Oxidative Stress in Patients with Chronic Inflammatory Diseases in a Tertiary Health Care Setting in Africa

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Introduction

Rheumatoid Arthritis (RA) and Systemic Lupus (SLE) are multi-systemic, chronic autoimmune inflammatory diseases, associated with chronic disability, reduced quality of life, greater cardiovascular burden and mortality compared to the general population [1,2]. RA affects about 1-2% of the world’s population [3] while SLE affects about 0.5% [4]. Both conditions are more prevalent in females [3,4].

Free radical-mediated reactions have been recently identified as a possible mechanism for the pathogenesis of most chronic inflammatory diseases [5]. These are highly reactive species implicated in oxidative damage to biomolecules, proteins, lipids, DNA and membranes. Oxidative damage is kept in control by the body’s antioxidant system. Antioxidant provides major protection against oxidative damage by scavenging and neutralizing reactive species or by breaking oxidative chain reactions. Antioxidant species are enzymatic (superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT)) or non-enzymatic (glutathione (GSH), beta-carotene and vitamins A, C and E). Antioxidant status can be assessed by measuring these antioxidant species or by measuring the ferric reducing ability of plasma (FRAP) [6].

Oxidative stress refers to “an imbalance between oxidants and antioxidants in favour of the oxidants, leading to a disruption of redox signalling and control and/or molecular damage” [7]. Reactive oxygen species (ROS) have very short half-lives, therefore assessment of oxidative stress using in vivo measurements is difficult to achieve. However, lipids, proteins and nucleic acid that have been modified by ROS have longer half-life and are therefore ideal markers of oxidative stress. Lipid peroxidation, which is one of the major consequences of oxidative stress, results in the production of unstable hydroxyperoxides, which break down into various bioactive aldehydes such as malondialdehyde (MDA), 4-hydroxy-2-nonenal (HNE) and thiobarbituric acid-Reactive substances (TBARS) [8]. These are important markers of oxidative stress.

Abstract

Oxidative stress has been recently implicated in the pathogenesis of Rheumatoid Arthritis (RA) and Systemic Lupus (SLE). Little is known on oxidative status of these patients in Sub-Saharan Africa.

We assessed oxidative stress in 52 RA patients, 29 SLE patients and 72 healthy controls from measuring serum levels of malondialdehyde (MDA), glutathione (GSH) and ferric acid reducing ability of plasma (FRAP) and investigated association of these with disease activity. Oxidant status was high serum MDA or low GSH and antioxidant status was low plasma FRAP.

Patients had higher mean serum MDA compared to controls (0.44 ± 0.37 μmol/l; 0.19 ± 0.93 μmol/l); p = 0.036. Mean serum GSH values (0.37 ± 0.35 μmol/l) was lower in patients vs. (0.71 ± 0.20 μmol/l); p<0.001 in controls. Mean serum FRAP values was lower in patients vs. controls (199 ± 100 μmol/l; 212 ± 99 μmol/l; p = 0.420). There was a positive correlation between MDA and disease activity DAS-28 (r = 0.035, p = 0.040), SLEDAI (r = 0.136, p = 0.241); negative correlation between GSH and disease activity DAS-28 (r = -0.275, p = 0.024), SLEDAI (r = -0.063, p = 0.373); negative correlation between FRAP and disease activity (DAS-28 (r = -0.103, p = 0.233), SLEDAI (r = -0.033, p = 0.393).

Sub-Saharan African RA and SLE patients have higher oxidant status and lower antioxidant status compared to healthy individuals as described in other settings. Oxidative stress in these patients is associated to disease activity.

