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Oxidative Stress Levels, Metalloenzymatic Defense and Redox-Active Metals in Typhoid Perforation before and after Surgical Intervention

Ibrahim Hassan Garba* • Auwal Adamu Mahmoud

Chemistry Programme, School of Science, Abubakar Tafawa Balewa University, PMB 0248 Bauchi, Bauchi State, Nigeria

***Corresponding author*: * ihgarba2002@yahoo.com; hassanibrahimgarba0@gmail.com**

ABSTRACT

The serum concentration of two antioxidant metalloenzymes, superoxide dismutase (SOD) and catalase (CAT), as well as malondialdehyde (MDA) and the redox-active metals iron, copper, zinc and manganese were assessed in patients presenting typhoid perforation before and after surgical intervention. CAT and SOD activity decreased by 12.7 and 8.8% and by 40.16 and 52.70%, respectively during the pre- and post-surgical periods. There was a marginal elevation in the serum MDA concentration in the pre-surgical period and significant ($P < 0.05$) decline in MDA after surgical intervention. Significant perturbations were found in the serum concentration of iron, zinc and copper and the serum zinc/copper ratio. Serum copper concentration and Zn/Cu ratio were significantly elevated, while zinc and iron levels were significantly decreased in both pre- and post-surgical typhoid perforation relative to the control group ($P < 0.05$). These finding have potentially deleterious consequences on the ROS-scavenging ability of both CAT and SOD in typhoid perforation patients prior to surgical intervention, which has been found to have a positive effect in maintaining the homeostatic equilibrium of a significant number of these perturbed parameters.

Keywords: catalase, copper, dismutase, iron, manganese, zinc
Abbreviations: CAT, catalase; **MDA**, malondialdehyde; **OS**, oxidative stress; **ROS**, reactive oxygen species; **SOD**, superoxide dismutase

INTRODUCTION

Cellular oxidative damage is a well established general mechanism for cell and tissue injury. The cellular oxidative damage is primarily caused by free radicals and reactive oxygen species (ROS) (Burton and Jauneaux 2011). Free radicals have the ability to bind most normal cellular components, and particularly react with unsaturated bonds of membrane lipids, denaturing proteins and attack nucleic acids and initiating the phenomenon of oxidative stress (Trush and Kensler 1991). A disturbance of the balance between formation of ROS and its metabolites and the rate at which they are scavenged by enzymatic and non-enzymatic antioxidants is referred to as oxidative stress (OS) (Papas 1996). Prime targets of ROS are the poly-unsaturated fatty acids in cell membranes, causing lipid peroxidation which may lead to damage to cell structure and function (Floyd 1990). In addition the decomposition of lipid hydroperoxides yields a variety of end-products including malondialdehyde (MDA). OS is now recognized to play a central role in the pathophysiology of many different disorders, including carcinogenesis and pregnancy complications (Yeou-Li *et al*. 1999; Myatt 2010).

Metals play many roles in a wide variety of biological processes of living systems. Metal ion homeostasis maintained through tightly regulated mechanisms of uptake, storage and secretion is therefore critical for life and is maintained within strict limits (Bertini and Cavallaro 2008). The breakdown of metal ion homeostasis can lead to metal binding to protein sites different from those designed for that purpose or replacement of other metals from their natural binding sites. The consequence includes metal-DNA and metal-protein interactions leading to oxidative denaturation of biological macromolecules (Halliwell and Gutteridge 2007).

Disruption in metal homeostasis has been known to lead to a plethora of human and diseases, including hereditary hemochromatosis, cancer, anemia, Alzheimer's disease and Parkinson's disease as well as a host of inflammatory and degenerative diseases of the osteo-muscular system among others (Bush and Curtain 2008; Stanek *et al.* 2010). Metals are also known to modulate gene expression by interfering with signal transduction pathways which play important roles in cell growth and development (Valco *et al.* 2006). Since the generation of free radicals in living systems is closely linked with the participation of redox-active metals such as iron, copper, chromium and cobalt, the redox state of the cell is maintained within strict physiological limits. The disruption of metal ion homeostasis may lead to uncontrolled, metal-mediated formation of deleterious free radicals which in turn participate in the modification of deoxyribonucleic acid (DNA) bases, enhanced lipid peroxidation and altered calcium and sulphydryl homeostasis (Jamova and Valco 2011).

