Circulating antioxidants and lipid peroxidation products in untreated tuberculosis patients in Ethiopia

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ABSTRACT

Background: Knowledge of the antioxidant profile and its relation to lipid peroxidation in tuberculosis patients with or without accompanying HIV infection is scarce, particularly in developing countries.

Objective: The objective was to further investigate the interaction between HIV, tuberculosis, and antioxidants and their relations with markers of oxidative stress in a large population of Ethiopians.

Design: In a cross-sectional study, we evaluated antioxidants and markers of oxidative stress in Ethiopian tuberculosis patients with (n = 25) and without (n = 100) HIV infection and in Ethiopian (n = 45) and Norwegian (n = 25) healthy control subjects.

Results: Concentrations of the antioxidant vitamins C and E and of vitamin A were significantly lower in tuberculosis patients than in healthy Ethiopians. Tuberculosis patients also had significantly lower thiols concentrations, particularly of the reduced forms. Tuberculosis patients, particularly those who were co-infected with HIV, had higher malondialdehyde concentrations than did control subjects. High malondialdehyde concentrations were associated with clinical severity as measured by the Karnofsky Performance Status Index and anthropometric scores. Ethiopian control subjects had lower concentrations of vitamin E and higher concentrations of malondialdehyde than did Norwegian control subjects.

Conclusions: Our findings further support a link between oxidative stress, tuberculosis, and HIV infection. However, whether antioxidant supplementation will improve tuberculosis outcome or is of importance for its prevention should be further examined in future prospective studies. Am J Clin Nutr 2003;78:117–22.

KEY WORDS Tuberculosis, HIV, malnutrition, glutathione, antioxidants, oxidative stress

INTRODUCTION

HIV infection is characterized by progressive immunodeficiency. The immunologic hallmark of HIV infection is a numerical and functional decline in CD4+ T cells, which over time leads to the development of AIDS. Several factors seem to be involved in the pathogenesis of HIV infection, and we and others have suggested that oxidative stress may play an important role in this process (1–3). Several studies have reported decreased antioxidant concentrations, disturbed glutathione metabolism, and enhanced spontaneous generation of reactive oxygen species (ROSs) in HIV-infected patients. Moreover, it was shown that supplementation with glutathione or antioxidants may improve immunologic and virologic indexes in HIV-infected persons (4).

An alarming epidemic of tuberculosis has followed the rapid rise in HIV/AIDS cases, particularly in developing countries such as Ethiopia. Mycobacterium can induce ROS production by activating phagocytes (5–7), and although an important part of the host defense against mycobacteria, enhanced ROS generation may promote tissue injury and inflammation. This further contributes to immunesuppression (8–10), particularly in those with impaired antioxidant capacity, such as HIV-infected patients (1, 2, 4). Moreover, the malnutrition that commonly occurs in Ethiopian patients with tuberculosis or HIV infection may further contribute to the impaired antioxidant capacity in these patients.

Examination of antioxidants in patients with HIV infection and tuberculosis may identify deficiencies that predispose to severe oxidative injury and immunodeficiency. However, our knowledge of the antioxidant profile and its relation to lipid peroxidation in tuberculosis patients with or without accompanying HIV infection is scarce, particularly in developing countries. Thus, to further study the interaction between HIV, tuberculosis, and antioxidants, we investigated antioxidant status and its relation to lipid peroxidation products in Ethiopian tuberculosis patients with or without HIV infection.

