Original Article

Community Health

Diminution in Concentration of Free Radical Scavenger System in *Salmonella typhii* Infections in some Fishing Communities of Cross River State, Nigeria

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ABSTRACT [ENGLISH/ANGLAIS]

The involvement of free radicals in the pathogenesis of some disease has been highlighted in recent years. The status of free radicals scavenging and antioxidant enzyme system was therefore assessed in both patients diagnosed with Salmonella typhii infection and infection free persons as control. This study therefore aimed at investigating the possible consumption of antioxidants during the process of disease. Seventy eight (78) persons were grouped as: untreated patients (20), 3-day on-treatment (17), treated (16) and the control (25). The assays were done using Randox kits. Results revealed significant diminution ($p<0.05$) in the concentrations of uric acid, total lipids and ascorbic acid in the untreated group when compared with that of the control groups. Furthermore, significant decrease was also observed in the activity of super oxide dismutase (SOD) when compared to that of the control group. However, insignificant diminutions were observed in the mean plasma concentrations of the antioxidants and the activity of SOD in on-treatment and treated groups when compared to control group.

Keywords: Reactive Oxygen Species (ROS), *Salmonella typhii*, free radical scavengers, fish production, fish supply, Cross River State

RÉSUMÉ [FRANÇAIS/FRENCH]

L'implication des radicaux libres dans la pathogénie de certaines maladies a été mis en évidence ces dernières années. Le statut de radicaux libres et le système de balayage enzyme antioxydante a donc évalué à la fois les patients diagnostiqués avec Salmonella typhii infection et l'infection des personnes sans titre de témoin. Cette étude vise donc à enquêter sur l'éventuelle consommation d'antioxydants pendant le processus de la maladie. Soixante-dix personnes huit (78) ont été regroupées comme suit: les patients non traités (20), 3 jours sur le traitement (17), traitée (16) et le contrôle (25). Les analyses ont été faites en utilisant des kits Randox. Les résultats ont révélé une diminution significative ($p <0,05$) dans les concentrations d'acide urique, lipides totaux et en acide ascorbique dans le groupe non traité par rapport à celle des groupes témoins. Par ailleurs, une diminution significative a également été observée dans l'activité des super oxyde dismutase (SOD) par rapport à celle du groupe contrôle. Cependant, des diminutions négligeables ont été observées dans les concentrations plasmatiques moyennes d'antioxydants et de l'activité de la SOD dans le traitement et les groupes traités par rapport au groupe témoin.

Mots-clés: Reactive Oxygen Species (ROS), Salmonella typhii, production de radicaux libres poissons charognards, l'approvisionnement en poisson, État de Cross River

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INTRODUCTION

Oxygen derived free radicals are known to play a vital role in pathogenesis of many disease states and disorders. A free radical is any molecules capable of independent existence that contains one or more unpaired electrons [1]. Reactive oxygen species (ROS), free radicals, include superoxide anion (O_2) hydroxyl radical (OH), hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl) and peropxynites (ON00-). Their role in these disease state are complex ranging from immune surveillance of the host by destroying intercellular .parasites [2], [3], to causing damage to host cell and tissues [4],[5], by destroying carbohydrates [6], proteins [7] and deoxyribonucleic acids (DNA) [8]. Fortunately, the deleterious effects of the ROS are nullified by various free radical scavengers (such as ascorbic acid, thiols and uric

acid) and free radicals scavenging enzymes (such as superoxide dismutase (SOD and catalase). A significant increase of antioxidant capacity has been reported to occur after supplementation with ascorbic acid and antioxidant enzyme [9].

The increase in cellular death affects tissues not only in increasing the production of reactive oxygen species but also affects antioxidant reactions catalyzed by reacting oxygen species scavenging enzymes [10]. Since superoxide dismutase (SOD) is present in all aerobic organism and most (if not all) subcellular compartment that generate activated oxygen. It has been assumed that SOD has central role in the defense against free radical mediated damage to tissues and cell [8]. In the present study, we have assayed the production of free radical and the statues of serum free radicals scavengers (ascorbic acid and uric acid) as well as serum total lipid and SOD activity in *Salmonella typhii* infection.

