions on COBAS INTEGRA 400 analyzer (Roche Diagnostic®, Germany). HDL and LDL cholesterol were determined on the same instrument by direct methods. Oxidized LDL was quantified by ELISA method using kits marketed by Mercodia (Mercodia®, Sweden). Mercodia oxidized LDL ELISA is an enzyme immunoassay based on a direct sandwich technique utilizing anti-oxLDL antibodies as first antibody. Before the ELISA test, serum was diluted in order to obtain the same concentration of total LDL (0.5 g/L). This normalization concerns both patients and control subjects.

Statistical analysis

The results were expressed as mean ± standard deviation. Statistical analysis was carried out using EPI INFO 6. Student's test was used for comparing the different concentrations obtained. A difference to the value of $p < 0.05$ was considered significant.

RESULTS

This study population is composed of 118 individuals aged 15 to 36 years divided into three groups according to the electrophoretic patterns of hemoglobin and the Emmel test: 43 homozygous sickle cell patients (SS), 33 heterozygous sickle cell patients (AS) and 42 control subjects (AA). The individuals in the various groups were matched for age and sex. The lipid plasma concentrations (total, HDL and LDL cholesterol and triglycerides) of the three groups are shown in Table 1. Total, LDL and HDL cholesterol are significantly lower in homozygous SCA patients compared to the control groups ($p < 0.001$); triglycerides are significantly higher in SCA patients than in control group (Table 1). The decrease in LDL cholesterol is associated to an increase in LDL particle oxidized fraction (Table 1). Really, the oxidized LDL level per Gramm of total LDL is significantly higher in SCA patients than in the control groups ($p < 0.05$). Table 1 shows also the results of lipid indexes. The LDL/HDL ratio is not significantly different from one group to another among the study participants. In contrast, the ratio of triglycerides to HDL-c (TG/HDL- ratio) is significantly higher in SCA patients than in controls.

Table 2 presents the lipid data of the AA and SS groups according to the sex: apparently the differences between control subjects and SCA patients are more pronounced in males for all parameters but HDL- cholesterol and triglyceride levels show a more pronounced difference in females.

DISCUSSION

The hypocholesterolemia (total and LDL) observed in this study SCA patients confirms the results of a number of studies (El-hazim et al., 1987; Oztaz et al., 2011; Rahimi et al., 2006; Shores et al., 2003; Vanderjagt et al., 2001; Mokondjimob et al., 2012); the mean cholesterol concentration in this study patients is however particularly low (1.18 g/L).

The more pronounced difference observed in males has also been already reported by Shores et al. (Shores et al., 2003). The decrease of both LDL and HDL status attenuates the variation of the LDL-c/HDL-c ratio. In this case, the lipid index losses in this study the clinical utility reported by Fernandez and Webb (2008).

Hypocholesterolemia may be caused by several mechanisms. Plasma cholesterol is essential to the renewal of erythrocyte membranes and it is mobilized from plasma

Table 2. Sex-related Lipid variations among Sickle hemoglobin carriers compared to healthy controls.

Biological parameter	Males (n=44)		Females (n=74)	
	AA	SS	AA	SS
Chol (mg/ml)	1.60 ± 0.37	1.30 ± 0.21*	1.64 ± 0.45	1.36 ± 0.32*
HDL-c (mg/ml)	0.42 ± 0.10	0.38 ± 0.09*	0.50 ± 0.14	0.38 ± 0.10*
LDL-c (mg/ml)	1.00 ± 0.33	0.76 ± 0.20*	0.97 ± 0.30	0.87 ± 0.25*
Triglyceride (mg/ml)	0.80 ± 0.20	0.85 ± 0.25*	0.78 ± 0.20	0.90 ± 0.21*
Triglycerides/HDL	1.58 ± 0.66	2.83 ± 1.15	1.83 ± 0.71	3.16 ± 2.41*

The values are expressed as mean ± standard deviation. *significantly different from the control group (AA) ($p < 0.05$).

for this function. This turnover is exacerbated in SCA as a result of the alteration of erythrocyte components by intense oxidative stress (Diatta et al., 1999, 2002; Rice et al., 1986; El-hazmi et al., 1987; Ngogang et al., 1989). Hypocholesterolemia may also result from hemodilution caused by the decreased size of red blood cells resulting in an increased plasma volume and having thus a dilution effect on plasma constituents (Shores et al., 2003).

