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Original Article

Role of glutathione S-transferases polymorphisms and monocyte CD64 expression in Egyptian patients with systemic lupus erythematosus

Aml S. Nasr [a,*], Hanan Darweesh [b], Engy El Khateeb [a], Hala L. Fayed [b], Al-Hussein El-Dakrony [b]

[a] Clinical and Chemical Pathology Department, Faculty of Medicine, Cairo University, Egypt
[b] Rheumatology and Rehabilitation Department, Faculty of Medicine, Cairo University, Egypt

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ABSTRACT

Aim of work: To study the genetic variants of glutathione S-transferases and monocyte CD64 expression in systemic lupus erythematosus patients and to evaluate their role in disease susceptibility, activity and damage.

Patients and methods: Forty female SLE patients and 40 age matched controls were genotyped for GSTP1, GSTM1 and GSTT1 gene polymorphisms using polymerase chain reaction-restriction fragment length polymorphism, conventional PCR and were assessed for monocyte CD64 expression level using flow cytometry. SLE disease activity index (SLEDAI) and the systemic lupus international. collaborating clinics/damage index (SLICC DI) were considered.

Results: The patients mean age was 28.13 ± 4.56 years and disease duration of 6.4 ± 4.9 with a SLEDAI of 14.4 ± 7.1 and SLICC/DI 3.7 ± 1.5. The frequency of GSTM1 null genotype tended to be higher (55%) in SLE patients compared to the controls (and 42.5%) ($p = 0.09$). The frequency of GSTT1 null genotype was significantly higher in SLE patients (25%) compared to controls (12.5%) ($p < 0.001$) and with a 1.7-fold risk. The genotypes frequencies of GSTP1 polymorphism were comparable between SLE patients and controls. The monocyte CD64 expression was significantly increased in the patients (MFI: 46.23 ± 4.56) compared to the control (MFI: 14.05 ± 2.01) ($p = 0.001$). The GSTM1 and GSTT1 as well as CD64 significantly correlated with the serum creatinine ($p = 0.005$, $p = 0.01$ and p-0.001, respectively).

Conclusion: The GST gene polymorphisms together with monocyte CD64 expression level could have a significant relation with SLE and with increased risk in Egyptian patients. The GST gene polymorphisms and monocyte CD64 may form potential biomarkers for renal function.

1. Introduction

Systemic Lupus Erythematosus (SLE) is a prototypical chronic and systemic autoimmune disease with heterogeneous clinical manifestations and various immune dysfunctions [1]. Genetic factors play an important role in the susceptibility to SLE [2]. Although the etiology of SLE remain unknown, it is possible that environmental and epigenetic factors contribute to its pathogenesis. It frequently develops in people with a family history of the disease, and a number of likely candidate genes have been investigated [3]. Previous studies on Egyptian SLE patients found gene polymorphisms for cytokines as TNF-α [4] and IL-6 [5] as well as for various factors as signal transducer and activator of transcription 4 [6], Mannose binding lectin [7] and vitamin D [8].

Environmental stimulators such as ultraviolet radiation and xenobiotic compounds have critical roles in the onset and progression of SLE [9]. Environmental carcinogens including smoking, air contamination and occupational exposures have strong influences on individual factors [10]. There are several known enzymes involved in metabolic activation and detoxification of carcinogens including polycyclic aromatic hydrocarbons and aromatic amines. Therefore inter-individual differences in ability to activate and detoxify carcinogens might affect the risk of developing SLE [11].

Glutathione S-transferases (GSTs) are a superfamily of metabolic enzymes which include GSTα (GSTA1), GSTμ (GSTM1), GSTΘ (GSTT1) and GST π (GSTP1). These enzymes catalyze detoxification of a wide variety of potentially toxic and carcinogenic electrophiles in human environment, by conjugating to glutathione (GSH) [11,12]. Gene clusters of GSTμ (GSTM1, M2, M3, M4, and M5) and GST Θ (GSTT1 and T2) are located on chromosomes 1 and 22, respectively [13]. Independent gene deletions exist at both GSTM1 and GSTT1 loci, resulting in a lack of active protein in

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* Corresponding author.
E-mail address: amlsoliman78@yahoo.com (A.S. Nasr).

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50% and 20% of Caucasians, respectively [14]. GST π or GSTP1, encoded by a single locus (GSTP1) on chromosome 11, is also subject to polymorphic variation [15]. The GSTP1 variant consists of an A-to-G substitution at base pair 313 at codon 105 resulting in an amino acid difference, from isoleucine to valine. It affects substrate specific catalytic activity and thermal stability of the encoded protein. [16].

Polymorphisms within genes that encode GSTs have been associated with susceptibility to nonmalignant [17] and malignant human diseases [18]. GSTs polymorphisms have been associated with SLE and its manifestations [19,20].

Although many biomarkers such as anti-DNA antibody, complements and so on, are useful for evaluating the disease activity of SLE, so far no single biomarker has been identified. Recently, the analysis of pooled indices of several parameters has been developed for evaluating disease activity in SLE [21].

It has been reported that CD64 (FcgRI) on circulating monocytes/macrophages is induced by certain cytokines, and CD64 expression on human peripheral blood mononuclear cells (PBMCs) is up-regulated at both the mRNA and protein levels after stimulation with interferon (IFN-α) [22]. Taken together, this suggests that monocytic expression of CD64 reflects serum IFNα level and thus could be considered as a surrogate marker.

The aim of the present work was to study the genetic variants of glutathione S-transferases (GSTM1, GSTT1 and GSTP1) and monocyte CD64 expression in SLE patients and to evaluate their role in disease susceptibility, activity and damage.

