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Original Article

Non-enzymatic antioxidants status of leprosy patients in a leprosarium settlement in Nigeria

[1]Humphry B. Osadolor and [2]Romanus Okosun

[1]Department of Medical Laboratory Science, University of Benin, P.M.B 1154, Benin City, Edo State, Nigeria.

[2]Department of Chemical Pathology, University of Benin Teaching Hospital, Benin City, Edo State, Nigeria.

*Corresponding author: Humphry Osadolor. Email: Humphrey.osadolor@uniben.edu; Tel.; +2347060813792

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ABSTRACT: The plasma levels of vitamin C, vitamin E and uric acid were determined in leprosy subjects from in Ossiomo leprosorium settlement in Edo state, Nigeria. In this study, antioxidant status were determined in a total of 86 subjects that comprised of 31 leprosy patients on multidrug therapy (MBT), 40 leprosy patients relieved from therapy (RFT) in Ossiomo leprosorium settlement and 15 normal individuals who served as control subjects. Of the MDT group, 10 subjects were paucibacillary (PB) leprosy patients while 21 were multibacillary (MB) patients. There were significant decreases (P<0.05) in the plasma vitamin C and vitamin E levels relative to the controls. However the uric acid level was higher in the leprosy patients (P<0.05) compared with the control subjects. The uric acid levels determined in the RFT patients (10.74±0.99) obtained was significantly higher than those of MDT patients (2.90±0.29) and controls (6.69±0.32). The observed decrease in the vitamin C and vitamin E levels in the leprosy patients could be as a result of the free radicals produced during metabolism of the chemotherapeutic agents administered to the patients and the chronic pathological effects of *Mycobacterium leprae* on the population studied.

KEYWORDS: leprosy, multidrug therapy, antioxidants, vitamin C, vitamin E, Uric acid.

INTRODUCTION

Leprosy, also known as Hansen's disease is caused by *Mycobacterium leprae*. It is characterized by damage to the skin, peripheral nerves and the lining of the upper respiratory tract. As a result of the nerve damage, there may be paralysis, deformity and ulceration (Macleod, 1984). World Health Organization (WHO 1998) listed Nigeria along with other countries such as Bangladesh, Brazil, India, Indonesia and Myanmar where the disease is still endemic (Jacobson and Krahenbuhl 1999).

For treatment purposes, leprosy patients are classified into three groups: paucibacillary single-lesion leprosy, paucibacillary leprosy and multibacillary leprosy. Skin smears were originally used to distinguish between paucibacillary and multibacillary leprosy. However, because services for processing skin smears are not always available, and also because their reliability is often doubtful, in practice most leprosy programmes classify and choose the appropriate regimen for a particular patient using clinical criteria, which uses the number of skin lesions and nerves involved to classify leprosy patients into paucibacillary single-lesion leprosy (one skin lesion), paucibacillary (PB) leprosy (2-5 skin lesions) and multibacillary (MB) leprosy (more than five skin lesions). When skin smears are available and are dependable, any patient with a positive skin smear, irrespective of the clinical picture, must be classified as multibacillary leprosy and must be treated with the multidrug therapy regimen for multibacillary leprosy.

Several drugs are used in combination in the treatment of leprosy as part of a multidrug therapy regimen (WHO, 1998).

Dapsone**,** which is bacteriostatic or weakly bactericidal against *M. leprae,* was the mainstay treatment for leprosy for many years until widespread resistant strains appeared. Combination therapy has become essential to slow or prevent the development of resistance. Rifampicin is now combined with dapsone to treat paucibacillary leprosy, while rifampicin and clofazimine are now combined with dapsone to treat multibacillary leprosy (WHO, 1998).

