ORIGINAL ARTICLE

Glutathione S-transferase, catalase, and mitochondrial superoxide dismutase gene polymorphisms modulate redox potential in systemic lupus erythematosus patients from Manaus, Amazonas, Brazil

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Abstract

Objective To investigate the frequency of glutathione S-transferase (GST), catalase, and SOD2 genetic polymorphisms and their correlation with SLE.

Methods A total of 290 females (patients = 151; controls= 139) were recruited. Multiplex PCR was performed for genotyping GSTM1 and GSTT1 genes, whereas real-time qPCR was used for determination of SNPs: CAT C262T, SOD2 C47T, GSTP1 A313G and GSTP1 IVS6 -C16T.

Results Thiol levels are decreased in SLE patients ($p<0.001$), while MDA levels were significantly higher ($p<0.001$) and those carrying the polymorphisms had higher rates of oxidative stress. Patients with double null deletion GSTT1^{null}/GSTM1^{null} had a frequency almost five times higher than the controls ($p<0.001$, OR 4.81, CI 1.98–12.11). SLE patients had a lower wild-type frequency of SOD2^{CC} allele compared to controls (12.4% vs 27.3%). Statistical significances were observed on the association between the GSTT1^{null} and GSTM1^{null} with SOD2^{mut} ($p<0.001$, OR 0.15, CI 0.05–0.47), with GSTP1 A303G ($p=0.012$, OR 0.19, CI 0.05–0.69), and with GSTP1 IVS6 ($p=0.008$, OR 0.14, CI 0.03–0.63). The same was observed between SOD2 C47T with GSTP1 A303G ($p=0.09$, OR 0.27, CI 0.09–0.74) and GSTP1 IVS6 ($p=0.036$, OR 0.41, CI 0.18–0.92).

Conclusions The deletion GSTT1^{null}/GSTM1^{null} may contribute to the increased of the oxidative stress in SLE patients. Isolated GSTP1 and CAT polymorphisms do not seem to influence the increased oxidative stress, neither SLE clinical manifestations. SOD2 47$^{CT/TT}$ allele may have greater oxidative stress due to structural change in the protein and decreased H_2O_2 production. The combination of polymorphic genes may be involved in the pathogenesis of the disease.

Key points
- Major question of our paper: *Many studies have shown that the antioxidant status levels are decreased in patients with SLE, especially in severe stages of disease. We believe that this paper will be of interest to the readership of your journal had the involvement of polymorphisms and mutations in several genes that contribute to the genetic etiology of SLE, suggesting that these may influence the mechanisms of disease.*
- Our results. *Thiol level was significantly ($p<.001$) lower and MDA level significantly increased ($p<.001$) among SLE patients. Those carrying the polymorphisms had higher rates of oxidative stress. SLE Patients had a frequency almost five times higher of double null deletion GSTT1null/GSTM1null than the controls. SLE Patients had a lower wild type frequency of SOD2CC allele compared to controls (12.4% vs 27.3%).*

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We believed the deletion GSTT1null/GSTM1null may contribute to the increased of the oxidative stress in SLE patients while carriers of the mutant SOD2 47CT/TT allele may have greater oxidative stress due to structural change in the protein and decreased H2O2 production. The combination of polymorphic genes may be involved in the pathogenesis of the disease.

• Implications of our results: *Evidence for the involvement of genetic factors in severe clinical to lupus is compelling. This manuscript shows genetic insights in pathogenic pathways that may lead to severe clinical implications to LES. Therefore, it is necessary to understand their impact on overall disease pathogenesis and prognosis in these patients. We understand from general consensus about environmental factors can modify disease, however, maybe just in individuals who have a permissive genetic background. Even that no single gene predisposes some individuals to LES, we believe the genetic factors described in this manuscript are important elements in susceptibility to severe clinical to LES*

Introduction

Systemic lupus erythematosus (SLE) is a chronic inflammatory autoimmune disease. The formation of immune complexes leads to multisystemic disorders, with specific signs and symptoms depending on the affected organ, characterized by periods of exacerbation and remission. The inflammatory nature of the disease implies the existence of increased oxidative stress in these patients and can contribute to immune dysfunction and damage of biological molecules such as DNA, RNA, proteins, and antioxidants. Although the etiology is not yet fully understood, studies show that combinations of polymorphic genes can predispose to the disease, allowing environmental factors to trigger immunopathologic responses in these patients [1].

