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Os polimorfismos *LGALS3* + 191A e + 292C estão associados à redução dos níveis séricos de gal-3, mas não aos eventos clínicos de indivíduos com anemia falciforme

LGALS3 +191A and +292C polymorphisms are associated with a reduction in serum gal-3 levels, but not with the clinical events of individuals with sickle cell anemia

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Resumo

Objetivo: Este estudo teve como objetivo avaliar se os polimorfismos de nucleotídeo único (SNPs) +191 C>A (rs4644) e +292 A>C (rs4652) do gene *LGALS3* e os níveis séricos da galectina-3 (gal-3) estão associados com os eventos clínicos de pacientes com anemia falciforme (AF). Métodos: Os polimorfismos +191 e +292 no *LGALS3* foram estudados usando o sistema de PCR em tempo real, pela metodologia TaqMan; os níveis séricos da gal-3 foram medidos pela técnica de ELISA. O estudo incluiu 322 pacientes com AF, média de idade 36 (21-84). Resultados: Os genótipos AA e CA da região +191 foram relacionados a níveis mais baixos de gal-3 quando comparados ao genótipo CC (*p = 0,0296*). Níveis mais baixos de gal-3 também foram associados aos diplótipos +191/+292 (AA/CC; CA/CC) (*p = 0,0137*) em comparação com os diplótipos (CC/AA; CC/CC; CC/AC; CA/AC). Não houve associação entre os níveis séricos da gal-3 e as frequências genotípicas dos SNPs *LGALS3* +191 e +292 com os eventos clínicos na AF. Conclusão: Os SNPs +191 e +292 do *LGALS3* estão associados à diminuição dos níveis séricos de gal-3. No entanto, não foi observada associação de polimorfismos e níveis séricos da gal-3 com os eventos clínicos em pacientes com AF.

Palavras-chave: Galectin-3; *LGALS3*; Polimorfismo; Anemia falciforme.

Abstract

Objective: This study aimed to evaluate whether the single nucleotide polymorphisms (SNPs) +191 C>A (rs4644) and +292 A>C (rs4652) of the *LGALS3* gene and the serum levels of galectin-3 (gal-3) are associated with clinical events in patients with sickle cell anemia (SCA). Methods: SNP +191 and +292 of the *LGALS3* gene were detected by the TaqMan PCR system in real time. Gal-3 levels were measured in serum by ELISA. The study included 322 patients, mean age 36 (21-84). Results: AA and CA genotypes of the +191 region were related to lower levels of gal-3 when compared to CC genotype (p=0.0296). Lower level of gal-3 was also associated with the +191/+292 (AA/CC; CA/CC) diplotypes (p=0.0137) compared to the diplotypes (CC/AA; CC/CC; CC/AC; CA/AC). There was no association between serum levels of galectin-3 and genotype frequencies of the *LGALS3* +191 and +292 polymorphisms with clinical events in SCA. Conclusion: The polymorphisms +191 and +292 of the *LGALS3* are associated to decrease in serum levels of gal-3. However, no association of polymorphisms and levels of gal-3 with clinical events was observed in patients SCA.

Keywords: Galectin-3; *LGALS3*; Polymorphism; Sickle cell anemia.

Resumen

Objetivo: Este estudio tuvo como objetivo evaluar si los polimorfismos de un solo nucleótido (SNPs) +191 C>A (rs4644) y +292 A>C (rs4652) del gen *LGALS3* y los niveles séricos de galectina-3 (gal-3) están asociados con los eventos clínicos de pacientes con anemia falciforme (AF). Métodos: SNPs +191 y +292 en *LGALS3* se estudiaron utilizando el sistema de PCR en tiempo real TaqMan; Los niveles séricos de gal-3 se midieron por ELISA. El estudio incluyó a 322 pacientes con AF, edad media 36 (21-84). Resultados: los genotipos AA y CA de la región +191 se relacionaron con niveles más bajos de gal-3 en comparación con el genotipo CC ($p = 0.0296$). El nivel más bajo de gal-3 también se asoció con los diplotipos + 191 / + 292 (AA / CC; CA / CC) ($p = 0.0137$) en comparación con los diplotipos (CC/AA; CC/CC; CC/AC; CA/CA). No hubo asociación entre los niveles séricos de galectina-3 y las frecuencias de genotipo de los polimorfismos *LGALS3* +191 y +292 con eventos clínicos en SCA. Conclusión: Los polimorfismos +191 y +292 de *LGALS3* están asociados con una disminución en los niveles séricos de gal-3. Sin embargo, no se observó asociación de polimorfismos y niveles séricos de gal-3 con eventos clínicos en pacientes con AF.