Krinsky (1992) describes antioxidants as compounds that protect biological systems against the potentially harmful effects of processes or reactions that can cause excessive oxidation (such as produced by this disease and drugs for its treatment). The destructive potentials of *M. leprae* (Macleod, 1984) and metabolic effects of the antileprosy drugs are capable of producing oxidative damage to macromolecules, such as lipids, protein, carbohydrates and nucleic acid, ultimately leading to cells necrosis in patients (Halliwell and Gutteridge, 1989). Humans have antioxidant defense mechanisms against reactive oxygen species; among the oxidants are vitamins A, C and E, enzymes such as superoxide dismutase and catalase. (Waring, 2002).

One of the roles of Vitamins C, A and E is that of scavenging for free radicals in the aqueous phase of cells and the circulatory system to mop up generated reactive oxygen species (Beyer, 1994; Chamorro *et al*, 2002; Waring, 2002; Squadrito *et al*, 2000). One of the multidrug therapy (MDT) drugs for leprosy dapsone is strongly oxidative in a way that damages the membranes red blood cells and results in haemolysis. It was shown that combined therapy of Vitamin C and Vitamin E confers partial protective effects against dapsone-induced haemolysis in patients with dermititis and herpetiformis (Lardo *et al*. 1997). In a similar manner, Vitamins A and E were used to relief mouth sores arising as common sides effects of chemotherapy (Wadleigh *et al* 1992).

For many years, uric acid has been used in clinical practice as a marker of several metabolic disturbances, although, until recently, its oxidants properties had not been considered (Chamorro *et al*, 2002). It has been specifically postulated that uric acid, the naturally occurring product of urine metabolism, may provide endogenous natural protection against the oxidative injury occurring as a result of peroxynitrtate exposure by acting as an endogenous free radical scavenger or oxidant (Waring 2002).

Leprosy imposes oxidative stress on its victims, which is further compounded by chemotherapy. In the more developed nations of America and Europe, the contributions of chemotherapy and antioxidants such as vitamin C, and vitamin E and in the treatment of leprosy have been well documented and dietary modification is fast becoming an acceptable part of the management protocol for this disorder in developed parts of the world (Trimbake et al., 2013).

However, information on the oxidative status of Nigerian leprosy patients is scare. The role of antioxidants (especially uric acid) in leprosy has received limited attention in this part of the world. In this study, the effects of leprosy on non-enzymatic antioxidants (vitamin C, E and uric acid) were studied in Ossiomo leprosorium settlement in Edo state.

MATERIALS AND METHODS

Subjects

A total of 86 subjects comprising of seventy-one leprosy patients from Ossiomo leprosarium specialist hospital, Ogan, Edo state, Nigeria were involved in this study. A different set of fifteen non-leprous subjects, within the same age and sex and in similar socio-economic and environmental status served as control. Of the seventy-one leprosy patients, thirty-one of them were on multidrug therapy (MDT) while forty were leprosy patients relieved from therapy (RFT). The MDT group were further subdivided into (a) Ten Paucibacillary (PB) leprosy patients and (b) twenty-one multibacillary (MB) leprosy patients. Six month regimen for Paucibacillary (PB) leprosy adult of 50-70 kg, was dapsone 100 mg and rifampicin 600 mg daily while the 12 month regimen for Multibacillary (MB) Leprosy adult of 50-70 kg was dapsone 100 mg and rifampicin 600 mg daily and clofazimine 50 mg once a month. Since these drugs are not recommended for pregnant women and children, individuals from these two groups were not included in the study. Informed consent was obtained from all subjects prior to the commencement of the experiment and sample collection. The subsequent biochemical analyses were carried out within two hours of sample collection.

Biochemical Assays

All parameters (vitamin C, vitamin E, and Uric acid) were determined using standard procedures (Wadleigh; *et al* 1992; Sauberlich, *et al* 1974; Roe and Kuether, 1943). The parameters were determined using test kits from Randox Laboratories, U.K. The protocols used were based on the kit manufacturer's instructions.

Statistical Analysis

The group mean ± SEM was calculated for each analyte and significant differences between means evaluated using the student t-test, with $P<0.05$ considered as statistically significant.