SUBJECTS AND METHODS

Patient selection

From July to December 1995 we studied 239 consecutively identified pulmonary tuberculosis patients in Ethiopia (11). Tuberculosis was considered proven when a patient had signs of clinical and radiologic pulmonary tuberculosis, including positive
Ziehl Neelsen staining of sputum showing acid-fast bacilli (12). In a cross-sectional study, we selected patients on the basis of a coin toss. After the patients were recruited for study, they were randomly assigned for analysis of antioxidants and lipid peroxidation products depending on the results of the coin toss. The patients included in the antioxidant substudy were later compared with the whole study population with respect to variables such as age, sex, HIV serostatus, malnutrition, and treatment outcomes, and the 2 groups were not significantly different regarding these variables. Forty-five healthy blood donors (38 men, 7 women; \( \bar{x} \pm \text{SD age: } 31 \pm 11.6 \text{ y} \)) from the same area were recruited as control subjects. As an additional control group, we included blood samples from 25 healthy Norwegian blood donors (21 men, 4 women; \( \bar{x} \pm \text{SD age: } 34.7 \pm 10.6 \text{ y} \)). All blood donors identified themselves as healthy volunteers for blood donation. After a routine interview to exclude individuals with all types of acute or chronic disease, the blood donors underwent structured clinical and some basic laboratory examination to exclude those with HIV infection or diseases that can be transmitted with blood transfusion, such as hepatitis.

None of the tuberculosis patients or the Ethiopian control subjects were using any kind of treatment or prophylaxis for chronic disease, such as hypertension, diabetes mellitus, coronary artery disease, HIV infection, or other diseases that could affect the results of our analysis. In the patient group, all blood samples were taken before the start of tuberculosis treatment.

Informed consent for participating in the study was obtained from all patients and control subjects. The study was approved by the National AIDS Control Program and the National Tuberculosis Control Program of Ethiopia.

Treatment, follow-up, and recording of treatment outcomes

After their tuberculosis was diagnosed, the patients were treated according to standard protocol at the hospital (13). Patients in whom supervised therapy was feasible were given short-course chemotherapy DOTS (directly observed treatment—short course), ie, rifampicin, isoniazid, pyrazamide, and streptomycin, for 2 mo followed by 6 mo of treatment with isoniazid and thioacetazone. The other patients were given long-course chemotherapy (2 mo with isoniazid, thioacetazone, and streptomycin followed by 10 mo with isoniazid and thioacetazone). Short-course chemotherapy was given to all patients with previous treatment failure on long-course chemotherapy.

We assessed and categorized treatment outcomes as cured \((n = 25); \) patients who were smear-negative at the completion of treatment and on at least one previous occasion), treatment completed \((n = 24); \) patients who had completed treatment but without proof of cure), treatment failure \((n = 1); \) patients who remained or became smear-positive 5 mo or later during treatment), died \((n = 5); \) patients who died for any reason during treatment), treatment interrupted ("defaulter,” \( n = 29); \) patients whose treatment was interrupted for \( \geq 2 \text{ mo} \), and transferred out \((n = 41); \) patients who were transferred to another treatment unit and for whom the treatment outcome was unknown) (13).

Investigation of patients

Standardized procedures were used to measure body weight, height, and midupper arm circumference (14). The weighing scales were calibrated regularly, and all subjects were weighed while wearing minimal clothing. Body mass index (BMI; in kg/m\(^2\)) values of 18.5, 17.0, and 16.0 were used as the thresholds below which patients were classified as having mild, moderate, or severe malnutrition (14). Patients with a midupper arm circumference < 24 cm for men and < 23 cm for women were considered malnourished (14). In addition, dietary intakes were estimated by a dietary and history interview procedure with the use of a modified food-frequency questionnaire (15) that included a food list adapted to include foods commonly consumed in south Ethiopia. The subjects were specifically questioned about the number of daily eating occasions and how often they consumed meat, milk, cereals, fruit, legumes, vegetables, and enset (Ensete ventricosum, or false banana). Enset is the most common traditional food in this rural area. Functional status assessments were based on the Karnofsky Performance Status Index, which rates functional status at 10-point intervals from 0 to 100.

Protocol for blood sampling, transport, and storage

Peripheral venous blood was drawn into sterile vacuum tubes without additives between 0800 and 1000 after the subjects had fasted overnight. The tubes were kept at 4 °C until clotting occurred (< 1 h) and were then centrifuged (400 \( \times \) g, 10 min, 4 °C); serum was stored at \(-80 \text{ °C} \) (Norway) or \(-20 \text{ °C} \) (Ethiopia). After storage at \(-20 \text{ °C} \) for < 3 wk, the Ethiopian samples were transported on dry ice to Armauer Hansen Research Institute (Addis Ababa, Ethiopia) for storage at \(-80 \text{ °C} \). Samples were later transported on dry ice to Bergen, Norway, and were stored at \(-80 \text{ °C} \) until analyzed. All samples were protected from light with aluminum foil during transport and processing and were thawed only once.