The concomitant decrease in HDL cholesterol is consistent with data reported by several authors (Vanderjagt et al., 2001; Mokondjimobe et al., 2012; Seixas et al., 2010; Nnodim et al., 2012). Other reports describe either no difference (Shores et al., 2003) or an increase in SCA patients compared to controls (Rahimi et al., 2006).

It is well known that HDL levels are associated with age. In women, HDL-Cholesterol levels increase progressively to the fifth decade and then decrease with menopause (Kim et al., 2000). This mechanism may influence the data on HDL Cholesterol concentrations reported by Rahimi et al. (2006): this study concerns women (with SS or AS phenotypes) who are older than the control subjects (23.7, 34.8 and 20.6 years respectively). This is probably the reason for the increase in HDL cholesterol in SCA patients observed by Rahimi et al. (2006). Another finding of this study is the higher triglyceride concentration observed in SCA patients. The atherogenic link between triglycerides (TG) and low HDL-c is better demonstrated, in this study, by the ratio of triglycerides to HDL-cholesterol (TG/HDL-c ratio). The raise in this index and in the triglycerides, strongly implycated in the atherogenic process (Gotto, 1998; Da Luz et al., 2008) suggests an increased risk of atherosclerosis during SCA**.**

In the studied population, the depletion in HDL cholesterol, recognized as a protective factor (Filippatos and Elisaf, 2013; Besler et al. 2010), raises the issue of a possible atherosclerotic risk hidden by hypocholesterolemia in SCA patients. This cardiovascular risk, little studied in sickle cell disease, is underpinned in this study by low HDL-cholesterol levels associated both with a rise in the fraction of oxidized LDL, in the serum triglycerides and in the TG/HDL-c ratio. The atherogenic potential of these lipid disorders has been reported by several authors (Mokondjimobe et al., 2012; Daugherty and Roselaar, 1995; Gotto, 1998; Da Luz et al., 2008).

Indeed the role of oxidized LDL or Ox LDL in atherogenesis is well known (Belcher et al., 1999; Huang et al., 2008; Itabe, 2009). Their internalization mediated by macrophage receptors leads to the formation of foam cells (Nagy et al., 1998). The release of the contents of these cells induces inflammation which, in synergy with cytokines, causes the formation of atherosclerotic plaque (Daugherty and Roselaar, 1995; Berliner et al. 1995; Chilsom et al., 1999).The outgrowth obtained then increases to the point of obstructing the lumen. In parallel, protein fibrous cap overlying the atheroma is degraded by the metalloproteinases released by macrophages and smooth muscle cells. This erosion promotes thrombus formation. Thus, the large fraction of oxidized LDL in this sickle cell population appears to be a significant risk factor for atherosclerosis.The presence of an atherogenic phenotype in SCA patients has been already reported (Seixas et al., 2010).

The combined effects of decrease in HDL-cholesterol, increased oxidized LDL status, raised triglyceridemia and TG/HDL-c ratio in SCA patients, despite the global hypocholesterolemia, could present a not negligible cardiovascular risk.

Conclusion

The exploration of classical lipid parameters and oxidized LDL in the sickle cell disease shows that in addition to the qualitative abnormality of hemoglobin, an abnormal lipid profile which includes a decrease in HDL cholesterol level, an increase in triglycerides, in TG/HDL-c ratio and a high rate of oxidized LDL is observed in these patients. These lipid disorders by their powerful atherogenic potential can possibly carry an early cardiovascular risk. Without care, the atherogenic risk could be an obstacle to the improvement of the survival of patients with sickle cell disease.

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