Palabras clave: Galectin-3; LGALS3; Polimorfismo; Anemia falciforme.

1. Introduction

Sickle cell anemia (SCA), the most prevalent monogenic disease in the world, which causes structural changes in hemoglobin and erythrocyte, characterized by chronic inflammatory manifestations. (Marques *et al*, 2012; Jorgensen *et al*, 2016; Domingos *et al*, 2020). It is a public health problem in Brazil, with the highest frequency in the Northeast region of the country. This disease has a great heterogeneity in terms of clinical manifestations, ranging from almost asymptomatic patients to clinically severe forms. (Serjeant, 1997; Higgs & Wood, 2008).

In SCA patients, the process of vasoocclusion, together with ischemia and hemolysis leads to endothelium activation, chemokine production, leukocyte recruitment, and pro and anti-inflammatory cytokine production. These events determine most of the signs and symptoms present in these patients, such as leg ulcer, vasoocclusive crisis (VOC), stroke, acute chest syndrome (ACS), as well as an increase in the susceptibility to respiratory infections (Marques *et al*, 2012; Rêgo *et al*, 2015; Kosaraju, *et al*, 2017). These clinical manifestations are multifactorial and molecules are involved in these processes, such as IL-6,

IL-8, TNF-a and inflammatory receptors (Pitanga *et al*, 2016; Abd El-Kader *et al*, 2018; Domingos *et al*, 2020).

Galectin-3 (gal-3) is a protein that acts in several biological processes that are common in clinical events of SCA, such as cell adhesion, proliferation, differentiation, apoptosis, in addition to inflammatory processes (Rabinovich *et al*, 2002; Romaniuk *et al*, 2011; Mendonça-Belmont *et al*, 2016). Thus, gal-3 has been studied in several diseases of a chronic inflammatory character, such as rheumatoid arthritis (RA) (Hu *et al*, 2011; Atabaki *et al*, 2017), cardiovascular diseases (George *et al*, 2015; Numano *et al*, 2015; Zhang *et al*, 2018), infections (Cruz *et al*, 2015; Mendonça-Belmont *et al*, 2016), different types of cancer (Huttle et al, 2015), and VOC in SCA (Mendonça-Belmont *et al*, 2016). Therefore, it is important to study gal-3, in order to understand its role in the pathophysiology of the SCA.

In addition to studying the functions of gal-3 protein plays in inflammatory diseases, two single nucleotide polymorphisms (SNPs) in the gene encoding gal-3 (*LGALS3*) have also been studied as possible markers associated with these diseases: SNP +191 C>A (rs4644) and SNP +292 A>C (rs4652), both related to the reduction in serum levels of gal-3 (Hu *et al* 2011; Mendonça-Belmont *et al*, 2016). So, in addition to assessing protein functions in SCA, these SNPs could play an important role in the disease. Thus, the objective of this study was to evaluate the existence of association between the SNPs +191 and +292 of *LGALS3* and serum levels of gal-3 with clinical manifestations in SCA patients, in order to find possible biomarkers that could contribute to a better understanding of the pathophysiology of SCA.

2. Methods

Patients and study location

Three hundred twenty-two eligible SCA patients aging over 18 years regularly followed at a single reference center in Northeast Brazil (HEMOPE-Hematology and Hemotherapy Foundation of Pernambuco) were enrolled, between March 2018 and December 2019. The diagnosis of SCA (presence of Hemoglobin S homozigosity) was performed following standard molecular procedures (Sanchaisuriya *et al*, 2004).

Were divided two groups, case group, composed of patients who presented at least one of the following events: leg ulcer, stroke, and ACS; and the control group, composed of patients that did not present the described clinical events. Pain crises resulting from vascular obstruction were assessed according to the VOC frequency, which was defined by the total

number of VOC episodes divided by the age of the patients. (group 1: VOC<3/year; group 2: VOC 3-6/year; group 3: VOC>6/year). The clinical data were collected from the records in the medical files of the HEMOPE Foundation. Patients presenting diabetes were excluded. For the analysis of gal-3 serum levels, were excluded, patients who had a history of transfusion in the last 90 days and hemolysis in serum samples. The study was approved by the local Research Ethics Committee of the HEMOPE-Pernambuco Foundation (number 2,108,063) and, according to the Declaration of Helsinki, consent was obtained from all patients, or, when applicable, from their parents, prior to study commencement.