Biochemical analysis

 Hematocrit, albumin, alkaline phosphatase, total bilirubin, \( \gamma \text{-glutamyltransferase}, \) creatinine, triacylglycerol, and cholesterol were measured by standard techniques. C-reactive protein measurement was based on liquid-phase immunoprecipitation (Orion Diagnostic, Espoo, Finland). Ferritin was measured by heterogeneous sandwich magnetic separation assay (Bayer, New York).

Measurements of vitamins A, C, and E

Serum retinol and \( \alpha \)-tocopherol were measured simultaneously by an HPLC method (16). For measurement of ascorbic acid, serum was stabilized with an equal volume of 10% meta-phosphoric acid. The precipitate was removed by centrifugation for 5 min (4000 \( \times \) g at 4 °C), and the supernatant fluid was kept at \(-80 \text{ °C} \) until the analysis of ascorbic acid by HPLC with colorimetric detection (17, 18).

Thiol analyses

Total and reduced glutathione, cysteine, and cysteinylglycine were quantified after derivitization of samples with the fluorescent agent monobromobimane (Molecular Probes, Eugene, OR) by reversed-phase HPLC as described (19).

Malondialdehyde measurement

Plasma concentrations of malondialdehyde (MDA) as thiobarbituric acid complexes were measured by HPLC (20). To prevent peroxidation during the assay, the chain-breaking antioxidant butylated hydroxytoluene was added to the samples at the start of the assay.

Statistical analysis

Statistical analyses were performed by using SPSS for WINDOWS, version 9.0.0 (21). In addition, SIGMAPLOT
graphing software (SPSS Inc, Chicago) was used to compare groups by using means and CIs. Data are presented as means with 95% CIs or as means ± SDs unless stated otherwise. To obtained normal distributions, some variables (eg, ascorbic acid, vitamin A, total bilirubin, total and reduced cysteine, γ-glutamyltransferase, and ferritin) were log-transformed. Statistical comparisons between the groups were assessed by one-way analysis of variance (ANOVA) for normally distributed and log-transformed data followed by Tukey’s test. Correlations between variables were calculated by Pearson’s correlation test for normally distributed variables and by the Spearman rank test for variables that were not normally distributed. *P* values are two-sided and were considered statistically significant when the α level was < 0.05.

**RESULTS**

Values for the clinical, hematologic, and biochemical indexes measured in the study group are shown in Table 1. Tuberculosis patients with or without HIV infection had significantly lower values for anthropometric (eg, weight, BMI, and midupper arm circumference), functional (Karnofsky Performance Status Index), and some biochemical (eg, albumin and hematocrit) indexes than did healthy Ethiopian control subjects. Tuberculosis patients with or without HIV infection also had significantly higher concentrations of ferritin, alkaline phosphatase, γ-glutamyltransferase, and C-reactive protein than did the healthy control subjects, with particularly high ferritin and C-reactive protein concentrations in the HIV-infected group. This latter group also had significantly higher concentrations of γ-glutamyltransferase than did the HIV-seronegative tuberculosis patients.

**Antioxidant concentrations**

As shown in Table 2, serum concentrations of the antioxidant vitamins C and E and of vitamin A, a vitamin with no antioxidant capacity, were markedly lower in patients with tuberculosis than in healthy Ethiopian control subjects. Thiols, particularly glutathione, representing the major intracellular redox buffering compounds, were also significantly lower in tuberculosis patients, with particularly low concentrations of the reduced thiol forms. Notably, these alterations in vitamins and thiols were seen in both HIV-seropositive and HIV-seronegative tuberculosis patients, with no significant differences between these groups (data not shown).