Oxidative damage can be propagated through endogenous and exogenous pathways. Decreased levels of reduced glutathione, formation of superoxide anion (O2-) via mitochondrial dysfunction, and formation of hydroxyl radicals (OH-), for example, are endogenous causes of the oxidative stress. This state is also observed in the decrease of antioxidant defense, caused by the decrease or inactivity of the enzymes that participates in redox equilibrium [2, 3]. The products of these oxidative modification cascades, such as serum levels of malondialdehyde (MDA), resulting from lipid peroxidation, are associated with the activity, organ damage, and comorbidities in SLE. Inversely, decreased levels of serum thiol have been used as markers of oxidative stress [4]. Studies demonstrate that enzymes such as glutathione S-transferase (GST), catalase (CAT), and mitochondrial superoxide dismutase 2 (SOD2) are altered in SLE. These enzymes have polymorphic genes that alter the structure or activity, thus contributing to the increased oxidative stress [5, 6]. The mundial literature showed lupus nephropathy is a severe clinical of SLE. Recently, Bona and colleague (2020) showed imbalance in the redox status between lupus nephritis types, principally in active lupus nephritis patients, with potential lipid peroxidation and could also affect renal tubular function in these patients [7].

Glutathione S-transferase (GST) is a superfamily of phase II enzymes that act catalyzing the detoxification of hydrophobic and electrophilic compounds with reduced glutathione [8]. Products of GSTM1 and GSTT1 genes are involved in the detoxification of aromatic hydrocarbons, mutagens, and compounds such as MDA [8, 9]. Both genes have homozygous null variants (M1[null] and T1[null]) resulting in the absence of the enzymes and they are investigated in the susceptibility to diseases such as cancers, atherosclerosis, and hypertension [10–12]. Glutathione S-transferase P1 (GSTP1) is of importance for oxidative stress research because of its role mainly in detoxifying xenobiotics and metabolizing any drugs and because of its involvement in cell cycle and apoptosis regulation [13, 14]. Two common GSTP1 genetic polymorphisms are studied extensively. Many common GSTP1 genetic polymorphisms are studied extensively; however, functionally, the single nucleotide polymorphisms (SNP) rs1695 (A313G) and rs1871042 (IVS6 -C16T) express a protein with altered thermal instability and catalytic activity [15–18].

Mitochondrial superoxide dismutase 2 along with catalase comprises the primary enzyme system defense against reactive oxygen species (ROS) [19], whereas SOD2 acts in the conversion of O2 anion to the less toxic hydrogen peroxide (H_2O_2) and then catalase catalyzes the conversion of H2O2 into water and oxygen [20]. The SOD2 C47T SNP (rs4880) alters the structure of the enzyme, decreasing the efficiency of entry into the mitochondrial matrix and consequently decreases the production of H_2O_2 and dismutation. Catalase is expressed in all tissue types, predominantly in the liver, kidney, and erythrocytes [21]. The CAT C262T SNP (rs1001179) is found in the promoter region of the gene and is associated with low enzyme activity [22].

Studies have demonstrated the involvement of polymorphisms and mutations in several genes that contribute to the genetic etiology of SLE. This suggests that SNPs and deletions can influence the mechanisms of disease [23, 24]. Recent studies have shown that the antioxidant status levels are decreased in patients with SLE, especially in severe stages of disease [25], and an Egyptian study demonstrated a strong association of the SOD2 rs2758332 not GSTP1 rs1695 polymorphism with the risk of SLE disease [26]. The aim of our study was to investigate the polymorphisms of glutathione s-transferases (T1[null], M1[null], P1 A313G, and P1 IVS6 -C16T), superoxide dismutase (C47T), catalase (C-262T), and their correlation with SLE.

Materials and methods

Study population

This was a case-control study conducted in the period from 2009 to 2014. A total of 348,187 patients and 195 healthy volunteers (controls) attending Rheumatology Department of Federal University of Amazon (UFAM), Brazil, were evaluated. All the patients as well as controls were only females. The control group consisted of people without consanguinity with patients, non-smoking, without inflammatory symptoms, and diseases. Patients and controls were excluded if they exhibited diseases, condition, or behavior that influences the rise of ROS, such as infectious diseases, primary diabetes, HIV, smoking, and pregnancy. The protocol was approved by the Institutional Ethical Committee from UFAM (CAAE n° 0286.0.115.000-11) and confirms to the ethical guidelines of Declaration of Helsinki 1975, as revised in 2000.

Diagnosis of SLE was based on clinical and laboratory criteria of the American College of Rheumatology (ACR) and was evaluated according to disease activity index SLEDAI at the time of sample collection and divided into three groups: inactive lupus (IL) (n=71) consisted of individuals who did not show lupus activity (SLEDAI = 0) for at least 1 year and without laboratory results characteristic of kidney injury; active lupus (AL) ($n = 47$) composed of active lupus patients (SLEDAI $\geq$ 4) without renal manifestation; and lupus nephritis (LN) (n=69) of active lupus patients (SLEDAI $\geq$ 4) diagnosed with kidney injury (persistent proteinuria greater than 500mg/24h or $\geq$3 + in urinary sediment) [27]. It is important to emphasize that 85% of our LN patients were classified of group IV, patients with diffuse nephritis [28].

According to a 2013 genetic study, the ancestry of the inhabitants of Manaus is 45.9% European, 37.8% Native American, and 16.3% African [29]. The authors do not speculate about the race of the participants due to the strong regional ancestral mix found in the Amazonian caboclos, which originate with the arrival of Caucasians and blacks in indigenous lands. However, in the interview and signing of the informed consent form, we asked each patient/volunteer (control) to answer variables such as weight, disease time, and skin color (white, black, Brown, and indigenous). Brown color was the most frequent in both groups, with 82% in patients and 78% in controls, followed by 12 and 17% for white, and 6 and 5% black, respectively. No indigenous people participated in the study.