Due to water borne ailments in the fishing and rice farming communities due to frequent contaminations [11], the role of the ailments are important in fish production. It was thought possible to feed the world population from capturing of natural food resources alone. But with an ever increasing population and the dwindling resources, it has been realized that man must manage his resources well and wisely culturing preferred species at the expense of others less valuable ones [12]. Most of the productive population especially those involved in alternative animal protein production (fish) lives in riparian areas where contaminated water abounds. This disease is debilitating and lethal and therefore reduces fish production [13]. To achieve this, a healthy population is necessary. And this could be achieved, by firstly understanding the dynamism of these reactive radical species and how they are being consumed by scavengers leading to curing the victim.

MATERIALS AND METHODS

A population of 78 individuals in fishing communities spread over Calabar (Anantiga), Akamkpa (Akaisiko) and Obubra (Ovonum) Local Government Areas of Cross River State, Nigeria were randomly sampled. These included those involved in capturing, culturing and marketing of fish products. They were grouped into untreated confirmed typhoid patients (20), 3 days on-treated patients (17), confirmed treated persons (16) and healthy person to act as control (25). All persons (volunteers) involved in this experiment as both control and treated were non-smokers and non –alcoholic and are known to have no infection by time blood sample were drawn from them. Only those typhoid patients who had undergone treatment for only three days were placed in the on-treatment group

Blood samples (5.0 ml) were collected from the test patients by venipuncture. The blood was centrifuged at 3000x g for 10 minutes. The resulting serum was collected and stored in 200 ul aliquot at-4^{0}c for analysis

Ascorbic acid concentrations were assayed by the method of Wallin et al. [14]. Freshly prepared metaphosphoric acid concentration (2.0 ml) was added to 0.5 ml of plasma, mixed thoroughly and centrifuged for 10 minutes at 3000x g. Dinitrophnyl-hydrazine, thiourea, copper sulphate (DTCS) (0.4 ml), prepared by mixing 5 ml of the already prepared, 5 ml of copper sulphate solution and 100 ml of the 2,4–dinitrophenlhydrazine reagent, was added to 1.2 ml of the supernatant, mixed and incubated in water bath at 37^{0}c for 3 hours. The mixture was chilled for 10 minutes in an ice bath and 2.0 ml of cold sulphuric acid added slowly and allowed to stand for 30 minutes at room temperature. The absorbance was then read at 520 nm against metaphoric acid as blank.

Total lipid was assayed by the method of Strove and Makarova [15]. To 0.1 ml of plasma 2.9 ml concentration H_2SO_4 was added mixed thoroughly and boiled for 10 minutes in boiling water bath. The tubes were then cooled and 5ml of phosphovanillin reagent added, incubated in the dark for 45 minutes at room temperature and the absorbance read at 45 minutes at room temperature and the absorbance read at 450nm.

Uric acid assayed using commercial Randox kit.SOD activity was assayed using Randox commercial Kit. One unit of enzymes activity has been defined to cause 50% inhibition of the rate of reduction of 2- (4- idophenyl)-3-(4-nitrophenol) –s –phenyltetrazolinum chloride (I.N.T).

One-way ANOVA using computer package SPSS version 13.5 was used to evaluate significant levels between the different groups of persons. The data were expressed as mean $\pm$ SD. $p < 0.05$ has been considered as significant level.

RESULTS

The results show that there were significant diminution in the level of free radicals scavengers (uric acid and ascorbic acid) in the untreated and 3 day on- treatment groups when compared with the healthy control and the treated groups as it is displayed in figures 1 and 2 respectively. Also the concentration of total lipids showed significant

decrease ($p < 0.05$) in the untreated and 3 days on-treatment groups than in the control group as shown in Figure 3. Similarly, the concentration of the free radicals scavenging enzymes (SOD) revealed a significant ($p < 0.05$) diminution in the untreated and 3 days on treatment *Salmonella typhii* groups than in the control groups (see figure 4) in the figures, means carrying the same letters are statistically the same while those carrying different letters are statistically significant at $p < 0.05$.