**Concentrations of malondialdehyde and its association with circulating antioxidants**

As shown in Figure 1, serum MDA concentrations, as a measure of lipid peroxidation reflecting the degree of oxidative stress, were significantly higher in patients with tuberculosis than in healthy Ethiopian control subjects, with particularly high concentrations in those who were co-infected with HIV. In tuberculosis patients, there were weak but significant inverse correlations between MDA and vitamin A (*r* = −0.23, *P* < 0.01), reduced glutathione (*r* = −0.27, *P* < 0.01), and reduced cysteine (*r* = −0.21, *P* < 0.01).

**Oxidative stress and antioxidants in relation to nutritional state**

Malnutrition may influence antioxidant concentrations and oxidative stress. As shown in Table 3, in patients with tuberculosis, but not in healthy Ethiopian control subjects, there was a weak but significant inverse association between low BMI and low weight and high MDA concentrations. Moreover, BMI was positively correlated with concentrations of vitamin E and the reduced

**TABLE 1**

Clinical, hematologic, and biochemical indexes in the study group

<table>
<thead>
<tr>
<th>Index</th>
<th>HIV-positive TB patients (n = 17 M, 8 F)</th>
<th>HIV-negative TB patients (n = 71 M, 29 F)</th>
<th>Healthy control subjects (n = 38 M, 7 F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>29.5 ± 9.4†</td>
<td>29.5 ± 9.4†</td>
<td>31.9 ± 11.6†</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>46.8 ± 9.4†</td>
<td>45.9 ± 8.9†</td>
<td>61.2 ± 6.2†</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>168.2 ± 8</td>
<td>166.1 ± 9.4</td>
<td>169.4 ± 7.4</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>16.5 ± 2.3†</td>
<td>16.6 ± 2.4†</td>
<td>21.6 ± 2.3†</td>
</tr>
<tr>
<td>MUAC (cm)</td>
<td>19.6 ± 2.7†</td>
<td>19.2 ± 2.7†</td>
<td>25.0 ± 2.1†</td>
</tr>
<tr>
<td>KPS</td>
<td>512.2 ± 23.7†</td>
<td>651.5 ± 14.2†</td>
<td>1000.0 ± 50†</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>26.5 ± 7.1†</td>
<td>29.7 ± 7.7†</td>
<td>42.1 ± 2.9†</td>
</tr>
<tr>
<td>Ferritin (µg/L)</td>
<td>509.3 ± 400.0†</td>
<td>255.5 ± 271.7†</td>
<td>96.7 ± 125.0†</td>
</tr>
<tr>
<td>Median (IR)</td>
<td>461.0 (69.3–1000)</td>
<td>157.0 (64.8–356.3)</td>
<td>56.0 (31–124)</td>
</tr>
<tr>
<td>Bilirubin (µmol/L)</td>
<td>13.8 ± 24.0</td>
<td>7.5 ± 8.7</td>
<td>9.4 ± 4.0</td>
</tr>
<tr>
<td>Median (IR)</td>
<td>8.0 (5.8–9.8)</td>
<td>6.0 (4.8–7)</td>
<td>9.0 (7–11)</td>
</tr>
<tr>
<td>ALP (U/L)</td>
<td>470.0 ± 429.4†</td>
<td>316.1 ± 307.7†</td>
<td>187.7 ± 48.4</td>
</tr>
<tr>
<td>γ-GGT (U/L)</td>
<td>83.0 ± 70.6†</td>
<td>58.3 ± 61.8†</td>
<td>26.0 ± 23.4</td>
</tr>
<tr>
<td>Median (IR)</td>
<td>60.0 (27.8–127.5)</td>
<td>31.0 (20.8–58)</td>
<td>170.0 (13–32)</td>
</tr>
<tr>
<td>Creatinine (µmol/L)</td>
<td>80.8 ± 19.3†</td>
<td>67.6 ± 17.6†</td>
<td>82.0 ± 10.6</td>
</tr>
<tr>
<td>Cholesterol (µmol/L)</td>
<td>2.4 ± 0.89†</td>
<td>3.0 ± 1.9</td>
<td>3.6 ± 0.8</td>
</tr>
<tr>
<td>Median (IR)</td>
<td>2.1 (1.8–3.4)</td>
<td>2.7 (2.2–3.5)</td>
<td>3.4 (3.0–4.2)</td>
</tr>
<tr>
<td>Triacylglycerol (mmol/L)</td>
<td>1.6 ± 0.4</td>
<td>1.3 ± 0.5</td>
<td>1.4 ± 1.3</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>33.4 ± 5.0†</td>
<td>33.2 ± 5.1†</td>
<td>44.6 ± 6.2</td>
</tr>
<tr>
<td>CRP (ng/mL)</td>
<td>108.5 ± 81.7†</td>
<td>79.3 ± 44.4†</td>
<td>4.9 ± 7.1†</td>
</tr>
</tbody>
</table>