Sample collection and clinical and laboratory analysis

Peripheral blood samples (2–3 ml) were collected in tubes containing EDTA for hematological and molecular analysis and in tube without anticoagulant for serum analysis. All the hematological, molecular, and biochemical analysis were carried out at the Laboratory of Hematology and Molecular Biology (LAEBM), Faculty of Pharmaceutical Sciences (FCF-UFAM), Manaus, Amazonas, Brazil.

Serum biomarkers

Serum urea, creatinine, urea, uric acid, and total proteins were quantified using Cobas Mira Plus® (Roche Diagnostic Systems®, Inc., Branchburg, NJ). Quantitative measurement thiols and MDA were performed as described previously [4]. Total ROS stress index and total antioxidant capacity quantification were performed using established methods [30, 31].

Sample size and power analysis

To estimate sample size for our study, we chose the total serum thiols as the central study variable. From previous results of our group [4], it was demonstrated a detectable of 80±50µmol/L in serum thiol levels. It was then defined a significance of 0.05 and power at 90%, we calculated an effect size of 1.98 and estimated a sample size of 108 patients for each study group. Sample size calculation and power analysis were performed by G*Power: Statistical Power Analyses v3.1.9.2 [32].

DNA extraction and genotyping

The DNA was extracted from leukocytes using Brazol® technique according to the manufacturer's instructions. Homozygous null deletion polymorphisms in M1 and T1 genes were determined by multiplex PCR using specific primers [33]. SNP genotyping was conducted using TaqMan® SNP genotyping assays (Applied Biosystems, Foster City, CA) on a StepOnePlus™ real-time PCR system (Applied Biosystems). The probes used were GSTP1 A303G (rs1695), GSTP1 IVS -16CT (rs1871042), CAT C267T (rs1001179), and SOD2 C47T (rs4880).

Statistical analysis

Analysis of the distribution of variables was performed using the Kolmogorov-Smirnov test together with ANOVA parametric tests or nonparametric Kruskal-Wallis tests. The ANOVA parametric test was used to analyze the distribution of the means of quantitative variables with normal distribution within categories. The nonparametric Kruskal-Wallis test was used for the off-normal distributions.

The analysis of qualitative or categorical variables of three or more groups was performed by nonparametric chi-square (χ2), corrected by the Mantel-Haenszel test and Yates. The analysis values of less than 4 were performed by Fisher's exact test. Genotype frequencies of the SNPs observed were analyzed by χ2 test for association analysis of the variables (SLE

× controls). The odds ratio (OR) was calculated to estimate the risk, and the adopted confidence interval was 95%. When values less than 4 were obtained, a Fisher's exact test was used.

The data analysis was performed using IBM SPSS Statistics, version 22.0; GraphPad Prism version 5.0; and Epi Info™ 7.1.5.2. A p value of less than 0.05 was considered statistically significant. The genotypic frequencies for polymorphisms were analyzed in accordance with Hardy-Weinberg equilibrium.

Results

The genotypic frequencies of polymorphisms were 34.5% to GSTT1[null] and 15.8% to GSTM1[null]; 44.4% to GSTP1 313[AG] and 16.9% to GSTP1 313[GG]; 27.8% to GSTP1 IVS6[CT] and 6.7% to GSTP1 IVS6[TT]; 48.9% to SOD 47[CT] and 33.8% to SOD 47[TT]; and 14.8% to CAT 262[CT] and 0.9% to CAT 262[TT].

All individuals in our study (patients and controls) were women. The average age was 32.7± 6.8 in the control group ranging from 18 to 54 years and 33.7 ± 9.8 in patients ranging from 15 to 64 years. Photosensitivity was prevalent in most patients (88.4%), followed by erythematous rash (72.5%), renal disease (46.7%), hypertension (high blood pressure) (41.7%), oral ulcers (34.8%), fibromyalgia (14.9%), and antiphospholipid syndrome (12.5%). Other clinics as diabetes, cardiac failure, and asthma had a frequency below 5%. Demographic data of patients and controls and the two most frequent medications used by patients are showed in Table 1. It is important to note that patients involved in this study were being treated with prednisone (20–60 mg/day) and chloroquine (150–400 mg/day).

Those patients who were being treated with concentrations above of these were excluded from the study. Corticosteroid use (prednisone) was categorized as follows: low (≤7.5mg/day), medium (7.5–30 mg/day), and high dose (>30mg/d) [34].

In Fig. 1, we can observe the involvement of the mutations on the oxidative stress index, while Table 2 demonstrates the hematological, biochemical, and oxidative stress parameters among SLE patients and controls. Hematocrit, hemoglobin, as well as platelet counts were found to be significantly lower ($p<0.001$) in patients as compared to controls.