Keywords: Oxidative stress; Rheumatoid arthritis; Systemic lupus; Malondialdehyde; Glutathione; Ferric acid reducing ability of plasma; Africa
It is well established that oxidative stress plays a major role in most chronic diseases such as neurodegenerative diseases, cardiovascular diseases [9], diabetes, metabolic syndrome [10], chronic kidney disease [11] and cancers [12]. Many studies support an interdependent relationship between oxidative stress and chronic inflammation [13]. Multiple case control studies have demonstrated an increased oxidative stress in patients with RA [14] and SLE [15,16] compared to healthy controls, with greater oxidative stress occurring in RA [17]. Oxidative stress may participate in the pathogenesis of RA as there are reports of oxidative damage in synovial fluid, increased lipid peroxidation, increased carbonyl resulting from protein oxidation, DNA modifications, alongside structural changes in hyaluronic acid, cartilage, and collagen in RA [18]. Also, oxidative stress influences the pathogenesis of SLE as it causes altered T-cell signalling, autoantibody production [19] and contributes substantially to cardiovascular diseases, a major cause of morbidity and mortality in SLE [20]. Furthermore, intake of antioxidants have been shown to significantly relieve clinical symptoms in RA [21] and SLE [22].

Very little is published on the oxidative stress of patients with chronic inflammatory diseases in sub-Saharan Africa. The aim of this study was to assess oxidant/antioxidant status of patients with chronic inflammatory diseases at the Douala General Hospital, Cameroon.

Methods

Study population and sampling

After prior ethical clearance from Institutional Review board, we carried out a case control study in the Rheumatology unit of the Douala General Hospital (DGH) from January to April 2015. We systematically included all RA and SLE patients coming for follow-up visits during the study period. The control group consisted of volunteer age and sex-matched patient care-takers and health workers at the hospital. Were excluded those aged less than 16 years, chronic smokers, those with cancer, active infections or history of intake of an antioxidant supplement within the past month.

Clinical assessment

Socio-demographic data and past medical history were obtained from all participants through an interviewer-administered questionnaire. Anthropometric data were obtained with the use of a weighing scale (BRN 9311) and a stadiometer; obesity was considered as a BMI ≥ 30.0 kg/m². After rest of 15 min, blood pressure values were obtained for both using an automatic blood pressure machine (OMRON® M2, HEM-7121-E) and the average value recorded.

Disease activity was assessed for RA patients using disease activity for 28 joint indices score (DAS-28) [23], and for SLE patients using systemic lupus erythematosus disease activity index (SLEDAI) [24].

Biochemical assessment

After 48 h of abstaining from alcohol intake, cigarette smoking and vigorous exercise, and after 8 h of fasting, blood was collected from the study participant. Venous Blood was obtained for assessment of lipid profile and of oxidant/antioxidant status.

Lipid profile (triglycerides (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-c) and high-density lipoprotein cholesterol (HDL-c)) were evaluated using commercially available test kits (Cypress Diagnostic®).

Oxidant/antioxidant status was evaluated using spectrophotometry (Biomate-6, UV-VIS, Madison, WI, USA). Serum MDA was assayed based on the principle that reaction between MDA and thiobarbituric acid (TBA) leads to the formation of a pink coloured complex, having a maximum absorption of 532 nm [25]. Serum GSH was assayed based on the principle that glutathione reductase catalyses the reduction of oxidised glutathione by NADPH to reduced glutathione. Serum antioxidant capacity (FRAP) was assayed based on the capacity of the serum to reduce iron to a low pH (3.6). The absorbance is measured at 596 nm [26].

Statistical analysis

Data were entered into a Microsoft Excel 2013 database and exported to Statistical Package for Social Sciences (SPSS), version 23 Inc., Chicago, Illinois, USA) for all statistical analysis.

Continuous data were summarised as means and standard deviations while categorical data were summarised as frequencies and proportions. Hypothesis testing for statistical significance of the difference between proportions was done using the Pearson’s Chi-Square Test, and between means using Student’s T-test. Bivariate correlation between continuous variables was assessed using Spearman’s correlation index. Statistical significance set at α ≤ 0.05. The study power was set to the default 0.05 (5%).

Results

Baseline characteristics

A total of 52 RA patients (41 females, 11 males), 29 SLE patients (all females) and 72 healthy controls were involved in the study.

The mean age of patients was 47.5 ± 13.5 years and 44.6 ± 10.2 years for controls. Male-to-female sex ratio was 1:6.4 for patients and 1:6.2 for controls. There was no significant difference in age (p=0.133), sex (p=0.998) and body mass index (p=0.056) between patients and controls.

