Meta Gene 10 (2016) 27–31

Contents lists available at ScienceDirect

Meta Gene

journal homepage: www.elsevier.com/locate/mgene

Leptin −2548 G > A gene polymorphism is associated with lipids metabolism and TGF-β alteration in sickle cell disease

Camylla Vilas Boas Figueiredo [a,b], Magda Oliveira Seixas Carvalho [c], Rayra Pereira Santiago [a,b], Sânzio Silva Santana [a,b], Rodrigo Mota de Oliveira [b], Luciana Magalhães Fiuza [a,b], Caroline Conceição da Guarda [a,b], Milena Magalhães Aleluia [a,b], Valma Maria Lopes do Nascimento [d], Isa Menezes Lyra [c,d], Junia Raquel Dutra Ferreira [a,b], Marilda de Souza Goncalves [a,b,*]

[a] *Centro de Pesquisa Gonçalo Moniz/FIOCRUZ, Rua Waldemar Falcão, 121, Candeal, CEP: 40.296-710 Salvador, Bahia, Brazil*
[b] *Universidade Federal da Bahia, Av. Barão de Geremoabo, Campus Universitário de Ondina, CEP: 40.000-000 Salvador, Bahia, Brazil*
[c] *Complexo Hospitalar Universitário Professor Edgard Santos, Rua Augusto Viana, sn°, Canela, CEP: 40110-060 Salvador, Bahia, Brazil*
[d] *Fundação de Hematologia e Hemoterapia da Bahia (HEMOBA), Av. Vasco da Gama, s/n° Rio Vermelho, CEP: 40.240-090 Salvador, Bahia, Brazil*

A R T I C L E   I N F O

Article history:
Received 21 December 2015
Revised 6 September 2016
Accepted 3 October 2016
Available online 5 October 2016

Keywords:
Sickle cell disease
Leptin
TGF-β
Gene polymorphism

A B S T R A C T

Background: Leptin is a protein with regulatory role in several body systems such as the immune system, and energy balance. Given that patients with sickle cell disease (SCD) have changes in cellular immunity and lipid metabolism, it is important to conduct research aimed understand the role of leptin in the pathophysiology of SCD.
Results: We studied 103 patients with SCD from Northeast of Brazil in a case-control study. The investigation of the *leptin* −2548 G > A polymorphism in SCD individuals shows the frequency of 60.20% (62/103) for the wild genotype (GG); 34.95% (36/103) for the heterozygous genotype (AG) and 4.85% (5/103) for the variant homozygote genotype (AA). In the healthy volunteers group the polymorphism investigation indicated the frequency of 58.24% (53/91) for the wild genotype (GG); 37.36% (34/91) for the heterozygous genotype (AG) and 4.40% (4/91) for the variant homozygote genotype (AA). The AA genotype was associated with increased levels of very-low-density lipoprotein cholesterol (VLDL-C) and triglycerides among SCD patients. Furthermore, the presence of allele A was associated with the highest levels of transforming growth factor beta (TGF-β) in SCD patients.
Conclusion: The results suggest that the presence of the variant allele may influence the disturbances in lipid metabolism and serum levels of TGF-β described in SCD patients.

© 2016 Published by Elsevier B.V.

1. Introduction

Sickle cell disease (SCD) designates a group of diseases that has in common the presence of the beta S allele (β^s) that can be found in homozygous state called sickle cell anemia (SCA) or in heterozygous paired with other alleles from variants hemoglobin. The hemoglobin S (HbS) is a variant hemoglobin resulting from GAG → GTG point mutation in the sixth codon of the beta globin gene (*HBB*) where valine replaces glutamic acid on the beta polypeptide chain (Silla, 1999; Steinberg, 2009). Clinical symptoms associated with the SCD are heterogeneous, with the presence of severe hemolytic anemia, pain crises, vaso-occlusive events, high susceptibility of infection, pulmonary hypertension, priapism, leg ulcers, and stroke among other clinical events (Ghosh et al., 2014).