Considering the need to maintain the delicate and crucial balance between ROS homeostasis on one hand and metal ion balance on the other and in order to protect cells against ROS/metal- induced oxidative stress, nature has evolved an array of elaborate mechanisms for protecting cells and tissues. The defense system against intracellular ROS production and propagation, termed antioxidant defense, is controlled by a highly complex and integrated antioxidant system. This system enables mammalian cells to cope with oxidative environments. The composition of this system includes enzymatic antioxidants, the thiol tripeptide glutathione (GSH) and various non-enzymatic dietary antioxidants (Al-Gubory *et al.* 2010). The notable tissue enzymatic antioxidants include the superoxide dismutases (SODs), catalase (CAT), glutathione reductase (GR), glutathione peroxidases (Gpx) and glutathione-*S*-transferase

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Short Communication

(GST) all of which are important in maintaining redox balance and healthy state of the human body (Zhou *et al.* 2002; Maffei *et al.* 2011).

Considering the present state of knowledge on the role of ROS in the pathogenesis of many biological processes and diseases, and taking into account the facts stated above, the aim of this study was to assess the redox-active metals concentration, OS levels, CAT and SOD activities and the relationship between these parameters in typhoid perforation, as to the best of our knowledge not much work has been done on this aspect of typhoid infection.

MATERIALS AND METHODS

Study design

Patient selection and pre-qualification was done using simple random sampling of individuals admitted and booked for surgery due to typhoid perforation at the male and female surgical wards of the Specialist Hospital, Bauchi, Bauchi State Nigeria. Patients booked and presenting concomitantly with a history of alcoholism, tobacco smoking, self-medication and drug addiction within the prescribed period of clinical presentation (2-4 weeks) were not included in the study. Based on these criteria a total of 20 patients comprising of 15 males and 5 females were enrolled for the study. The control group consisted of 20 age and sex-matched healthy adult males and females. The age range for both the patients and controls was 15-45 years.

Collection and preparation of serum samples

Blood samples (5 ml) was collected from each of the patients 24 h before surgery (pre-surgical samples) and 24 h after surgery (post-surgical samples) by venepuncture of the antecubital vein into clean, sterile metal-free plastic centrifuge tubes. The samples were centrifuged at 3500 × *g* for 5 min and the separated sera were collected by careful and gentle aspiration using a Pasteur pipette. Samples were frozen at -4°C and analyzed within one month after collecting an aliquot of 500 µl for MDA, CAT and SOD analysis, which were done on the day of sample collection.

Determination of serum MDA concentration

MDA concentration was determined according to the fluorometric method described in Hedriks and Assman (1988). This method involves the reaction of MDA with thiobarbituric acid (TBA) at 100°C under acidic conditions. The concentration of the MDA-TBA adduct was then measured fluorometrically at an excitation wavelength of 525 nm and an emission wavelength of 547 nm.

Enzyme assays

CAT activity was assayed according to the method described in Sinha (1972) and one enzyme unit as defined as the amount of enzyme which catalyzed the oxidation of 1 µmol of H_2O_2/min under assay conditions. SOD activity was assayed using the Cayman's SOD assay kit (Cayman Chemical Co., Ann Arbor, MI, USA). Briefly, this assay method utilizes a tetrazolium salt for the detection of superoxide radicals generated by xanthine oxidase and hypoxanthine. One unit of SOD is defined as the amount of enzyme needed to exhibit 50% dismutation of the superoxide radical.

Determination of metal concentrations

Serum sample (0.5 ml) was diluted 10-fold with 0.5% (v/v) solution of triton-X-100 and the concentration of each metal sample determined using a Buck Scientific Atomic Absorption Spectrophotometer 200 (Buck Scientific Inc., East Norwalk, CT).

Ethics

This work was conducted in accordance with the following ethical declarations: World Medical Association's Declaration of Helsinki (1996) and the CIOMS/WHO International Guidelines for the Conduct of Research Involving Human Subjects (1993).

Statistical analyses

Data was analyzed using Stratigraphics statistical software version 3.0 (Statistical Graphics Corporation, U. S. A.). Pre-surgical, post-surgical and control samples were compared using one-way analysis of variance. The least significant difference (LSD) was used to test for differences between pair means where an ANOVA P value < 0.05 was considered significant.