†TB, pulmonary tuberculosis; MUAC, midupper arm circumference; KPS, Karnofsky Performance Status Index; IR, interquartile range; ALP, alkaline phosphatase; γ-GGT, γ-glutamyltransferase; CRP, C-reactive protein.

*Significantly different from healthy control subjects (ANOVA and Tukey’s test): †P < 0.001, ‡P < 0.05.

*Significantly different from healthy control subjects (ANOVA and Tukey’s test): †P < 0.01, ‡P < 0.05.*
thiols. Low vitamin A concentrations were strongly correlated with low values of all anthropometric variables, suggesting a strong association between vitamin A deficiency and malnutrition in these patients.

**Oxidative stress in relation to clinical severity and outcome**

Higher MDA concentrations were associated with poor clinical performance status (Karnofsky Performance Status Index) in the patient group as whole ($r = -0.23, P < 0.01$), regardless of accompanying HIV infection. There were no significant differences between the different treatment outcome groups (see Subjects and Methods) in serum concentrations of MDA, thiols, or vitamins (data not shown).

**Comparison between healthy blood donors in Ethiopia and Norway**

To further investigate the relations between antioxidants and oxidative stress in the Ethiopian population, we compared these variables in Ethiopian and Norwegian healthy blood donors. As shown in Table 2, concentrations of the antioxidant vitamin E, but not of vitamins A and C, were significantly lower and MDA concentrations were significantly higher (Figure 1) in Ethiopian than in Norwegian blood donors. Several thiol variables, especially the reduced form of cysteine, were also significantly lower in Ethiopian than in Norwegian healthy control subjects (Table 2). Several of the measured variables (eg, vitamin C) are sensitive to blood sampling and storage procedures. However, our findings of low vitamin E and some thiols but not of vitamin A and vitamin C in Ethiopian blood donors suggest that the differences in concentrations of antioxidants and MDA between the Ethiopian and Norwegian blood donors do not merely reflect differences in blood sampling protocols (see Subjects and Methods).

**DISCUSSION**

To our knowledge, the present study is the most comprehensive evaluation to date of circulating concentrations of antioxidants and markers of oxidative stress in tuberculosis patients in developing countries. Our results show lower antioxidant potential and enhanced lipid peroxidation in these patients, with particularly high concentrations of lipid peroxidation products (ie, MDA) in those who were co-infected with HIV. Moreover, we found that healthy Ethiopian control subjects had lower antioxidant and higher MDA concentrations than did healthy Norwegian control subjects. Our findings further support a role for oxidative stress in the pathogenesis of tuberculosis and suggest lower antioxidant capacity and higher oxidative stress in the Ethiopian than in the Norwegian population.

We did not systematically control for all of the possible confounding factors that could influence the concentrations of antioxidants and markers of oxidative stress. Some of these factors, however, such as the use of medications and differences in diet, may have had little effect in our study because none of the tuberculosis patients or Ethiopian control subjects were using any kind of...
Tuberculosis has been reported to enhance HIV replication and the progression to AIDS in dually infected patients, possibly involving enhancement of inflammatory cytokines such as tumor necrosis factor involving enhancement of inflammatory cytokines such as tumor necrosis factor and the lipid-soluble vitamin E may act synergistically to protect cells from oxidative-stress-induced damage (38). Accordingly, the combined deficiency of these antioxidants may markedly increase oxidative stress, possibly adversely affecting the immune response and predisposing to drug toxicity (39).