Inflammation on SCD is also driven by several cytokines, such as transforming grow factors-beta (TGF-β), which are a pleiotropic cytokine family that can acts in both pro-inflammatory and anti-inflammatory pathways. Episodes of pain, occurrence of infection, stroke, leg ulcers, priapism, acute chest syndrome, pulmonary hypertension and renal failure are important clinical manifestations, being associated with higher levels of TGF-β (Nolan et al., 2006; Pereira et al., 2014). Lipids are also involved on the inflammatory milieu of SCD, and dyslipidemia has been described among SCD patients. Alterations in plasma cholesterol concentrations were reported in SCD, and studies demonstrated the association between decreased levels of high-density lipoprotein cholesterol (HDL-C) and increased levels of very-low-density lipoprotein cholesterol (VLDL-C) and triglycerides as biomarkers related to inflammation among this patient group (Seixas et al., 2010; Zorca et al., 2010).

Leptin is a peptide hormone secreted by adipocytes, formed by 167 amino acids, has a molecular weight of 16 kDa, transcribed from the *ob* gene in mice, and serves as an integral component in the physiological system, regulating the storage, balance and the use of energy by the body (Negrão and Licinio, 2000). Studies suggest that leptin also has the role of modulating the immune response, acting on inflammatory processes and immune-mediated pathologies.

* Corresponding author at: Centro de Pesquisas Gonçalo Moniz/FIOCRUZ, Rua Waldemar Falcão 121. Brotas, CEP: 40.295-001 Salvador, Bahia, Brazil.
E-mail address: mari@bahia.fiocruz.br (M.S. Goncalves).

http://dx.doi.org/10.1016/j.mgene.2016.10.001
2214-5400/© 2016 Published by Elsevier B.V.

Leptin acts in the immune system by a pro-inflammatory pattern, mediating increased production of cytokines, macrophages adhesion and phagocytosis, and stimulate proliferation of T cells, with increased immunological competence (Silveira et al., 2009). In other tissues, leptin has important metabolic functions such as the secretion of insulin by the pancreas, hepatic glucose production and glucose uptake by muscle. Since changes for adipose tissue, such as obesity, affect the production/secretion of this hormone, there is an association between such changes and metabolic disorders, including also the increased risk of cardiovascular damage (Guimarães et al., 2007).

The polymorphism −2548 G > A is characterized by a single nucleotide exchange replacing an adenine (A) by a guanine (G) at the promoter region of the *leptin* gene (*LEP*), and the presence of this polymorphism has been associated with plasmatic leptin variations and body mass index (BMI) in both obese and non-obese individuals (Zhang et al., 2014).

A study performed by Nigerian researchers, involving 55 SCA patients aged 5–35 years and 22 non-SCD control subjects, demonstrated an association between decreased levels of plasma leptin and occurrence of inflammatory events as well as decreased on reticulocyte count in SCA patients. BMI was calculated of each study participant; however was found no significant correlation between plasma leptin level and BMI in SCA patients (Iwalokun et al., 2011).

The aim of this study was to investigate the association between the polymorphism −2548 G > A in the leptin gene related to lipid metabolism and the occurrence of changes in lipid profile, hematological and the inflammatory marker TGF-beta in individuals with SCD and healthy volunteers.

2. Methods

2.1. Subjects

We studied 103 patients with SCD from Northeast of Brazil attending the outpatient clinic of the Fundação de Hematologia e Hemoterapia do Estado da Bahia (HEMOBA) in a case-control study. All patients were in the steady state of the disease that was characterized as a time of three months without any acute clinical events and without receiving hemocomponents prior blood sampling. Exclusion criteria were the presence of infectious diseases and hemoglobin (Hb) profiles other than HbSS and HbSC. It was also investigated a group of 91 healthy volunteers, attended at the Laboratório de Análises Clínicas da Faculdade de Farmácia (LACTFAR) of Universidade Federal da Bahia (UFBA).

The Centro de Pesquisas Gonçalo Moniz of the Fundação Oswaldo Cruz (FIOCRUZ) research board approved this study, and all patients and their guardians provided written informed consent, in according to the Declaration of Helsinki of 1975, and its revision.