LGALS3 genotyping

DNA was extracted of peripheral blood (EDTA), as previously described (Davis et al, 1986). The analysis of the SNPs +191 (rs4644) and +292 (rs4652) of *LGALS3* was done by RTq-PCR, using QuantStudio5 equipment (Applied Biosystems, Foster City, CA, USA), using the genotyping Taqman assays methodology (Applied Biosystems, CA, USA).

Galectin-3 levels

Gal-3 concentrations were measured in 48 SCA patients by the commercial enzyme immunoassay kit (ELISA), Human LGALS3/galectin-3 (Sigma Aldrich, USA), according to the instructions presented by the manufacturer. Epoch (Biotek Instruments Inc.), a microplate reader, was used to read gal-3 concentrations. The minimum concentration detected by the kit is 0.47 ng/ml.

Statistical analysis

Categorical variables were compared by chi-square test or Fisher's exact test ($\chi 2$) with Yates correction. The risk chance was estimated by the odds ratio (OR) with a 95% confidence interval (IC), considering $p < 0.05$ as significant. Regarding the continuous quantitative variables, Student t or Mann-Whitney tests were applied for comparisons between two groups. For three or more groups, Kruskal-Wallis test or ANOVA was applied when appropriate. The GraphPad PRISM software (GraphPad Software, San Diego, California, USA) for Windows was used for these analyzes. Hardy-Weinberg equilibrium was tested using the Haploview software (version 4.2).

3. Results

VOC resulting from vascular occlusion affects almost all SCA patients, as it has a significantly high frequency and heterogeneity. In our population of 322 individuals with SCA, median age 36 (21-84) and 45.6% male, 56% had FVOC> 3. The other most frequent event in these patients was leg ulcer (43%) followed by osteonecrosis (20%), ACS (15%) and stroke (14%). The number of patients in each group, as well as the occurrence of clinical events are described in Table 1.

Table 1. Frequency of polymorphisms +191(rs4644) and +292(rs4652) of the *LGALS3* gene in patients with sickle cell anemia Followed up at the Hemope Foundation-Recife/Brazil.

rs4644 (+191)	Total (322)	VOC <3 (143)	VOC 3-6 (119)	VOC >6 (60)	Stroke+ (46)	Stroke - (276)	ACS + (49)	ACS - (273)	Leg ulcer + (138)	Leg ulcer - (184)
AA	46 (14)	23 (16)	18 (15)	5 (8)	4 (9)	42 (16)	7 (14)	39 (15)	19 (14)	27 (15)
CA	134 (42)	56 (39)	51 (43)	27 (45)	20 (43)	114 (41)	18 (37)	116 (42)	57 (41)	77 (42)
CC	142 (44)	64 (45)	50 (42)	28 (47)	22 (48)	120 (43)	24 (49)	118 (43)	62 (45)	80 (43)
CA+CC	276 (86)	120 (84)	101 (85)	55 (92)	42 (91)	234 (84)	42 (86)	234 (85)	119 (86)	157 (85)
A	226 (35)	102 (36)	87 (37)	37 (31)	28 (30)	198 (36)	32 (33)	194 (36)	95 (34)	131 (36)
C	418 (65)	184 (64)	151 (63)	83 (69)	64 (70)	354 (64)	66 (67)	352 (64)	181 (66)	237 (64)
rs4652 (+292)	**Total (322)**	**VOC <3 (143)**	**VOC 3-6 (119)**	**VOC >6 (60)**	**Stroke+ (46)**	**Stroke - (276)**	**ACS + (49)**	**ACS - (273)**	**Leg ulcer + (138)**	**Leg ulcer - (184)**
AA	49 (15)	15 (10)	22 (19)	12 (20)	8 (17)	41 (15)	9 (18)	40 (15)	22 (16)	27 (15)
AC	135 (42)	65 (46)	49 (41)	21 (35)	15 (33)	120 (43)	19 (39)	116 (42)	57 (41)	78 (42)
CC	138 (43)	63 (44)	48 (40)	27 (45)	23 (50)	115 (41)	21 (43)	117 (43)	59 (43)	79 (43)
AC+CC	273 (85)	128 (90)	97 (81)	48 (80)	38 (83)	235 (85)	40 (82)	233 (85)	116 (84)	157 (85)
A	233 (36)	95 (33)	93 (39)	45 (37,5)	31 (34)	202 (37)	37 (38)	196 (36)	101 (37)	132 (36)
C	411 (64)	191 (67)	145 (61)	75 (62,5)	61 (66)	350 (63)	61 (62)	350 (64)	175 (63)	236 (64)

