Systemic Lupus Erythematosus activity and serum bilirubins

Brenda Hernandes dos Santos[1], Camila Monteiro de R Almeida[1], Thelma L Skare[1]

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ABSTRACT

Background: Serum bilirubins play an important role in controlling oxidative stress; there is increased oxidative stress during activity of rheumatic diseases such as systemic lupus erythematosus (SLE).
Objective: To study bilirubin levels in SLE (Systemic Lupus Erythematosus) patients and relate them to disease activity.
Methods: We analyzed levels of total bilirubins (TB), direct bilirubins (DB) and indirect bilirubins (IB), sedimentation rate (ESR) and C reactive protein (CRP) in 143 SLE patients. Data were collected on the clinical and autoantibody profiles and patients underwent measurement of SLEDAI and SLICC .
Results: Correlation of indirect bilirubin values with SLEDAI was negative (p=0.02; Spearman rho=-0.18). Comparing the levels of IB according to the clinical activity profile we observed associations with increase of anti DNA titer (p=0.027) and with decrease in complement levels (p=0.017). ESR correlated negatively with IB levels (p=0.01) but CRP did not (p=0.15). In a multiple linear regression analysis only the increase in ESR titer remained significant. SLICC values were not correlated with TB (p=0.30), DB (p=0.12) or IB (p=0.31).
Conclusions: We conclude that IB levels in SLE correlate negatively with disease activity. IB levels are lower in patients with higher ESR.

Keywords: Systemic lupus erythematosus; Oxidative stress; bilirubins.

1. Rheumatology Unit - Evangelic University Hospital, Curitiba, PR-BRAZIL

INTRODUCTION

Oxidative stress is a state in which there is an increase in reactive oxygen species (ROS) as result of an increase in its production or because of reduced antioxidative defenses of the body[1]. This imbalance is associated with a variety of inflammatory conditions or associated with the aging process and results in oxidation of cellular components such as DNA, proteins, lipids and carbohydrates[1]. In a normal physiological state there is a balance between oxidizing and reducing agents, including the generation of active oxygen radicals and antioxidant defenses[1,2]. Substances such as alpha-tocopherol, beta carotene, retinol and bilirubins act as antioxidants and avoid the deleterious consequences of oxidative stress such as accelerated atherogenesis[2-4].

Interpretation of the role of serum bilirubins has changed recently. Initially considered just the final product of heme metabolism, they have emerged as substances with potent cytoprotective action due to their antioxidant, anti-inflammatory and immunosuppressive roles when at low concentrations[5,6]. When indirect bilirubin (IB) reacts with an oxidizing agent, this formed oxidized IB is excreted in the urine[7]. Lower bilirubin serum levels have been demonstrated in patients with peripheral artery disease, increased intima-media thickness of carotid artery and with coronary calcification[8]. Their protective role has also been studied in the context of metabolic syndrome X, myasthenia gravis, and systemic lupus erythematosus (SLE)[3,4,9,10].

SLE is an autoimmune multisystemic inflammatory disease characterized by cyclic periods of activation and remission. During periods of activity, there is vascular inflammation and increased oxidative stress[4]. Vitek et al[4] studying the levels of TB in SLE patients noticed that they were lower in SLE than in controls and that there was an inverse correlation between serum levels and disease activity. Yang et al[3] confirmed that serum bilirubins were lower in SLE patients and that their values

were negatively associated with C-reactive protein and renal involvement.

In the present study we analyzed serum bilirubins of patients with SLE looking for its association with disease activity measured by SLEDAI and cumulative damage measured by SLICC.

METHODS

This study was approved by the Ethics Committee in Research of the local institution and written consent was obtained from all participants.

We evaluated 143 patients with SLE, chosen in order of arrival to the Clinic of Rheumatology and willing to participate in the study, between September 2010 and September 2011. All participants had been diagnosed with SLE according to the classification criteria of the American College of Rheumatology (ACR)[11]. We excluded patients who had evidence of liver injury, use of potentially cholestasis inducing medication, patients younger than 18 years, smokers, with diabetes mellitus, renal failure, infections, hemolytic anemia at the time of data collection. Patients with tumors, pregnancy and alcohol users were also excluded.