2.2. Hematological analysis

The hemoglobin profile confirmation was carried out by High Performance Liquid Chromatography (HPLC) using Variant II (Bio-Rad, California, USA) equipment. It was also performed the blood count of all individuals participating in the study by the automated method using an electronic cell counter, ABX Pentra 80 (HORIBA Medical, California, USA).

2.3. Biochemical analysis

Total cholesterol, HDL-C, low-density lipoprotein cholesterol (LDL-C), VLDL-C and triglycerides plasma concentrations were performed by automated method in according to the manufacturer's instruction (A25 system, BIOSYSTEMS SA, Barcelona, Spain).

2.4. Polymorphisms genotyping

Genetic analysis was performed by studying the genomic DNA extracted from white blood cells using the QIAamp DNA Blood Mini Kit (Qiagen, California, San Francisco, USA). The polymorphism *leptin* −2548 G > A (rs7799039) was investigated by polymerase chain reaction (PCR) using a combination of specific primers (Le Stunff et al., 2000).

Digestion of the PCR product was performed by restriction fragment length polymorphism technique (RFLP) using the restriction enzyme *CfoI* (PROMEGA, Madison, Wisconsin, USA). The analysis of the obtained fragments was performed in 8% polyacrylamide gel, and by fragments visualization by SYBR® Green dye (Sigma-Aldrich Corp., St. Louis, Missouri, USA) and observation under ultraviolet illumination.

Table 1
Hematological and lipid profile of the SCD patients' and healthy volunteers.

	HbSS individuals (n = 88)			HbSC individuals (n = 15)			Healthy volunteers (n = 91)		
	Wild allele (n = 52)	Variant allele (n = 36)	*p*	Wild allele (n = 10)	Variant allele (n = 5)	*p*	Wild allele (n = 53)	Variant allele (n = 38)	*p*
	Mean ± Std. Deviation	Mean ± Std. Deviation		Mean ± Std. Deviation	Mean ± Std. Deviation		Mean ± Std. Deviation	Mean ± Std. Deviation	
Age (years)	11.12 ± 7.28	12.06 ± 6.07	0.1469	10.20 ± 3.36	8.47 ± 4.89	0.8272	8.43 ± 2.97	8.24 ± 3.55	0.6134
RBC, 10^6/mL	2.77 ± 0.64	2.74 ± 0.68	0.9670	4.04 ± 0.71	4.03 ± 0.60	0.8531	4.73 ± 0.32	4.68 ± 0.36	0.2887
Hemoglobin, g/dL	8.34 ± 1.46	8.59 ± 1.75	0.7029	10.98 ± 1.22	10.20 ± 0.84	0.3540	12.89 ± 0.89	12.71 ± 0.93	0.3263
Reticulocyte, %	7.34 ± 3.24	7.79 ± 3.23	0.7717	4.57 ± 2.54	2.36 ± 1.42	**0.0465**	0.86 ± 0.26	0.80 ± 0.21	0.3373
Hematocrit, %	24.53 ± 4.11	25.15 ± 5.31	0.8840	34.31 ± 3.68	31.94 ± 2.84	0.3263	38.69 ± 2.50	38.23 ± 2.59	0.3512
Leukocyte count, (10^9/L)	13,958.0 ± 4444.0	14,694.0 ± 5018.0	0.1720	11,700.0 ± 9829.0	9160.0 ± 3076.0	0.9770	7443.0 ± 2365.0	6839.0 ± 197.0	0.2356
Platelet count, ($\times 10^9$/L)	490.0 ± 162.1	457.3 ± 152.9	0.3736	328.0 ± 119.3	405.8 ± 242.2	0.9451	311.9 ± 68.92	303.2 ± 62.33	0.5845
Total cholesterol, mg/dL	122.9 ± 24.98	123.3 ± 29.89	0.6590	116.9 ± 25.30	112.0 ± 16.78	0.7173	163.6 ± 37.96	163.7 ± 28.85	0.7856
HDL-C, mg/dL	31.40 ± 7.73	29.56 ± 7.48	0.1766	38.70 ± 12.09	39.40 ± 14.29	0.8821	47.96 ± 12.56	49.78 ± 15.58	0.9947
LDL-C, mg/dL	73.13 ± 24.31	72.17 ± 26.07	0.5728	62.20 ± 16.83	55.20 ± 17.33	0.2674	95.63 ± 36.29	94.42 ± 31.30	0.9700
VLDL-C, mg/dL	18.37 ± 7.80	21.53 ± 9.0	0.0732	16.0 ± 4.47	17.40 ± 5.77	0.6134	19.22 ± 10.99	19.42 ± 12.58	0.8576
Triglycerides, mg/dL	91.85 ± 38.53	107.3 ± 44.84	0.0785	79.30 ± 22.78	87.40 ± 27.93	0.5921	94.94 ± 55.55	97.28 ± 62.15	0.9348