Each cell represents the absolute value (%). * Chi-square test ($\chi 2$) with Yates correction (OR CI 95%). P <0.05. There were no significant p values in this table. Leg ulcer +=group with the presence of leg ulcer; leg ulcer-=group with no leg ulcer; ACS+=presence of acute chest syndrome; ACS-=Absence of acute chest syndrome; VOC<3=up to three episodes of vasoocclusive crisis; VOC 3-6=three to six episodes of vasoocclusive crisis; VOC>6=more than six episodes of vasoocclusive crisis; Stroke+=presence of stroke; Stroke-=Absence of stroke. Source: Author.

The studied groups were in Hardy-Weinberg equilibrium. The frequencies of the genotypes for the +191 (rs4644) polymorphism, in the total patient population were: 14% (N=46) AA; 42% (N=134) CA; 44% (N=142) CC; and for the +292 (rs4652) region were:

15% (N=49) AA, 42% (N=135) AC and 43% (N=138) CC, as shown in **Table 1**. No association was observed between the allelic and genotypic frequencies of these SNPs in the *LGALS3* gene with clinical events in SCA.

In Table 2, there are the diplotypes of the +191/+292 regions of the *LGALS3* and their respective frequencies in the different events, however, no association was found between the diplotypes and the clinical events studied in these patients.

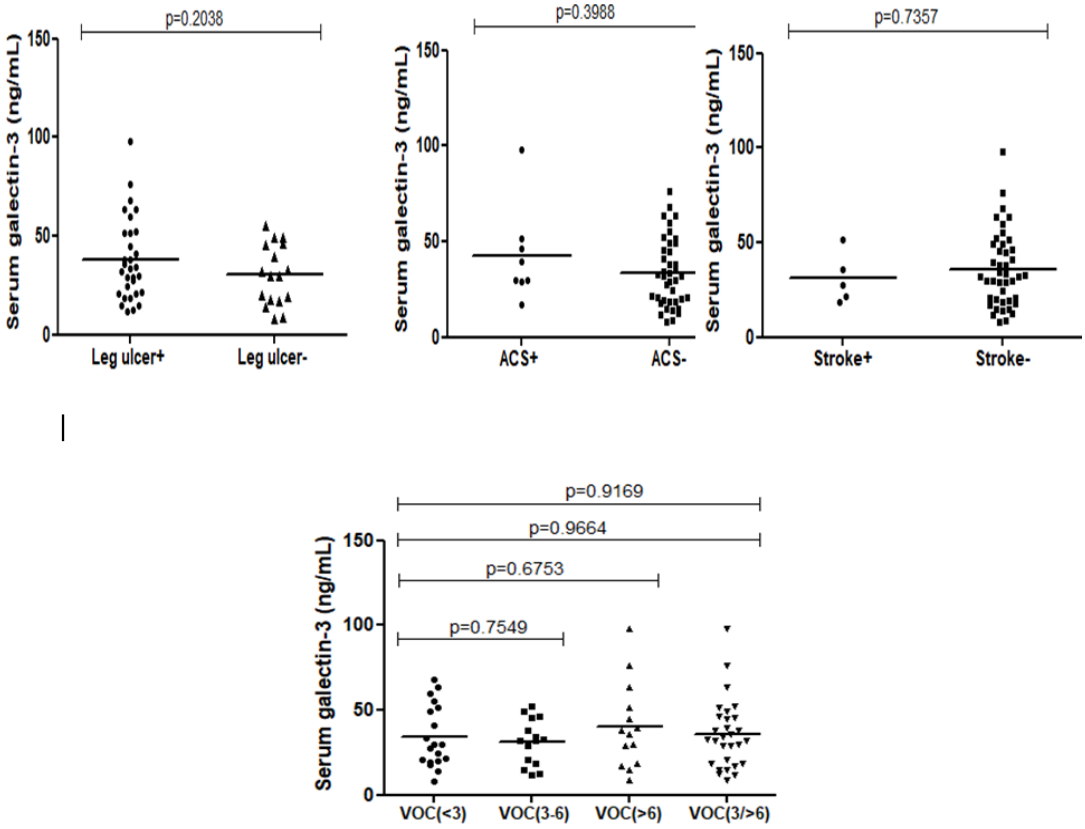
Table 2. Diplotypes + 191/+292 of the *LGALS3* gene in the clinical events of patients with sickle cell anemia followed up at the Hemope Foundation-Recife/Brazil.

