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Status of Oxidative Stress in Typhoid Fever Patients among Fishers of Calabar, Cross River State, Nigeria

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ABSTRACT [ENGLISH/ANGLAIS]

Oxidative stress had been known to contribute to some physiological damages in host infected with some bacteria and viruses. In view of this, the extent of lipid peroxidation and then status of endogenous antioxidants (ascorbic acid, B- carotene) and total cholesterol were (investigated) in fishers in Calabar confirmed to be infected with *Salmonella typhi* with a view to assessing the status of oxidative stress during the progress of the disease. Eighty six residents of Calabar, a coastal city in cross river state, were involved in this study. They consisted of 23 untreated, 22 on - treatment for 3 days, 22 confirmed treated patients and 23 persons tested to have no ailment by the time their blood sample were drawn to serve as control. Results reveal significant plasma malondialdehyde (MDA) elevation ($p < 0.05$) but significant decrease ($p < 0.05$) of the oxidants and total cholesterol concentrations in patients not yet treated, when compared with those of mean concentrations in control group. The results further showed insignificant MDA elevation and antioxidant concentration diminution in on - treatment and treated groups. These results suggest the generation of oxidative stress during the progress of typhoid fever.

Keywords: Oxidative stress, lipid peroxidation, *Salmonella typhi*, fishers, Cross River State

RÉSUMÉ [FRANÇAIS/FRENCH]

Le stress oxydatif a été appelé à contribuer à certains dommages physiologiques dans l'hôte infectées par des bactéries et des virus. Dans cette perspective, la mesure de la peroxydation lipidique et le statut des antioxydants endogènes (acide ascorbique, B-carotène) et de cholestérol total étaient (enquête) chez les pêcheurs à Calabar a confirmé d'être infecté par la bactérie *Salmonella typhi* en vue d'évaluer l'état de le stress oxydatif au cours de la progression de la maladie. Quarante-six résidents de Calabar, une ville côtière dans Etat de Cross River, ont été impliqués dans cette étude. Ils se composaient de 23 non traitée, 22 sur - le traitement pendant 3 jours, 22 ont confirmé les patients traités et 23 personnes testées ne pas avoir de mal au moment où leur échantillon de sang ont été établis pour servir de témoin. Les résultats révèlent significative plasmamalondialdéhyde (MDA) d'élévation ($p < 0,05$), mais diminution significative ($p < 0,05$) des oxydants et des concentrations de cholestérol total chez les patients non encore traités, comparativement à celles des concentrations moyennes dans le groupe contrôle. Les résultats ont également montré d'élévation insignifiante MDA et la diminution de concentration en antioxydant sur - le traitement et les groupes traités. Ces résultats suggèrent la génération du stress oxydatif au cours du déroulement de la fièvre typhoïde.

Mots-clés: Stress oxydatif, peroxydation lipidique, *Salmonella typhi*, les pêcheurs, Etat de Cross River

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INTRODUCTION

Typhoid fever is an infectious disease caused by the bacterium *Salmonella typhi*. The disease has several symptoms in the digestive system in the second phase of the illness [1, 2]. It is a multisystem illness, characterized by the classic prolonged fever, sustained bacteremia with endothelial or endocardial involvement; and bacterial invasion and multiplication within the mononuclear phagocytic cells of the liver, spleen, lymph nodes and Payer patches [3]. It is debilitating and potentially fatal if

untreated. It passes from one person to the other through contaminated water. Typhoid fever is most prevalent in many parts of Asia, Africa, Central and South America, where it occasionally causes epidemics [4]. This bacterium therefore poses problem to fisheries industry since fish can only be produced in riparian areas. Earlier investigations have revealed that oxidative stress has been implicated in a number of degenerative diseases [5]. Lipid peroxides, which are free radicals, originate from the per oxidations of lipids presumably from plasma

membrane. Peroxyradicals have been implicated in the pathogenesis of many disease states such as malaria [6,7], male infertility [8], sickle cell anaemia [9], hypertension [10], tuberculosis [11], trypanosomiasis [12] and many other disease states [13,14]. In the present study we investigated the extent of lipid peroxidation indexed by malondialdehyde (MDA) and the status of endogenous antioxidants (ascorbic acid, B- carotene and total cholesterol) in volunteers confirmed to be infected with *Salmonella typhi* with a view to assessing the status of oxidative stress during the progress of typhoid fever,

MATERIALS AND METHODS

In all subjects blood samples (5-10 ml) were collected by venipuncture into polystyrene tubes and allowed to clot. Serum was prepared by centrifuging at 3000 X g (Wesper Bench centrifuge) for 20 minutes; the upper layer (serum) was collected and used immediately or stored at 20 °C until used.