RBC: red blood cells; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; VLDL-C: very-low-density lipoprotein cholesterol; TGF-β: transforming growth factor beta. Significant *p* values are shown in bold.

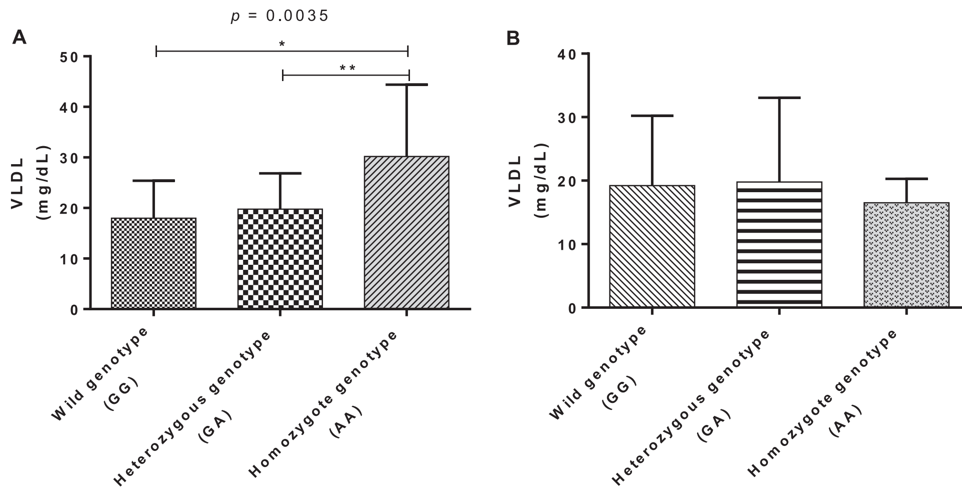

Fig. 1. A- Analysis of VLDL-C levels and genotypes for the polymorphism *leptin* −2548 G > A among SCD patients (ANOVA test). B- Analysis of VLDL-C levels and genotypes for the polymorphism *leptin* −2548 G > A among healthy individuals group (ANOVA test). *$p = 0.0027$, **$p = 0.0159$ (Bonferroni post-hoc).

2.5. Cytokine assay

The TGF-β quantification was performed in the serum of patients with SCD by Enzyme Linked Immunosorbent Assay (ELISA) methodology in according to the manufacturer's instruction (BD Biosciences Pharmingen Kit, San Diego, California, USA).

2.6. Statistical analysis

The Hardy-Weinberg equilibrium (HWE) for the polymorphism *leptin* −2548 G > A was calculated using the online calculator program at Tufts University, USA (www.tufts.edu). Considering $p > 0.05$ consistent with HWE.

Data analyses were conducted using the software programs SPSS version 18.0 (IBM Software, New York, USA) and GraphPad Prism version 6.0 (Graphpad Software, California, USA). The p values < 0.05 were considered significant for the analyses. The analysis of normal distribution of quantitative variables was performed using the Kolmogorov-Smirnov test and from this information, the ANOVA parametric test was used to analyze the distribution of the means of quantitative or numerical variables with normal distribution with more than three categories.