Plate 1: This plate shows a map of Cross River State showing sampling areas

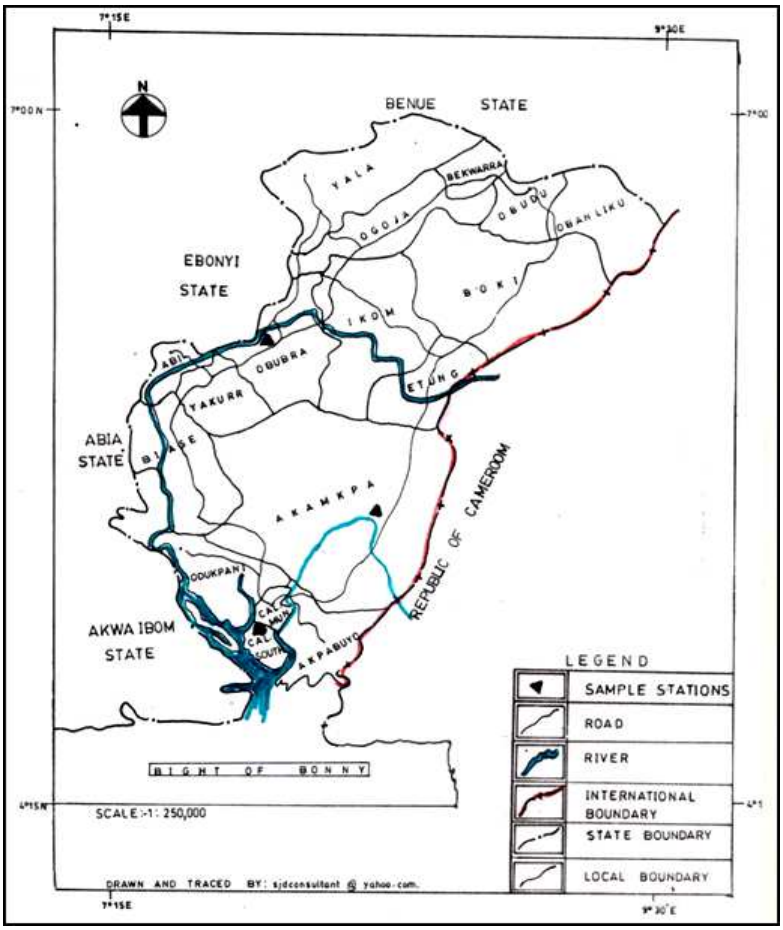


Figure 1: This figure shows mean plasma ascorbic acid concentration (mg/dl) of different groups. Means carrying different letters are significantly different

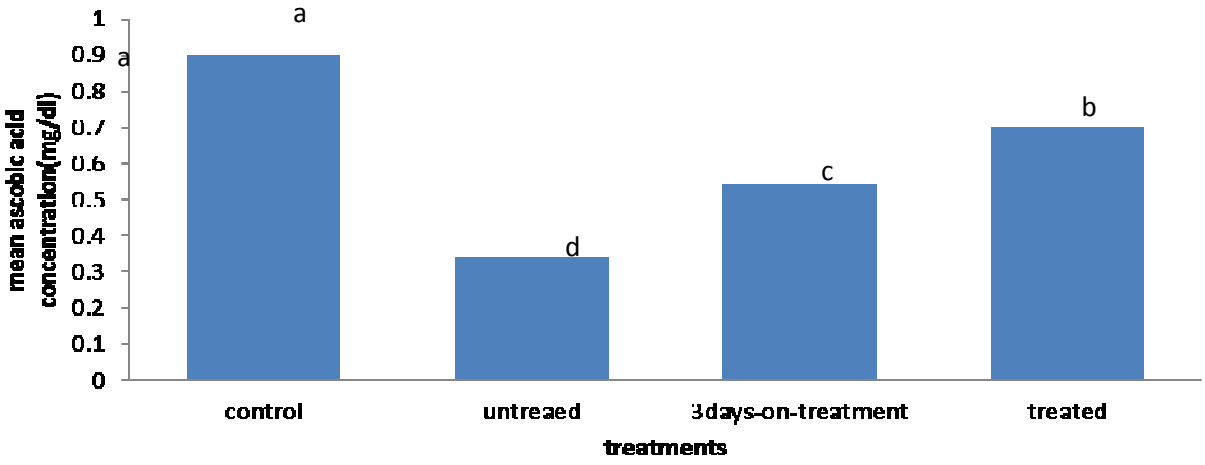


Figure 2: Uric acid concentration in different groups of typhoid fever patients. Means carrying different letters are statistically different