Diplotypes	Total (322)	VOC <3 (143)	VOC 3-6 (119)	VOC >6 (60)	Stroke + (46)	Stroke - (276)	ACS + (49)	ACS - (273)	Leg ulcer+ (138)	Leg ulcer - (184)
AA/CC	45 (0.14)	22 (15)	18 (0.15)	5 (0.08)	4 (0.09)	41 (0.15)	7 (0.14)	38 (0.14)	18 (0.13)	27 (0.15)
AA/AC	1 (0.003)	1 (0,006)	0	0	0	1 (0.004)	0	1 (0.004)	1 (0.007)	0
CA/AA	2 (0.007)	0	1 (0.008)	1 (0.02)	1 (0.02)	1 (0.004)	1 (0.02)	1 (0.004)	1 (0.007)	1 (0.005)
CA/AC	65 (0.20)	29 (0.20)	26 (0.22)	10 (0.17)	8 (0.17)	57 (0.21)	7 (0.14)	58 (0.21)	24 (0.17)	41 (0.225)
CA/CC	66 (0.20)	27 (0.19)	24 (0.20)	15 (0.25)	11 (0.24)	55 (0.20)	10 (0.20)	56 (0.20)	32 (0.23)	34 (0.18)
CC/AA	47 (0.16)	15 (0.10)	21 (0.18)	11 (0.18)	7 (0.15)	40 (0.14)	8 (0.16)	39 (0.14)	21 (0.15)	26 (0.14)
CC/AC	69 (0.21)	35 (0.24)	23 (0.19)	11 (0.18)	7 (0.15)	62 (0.22)	12 (0.24)	57 (0.21)	32 (0.23)	37 (0.20)
CC/CC	27 (0.8)	14 (0.10)	6 (0.05)	7 (0.12)	8 (0.17)	19 (0.07)	4 (0.08)	23 (0.08)	9 (0.076)	18 (0.10)

Chi-squared test with Yates correction (OR CI 95%). P<0.05. There were no significant p values in this table. Leg ulcer +=group with the presence of leg ulcer; leg ulcer-=group with no leg ulcer; ACS+=presence of acute chest syndrome; ACS-=Absence of acute chest syndrome; VOC<3=up to three episodes of vasoocclusive crisis; VOC 3-6=three to six episodes of vasoocclusive crisis; VOC>6=more than six episodes of vasoocclusive crisis; Stroke+=presence of stroke; Stroke-=Absence of stroke. Source: Author.

Fifty patients were randomly selected for the quantification of serum gal-3 levels. Among them, two patients were excluded for presenting inconclusive quantification after the experiment. The 48 remaining patients were distributed according to clinical events as follows: leg ulcer+, N=31 (65%), [37.88±20.76ng/mL] and leg ulcer-, N=17 (35%), [30.24±15.29ng/mL], (p= 0,2038); VOC<3, N=19 (40%), [34.43±18.19ng/mL], VOC 3-6, N=15 (31%), [31.35±13.47ng/mL] and VOC>6, N=14 (29%), [40.29±25.14ng/mL], (p= 0,9169); stroke+, N=5 (10%), [30.97±13.17ng/mL] and stroke-, N=43 (90%), [35.66±19.83 ng/mL], (p= 0,7357); osteonecrosis+, N=19 (40%), [32.47±17.83ng/mL] and osteonecrosis-, N=29 (60%), [35.99±19.83ng/mL], (p=0,6105); ACS+, N=8 (17%), [42.47±24.84ng/mL] and ACS-, N=40 (83%) [33.71±17.89ng/mL], (p= 0,3988). However, no association was found between serum levels of gal-3 and clinical events in these SCA patients (Figure 1).