2. Patients and methods

The current study was carried out on 40 female SLE patients and 40 age matched apparently healthy female volunteers. The cases were recruited from those attending the Rheumatology and Clinical Immunology outpatient clinic during the period between 2013 and 2015. All patients fulfilled the Systemic Lupus International Collaborating Clinics (SLICC) classification criteria for SLE [23]. Full history taking, thorough examination and relevant laboratory investigations were performed for all the patients. Disease activity was assessed for all the patients using the SLE disease activity index (SLEDAI) [24]. Furthermore, systemic lupus international. collaborating clinics/American College of Rheumatology damage index (SLICC/ACR DI) was also assessed [25]. Patients with an overlap syndrome or mixed connective tissue disease were excluded. Local institutional research ethical board approval and oral informed consents were taken from all the participants prior to the study. Also the study conformed to the provisions of the World Medical Association's Declaration of Helsinki.

In addition to the routine laboratory investigations including complete blood count, liver and kidney function tests and 24 h urine protein, the lipid profile, antinuclear antibody (ANA), anti-double stranded DNA (anti-ds DNA), complement (C3 and C4), lupus anticoagulant and anticardiolipin were also measured.

2.1. Genotyping for GSTP1, GSTM1 and GSTT1 gene polymorphisms

It was performed for all subjects using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and conventional PCR respectively and monocyte CD64 expression level was assayed using flow cytometry. Three ml of blood were withdrawn in a sterile ethelene diamine tetra acetic acid (EDTA) vacutainer. DNA was extracted from the whole blood using DNA extraction kit (QIAamp Blood Kit (Cat. No. 51106; Qiagen Inc., Valencia, CA) according to the manufacturer's instructions.

For GSTP1 polymorphism, we used a PCR-RFLP method according to Harries et al. [26] The PCR was carried out in a total volume of 25 μL containing 12.5 μl master mix (Fermentas, Lot: #K0171), 2 μl forward primer (20 pmol) (5′ ACC CCA GGG CTC TAT GGG AA 3′), 2 μl reverse primer (20 pmol) (5′ TGA GGG CAC AAG AAG CCC CT 3′), 3.5 μl nuclease-free water and 5 μl genomic extracted DNA. The PCR products were incubated with BsmAI (New England Biolabs, Hertfordshire, United Kingdom) for 4 h at 37 °C and loaded on a 2.5% agarose gel stained with ethidium bromide. The wild type (313 AA) showed a single band at 176 bp. The presence of the 'G' allele introduces a restriction site. Three bands of 176 bp, 91 bp and 85 bp were seen heterozygous (313AG). The homozygous (313GG) had two bands of 91 bp and 85 bp. The size of the amplified product was read with a DNA ladder of different molecular weights (fermentas, NoLimits™ 100 bp DNA Fragment, catalogue number SM1441).

Homozygous deletions of GSTM1, resulting in absence of specific enzymes, were studied using conventional PCR technique [27] including the housekeeping gene BCL-2 as internal control. Primers used for GSTM1 were as follows: 5-GAACTCCCTGAAAAGCTAAAGC-3 and 5-GTTGGGC TCAAATATACGGTGG-3. Primers used for BCL-2 were as follows; 5-GCAATTCCGCATTTAATTCATGG-3 and 5-GAA ACAGGCCACGTAAAGCAAC-3 .PCR reaction was carried out in a total volume of 25 μl. Presence of GSTM1 allele, identified by a 219-bp fragment, or its complete deletion (null genotype), was analyzed by electrophoresis on a 2.5% agarose gel. The absence of amplifiable GSTM1 indicates a null genotype. BCL 2 gave a band at 154 bp fragment.

Homozygous deletions of GSTT1, resulting in absence of specific enzymes, were studied using conventional PCR technique [27] including the housekeeping gene BCL-2 as internal control. The primers used for GSTT1 were as PCR was performed in 25 μL .The presence of GSTT1 alleles was identified by a 480 bp fragment.

2.2. Quantitative measurement of CD64 expression using flow cytometry

CD64 expression on monocytes was measured by CD64 PE / CD45 FITC monoclonal antibodies (Becton-Dickinson, CA, USA) according to the manufacturer's instructions. At room temperature, 10 μL of CD64PE/CD45 FITC was added to 50 μL of whole blood and incubated for 60 min in the dark. This was followed by lysis of red blood cells by using 250 μL of OptiLyse C (Beckman Coulter), with washing. These specimens were analyzed using a FC500 flow cytometer (Beckman Coulter).

2.3. Statistical analysis

All statistical analysis was performed with SPSS Version 20. Demographic differences between groups were examined by $\chi 2$ test or an independent Student's *t*-test whenever appropriate. Frequencies genotypes were compared between patients and control groups by the $\chi 2$ test or Fisher's exact test. The odds ratio (OR) and 95% confidence intervals (CI) were also estimated. Correlation coefficients were calculated using Spearman correlation coefficient. Chi-square ($\chi 2$) test was performed to assess deviation from Hardy–Weinberg equilibrium in controls. $p < 0.05$ was considered significant.

3. Results

The age of the patients mean age was 28.13 ± 4.56 years (19–45 years) and disease duration of 6.4 ± 4.9 years (1–19 year). The mean age of the matched controls was 27.33 ± 7.37 years (17–51 years) ($p = 0.86$). Clinical and laboratory data of the patients are summarized in Table 1. The monocyte CD64 expression was significantly increased in the patients (Mean fluorescent