RESULTS AND DISCUSSION

In this study, the non-enzymatic antioxidants (vitamin C, vitamin E and Uric acid) status of eighty-six subjects was investigated. There was a general decrease in the levels of vitamins C and E in leprosy patients (Figure 1).

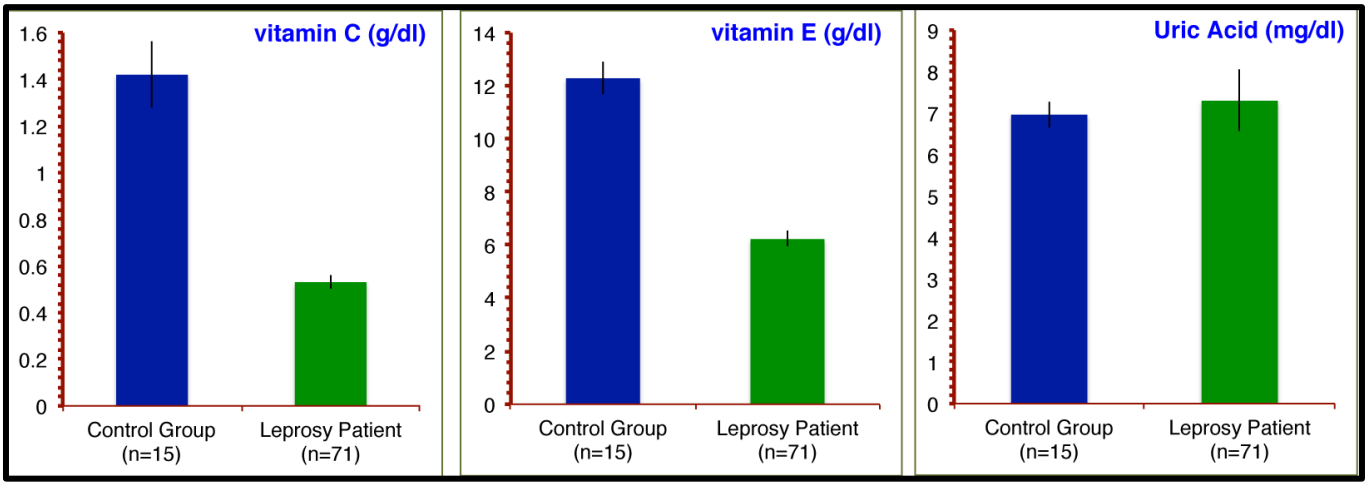

Figure 1: Levels of serum antioxidants (vitamin C, E and Uric Acid) in control subjects and all leprosy subjects irrespective of drug status. Each bar represents the mean ± SEM of the number of subjects as indicated.