Figure 1. Serum levels of galectin-3 in clinical events in sickle cell anemia. Leg ulcer +=group with the presence of leg ulcer; leg ulcer-=group with no leg ulcer. Leg ulcer+ vs leg ulcer- p=0.2038. ACS+=presence of acute chest syndrome; ACS-=Absence of acute chest syndrome, ACS+ vs ACS- p=0.3988. VOC<3=up to three episodes of vasoocclusive crisis; VOC 3-6=three to six episodes of vasoocclusive crisis; VOC>6=more than six episodes of vasoocclusive crisis, VOC<3vsVOC 3-6vsVOC>6, p=0.9169. Stroke+=presence of stroke; Stroke-=Absence of stroke, Stroke+ vs Stroke-, p=0.7357. Osteonecrosis+=presence of osteonecrosis; Osteonecrosis-=Absence of osteonecrosis, Osteonecrosis+ vs Osteonecrosis-, p= 0.6105. Mann-whitney test and ANOVA test.

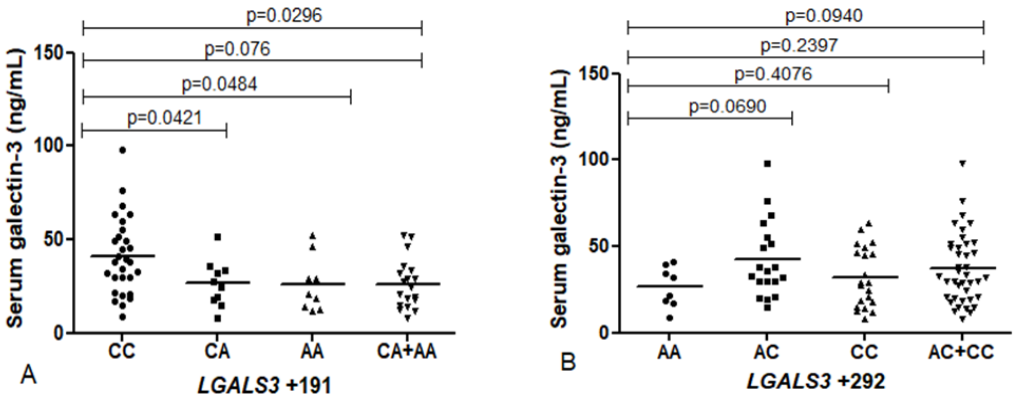


Source: Author.

As seen in Figure 1, the serum levels of gal-3 were not differentiated in the groups of patients separated according to each clinical event. Thus, serum levels of gal-3 are not associated with the severity of clinical events in these SCA patients.

The Figure 2 shows the distribution of the levels of gal-3 in the different genotypes of the SNPs *LGALS3* +191 and +292. An association was observed between the serum levels of gal-3 and the region +191 *LGALS3* (p=0.0296), in which the genotypes AA (n=9) [20.62 (11.95–52.15) ng/mL] and CA (n=10) [25.86 (8,310-51,38) ng/mL] were associated with low serum levels of gal-3, when compared to the CC genotype (n=29) [38,36 (8,590–97.74) ng/ml]. There was no association between the serum levels of gal-3 and the genotypes of the +292 *LGALS3* region.

Figure 2. Serum levels of galectin-3 with *LGALS3* polymorphisms +191(A) and +292(B). +191*LGALS3*:C=reference allele; A=variant allele. CCvsCA: p=0.0421(t-student test); CCvsAA, p=0.0484(t-student test); CCvsCA+AA: p=0.0076, (t-student test); ANOVA test p=0.0296 +292*LGALS3*: A=reference allele; C=variant allele; p=Mann Whitney tests. Region +191:AA(n=9) [20.62(11.95–52.15) ng/mL]; CA(n=10) [25.86(8.310-51.38) ng/mL]; CC(n=29) [38.36(8.590–97.74) ng/mL]. Region +292:AA(n=8) [26.82(8.590-40.87) ng/mL]; AC(n=19) [35.95(14.46-97.74) ng/mL]; CC(n=21) [28.97(8.310-63.59) ng/mL].

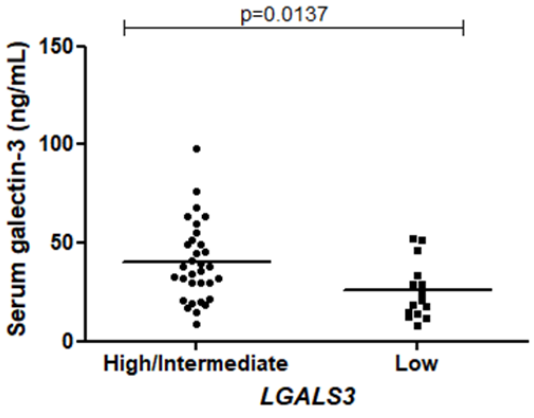


Source: Author.