Serum urea, creatinine, and uric acid levels were increased among patients, while the levels of total protein and albumin were decreased in the present study. We also evaluated serum oxidative markers such as thiol, ROS, and MDA between SLE patients and controls. Whiles thiol level was significantly ($p<0.001$) lower in SLE patients, MDA on the other hand was significantly increased ($p<0.001$) among SLE patients.

Frequencies of GSTM1 and GSTT1 genotypes between the study groups by index SLEDAI (IL, AL, and LN) were also evaluated (data not shown). We found that 20 individuals carrying the double null deletion (GSTT1[null]/GSTM1[null]) are in IL group. The comparison between the frequencies of GSTT1 and GSTM1 genotypes in patients and controls is shown in Table 3. No significant differences were observed in the frequencies of GSTT1[null] and GSTM1[null] genotypes between patients and controls. However, we observed significant differences among patients carrying GSTT1[null]/GSTM1[null] double null deletion and controls (OR 4.81, CI 1.98–12.11, $p<0.001$).

The SOD2 C47T genotypic frequency demonstrated that patients had a lower frequency of the wild allele than the controls (12.4% vs 27.3%, $p=0.026$). We found that frequency
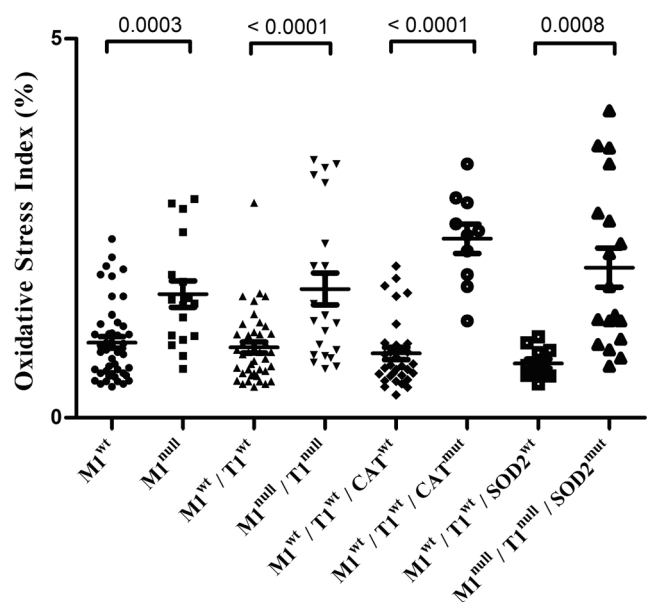
Table 1 Demographic data of study populations

Characteristics	SLE	Controls
Number	151	139
Median age, years	33.7 ± 9.8	32.7± 6.8
Smokers	04 (2.65)	0
SLEDAI, median	12	--
SLEDAI, n (%)		
0	0	--
1–5	3 (2.0)	--
6–10	64 (42.38)	--
11–19	72 (47.68)	--
≥ 20	12 (7.94)	--
Medications (mg/day)		
Prednisone (≤ 7.5) n (%)	40 (26.50)	--
Prednisone (7.5–30) n (%)	50 (33.10)	
Prednisone (> 30) n (%)	41 (27.20)	
Chloroquine (100–200) n (%)	63 (41.70)	--
Chloroquine (400) n (%)	42 (27.80)	

SLE systemic lupus erythematosus, *n* numbers

Fig. 1. Positive correlations between oxidative stress index and genotypes from SLE patients

Table 2 Hematological, biochemical, and oxidative stress parameters among SLE patients and controls

SLE		Controls	p value
Leukocytes (1000/mm3)	7.87 ± 2.84	7.48 ± 2.10	0.405
Platelets (1000/mm3)	263.94 ± 79.89	336.53 ± 61.73	<0.001
Hematocrit (%)	35.13 ± 4.49	38.48 ± 3.21	<0.001
Hemoglobin (g/dL)	11.65 ± 1.37	13.4 ± 1.24	<0.001
Urea (mg/dl)	40.29 ± 27.16	25.41 ± 6.71	<0.001
Creatinine (mg/dl)	0.89 ± 0.73	0.51 ± 0.18	0.001
Uric acid (mg/dl)	5.51 ± 2.89	3.72 ± 1.08	<0.001
Total protein (g/l)	5.87 ± 1.26	7.89 ± 0.54	<0.001
Albumin (g/l)	4.11 ± 0.69	4.62 ± 0.38	0.008
Total ROS (μmol/l)	10.32 ± 5.42	27.73 ± 34.81	<0.001
Thiols (mmol/L)	178.36 ± 104.71	463.00 ± 156.48	<0.001
Malondialdehyde (mmol/L)	4.67 ± 1.24	0.65 ± 0.32	<0.001

SLE systemic lupus erythematosus

of the mutant allele was nearly two times higher in patients compared to wild-type allele.

Frequency analysis between two polymorphisms was performed in order to verify the joint involvement of the mutations with the disease (Table 4). Associations of protection of the combined GSTs and SOD2 genotypes were observed in controls when compared to SLE patients.