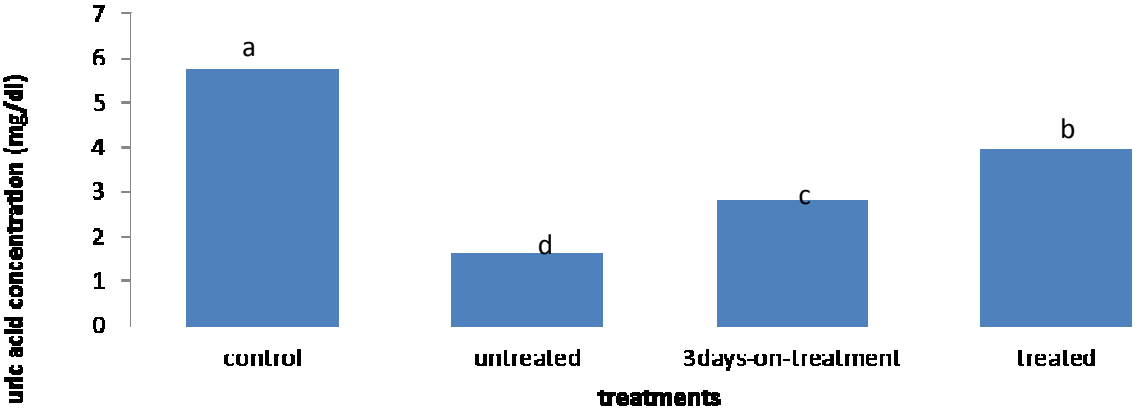


Figure 3: Total plasma lipids; means carrying different letters are statistically different

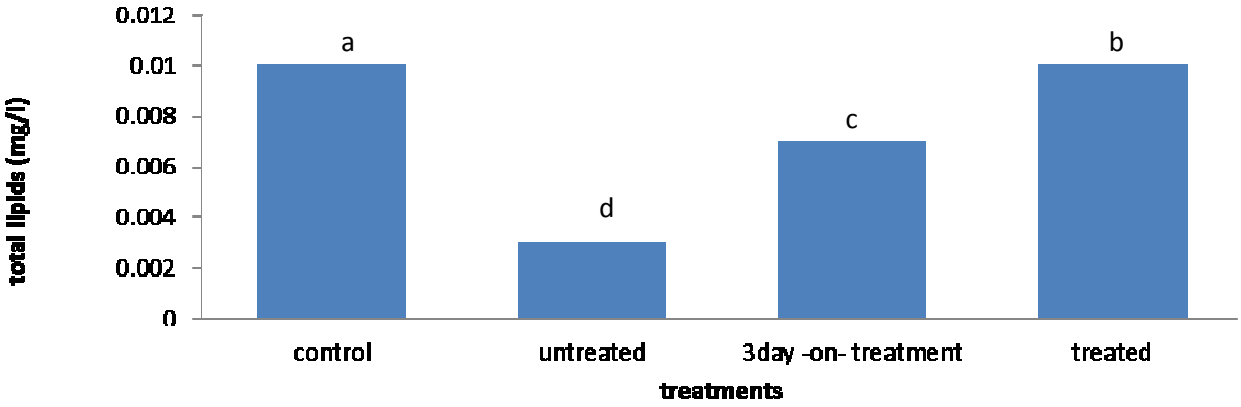
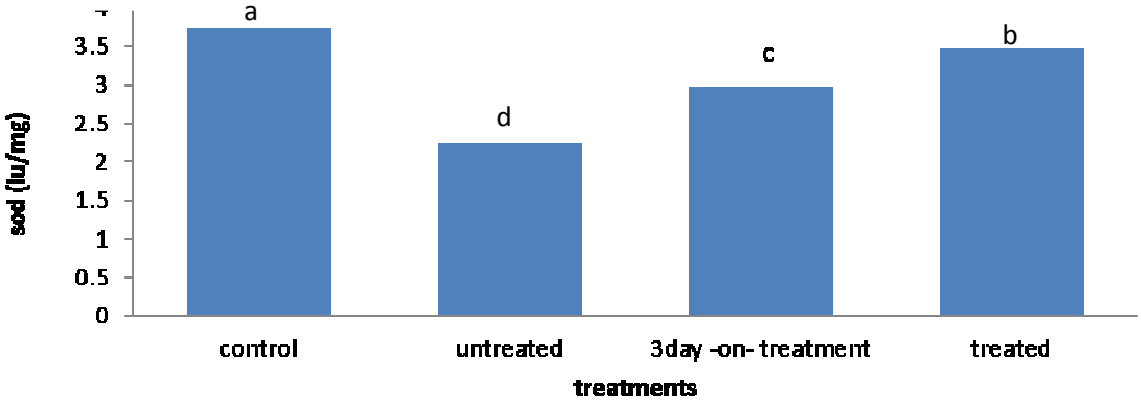