Figure 2 highlights that the SNP of the LGALS3 +191 CC gene can cause changes in the serum levels of gal-3 in these patients, giving higher serum levels of gal-3 when compared to the other genotypes. The same was not observed in the SNP of the +292 region.

We categorized the groups of the diplotypes *LGALS3* +191/+292 according to the serum levels of gal-3, by classifying them in high (CC/AA), intermediate (CC/AC; CA/AC; CC/CC) and low (AA/CC; CA/CC), also based on other works in the literature (Figure 3).

Figure 3. Serum levels of galectin-3 and the +191/+292 *LGALS3* diplotypes in patients with sickle cell anemia. Intermediate +High vs Low:p= 0.0137, Mann Whitney test. Diplotypes: High/Intermediate (CC/AA; CC/CC; CC/AC; CA/AC) N=32(39.92±19.85 ng/mL), low (AA/CC; CA/CC), N=16 (25.68±13.97ng/mL).



Source: Author.

It was observed an association between the levels of gal-3 with the diplotypes (AA/CC;CA/CC) +191/+292 of *LGALS3* that were related to the low levels of gal-3, N=16 (25.68±13.97ng/mL) when compared to the diplotypes (CC/AA; CC/CC; CC/AC; CA/AC) N=32 (39.92±19.85ng/mL) (p=0.0137) (Figure 3).

4. Discussion

The pathophysiology of these events, as well as factors that can predict their development, are not completely understood, although they are important for the clinical prognosis of patients. Studies suggest that vascular occlusion, genetic factors, hemolysis and genetic factors are the most important processes related to the occurrence of clinical events in SCA patients (Bartolucci *et al*, 2012; Minniti *et al*, 2016; Senet *et al*, 2017). The vessel occlusion leads to tissue hypoxia and with the reduction of oxygen, the process of sickling increases. When this occlusion is prolonged, it causes hemolysis, releasing inflammation mediators such as IL-6, IL-1b, TNF, IL-8, among other molecules capable of promoting the proliferation, recruitment, and adhesion of erythrocytes, leukocytes, and platelets to the endothelium. This promotes a reduction in blood flow, as a result, ischemia and damage to several organs may occur (Minniti *et al*, 2010; Senet *et al*, 2017; De Carvalho-Siqueira *et al*, 2019).

In this study, it was observed that patients with the + 191AA variant genotype had lower serum levels of gal-3 when compared to the wild (CC) genotype (**Figure 2 A**). However, there were no significant differences between the levels of gal-3 and the genotypes of the +292. in addition, the diplotypes (AA/CC; CA/CC) of the polymorphisms + 191/+ 292 LGALS3 were shown to be associated with low levels of gal-3 when compared to the diplotypes (CC/AA; CA/AC; CC/AC; CC/CC). This highlights that these polymorphisms are capable of altering the availability of this protein in serum of the patients (**Figures 2 and 3**), as it has been described in the literature (Hu *et al* 2011; Mendonça-Belmont *et al*, 2016).

Mendonça-Belmont *et al*. (2016), also found an association between the +191 polymorphism (genotypes AA and CA) and lower serum levels of gal-3 when compared to the CC genotype, p=0.0001, corroborating our findings. However, these authors also found an association between the +292CC polymorphism and low serum levels of gal-3, followed by AC with intermediate levels and AA with high levels, p=0.0169, which confirms the modification in serum levels of gal-3 also due to the SNP of the +292 region.

Hu *et al*. (2011), evaluating *LGALS3* in 72 patients, Chinese population, with rheumatoid arthritis (RA), inflammatory and chronic disease, observed that patients with *LGALS3* +292CC and AC genotypes presented a decrease in serum levels of gal-3 when compared to patients with AA genotype (p=0.006), unlike our study. This may be due to the difference in sample size since our study evaluated only 48 subjects.

Understanding the influence of polymorphisms on the LGALS3 gene is important, since altered levels of the gal-3 protein have been shown in several diseases, such as some types of cancer and inflammatory diseases. no association was found between the serum levels of gal-3 with the clinical events of these SCA patients in this study (**Figure 1**). Rebholz *et al*. (2018) observed that high levels of gal-3 in plasma are associated with an increased risk of developing chronic kidney disease. Also, Shen et al. (2019) when studying gal-3 in pulmonary arterial hypertension (PAH), in patients with congenital heart disease, showed that gal-3 could aggravate PAH by activating the immune response. This reinforces the argument that gal-3 also influences other vascular and inflammatory diseases, such as SCA.