The possible influence of the polymorphisms, separate and in combination, with the levels of thiols was also evaluated (Fig. 2). It was shown that SLE patients with of mutation have a higher concentration of these antioxidants, suggesting a protective effect mainly changing by GSTT1[null]/GSTM1[null] patients. Figure 3 shows greater peripheral pancytopenia in patients with SNPs and SLE, mainly involving the GSTs genotypes Mu, Theta, and Pi.

Discussion

The majority frequency of women in our study is in agreement with the literature that demonstrates that SLE is predominant in women of fertile age in the proportion 1:9 in relation to men [35, 36]. The fact that SLE as well as other autoimmune diseases are predominant in women is not fully understood, but recent study has shown that the X chromosome through independent hormones mechanisms can be one of the genetic causes of SLE based on the comparison in subject people with X trisomy and carriers of the disease [37].

The had higher rates of oxidative stress were found in individuals carrying the polymorphisms, therefore we believe that the mutations mainly in combination contribute to increased oxidative stress. Although they did not correlate genetic markers, the same results were found in rheumatoid arthritis patients [38] and lupus nephritis patients [25], which demonstrated high levels of oxidative stress in these patients.

The GST polymorphisms are distributed heterogeneously in different populations around the world [39, 40]. GSTT1[null] frequency was similar to other studies in Brazilian populations ranging from 23 to 30% [41]. However, GSTM1[null] frequency was different from other regions of Brazil ranging from 31 to 40% [42] but similar to other studies, particularly among Brazilian Indians and South African populations. The

Table 3 Frequency of GSTT1 and GSTM1 genotypes among SLE patients and controls

Genotypes	SLE N=144 (%)	Controls N=145 (%)	OR (CI)	p value
GSTT1[null]	46 (31.9)	44 (30.3)	1.08 (0.65–1.77)	0.799*
GSTT1[wt]	98 (68.1)	101 (69.7)		
	n=113 (%)	n=119 (%)		
GSTM1[null]	15 (13.3)	18 (15.1)	1.03 (0.51–2.09)	0.987*
GSTM1[wt]	98 (86.7)	101 (84.9)		
	n=126 (%)	n=107 (%)		
GSTT1[null]/GSTM1[null]	28 (22.2)	06 (5.6)	4.81 (1.98–1.12)	<0.001*
GSTT1[wt]/GSTM1[wt]	98 (77.8)	101 (94.4)		

SLE systemic lupus erythematosus, *wt* wild type; null, gene deleted

GSTT1 glutathione S-transferase theta 1, *GSTM1* glutathione S-transferase Mu 1

OR odds ratio, *CI* confidence interval, *Yates-corrected chi-square

distribution of A313G variants of GSTP1 shows data similar to a study conducted among indigenous populations of Amazonas; wild-type, heterozygous, and homozygous frequency were 35, 46, and 19%, respectively [43, 44]. Our study showed for the first time the frequency of GSTP1 IVS6 -C16T in the Brazilian population corroborating with databases like dbSNP (https://www.ncbi.nlm.nih.gov/snp/rs1871042).

The genotypic frequencies of SOD2 C47T and CAT C262T are also in accordance with data found in different Brazilian populations [45]. Taufer et al. (2005) demonstrated an approximated frequency of 60.6 and 24.4%, respectively, for CT and TT genotypes of SOD2 and it was a mixed ethnicity population, like our study [46].

Lower hematocrit and hemoglobin concentrations are expected in SLE patients. Studies have shown a correlation between low levels of hematocrit with the inflammatory response and disease activity. Also, thrombocytopenia is reported to be present in approximately 33% of SLE patients; however, levels below 100.000/mm3 were demonstrated only in 5% of cases [47, 48].

Thiol level results corroborated with earlier studies that suggest decrease in thiol levels may be an important biomarker of disease activity [3, 49]. The MDA marker is in accordance with the study by Pérez et al. (2012), which demonstrated a significant increase of MDA in SLE patients (OR 12,5; IC 3,7–41,5; $p<0.001$), indicating increased oxidative stress by lipid peroxidation in these patients [4].

In our study 86.8% of patients used prednisone as immunosuppressive ($p<.001$) and the high frequence of the double null deletion (GSTT1null/GSTM1null) found in IL group patients suggests the possibility that this double deletion is involved in a better response to treatment. In a study of patients with acute lymphoblastic leukemia, a better prognostic factor

Fig. 2. Correlations between polymorphisms and serum thiols from SLE patients

was the initial response to treatment with prednisone and, although it was not statistically significant ($p=.071$) GSTT1null deletion gave a decrease of 6.7-fold the risk of poor response to prednisone compared to wildtype individuals, while, GSTM1 and GSTP1 genotypes did not show any association [50]. Recent study, Salime and col. (2015) in an Iranian population showed high frequency to GSTT1 null in SLE patients than controls. This same study, the combination of GSTT1 null/ GSTM1 null genotypes as a risk factor for SLE (OR 2.8; CI 1.2-6.4, $p=.014$) [51]. We know that glutathione (GSH) is an important antioxidant, peforming concomitantly with glutathione S-transferase, important role in the detoxification via catalysis. This way, we understand that antioxidant activity of these enzymes exercise significant reduction of the formation of conjugates with endogenous dangerous compounds, avoiding the increased oxidative stress and facilitating metabolic detoxification of this xenobiotics.