Estimation of Lipid Peroxidation Index

Lipid peroxidation indexed by malondiadehyde (MDA), was estimated colorimetrically according to the method of Das et al. [15] as adapted by Uzoegwu [16]. A standard curve was plotted using serial dilutions of the chemical, 1, 1, 3, 3 – tetraethoxypropane (TEP) which reacts with hydrochloric acid releasing MDA. The MDA reacts with thiobarbituric acid (TBA) giving a red chromogen. The chromogen was extracted with butan 1-ol and its absorbance read at 532 nm using butan- 1- ol as blank.

Assay for Endogenous Antioxidants

Ascorbic acid (vitamin C) concentrations were measured by the method of Wallin et al. [17]. To 0.5 ml of serum, 2.0 ml freshly prepared metaphosphoric acid buffer was added, mixed with a vorlex mixer and centrifuged for 10 minutes at 3000xg. To 1.2 ml of the supernatant, 0.4 ml of dinitrophenylhydrazine-thiourea – copper sulphate (DTCS) was added, mixed and incubated in a water bath for 3 hours. The mixture was then chilled for 10 minutes in an ice bath and 2.0 ml of cold sulphuric acid (12 mol/l) added slowly. This was again mixed with vortex mixer, allowed to stand for 30 minutes at room temperature and the absorbance read at 520 nm against metaphosphoric acid as blank.

β – carotene was estimated by the method of Tietz [18]. To 0.5 ml of the test samples 0.5 ml of absolute ethanol was added drop-wise, then shaken to precipitate proteins.

Petroleum ether (1.0 ml) was added to each sample, mixed with a vortex mixer for 5 minutes, and centrifuged for 1 minute at 3000 X g. The separated upper layer was pipetted out and its absorbance read at 440 nm against petroleum ether as blank.

Assay for Serum Total Cholesterol

Total cholesterol was determined using commercial assay kit (purchased from Randox, UK)

Statistical Analysis

One way analysis of variance (ANOVA) Statistics for Social Sciences version 13.0 was used to evaluate significant levels between the different groups of persons. The results obtained are reported in Figures 2 – 5 and Table 1. These show statistically higher significant increase (p<0.05) in the concentration of MDA in the untreated and under- treatment groups compared to the control. The results also showed a significant decrease (p<0.05) in the concentrations of ascorbic acid, β-Carotene and total cholesterol in the untreated and under-treatment groups than in the control group. Furthermore, the results showed that MDA correlates negatively with the concentrations of β-carotene (-0.385) and ascorbic acid (-0.129) indicating inverse proportional relationship between them. The relationship of MDA and total cholesterol concentration was positive but not significant (p>0.05) (0.307).

Figure 1: This figure shows the map of Cross River State of Nigeria showing study area

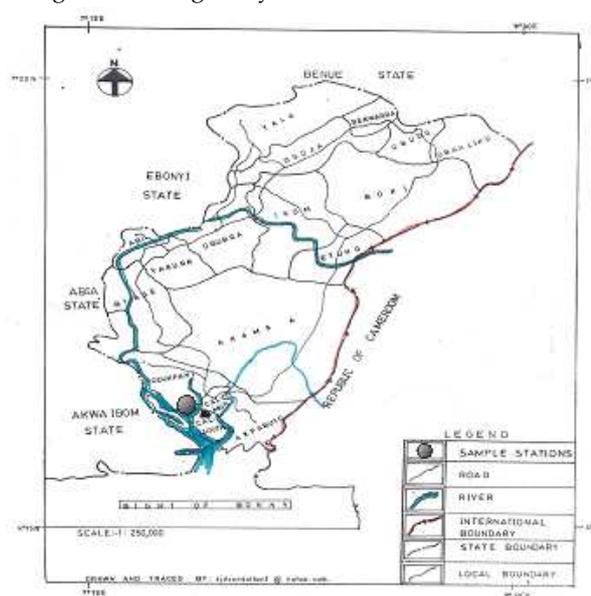


Figure 2: This figure shows the mean plasma β -carotene concentration ($\mu\text{g/ml}$) of different groups of typhoid fever patients and the control. Different letters show statistically different means