Wang *et al*. (2018) when studying the serum levels of gal-3 with the outcome of patients with acute ischemic stroke, observed an association between higher serum levels of gal-3 and increased risk of death or disability after the onset of stroke, suggesting that gal-3 may have a prognostic value in ischemic stroke. As stroke is a complication present in part of SCA patients, it is important to address further studies concerning gal-3 in this same event in SCA, with the inclusion of a healthy individuals group to assess its association in a higher number of SCA patients.

In addition to SCA, gal-3 levels, when increased, have already been associated with coronary remodeling promoting the development of coronary artery ectasia (CAE) (Aciksari *et al*, 2020). Increased serum levels of gal-3 have been observed in the patients with chronic obstructive pulmonary disease, as they were associated with systemic inflammation, which suggests that gal-3 could be a biomarker for this disease (Feng *et al*, 2017).

Regarding the genotypes of polymorphisms +191 and +292 of the *LGALS3* gene, no association was found between the frequency of the genotypes and the clinical events of these patients with SCA (Tables 1 and 2). Atabaki *et al*. (2017), in a study evaluating Iranian individuals, demonstrated that the genotype +292AC *LGALS3* was associated with RA (OR=11.622, 95% CI=4.473-28.656;p=0.001) and, even though this study was developed in a different population and disease, it shows the possible influence of this gene on inflammatory disease, as well as the importance of studying it as a biomarker.

Mendonça-Belmont *et al*. (2016), in a study conducted in the Northeast of Brazil, found an association between the +292 C polymorphism with higher frequencies of respiratory infection (p=0.032) and a vasoocclusive crisis (VOC) (p=0.023) in children with SCA, showing that this SNP influences the pathophysiology of these events since it leads to a reduction of serum protein levels.

Although these authors have also evaluated patients with SCA from northeastern Brazil, the population differs from our study, as it was performed only with children aging from 2 to 12 years, unlike the present study that evaluated adult patients aged 18 to 81 years. In addition to the difference in age, the high heterogeneity in the events can influence the association. However, even so, the influence of the *LGALS3* +191 and +292 polymorphisms in decreasing the serum levels of gal-3 was confirmed, as it is a determining genetic factor that does not depend on the age or clinical status of individuals.

Molecules such as gal-3 may act in the modulation of these frequent biological processes in the pathophysiology of SCA. This hypothesis is supported by previous studies, which show the different functions of this protein, such as inflammation, apoptosis, cell adhesion, and angiogenesis (Chen *et al*, 2015; Parker *et al*, 2019). High levels of galectin-3 in children with sickle cell disease, during the acute painful crisis, were indicators of subclinical myocardial injury (Wagdy *et al*, 2018). In another study, also in children with SCA, low serum levels of gal-3 were associated with respiratory infection and VOC (Mendonça-Belmont *et al*, 2020). This indicates variation in protein expression in different situations and shows that, although this study did not present an association between serum levels of this protein and clinical events in SCA, other studies highlight the role of gal-3 in SCA regarding the occurrence of VOC, respiratory infection and myocardial injury. However, there are not enough studies that address gal-3 in SCA, thus further studies concerning this subject are necessary.

A limitation of this study was the small sample size for the analysis of serum levels of gal-3 with clinical events and, due to the high heterogeneity and variability of these events in SCA, the inclusion of a healthy individuals group could complement a possible study in the future, which would better explain the real role of gal-3 in the pathophysiology of clinical events in SCA. Due to the functions of this protein, studying it as a biological marker is important for the development of better strategies for the treatment and prophylaxis of clinical events in SCA.

5. Conclusion

The results demonstrate that the +191 AA and AC polymorphism, as well as the +191/+292 (AA/CC; CA/CC) diplotypes of the *LGALS3* gene are able to reduce serum levels of gal-3. However, no association was observed between the frequency of polymorphisms and serum levels of gal-3 with clinical events in SCA patients.

To the best of our knowledge, our work is the first to evaluate gal-3 and its gene in adult SCA patients. Thus, further studies and new strategies are necessary to better clarify the role of these polymorphisms and gal-3 in clinical complications in SCA in order to contribute to a better understanding of the pathophysiological mechanisms in these patients. We encourage the performance of longitudinal and experimental studies, which could complement the understanding about these SNPs and gal-3 in SCA.

Conflict of interest

The authors declare no conflict of interest.

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