To assess the functional integrity of patients' liver, serum levels of aminotransferases (AST and ALT) and alkaline phosphatase were measured as well as serum bilirubins. Liver aminotransferases measurements were made by the method of Dry Chemical (QS)-System VITROS (Johnson & Johnson®) and their reference values were 17-59 U/L for AST in men, 14 to 36 U/L in women; 21-72 U/L for ALT in men and 9-52 U/L in women. Alkaline phosphatase was determined by the enzymatic method and its reference value is 38--126 U/L. With regards to bilirubin, we used the method of Dry Chemical (QS)-VITROS System (Johnson & Johnson ®). Both TB and DB were measured. The value of IB was obtained by subtracting the value of DB from TB. We considered as normal values: 0.2--1.3 mg/dL for the TB; 0.0 to 1.1 mg/dL for IB and 0 to 0.4 mg/dL for DB. At the same time sedimentation rate (ESR – by the Westergreen method) and C reactive protein (CRP by immunoturbidimetry; normal value <5 mg/L) were determined.

Patients underwent a demographic questionnaire and SLEDAI[12] and SLICC[13] measurements as tools for assessing respectively disease activity and cumulative damage of the disease. We also collected data on cumulative clinical and autoantibody profile. Data obtained were on joint involvement, skin (butterfly rash, discoid lesions, aphtae and photosensitivity), serositis, glomerulonephritis, leucopenia, lymphopenia, seizures and psychosis. The definition of each of these organ involvements followed those of the ACR classification criteria for SLE[11]. We also obtained an autoantibody profile, composed of anti-DNA, anti Ro, anti La, anti RNP, anti Sm, anti aCl IgG, IgM anti aCl, LAC and rheumatoid factor. In our institution dsDNA antibody is determined by indirect immunofluorescence method using Crithidia luciliae (IMMUNOCONCEPT, ALKA®), with a cutoff of 1/10. The values of anti Sm, anti Ro, La and anti Anti RNP are determined by ELISA kit (ORGENTEC, ALKA ®) and we considered positive values equal to or above 25 U/mL.

SLEDAI[12] is a composite index for the evaluation of 24 clinical and laboratory items with different weights, present until 10 days before the visit the value of which ranges from 0 (no clinical activity) to 105 (maximum activity). Values up to 4 are considered as disease with low activity, between 4 and 10 with moderate activity and above 10 with high activity. The SLICC[13] is a composite index of 12 items ranging from 0 to 46 and assesses the cumulative damage of SLE, zero meaning no damage and 46 the maximum damage.

Data on SLEDAI, SLICC, ESR, CRP, liver function and bilirubin levels were all obtained simultaneously, cross-sectionally. Data on population characteristics: clinical and auto antibody profile and treatment were obtained through chart review, retrospectively.

All data were grouped into frequency and contingency tables. Bilirubin levels of patients according to clinical and autoantibody profile were studied by t test or Mann Whitney test according to variable distribution. Correlation studies were made for testing the the Pearson s coefficient or Spearman s rank order coefficient according to variable distribution. Values that in univariate analysis showed $p < 0.1$ underwent multivariate analysis by linear multiple regression (stepwise). Calculations were made with the aid of the software Medcalc version 12.1.3.0. The level of significance adopted was 5%.

RESULTS

A) ANALYSIS OF THE STUDIED POPULATION:

The study sample consisted of 143 patients aged 18-69 years (average 37.29 ± 11.88 years) and duration of disease 0.3 to 39 years (median 6.5 years). Seven of 143

patients (4.89%) were male and 136 (95.31%) females. In relation to ethnicity, 4/143 (2.80%) were Asian descendents, 55/143 (16.08%) Afro descendents and 84/143 (58.74%) Caucasians.

Regarding the cumulative clinical profile 86/143 (60.13%) had joint involvement; 57/143 (39.86%) butterfly rash; 10/143 (6.99%) had discoid lesions; 62/143 (43.35%) oral ulcers; 96/143 (67.13%) photosensitivity; 8/143 (5.59%) pleuritis; 13/143 (9.09%) pericarditis; 12/143 (8.39%) hemolytic anemia; 46/143 (32.16%) had leucopenia; 9/143 (6.29%) lymphopenia; 12/143 (8.39%) seizures and 5/143 (3.49%) psychosis. With regards to glomerulonephritis 52/143 (36.36%) had had this manifestation: 8/52 (15.38%) were glomerulonephritis class II; 10/52 (19.23%) Class III; 21/52 (40.38%) Class IV; 9/52 (17.3%) Class V and 4/52 (7.6%) Class VI.