In the present study, concentrations of antioxidant vitamins and of several thiol compounds were lower and concentrations of MDA were higher in Ethiopian than in Norwegian control subjects. Malnutrition is more common in the Ethiopian community studied than in the Norwegian one, which may explain the differences in antioxidant concentrations. However, there was no significant difference in serum concentrations of vitamin A, a vitamin with no antioxidant effect, suggesting that the lower antioxidant concentrations in the Ethiopians may reflect more than merely malnutrition. It was previously reported that HIV-seronegative Africans are characterized by higher concentrations of inflammatory cytokines than those found in healthy Europeans (40, 41), and we showed that Africans also have indications of increased oxidative stress. This persistent immune activation and enhancement of oxidative stress may reflect 2 interacting phenomena possibly secondary to an increased infectious load in African populations.

In summary, we showed lower antioxidant potential and greater lipid peroxidation in Ethiopian tuberculosis patients than in Ethiopian control subjects, with particularly low concentrations of the reduced forms. Several factors—such as low food intake, nutrient malabsorption, and inadequate nutrient release from the liver; acute-phase response and infection; and an inadequate availability of carrier molecules—may influence circulating antioxidant concentrations (32–34). Our findings of a correlation between several indexes of malnutrition and low concentrations of antioxidants suggest the involvement of low food intake and nutrient malabsorption in the generation of oxidative stress in Ethiopian tuberculosis patients. Increased ROS generation was previously reported in patients with tuberculosis (5, 7, 8). Furthermore, our finding of a significant correlation between high MDA concentrations and low concentrations of some antioxidants suggests increased utilization by ROSs as an important contributing factor to the lower concentrations of antioxidants in tuberculosis patients. In fact, the combination of malnutrition leading to decreased “supplementation” of antioxidants and enhanced ROS generation leading to increased utilization of these compounds may represent a pathogenic loop that results in markedly enhanced oxidative stress during tuberculosis infection. Three of the antioxidants that were significantly reduced in tuberculosis patients, ie, glutathione, ascorbic acid, and α-tocopherol, are integral components of a regenerating redox cycle (35–37). Thus, a combined decrease in these antioxidants may markedly decrease antioxidant capacity in these patients. Moreover, water-soluble antioxidants such as glutathione and vitamin C and the lipid-soluble vitamin E may act synergistically to protect cells from oxidative-stress-induced damage (38). Accordingly, the combined deficiency of these antioxidants may markedly increase oxidative stress, possibly adversely affecting the immune response and predisposing to drug toxicity (39).

We thank Randi Sandvik, Svein Kruger, and Bjorn Netteland for excellent technical assistance and acknowledge all of the technical assistance received at Armer Hansen Research Institute, Addis Ababa, Ethiopia, and the Blood Bank at Yirga-Alem Hospital.

### Table 3

<table>
<thead>
<tr>
<th>Antioxidant</th>
<th>Weight</th>
<th>Height</th>
<th>BMI</th>
<th>MUAC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin A</td>
<td>0.603</td>
<td>0.269</td>
<td>0.561</td>
<td>0.307</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>0.145</td>
<td>0.023</td>
<td>0.181</td>
<td>−0.048</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>0.086</td>
<td>−0.024</td>
<td>0.084</td>
<td>−0.096</td>
</tr>
<tr>
<td>Glutathione (reduced)</td>
<td>0.274</td>
<td>0.022</td>
<td>0.316</td>
<td>0.04</td>
</tr>
<tr>
<td>Cysteine (reduced)</td>
<td>0.290</td>
<td>0.242</td>
<td>0.205</td>
<td>0.126</td>
</tr>
<tr>
<td>MDA</td>
<td>−0.165</td>
<td>−0.036</td>
<td>−0.168</td>
<td>0.131</td>
</tr>
</tbody>
</table>

1. MUAC, midupper arm circumference; MDA, malondialdehyde. Correlations between variables were calculated by the Pearson correlation test for normally distributed variables and by the Spearman rank test for variables that were not normally distributed (eg, vitamin C). None of the correlations in healthy Ethiopian subjects were significant.

2. P < 0.01.
3. P < 0.05.
REFERENCES