Deletions on the GST genes are widely studied for associations with diseases [52, 53]. Although some studies have shown the involvement of GSTT1[null] and GSTM1[null] deletions in SLE, the results are different in each population, while in our study, the influence of the polymorphisms was not observed separately; other studies have reported the risk of at least one of the genotypes in susceptibility to SLE. Rupasree et al. (2013) demonstrated that GSTT1[null] deletion may be associated with risk of SLE (OR 1.83, CI 1.11–3.01) as well as the association between polymorphisms of the enzymes GSTM1, GSTT1, and

Table 4 Associations of GSTs (M1, T1, P1-A313G, and P1-IVS6 -C16T) and SOD2 C47T genotypes among SLE patients and controls

Combined genotypes	SLE (%)	Controls (%)	OR (CI)	p value
T1[null] M1[null]/SOD2[mut]	12 (40.0)	27 (81.2)	0.15 (0.05–0.47)	<0.001*
T1[wt] M1[wt]/SOD2[wt]	18 (60.0)	6 (18.2)		
T1[null] M1[null]/P1 303[mut]	22 (62.9)	34 (89.5)	0.19 (0.05–0.69)	0.012**
T1[wt] M1[wt]/P1 303[wt]	13 (37.1)	04 (10.5)		
T1[null] M1[null]/P1 IVS6[mut]	44 (80.0)	54 (96.4)	0.14 (0.03–0.63)	0.008**
T1[wt] M1[wt]/P1 IVS6[wt]	12 (20.0)	2 (3.6)		
P1 303[mut]/SOD2[mut]	5 (6.1)	20 (19.6)	0.27 (0.09–0.74)	0.009*
P1 303[wt]/SOD2[wt]	77 (93.9)	82 (80.4)		
P1 IVS6[mut]/SOD2[mut]	12 (21.8)	30 (40.5)	0.41 (0.18–0.92)	0.036*
P1 IVS6[wt]/SOD2[wt]	43 (78.2)	44 (59.5)		

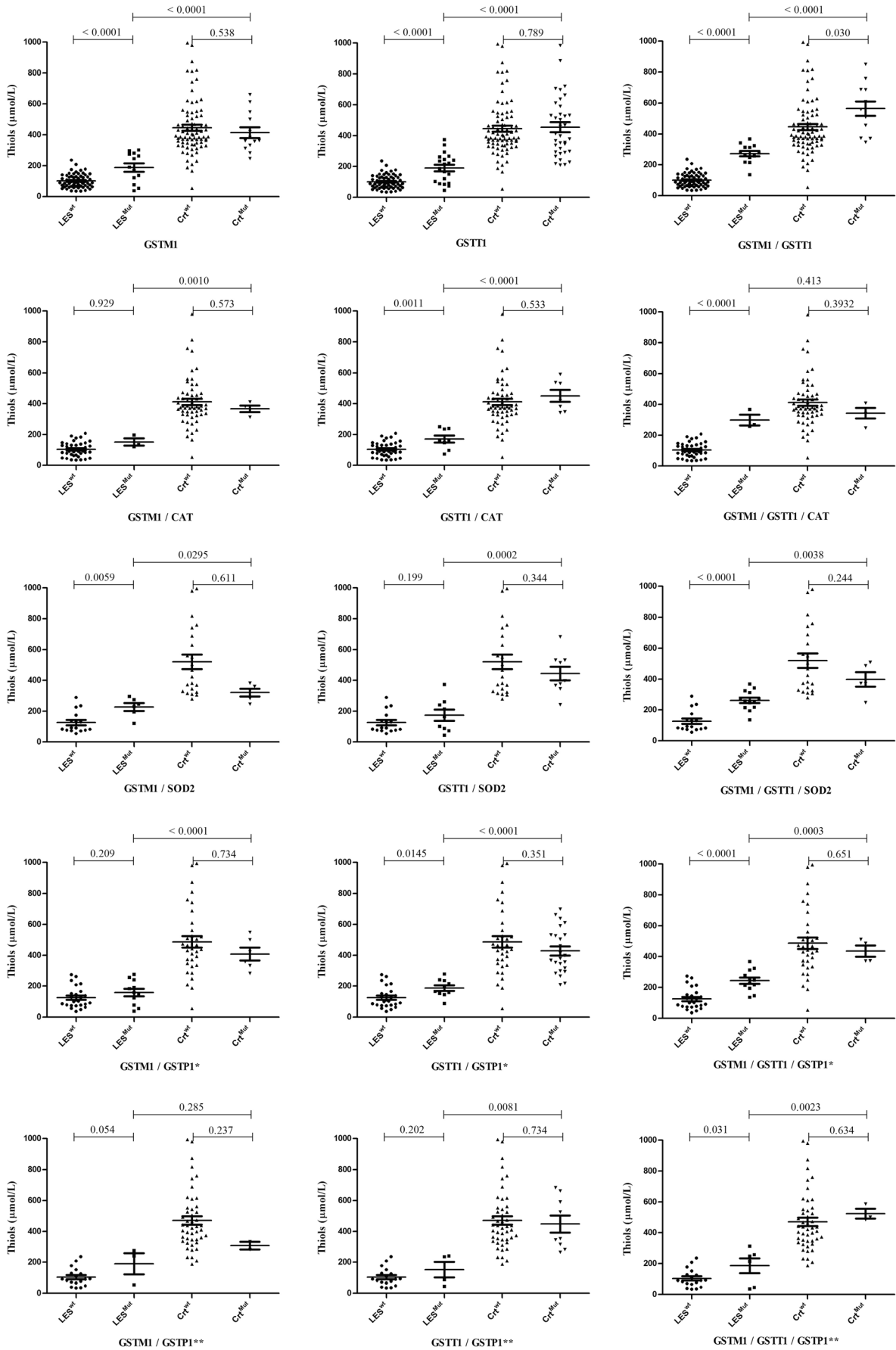T, glutathione S-transferase theta 1; M, glutathione S-transferase Mu 1