Figure 4: SOD of different groups, means carrying the same letters are not statistically different



DISCUSSION

The decrease ($p<0.05$) in antioxidant scavengers and antioxidant enzymes between untreated and 3-days on treatment groups could possibly be that these antioxidants are consumed by scavengers or attenuating the excess free radicals generated during the progress of the infection, in consonance with the report that the depletion of antioxidant systems in patients infected with *Trypanosomes brucei*, were associated with the neutralization of large amounts of free radicals generated

during the progress of the infection in rats [16]. Ascorbic acid (vitamin C) is very effective in protecting plasma lipoproteins from per oxidation during exposure to a wide spectrum of free radicals since semi dehydroscorbate anion radical (ASc) formed in the process is extremely unreactive, enhancing the antioxidant effectiveness of ascorbate [17]. A decrease in the mean serum ascorbate concentration in a system is thus a consequence of the ascorbate being used up to scavenge the free radicals produced. Thus ascorbate radical could serve as a gauge of ongoing oxidative stress in *Salmonella typhii* infection. Consequently, the diminution of ascorbic acid concentration observed in patients with *Salmonella typhii* infection was the result of its protective role against free radicals [18].

The observed diminution of urate concentration in untreated and 3 days on treatment groups could be due to the fact that most of it was used up for the protection of the body against the deleterious effects of free radicals produced. Our results is supported by the fact that lower serum uric acid concentration observed in aging and cancer patients was attributed to its being used up in scavenging free radicals produced in the patients [9].

Complex per oxidation of polyunsaturated fatty acids (PUFA) by oxygen free radicals produced in the typhoid fever could be responsible for a significant diminution of total lipids observed in untreated and 3 days on-treatment groups of the *Salmonella typhii* patients when compared with that of the control group. This result is in consonance with the report that oxidative damage to lipid components in patients with familial combined hyperlipidemia results in reduced total lipid levels [19]. Furthermore, this trend in the reduction of total lipid during tuberculosis in humans [20], also agrees with this result. Significant diminution ($p < 0.05$) in the mean serum superoxide dismutase (SOD) in the untreated and on-treatment groups was also revealed by the study. The highly significant diminution of SOD activity in the typhoid patients as observed in this study may either be due to accumulation of superoxide anion to a high level thereby causing inhibition of enzyme activity (high substrate inhibition) or the alteration in the conformation of SOD by free radicals, formed during *Salmonella typhii* infection, decreasing the activity of the enzyme, or the reduced synthesis of SOD in patients thereby rendering them more susceptible to oxidative damage. This result seem to agree with the report of a substantially reduce SOD activity in patients with Coronary artery disease [21] and also in patients with kidney failure [22, 23]. Furthermore, this study confirms the decrease of total SOD activity in whole placenta homogenates and mitochondrial and systolic fractions from women with pre-eclamptic pregnancy compared with those of the women with normal gestation [17, 24]. The decrease concentration of SOD was attributed to an increased generation of ROS in this disease state which were consequently consumed by the antioxidant enzyme thereby decreasing its concentration leading to low activity. These results suggest that the burst in oxygen consumption during *Salmonella typhii* infection could enhanced the generation of excess reactive Oxygen species (ROS), resulting in the decrease of the endogenous antioxidant system and enzymatic free radical scavengers. Consequently, excess ROS could mediate free radical damages of the host cells and tissues culminating; at least in part, in the damages association with *Salmonella typhii* infection.

CONCLUSION

In conclusion, this investigation revealed that during the progression of *Salmonella typhii* infection, there is copious generation of ROS, diminution in the concentrations of free radicals scavengers and depletion in as well as inhibition of the activity of antioxidant enzymatic system. These changes could probably be associated with increased generation of ROS coupled with antioxidant systems and consequently increased oxidative stress during the progress of typhoid fever disease. These changes are conducive to *Salmonella typhii* disease and may perhaps be treated faster by the combined therapy of *Salmonella typhii* medications as well as supplementation of antioxidant-rich diets.

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CONFLICT OF INTEREST

No conflict of interest was declared by authors