The independent *t*-test was used to analysis of two numerical variables, in the comparison between two groups of values inside the same variable, taking in consideration the distribution of each variable.

3. Results

3.1. Hematological and lipid profile analyses

Hematological and lipid profile of the 194 subjects included in the study in accord to the allele presence for the *leptin* −2548 G > A are shown in Table 1.

3.2. Polymorphisms frequencies

The investigation of the *leptin* −2548 G > A polymorphism in SCD individuals shows the frequency of 60.20% (62/103) for the wild genotype (GG); 34.95% (36/103) for the heterozygous genotype (AG) and 4.85% (5/103) for the variant homozygote genotype (AA). In the healthy volunteers group the polymorphism investigation indicated the frequency of 58.24% (53/91) for the wild genotype (GG); 37.36% (34/91) for the heterozygous genotype (AG) and 4.40% (4/91) for the variant

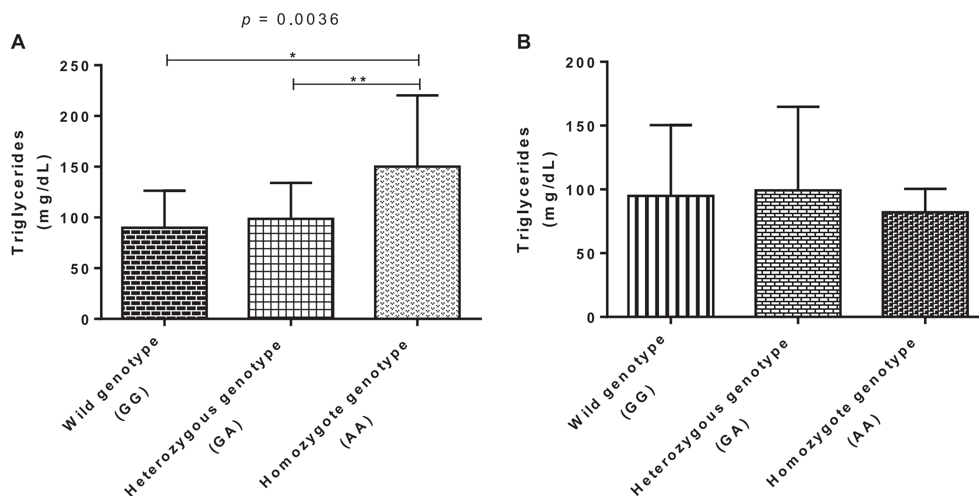

Fig. 2. A- Analysis of triglycerides levels and the presence of genotypes for the polymorphism *leptin* −2548 G > A among SCD patients (ANOVA test). B- Analysis of triglycerides levels and the presence of genotypes for the polymorphism *leptin* −2548 G > A among heathy individuals group (ANOVA test). *$p = 0.0028$, **$p = 0.0166$ (Bonferroni post-hoc).

Table 2
TGF-β levels of the SCD patients' and healthy volunteers.

	HbSS individuals (n = 21)			HbSC individuals (n = 14)			Healthy volunteers (n = 47)		
	Wild allele (n = 13)	Variant allele (n = 8)	*p*	Wild allele (n = 9)	Variant allele (n = 5)	*p*	Wild allele (n = 24)	Variant allele (n = 23)	*p*
	Mean ± Std. Deviation	Mean ± Std. Deviation		Mean ± Std. Deviation	Mean ± Std. Deviation		Mean ± Std. Deviation	Mean ± Std. Deviation	
TGF-β (ng/mL)	70.20 ± 21.31	89.35 ± 6.28	**0.0431**	50.09 ± 16.49	74.80 ± 29.86	0.1469	67.06 ± 22.83	62.35 ± 14.59	0.5944

TGF-β: transforming growth factor beta. Significant *p* values are shown in bold.

homozygote genotype (AA). The frequencies were in Hardy-Weinberg equilibrium.

The statistical analysis of the results showed that patients HbSC carriers of the wild type allele have higher reticulocyte count as compared to those of the variant allele ($p = 0.0465$). Patients HbSS carrying the wild type allele showed decreased serum levels of TGF-β when compared to patients with the variant allele ($p = 0.0431$).