Mean disease duration for patients was 7.0 ± 3.2 years; 7.3 ± 3.1 years in RA and 6.5 ± 3.2 years in SLE. Patients demonstrated higher cardiovascular risks (higher LDL-c, lower HDL-c, higher TC and higher TG) compared to healthy controls (Table 1).

Table 1: Baseline characteristics of study participants.
Markers of oxidative stress

Patients had higher mean serum MDA values (0.44 ± 0.37 µmol/l) compared to controls (0.19 ± 0.93 µmol/l) (p=0.036). Conversely, patients had significantly lower mean serum GSH values (0.37 ± 0.35 µmol/l) compared to controls (0.71 ± 0.20 µmol/l) (p<0.001); and lower mean serum FRAP values (199 ± 100 µmol/l) compared to controls (212 ± 99 µmol/l) (Table 2).

Table 2: Markers of oxidative stress and antioxidant status in study participants.

<table>
<thead>
<tr>
<th>Comorbidity</th>
<th>MDA (µmol/l)</th>
<th>GSH (µmol/l)</th>
<th>FRAP (µmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Obesity</td>
<td>0.46 ± 0.37</td>
<td>0.41 ± 0.34</td>
<td>194 ± 100</td>
</tr>
<tr>
<td>Hypertension</td>
<td>3.27 ± 0.73</td>
<td>3.13 ± 0.65</td>
<td>1.65 ± 0.62</td>
</tr>
<tr>
<td>Diabetes</td>
<td>13 (25.0)</td>
<td>17 (13.8)</td>
<td>6 (7.4)</td>
</tr>
<tr>
<td>Smokers</td>
<td>5 (9.6)</td>
<td>1 (3.5)</td>
<td>10 (18.5)</td>
</tr>
<tr>
<td>Kidney Disease</td>
<td>5 (9.6)</td>
<td>10 (34.5)</td>
<td>15 (18.5)</td>
</tr>
<tr>
<td>LDL-c (mmol/l)</td>
<td>3.27 ± 0.73</td>
<td>3.13 ± 0.65</td>
<td>1.65 ± 0.62</td>
</tr>
<tr>
<td>HDL-c (mmol/l)</td>
<td>1.12 ± 0.16</td>
<td>1.14 ± 0.16</td>
<td>1.74 ± 0.64</td>
</tr>
</tbody>
</table>

Disease duration and comorbidities were not associated to markers of oxidative stress. On bivariate analysis on logistic regression, it was shown that these possible confounders alongside other comorbidities did not significantly modify the markers of oxidative stress (Table 3).

Table 3: Association between comorbidities of study participants and markers of oxidative stress.

<table>
<thead>
<tr>
<th>Comorbidity</th>
<th>MDA OR</th>
<th>MDA P value</th>
<th>GSH OR</th>
<th>GSH P value</th>
<th>FRAP OR</th>
<th>FRAP P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Obesity</td>
<td>0.48</td>
<td>0.13</td>
<td>1.504</td>
<td>0.926</td>
<td>2.027</td>
<td>0.196</td>
</tr>
</tbody>
</table>
Correlation between disease activity and markers of oxidative stress

Disease activity was associated to oxidative stress in RA and SLE. There was positive correlation between MDA and DAS-28 (r=0.035, p=0.040); negative correlation between GSH and DAS-28 (r=-0.275, p=0.024) and significant negative correlation between FRAP and SLEDAI (r=-0.033, p=0.039) (Table 4).

Table 4: Correlation between disease activity and markers of oxidative.

<table>
<thead>
<tr>
<th>Marker</th>
<th>DAS-28</th>
<th>SLEDAI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA</td>
<td>r=0.035</td>
<td>-</td>
<td>0.04</td>
</tr>
<tr>
<td>GSH</td>
<td>r=-0.275</td>
<td>-</td>
<td>0.024</td>
</tr>
<tr>
<td>FRAP</td>
<td>r=-0.103</td>
<td>-</td>
<td>0.233</td>
</tr>
<tr>
<td>MDA</td>
<td>-</td>
<td>r=0.136</td>
<td>0.241</td>
</tr>
<tr>
<td>GSH</td>
<td>-</td>
<td>r=-0.063</td>
<td>0.373</td>
</tr>
<tr>
<td>FRAP</td>
<td>-</td>
<td>r=-0.033</td>
<td>0.039</td>
</tr>
</tbody>
</table>

MDA: Malondialdehyde; GSH: Glutathione; FRAP: Ferric Reducing Ability of Plasma; DAS-28: Disease Activity for 28 Joint Indices Score; SLEDAI: Systemic Lupus Erythematosus Disease Activity Index, r = Coefficient of Correlation.