The profile of autoantibodies can be seen in Table I.

Regarding treatment, 77/143 (53.8%) patients were on glucocorticoids (doses from 10 to 60 mg prednisone/day; median of 10 mg/day); 25/143 (17.8 %) on azathioprine; 29/143 (20.2%) on methotrexate; 13/143 (9.09%) on mofetil mycophenolate; 117/143 (81.8%) on antimalarials and 1/143 (0.69%) on cyclophosphamide at time of data collection.

Erythrocyte sedimentation rate (ESR) varied from 1 to 115 mm with a median value of 18 mm; CRP values varied from 0.9 to 49.70 mg/L (median value 5 mg/L).

The value obtained for SLEDAI varied from 0 to 18, with a median value of 2 and SLICC value varied from 0 to 14, with a median of 2.

Tests performed to verify liver integrity showed ALT values from 5 to 32 U/L (median 23 U/L); AST values from 6 to 59 U/L (median 22 U/L) and alkaline phosphatase from 38 to 123 (median 74 U/L). With respect to bilirubin results, they were: TB from 0.2 to 1.7 (mean 0.5), DB from 0 to 0.7 (mean 0.2) and IB from 0.1 to 1.3 (median 0.3).

TABLE I. AUTOANTIBODY PROFILE IN 143 PATIENTS WITH LUPUS ERYTHEMATOSUS

Autoantibodies	N	%
Anti-DNA	53/143	37.06%
Anti SM	28/143	19.58%
Anti Ro	45/143	31.46%
anti La	27/143	18.88%
Anti RNP	28/143	19.58%
Rheumatoid factor	14/143	9.79%
aCl IgG	16/143	11.18%
aCl IgM	16/143	11.18%
LAC	12/143	8.39%

aCl = anticardiolipin; LAC = lupus anticoagulant

B) STUDIES OF THE CORRELATION OF BILIRUBIN VALUES WITH SLICC, SLEDAI AND INFLAMMATORY ACTIVITY TESTS:

Analyzing the correlation of bilirubin values with SLEDAI, we observed that there was no correlation between this index with TB (p=0.17) and DB (p=0.47) (although) there was a negative correlation with IB (p=0.02, Spearman rho = -0.18, 95% CI -0.34 to -0.01).

Examining the SLEDAI scores, we found that at time of data collection there were: 3/143 (2.09%) patients with convulsions (2.09%), 1/148 (0.69%) with peripheral vasculitis, 1/143 (0.69%) with cranial nerve injury; 15/143 (10.48%) with active arthritis; 54/143 (37.7%) with muco-cutaneous manifestations (skin rash, alopecia and ulcers); 9/143 (6.29%) with hematological manifestations (leucopenia and thrombocytopenia); 17/143 (11.8%) with renal disease activity (increase in proteinuria, pyuria, hematuria and casts); 7/143 (4.8 %) with complement decrease and 21/143 (14.6%) with a 25% increase in the title of anti dsDNA. Comparing the levels of IB according to the clinical activity profile observed we obtained data shown in Table II.

TABLE II. COMPARISON OF INDIRECT BILIRUBIN LEVELS ACCORDING TO THE PROFILE OF CLINICAL ACTIVITY MEASURED BY SLEDAI.

Clinical profile (*)	With activity (**)	Without activity(**)	P
Arthritis	0.25 ± 0.20	0.33 ± 0.21	0.06
Muco-cutaneous	0.28 ± 0.17	0.35 ± 0.23	0.16
Hematological activity	0.23 ± 0.11	0.33 ± 0.21	0.23
Kidney	0.23 ± 0.11	0.33 ± 0.21	0.28
Decreased complement	0.24 ± 0.15	0.34 ± 0.22	0.01
Increased anti ds DNA titer	0.24 ± 0.13	0.34 ± 0.221	0.02

(*)Other items were not studied due to the low prevalence in the sample.
(**)Activity was determinate according to criteria used by SLEDAI determination [12].