According to the analysis of the results, the homozygous variant genotype (AA) and levels of VLDL-C and triglycerides were statistically significant when these variables were investigated among SCD patients. Figs. 1 and 2 show that the AA genotype is associated with increased levels of VLDL-C and triglycerides among SCD patients. There was no statistical significance in the same analysis for the healthy volunteers group.

3.3. Cytokine assay

TGF-β levels were statically significant when compared wild and variant alleles for the polymorphism *leptin* −2548 G > A among HbSS individuals (Table 2). The Fig. 3 shows that the presence of allele A is associated with the highest levels of TGF-β in SCD patients. However, there was no statistical significance in the TGF-β levels among the healthy volunteers.

4. Discussion

Our results obtained with the investigation of the polymorphism *leptin* −2548 G > A show that the AA genotype is associated with increased levels of VLDL-C and triglycerides. Increased levels of markers related to the lipid profile are factors associated with an increased oxidative stress, which contributes to endothelial dysfunction and clinical manifestations associated with worsening of clinical symptoms of SCD (Akinlade et al., 2014). Higher levels of HDL-C reduce the risk of hemolysis and endothelial dysfunction in these patients. Furthermore, high triglycerides levels may be involved in lipid oxidation and contribute to oxidative stress, leading to endothelial dysfunction (Seixas et al., 2010).

Results that we found in this study show that the presence of the allele A of the polymorphism *leptin* −2548 G > A is associated with higher levels of TGF-β. Higher levels of this cytokine may also, be linked to endothelial remodeling where increased extracellular matrix may lead to a reduction of the vessel lumen, contributing to the occurrence of vaso-occlusive crises (Nolan et al., 2006).

Compared to healthy volunteers, studies show that SCD patients have lower serum levels of leptin, with association to hemolytic events, sickling and inflammatory episodes, contributing to a worse prognosis (Hibbert et al., 2005; Iwalokun et al., 2011). Other studies also seek to find mechanisms that explain the participation of leptin in energy metabolism of SCD patients (Buchowski et al., 2000).

Le Stunff et al. (2000) have confirmed in a cohort study the hypothesis that different genotypes related to the polymorphism *leptin* −2548 G > A promote changes in leptin levels. In this study involving obese girls, leptin levels were 20–25% lower in AA genotype group compared to other genotypes.

A cohort study led by French researchers involving male and female volunteers with and without overweight, found that the G allele of the polymorphism −2548 G > A in *leptin* gene was more frequent in the group of overweight individuals. In the same study, male volunteers carrying the G allele had lower serum levels of leptin (Mammes et al., 2000).

Although many studies have demonstrated the association between the *leptin* −2548 G > A polymorphism and obesity risk, a meta-analysis involving nine case-controlled publications, including 2594 individuals, being 1235 obesity cases and 1359 controls, found no significant associations between this polymorphism and obesity risk (Yan et al., 2015).

Leptin levels increase in direct proportion to body mass and studies show that elevated serum levels of leptin in obese patients can promote obesity-related complications, in addition to playing an important role