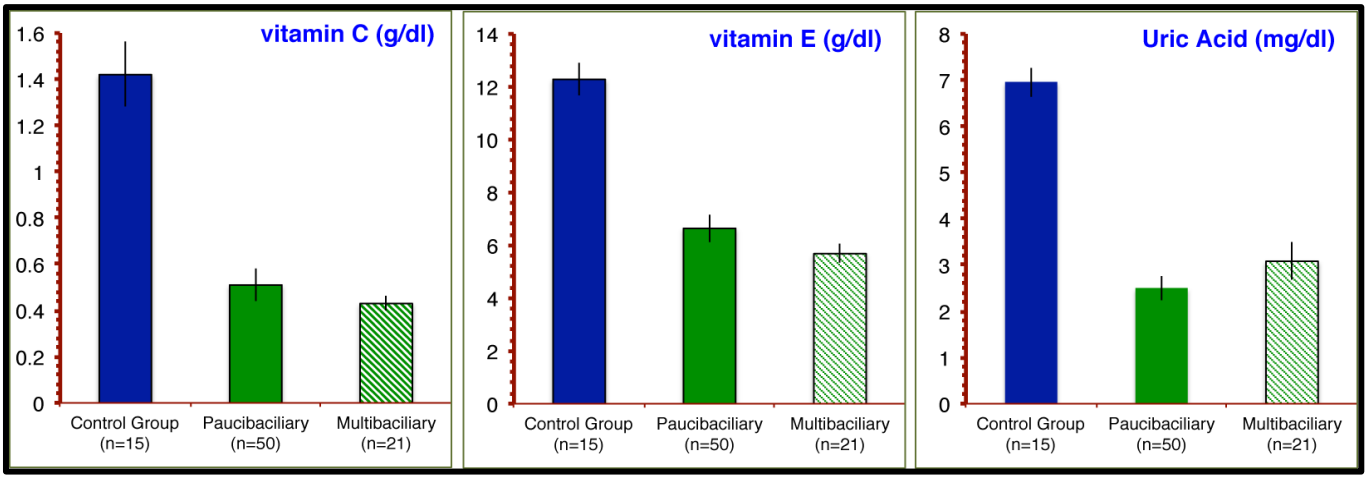

Figure 2: Comparison of the levels of serum antioxidants (vitamin C, E and Uric Acid) between paucibacillary (PB) and multibacillary (MB) leprosy patients and controls. Each bar represents the mean ± SEM of the number of subjects as indicated.

However, the level of uric acid in leprosy patients was not significantly different from that of the control subjects (P>0.05). In order to gain a better understanding of the results pattern, we compared the antioxidants level in paucibacillary and multibacillary leprosy patients with the control subjects. Figure 2 shows that vitamin C, E and Uric acid were significantly reduced (P<0.05) in both paucibacillary and multibacillary leprosy patients when compared with the control subjects. The negative influence of leprosy disease on the plasma levels of the antioxidants (vitamins C, E) studied as shown in Figures 1 and 2 above agrees with the work of (Vijayaraghavan *and* Panneerselvam, 2005) that antioxidants may provide first line defense against reactive oxygen species (ROS) generated in disease conditions. Hence, depletion of the antioxidants in the patients may reflect disease-combating mechanisms.

On the other hand, a pre-existing low antioxidant level could be a pre-disposing factor toward susceptibility to leprosy. Leprosy continues to afflict a large number of people globally as shown from several studies (Gandhi and Singh, 2004). The antioxidants (Vitamins C, E and Uric acid) status of the patients investigated in this study revealed a statistically significant decrease (p<0.05) when compared with the control subjects. This suggests that leprosy patients, irrespective of their drug therapy status or disease type, have significantly reduced levels of antioxidants. In an earlier study, Florence (1995) concluded that all leprosy patients had a mild to moderate lowering of antioxidants especially when nutrition and immunological status of the patients are compromised. This low antioxidant level appears to be associated with poverty, since all leprosy patients belonged to the low economic status.