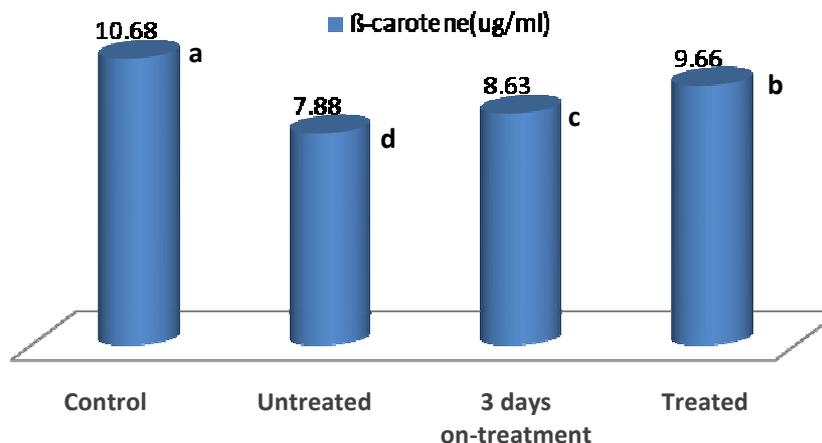


Figure 3: This figure shows the mean plasma Ascorbic acid concentration (mg/dl) of different groups of typhoid fever patients and control. Same letters show similar statistical means

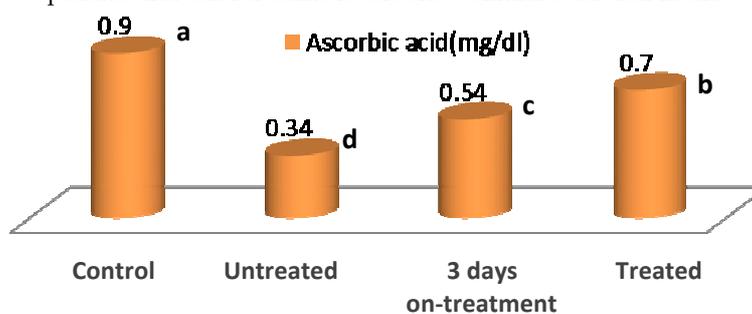
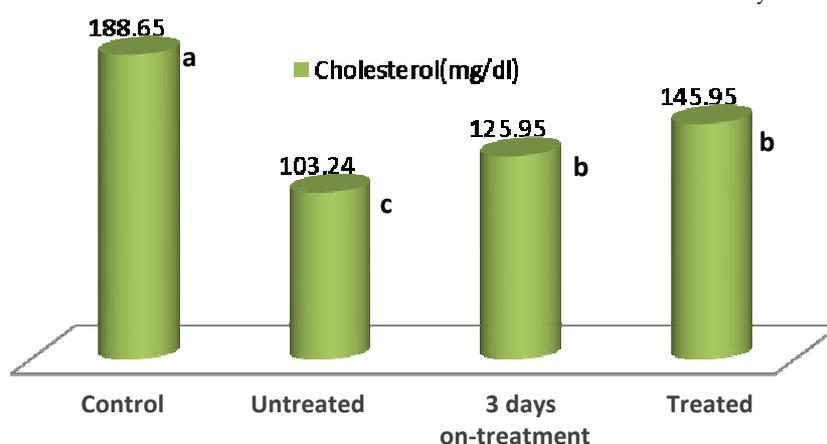


Figure 4: This figure shows the mean plasma cholesterol concentration (mg/dl) of different groups of typhoid fever patients and control. Different letters mean that the means are statistically different



DISCUSSION

The increased lipid per oxidation as depicted by the high concentration of MDA, may arise from a variety of factors such as enhanced generation of free radicals, reduced level of antioxidants available, enhanced consumption,

leakage or destruction of antioxidants, decreased protective capacity including antioxidants enzymes, leakage of electrons from the disrupted mitochondrial electron transport chain and phagocyte recruitment and activation [16].

Membrane lipids succumb easily to deleterious actions of reactive oxygen species. The measurement of lipid peroxidation is a convenient method to monitor oxidative damage [19]. In this study the results indicates that serum concentrations of MDA in the untreated and under-treatment typhoid patients was significantly higher than ($p < 0.05$) that of the control group. Higher concentrations of MDA in severe stages amounts of reactive oxygen species were generated as the disease progressed. This

corroborates earlier observation of Uzoegwu [16], who posits that excess reactive oxygen species could damage host tissues or cells and the damage to the host cells could precipitate cell death which is responsible for the elevation of MDA concentrations in malaria parasite infected persons. This result is also in consonance with the observation of the presence of a higher oxidative stress status in haemodialysis kidney patients when compared with healthy individuals [20].

Figure 5: This figure shows the mean plasma MDA (nmol/ml) of different groups of typhoid fever patients and control.