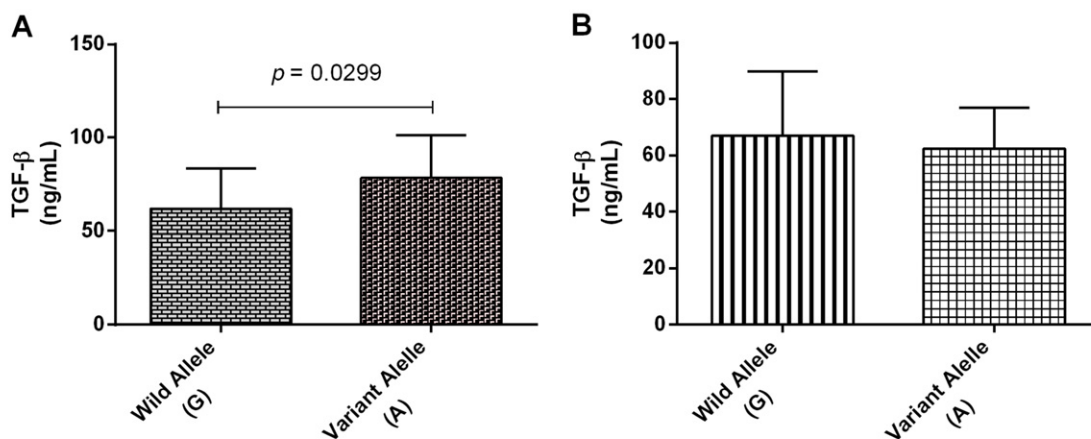

Fig. 3. A- Analysis of TGF-β levels and the presence of wild and variant alleles for the polymorphism *leptin* −2548 G > A among SCD patients (Independent *t*-test). B- Analysis of TGF-β levels and the presence of wild and variant alleles for the polymorphism *leptin* −2548 G > A among the healthy volunteers (Independent *t*-test).

in the development of breast cancer (Barreto et al., 2015). The TGF-β is a cytokine able to acts in different pathways, having an important role in cell proliferation and differentiation, and immune regulation, and plays role in the pathogenesis of several diseases, such as obesity and cancer. Studies have shown that the expression and secretion of TGF-β levels were higher in adipose tissue of obese subjects compared to healthy subjects. Furthermore, signaling pathways induced by TGF-β may act as both promoters and tumor suppressors (Pereira et al., 2014). Therefore, it is possible to state that serum leptin levels associated with TGF-β expression are important markers related to the prognosis of obesity and breast cancer development (Barreto et al., 2015).

Studies involving animal models showed that leptin stimulates cell proliferation and induces mRNA expression and protein secretion of TGF-beta in glomerular endothelial cells. The results obtained by the researchers demonstrated that leptin is able to induce TGF-beta system locally, inducing a cross-talk between the different cell types of the glomerulus, which could be associated with glomerulosclerosis observed in obesity (Wolf et al., 1999), which is a clinical complication that can also be found in SCD (Alhwiesh, 2014).

A study developed by Iranian researchers, involving 100 women who had breast cancer and 100 healthy female volunteers, found a high frequency of GG genotype for the polymorphism *leptin* −2548 G > A in cases of breast cancer when compared to healthy female volunteers (Mohammadzadeh et al., 2015).

Many studies have shown that serum levels of leptin and polymorphisms in *leptin* gene are involved in the pathogenesis of several diseases such as obesity and cancer, playing an important role in metabolic and inflammatory profile. The association of the polymorphism in *leptin* gene and TGF-β plasma concentrations has never been described in literature. Thus, the development of additional studies is needed to investigate the role of this protein in SCD, in order to confirm the results found in our study.

5. Conclusion

Our results emphasize the importance of research into new prognostic biomarkers in the SCD. The investigation of the polymorphism *leptin* −2548 G > A showed an association between the variant genotype (AA) and increased levels of VLDL-C and triglycerides, which are commonly investigated markers in the laboratory routine, easily accessible for monitoring and evaluation of the disease severity. Furthermore, we found an association between the presence of the variant allele and higher serum levels of TGF-β.

Conflict of interests

The authors declare no conflict of interests.

Acknowledgements

We thank the patients for their participation because without them, this study would not have been conducted. This work was supported by grants from the Brazilian National Council of Research (CNPq) (3065427/2007-5 and 484457/2007-1) (M.S.G.); the Foundation of Research and Extension of Bahia (FAPESB) (1431040053063 and 9073/2007) (M.S.G.); and MCD/CNPq/MS-SCTIE-DECIT (409800/2006-6), (M.S.G.). The sponsors of this study are public or nonprofit organizations that support science in general. They had no role in gathering analyzing, or interpreting the data.

References

Akinlade, K.S., et al., 2014. Defective lipid metabolism in sickle cell anaemia subjects in vaso-occlusive crisis. Niger. Med. J. 55 (5), 428–431.