Studying the correlation of IB levels with ESR and CRP we found a negative correlation for ESR (p=0.01; Spearman rho=-0.21; 95% CI=-0.37 to -0.046) but not for CRP (p=0.15).

In a multiple linear regression model where SLEDAI, joint activity data, reducing complement and increased anti dsDNA and ESR were placed, only the values of ESR remained significant (t=-2.81; B coefficient =-0.019; p=0.005).

The SLICC values were not correlated with the TB (p=0.30), DB (p=0.12) or IB (p=0.31).

DISCUSSION

The results of this study suggest that serum levels of IB correlate negatively with ESR. We speculate that this is probably due to a compensatory mechanism against oxidative stress resulting from inflammatory activity.

Bilirubins at physiological levels account for 10% of the total antioxidant capacity in adults[14]. Their antioxidant capacity is higher than that of alpha tocopherol, ascorbic acid and catalase that have been recognized for a long time as powerful antioxidants[2,4]. A study in the Framingham cohort has examined the link between serum bilirubin and coronary artery disease and found that higher bilirubin levels were associated with lower risk of myocardial infarction or other cardiovascular disease events[15]. It has also been demonstrated that patients with Gilbert disease, a genetic disorder associated with elevations of unconjugated bilirubins, have lower prevalence of ischemic heart disease than control population[16]. Smoking has also been associated with lower bilirubin levels and has attenuated its protective cardiovascular effect[17]. All these observations points to the fact that bilirubins might may have a role in alleviating oxidative stress in the blood.

In the same way it is also believed that bilirubins play an immunoregulatory role[18]. It is also known that they inhibit C1q complement activation by causing a pigment-protein interaction on C1q esterase[18]. As modification of DNA by ROS may turn it immunogenic, oxidative stress has been implicated in the appearance of auto antibodies[19]. This hypothesis is supported by the fact that anti-DNA antibodies have an increased reactivity against denatured DNA by ROS[19]. The protective action of IB might help decrease this immune stimulation.

Some authors have demonstrated that serum bilirubin levels are negatively related to lupus disease activity and extent[4]. Others, like Yang et al[3] have found a positive relationship of total and IB with CRP in patients with SLE. We did not establish any association with CRP in our current work; only ESR maintained a negative correlation. Characteristically in SLE, unlike other rheumatic diseases, there is a relative failure of acute phase CRP response during active disease despite evident tissue inflammation[20] justifying the classical observation that very high levels of CRP in a lupus patient suggest associated infection[21]. According to Batuca et al[22], CRP levels are elevated only in certain manifestations of disease activity such as pleuritis but not in other. None of our patients had serositis at the moment of the study. In the same way, anti CRP antibodies have been found in lupus patients mainly in those with renal activity[19] and may contribute to modify the serum measurement of this inflammatory marker. Differences in the spectrum of clinical activity and levels of anti CRP auto antibodies may explain the discrepancy of results between our study and those of the previously mentioned work.

Moreover, in the present study it was not possible to relate any specific form of clinical lupus activity with IB levels.

Therapeutic use of IB infusions has been applied in animals[17]. In perfused rat heart, it reverses the effects of ischemia in cardiac function[23]; intravenous administration of bilirubin ameliorates pulmonary fibrosis induced by bleomycin[24] and injury to liver grafts in rats is prevented by rinsing them with bilirubin[25].

A limitation of our study is the fact that most of our patients had low values of SLEDAI (mean 2.0) not allowing us to observe changes in bilirubin levels that may happen with a very active disease. Furthermore, some forms of activity such as serositis are underrepresented in our sample. Taking into account that cardiovascular events are a leading cause of death in SLE patients[26], it is very important to study the mechanisms involved in the defense of oxidative stress generated by disease activity in order to better understand these complications.

In conclusion, we have shown that that the levels of IB in SLE patients negatively correlate with ESR. No association could be found with any specific manifestation of disease activity.

CORRESPONDENCE TO
Thelma L Skare
Rua João Alencar Guimarães, 796
80310420 Curitiba PR – Brazil
E-mail: tskare@onda.com.br

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