P1: T: glutathione S-transferase Pi 1; SOD2, mitochondrial superoxide dismutase

wt, wild type; null, gene deleted; mut, mutated

OR, odds ratio; CI, confidence interval
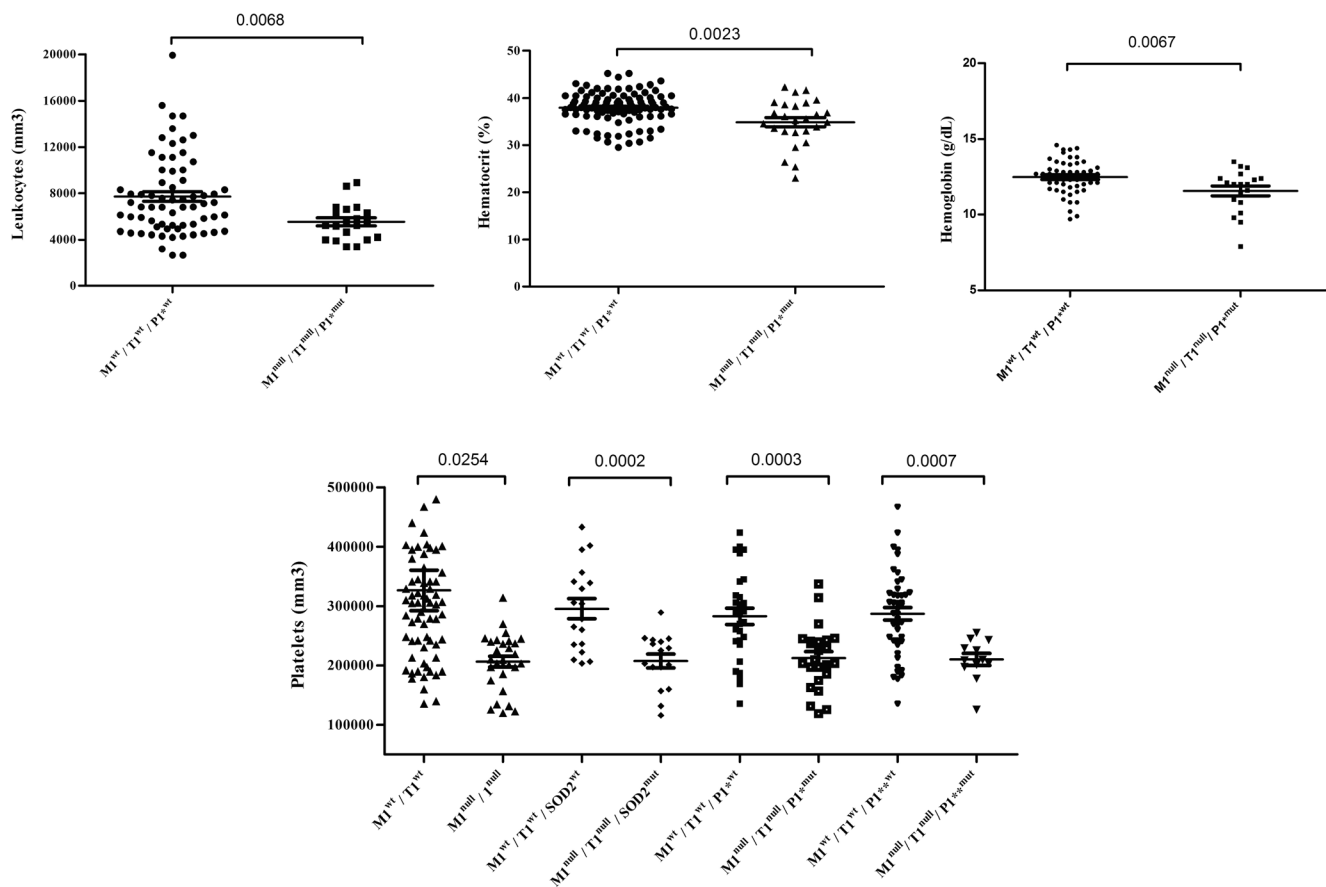
*Yates-corrected chi-square