Different letters mean that the means are statistically different

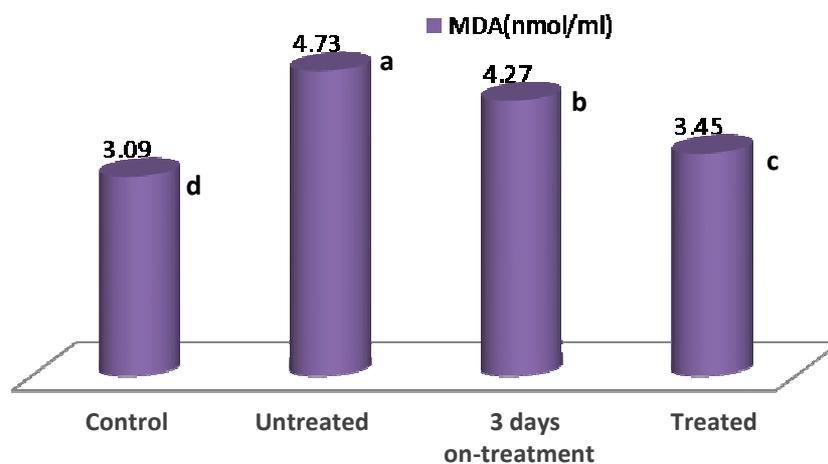


Table 1: This table shows the correlation between MDA and Ascorbic acid, β - carotene and total cholesterol

	Concentration of MDA (nmol/ml)	Concentration of ascorbic acid (mg/dl)	Concentration of β - carotene (microg/ml)	Concentration of Total cholesterol (mg/ml)
Concentration of MDA (nmol/ml)	1	-129	-385	0.307

Ascorbic acid acts as the first line of defence against oxidative stress during the destructive cycle of typhoid disease [21]. It is the only antioxidant in serum or plasma capable of completely inhibiting oxidative modification of the low density lipoprotein by aqueous peroxy radical [21]. Deficiency of ascorbate also has a direct association with increased atherosclerosis in guinea pigs and its intake has been shown to have an increased relationship to atherosclerosis in quail, rabbit and human [22]. Ascorbate administration exerts a protective role against peroxidative damage of lipid [22]. Significantly lowered levels of ascorbic acid concentration in the untreated and under-treatment groups of typhoid patients compared to the control group may be linked to the increased

consumption of ascorbic acid due to increased reactive oxygen species (ROS) as evident from enhanced MDA levels or failure of the system to recycle dehydroascorbic acid back to ascorbic acid. This corroborates the suggestion that oxidation inactivates and/ or depletes antioxidants and thus in the presence of ROS and subsequent lipid peroxidation, antioxidant vitamin concentration are diminished [9].

β - carotene reacts with lipid peroxidation products to terminate chain reactions [23] and also directly scavenge singlet oxygen and dissipate the energy as heat [24]. This study reveals significant diminution ($P < 0.05$) in the mean plasma β - carotene concentrations in the untreated group of typhoid fever patients when compared with the control

group while the on-treatment and treated group exhibited progressive increase of the β - carotene could affect the progress of the disease. This possibility could be supported by an observation that there was a significant decrease in the mean serum β - carotene concentration with increasing degree of breast cancer [25]. Furthermore, low concentration of β - carotene had been observed to reflect progression rather than the systemic inflammatory response in patients with prostate cancer [26].

Our study further showed a significant decrease in the mean serum cholesterol concentration in the untreated and on-treatment typhoid groups relative to that of the control groups, perhaps since cholesterol is one of the preferential targets of oxidation by free radicals. This observation is consistent with the report of Olabinri, et al. [25] who indicated that there was a significant decrease in the mean total cholesterol in patients with breast cancer than in the control. Similarly lower cholesterol level was reported in tuberculosis patients than in the control due to ROS attacks on cholesterol [11].

This study also reveals that MDA concentration correlates negatively with ascorbic acid and β - carotene concentrations as was similarly observed in tuberculosis patients [11]. It was also observed from the study that MDA correlates positively, though not significantly, with toya; cholesterol indicating that MDA concentration was directly proportional to this parameter. The insignificant positive correlation could be as a result of some vagaries introduced by the assay procedures of total cholesterol.

CONCLUSION

In conclusion, the decreased concentration of endogenous antioxidants along with elevated malondialdehyde concentration in typhoid patients could probably be associated with oxidative stress and/ or decreased antioxidant defense potential. The life threatening perforation of the patients' intestinal walls by *Salmonella typhii* organism is one of the most outstanding pathologic involvements of typhoid fever. Although, the implication of oxidative stress in the pathogenesis of typhoid fever is not investigated in this study, the observed generation of severe oxidative stress during this disease state could likely indicate that oxidative stress is an essential factor in the exacerbation and complications in the pathogenesis of this disease and therefore, a potential target for therapy. It could therefore be inferred that increasing the quantity of our daily antioxidant intake will invariably reduce the extent of lipid per oxidation thereby alleviating the

debilitating effect of oxidative stress generated during typhoid fever disease.

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CONFLICT OF INTEREST

No conflict of interests was declared by authors

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