Alhwiesh, A., 2014. An update on sickle cell nephropathy. Saudi J. Kidney. Dis. Transpl. 25 (2), 249–265 (Mar ISSN 1319-2442 (Print)).

Barreto, S.C., et al., May 2015. Extracellular matrix in obesity — cancer interactions. Horm. Mol. Biol. Clin. Invest. 22 (2), 63–77.

Buchowski, M.S., et al., Apr 2000. Plasma leptin association with body composition and energy expenditure in sickle cell disease. J. Am. Coll. Nutr. 19 (2), 228–236.

Ghosh, K., et al., 2014. Guidelines for screening, diagnosis and management of hemoglobinopathies. Indian J. Hum. Genet. 20 (2), 101–119.

Guimarães, D.E.D., et al., 2007. Adipocitocinas: uma nova visão do tecido adiposo. Rev. Nutr. 20 (5), 549–559.

Hibbert, J.M., et al., 2005. Proinflammatory cytokines and the hypermetabolism of children with sickle cell disease. Exp. Biol. Med. (Maywood) 230 (1), 68–74 (Jan).

Iwalokun, B.A., et al., 2011. Serum levels of leptin in Nigerian patients with sickle cell anaemia. BMC Blood Disord. 11, 2.

Le Stunff, C., et al., 2000. A common promoter variant of the leptin gene is associated with changes in the relationship between serum leptin and fat mass in obese girls. Diabetes 49 (12), 2196–2200 (Dec).

Mammes, O., et al., 2000. Association of the G-2548A polymorphism in the 5′ region of the LEP gene with overweight. Ann. Hum. Genet. 64 (Pt 5), 391–394 (Sep).

Mohammadzadeh, G., et al., 2015. The relationship between −2548 G/A leptin gene polymorphism and risk of breast cancer and serum leptin levels in Ahvazian women. Iran. J. Cancer Prev. 8 (2), 100–108 (Mar–Apr).

Negrão, A.B., Licinio, J., 2000. Leptina: o Diálogo entre Adipócitos e Neurônios. Arq. Bras. Endocrinol. Metabol. 44 (3).

Nolan, V.G., et al., 2006. Sickle cell leg ulcers: associations with haemolysis and SNPs in Klotho, TEK and genes of the TGF-beta/BMP pathway. Br. J. Haematol. 133 (5), 570–578.

Pereira, S., et al., 2014. Modulation of adipose tissue inflammation by FOXP3 + Treg cells, IL-10, and TGF-beta in metabolically healthy class III obese individuals. Nutrition 30 (7–8), 784–790 (Jul–Aug).

Seixas, M.O., et al., 2010. Levels of high-density lipoprotein cholesterol (HDL-C) among children with steady-state sickle cell disease. Lipids Health Dis. 9, 91.

Silla, L.M., 1999. Sickle cell disease: a serious and unknown problem of public health in Brazil. J. Pediatr. 75 (3), 145–146.

Silveira, M.R., et al., 2009. Correlation between obesity, adipokines and the immune system. Braz. J. Kinathrop. Hum. Perform. [S.l.] 11 (4), 466.

Steinberg, M.H., et al., 2009. Disorders of Hemoglobin: Genetics, Pathophysiology, and Clinical Management. Cambridge University Press.

Wolf, G., et al., 1999. Leptin stimulates proliferation and TGF-beta expression in renal glomerular endothelial cells: potential role in glomerulosclerosis [see comments]. Kidney Int. 56 (3), 860–872 (Sep. ISSN 0085-2538 (Print)).

Yan, J., et al., 2015. Lack of association between leptin G-2548A polymorphisms and obesity risk: evidence based on a meta-analysis. Obes. Res. Clin. Pract. 9 (4), 389–397 (Jul–Aug).

Zhang, L., et al., 2014. Association of leptin gene −2548 G/A polymorphism with obesity: a meta-analysis. Ann. Nutr. Metab. 64 (2), 127–136.

Zorca, S., et al., 2010. Lipid levels in sickle-cell disease associated with haemolytic severity, vascular dysfunction and pulmonary hypertension. Br. J. Haematol. 149 (3), 436–445.