**Fisher exact test

Fig. 3. Correlations between polymorphisms and hematological parameters from SLE patients

catecholamine methyltransferase (phase II enzyme) (OR 3.33, CI 2.30–4.82) [54]. Zhang et al. (2010) in a Chinese population showed significant differences between carriers of the GSTM1null, concluding the participation of the polymorphism on susceptibility to SLE [55]. Kang et al. (2005) did not observe any association between GST genes and susceptibility to SLE [56]. The authors suggest that the deletions may influence some clinical manifestations, the GSTM1null was associated with low frequency of hematological disorders ($p=0.042$), and GSTT1null was associated with low frequencies of discoid rash ($p=0.018$) and nephritis ($p=0.033$).

In present study, we also evaluated the influence of genotypes on clinical manifestations but no positive correlations were observed (data not shown). Fraser et al. (2003) evaluated the polymorphisms of GST in SLE patients with workers exposed to solar radiation. The authors in their study suggested GSTM1null can modify the effects of occupational exposure and risk to SLE in Caucasians. Similarly, our findings suggest that the double deletion can be a risk factor for SLE; however, our results show risk among African Americans (OR 3.9) and not in Caucasians (OR 0.8) [57].

Sobkowiak et al. (2008) observed association between SOD2TT and Raynaud's phenomenon (OR 12.0, CI 2.31–62.19, $p=0.001$) and immunologic manifestations (OR 2.9, CI

1.20–7.24, $p=0.022$) in patients with SLE, which suggests the participation of mutant homozygotes in clinical manifestations of the disease [58]. Despite the significant results found in our study, there were no correlations between the clinical manifestations and SOD2 C47T polymorphism (data not shown). SOD2 in C allele carriers are able to cross the two mitochondrial membranes, whereas T variant is incorporated only in the inner membrane of the mitochondrial matrix; therefore, carriers of TT genotype become 30 to 40% less effective in production of H_2O_2, favoring oxidative stress in these patients.

There was no positive correlation between SNPs of GSTP1 (A313G and IVS6 -C16T) and the risk to SLE (data not shown). Unlike our results, Glesse et al. (2014) demonstrated the protective role of heterozygous variant GSTP1 313 AG in European descent patients [58, 59]. We had the purpose of verifying the participation of haplotypes of GSTP1 A313G in patients with SLE. Unfortunately, few studies so far found in the literature describing possible correlations between GSTP1 IVS6 -C16T polymorphism and diseases (cognitive ability, asthma, allergies, hypertension, and acute myocardial infarction) [60–63]; however, there were no positive correlations, and our results are the first to cite these correlations.

Also, in present study, no positive correlation was observed between the CAT C262T polymorphism and SLE patients

(data not shown). These observations are supported by the study which suggested that the polymorphism is associated more with lower enzyme activity rather than the risk of the disease, hence confirming our results [64]. The same was observed by Eny et al. (2005) and D'Souza et al. (2008), suggesting that this polymorphism does not have a central role in the pathogenesis of SLE; however, the authors urge the need for studies with combinations of genotypes to determine whether combined mutations can influence the development of disease [65, 66].

Our study suggests that polymorphisms concomitantly may influence susceptibility to SLE. Different mechanisms associated with each mutation may be able to promote an exacerbated immune response by increased oxidative stress or low antioxidant capacity in these patients. It was shown that SLE patients with of mutation have a higher concentration of these antioxidants, suggesting a protective effect mainly changing by GSTT1^{null}/GSTM1^{null} patients. Studies have shown that elevated levels of thiols may reduce cellular damage caused by oxidative stress [1, 2, 53].

We understand that these data are important in the follow-up and treatment of patients, since spinal cord hypoplasia is a frequent finding in these patients and that these polymorphisms may aggravate clinical and severity, especially in those who use corticosteroids. We believe that this finding is of fundamental importance, since they reiterate the relevance of the hematological alterations associated with systemic lupus erythematosus.

Conclusion

Our study showed that patients with SLE have increased oxidative stress evidenced by increased lipid peroxidation markers and decreased levels of thiols. The double null deletion GSTT1^{null}/GSTM1^{null} may contribute to the increased oxidative stress in SLE, but has no influence on disease activity or nephritis. The isolated polymorphisms of SOD2 C47T and CAT C262T do not seem to be important in the increased oxidative stress or clinical manifestations of the disease; however, further studies are needed to determine the actual participation of polymorphisms in these patients.

Declarations

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