Discussion

The results of our study show increased oxidant status evidenced by higher MDA levels, and a diminished antioxidant status, evidenced by lower GSH and FRAP levels in patients with chronic inflammatory diseases (RA and SLE) compared to healthy controls. This supports reports of an interdependent relationship between oxidative stress and chronic inflammation [13]. Inflammatory cells liberate a number of reactive species at the site of inflammation causing an inflated oxidative stress [27], while reactive species can induce changes in transcription factors, as well as trigger intracellular signalling cascades which promote pro-inflammatory gene expression. If oxidative stress appears as the primary abnormality, inflammation ensues and further accentuates oxidative stress, while, if inflammation is the primary abnormality, oxidative stress ensues as a consequence and further exaggerates inflammation [28].

A major consequence of oxidative stress is lipid peroxidation, supported by our findings. Oxidative stress in chronic inflammatory diseases leads to oxidation of biomolecules such as lipids. Lipid peroxidation decreases membrane fluidity, increases membrane permeability, damages membrane proteins and impairs the activity of membrane receptors, enzymes and ion channels [29]. Lipid peroxidation results in production of various bioactive aldehydes such as MDA [8]. In our study, we recorded increased levels of MDA in patients compared to healthy controls, similar to findings in studies in RA [17,30-37] and SLE [16,17,36-41]. In addition, studies which assessed other markers of oxidative stress such as TBARS, protein carbonyls and Anti-HNE antibodies also reported increased oxidative stress in RA [30,42] and SLE [15] patients compared to controls. Furthermore, in their review article, Quiñones-Flores et al. showed an increased oxidative stress in RA patients, in any sample analyzed (serum, plasma, erythrocytes, urine, synovial fluid, and whole blood) [14]. In our study, levels of MDA correlated positively with disease activity in RA and SLE as seen in other studies in RA [17,31,32,37] and SLE [16,17,37,39,40] suggesting that MDA could be used as a marker of disease activity in RA and SLE.

We recorded decreased levels of GSH in patients compared to controls. This was similar to findings in other studies in RA [17,30,32,37] and SLE [16,17,37,40,41] patients compared to controls. GSH is a non-protein thiol molecule functioning as an intracellular reductant in redox reactions in the human body; it therefore, plays a key role in cellular resistance against oxidative damage and is an important component of the antioxidant defence system. Its importance is not only based on its abundance, but also on its versatility to counteract a wide range of reactive species. It either destroys reactive species directly, or indirectly as a co-substrate for glutathione peroxidase enzymes [43]. It has been described a decreased levels of other antioxidant status markers such as SOD, GPx, CAT, Vitamins A, C, E in RA [30-32,34,35] and SLE [15,38,44] patients compared to controls.
In our study, FRAP levels were decreased in patients compared to controls. This finding was consistent with findings by Mateen et al. [30] in India. This assay is based on the reduction of ferric to ferrous form by antioxidants present in the plasma. The observed decrease in FRAP of patients with chronic inflammatory diseases could either be due to depletion of antioxidant species by excessive reactive species, or an inherent deficiency of the antioxidant defence system of the body. Therefore, findings of a reduced antioxidant system must be interpreted with caution and must be combined with evaluation of the oxidant status. Combined evaluation of the oxidant/antioxidant status is a more reliable way to assess oxidative stress.

We also found a negative correlation between the markers of antioxidant status (GSH and FRAP) and disease activity in RA and SLE as described in other studies [16,17,32,37,40].

**Conclusion**

Sub-Saharan African patients with RA and SLE have higher oxidant status and lower antioxidant status compared to healthy individuals as described in other settings. Oxidative stress in these patients is associated to disease activity.

**Conflict of Interest**

Nothing to declare from all the contributing authors.

**References**