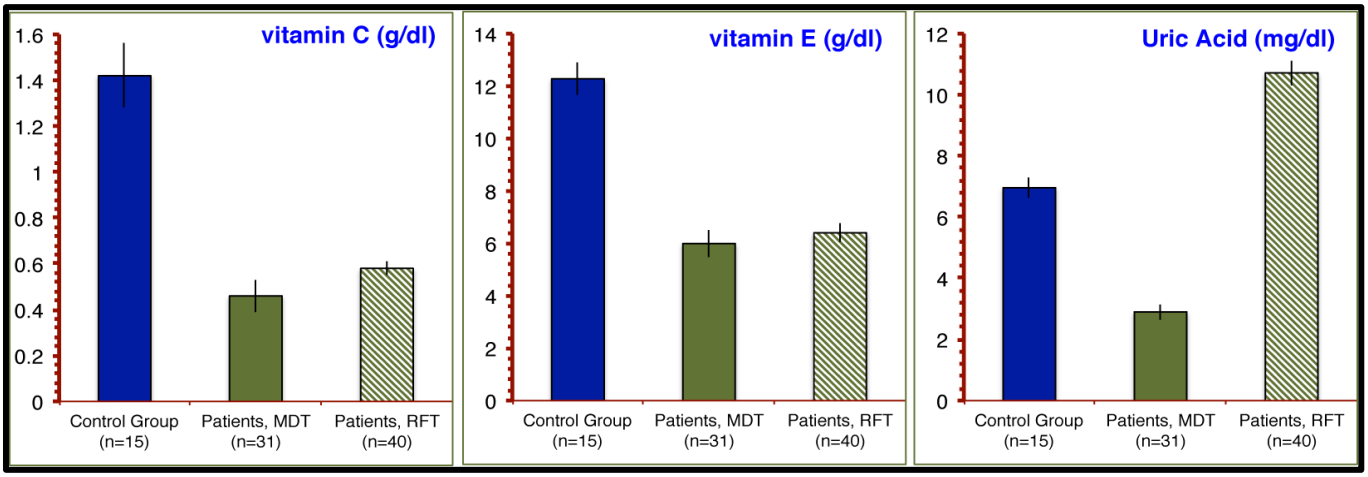

Figure 3: Comparison of the levels of serum antioxidants (vitamin C, E and Uric Acid) in all patients with leprosy on MDT and RFT. Each bar represents the mean ± SEM of the number of subjects as indicated.

Though the antioxidants levels were generally low among the leprosy patients, a closer look at the two disease types revealed that the antioxidants vitamin C and Vitamin E had higher concentrations in PB leprosy patients than those with MB ($p<0.05$). Hooper (2000) had shown that PB leprosy patients have higher immunity compared with the MB group.

This could be as a result of the low bacterial load of the causative organism *M. Leprae.* PB patients are better able to eliminate bacteria through cell–mediated immunity (Hooper *et al*; 2000; Lopez, 1994; Kelly *et al*; 1990).

Figure 3 shows that the levels of vitamin C and E in both patients on MDT and those in the RFT groups were markedly reduced ($P<0.05$) compared with the controls. This indicates that current or previous exposure to MDT generally had an effect on the antioxidant status of leprosy patients. Clear evidence have been provided that dapsone, one of the MDT drugs used in treating leprosy is strongly oxidative, and induces hemolysis (Kelly *et al*; 1990; Lardo *et al*, 1997). The increase in free radicals generated in the course of therapy could cause a reduction in the antioxidants. In this work, the level of uric acid (Figure 3) was found to be lower in patients on MDT compared with those RFT and normal controls (Lardo *et al*, 1997). Kong and Lillehi (1998) suggested from their work that over production of reactive oxygen species (ROS) following therapy exhausts the production of adaptive oxidants defenses.

Of particular interest in this study, is the observed difference in the uric acid levels obtained for the two leprosy groups (MDT and RFT). Leprosy patients on MDT had significantly decreased level of uric acid ($p<0.05$) compared with controls and those in the RFT group. The observed decrease in the uric acid level in all leprosy patients on MDT could be due to the presence of ROS resulting from the therapeutic drugs, which the uric acid tries to mop-up, thereby resulting in a decrease in antioxidant potential.

Uric acid is a strong peroxynitrite (ONOO-) scavenger demonstrated by the capacity to bind peroxynitrite but not nitric oxide (NO). Patients with multiple sclerosis have been shown to have significantly lower levels of Uric acid than controls (Chamorro *et al*., 2002) probably this is what is happening in this of leprosy. This study amongst other things suggests that Nigerian leprosy patients irrespective of whether they are on multi drug therapy (MDT) or relieved from therapy (RFT) have lowered plasma non-enzymatic antioxidants (vitamin C, E and Uric acid) except for those RFT whose Uric acid has a high level than those on MDT and control subjects. The free radical producing ability from drugs used in the MDT and the influence of *M. leprae* seemed to have a reducing effect on the antioxidants capacity of the patients.

Antioxidants are best supplied by balanced diet, but unfortunately leprosy patients are of the low socioeconomic status. Therefore, it is reasonable to say that there is need for intervention with antioxidants supplements.

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