RESULTS AND DISCUSSION

The results for changes in serum concentration of iron, copper, zinc and manganese are shown in **Table 1**. Serum iron concentration was found to decrease in both pre-surgical and post-surgical periods relative to the control value, $P < 0.05$, with the iron level being at its lowest during the post-surgical period. However the difference between pre-surgical and post-surgical serum iron concentrations was only marginal. Serum copper levels were found to increase significantly from the control through to both the pre-surgical and post-surgical conditions, $P < 0.05$.

Serum zinc values showed a significant decrease in both pre- and post-surgical periods relative to the control, $P < 0.05$. However no statistically significant difference exists between the serum zinc concentration in the pre- and post-surgical periods. Serum manganese levels were found to be stable across the three groups studied, $P > 0.05$. The serum copper/zinc ratio was also found to increase from the control to pre-surgical condition and peaking during the post-surgical period as shown in **Table 1**.

Table 2 shows the concentration of serum MDA, CAT and SOD in the control and pre-/post-surgical patients. Serum CAT activity decreased significantly relative to the control, within the pre-surgical period, $P < 0.05$ and showed a marginal increase during the post-surgical period. Serum SOD activity was significantly decreased relative to the control during both the pre-surgical and post-surgical periods, $P < 0.05$. Serum MDA increased marginally during the pre-surgical condition when compared to the control serum concentration. There was significant difference the observed decrease in MDA during the post-surgical condition relative to the control value.

Typhoid fever remains an important cause of morbidity globally, with an estimated annual incidence of 21 million cases, of which 1-4% ends fatally. The disease is caused by *Salmonella enteric serovar typhi (S. typhi).* It is characterized by high fever, colic pain, inflammation, hepatic injury and diarrhea. *S. typhi* has also been known to cause hepatic dysfunction and hepatic abscess (Khan 2009a). *S. typhi* enters the body via ingestion of food or water contaminated with excreta from typhoid fever cases or asymp-

Table 1 Serum concentration of iron, copper, zinc and copper/zinc ratio in control, pre-surgical/post-surgical typhoid perforation patients.

Parameters	Control	Pre-surgical	Post-surgical
Iron (mmol/dl)	0.24 ± 0.06 a	0.18 ± 0.05 ac	0.17 ± 0.02 ad
Copper (mmol/dl)	0.15 ± 0.03 a	0.18 ± 0.01 a	0.22 ± 0.03 a
Zinc (mmol/dl)	0.14 ± 0.01 a	0.06 ± 0.02 ab	0.04 ± 0.01 ab
Manganese (mmol/dl)	0.06 ± 0.02 a	0.06 ± 0.02 a	0.06 ± 0.02 a
Cu/Zn	1.07	3.0	5.5

Values are mean ± SEM. Values with the same letters in a row are significant at $P < 0.05$

Table 2 Serum concentration of malondialdehyde, catalase and superoxide dismutase in control, pre-surgical / post-surgical typhoid perforation patients.

Parameters	Control	Pre-surgical	Post-surgical
Catalase (µmol)	20.25 ± 0.45 a	17.67 ± 0.45 a	18.47 ± 0.97 c
Superoxide dismutase (µmol)	25.45 ± 1.57 a	15.23 ± 0.33 ab	12.04 ± 0.65 ac
Malondialdehyde (µmol/dl)	185.15 ± 3.11 a	189.75 ± 2.05 c	179.50 ± 0.86 ac

Values are mean ± SEM. Values with the same letters in a row are significant at $P < 0.05$

tomatic carriers of the bacterium. Although this disease is considered an endemic disease, epidemics do occur frequently as a result of breakdowns in water supplies and sanitation systems (Khan 2009b).

The pathogenesis of this protracted illness includes a bacteremic phase with fever and chills within the first week, widespread reticuloendothelial involvement with rash, abdominal pain and prostration in the second week and ulceration of Payer's patches with intestinal bleeding and perforation during the third week. Majority of the patients also present with longitudinal ulcers on the antimesentric border, situated within 45 cm of the ileocaecal valve (Karmacharya and Sharma 2006). The most lethal complications of typhoid are intestinal bleeding and ileal perforations, both arising from necrosis of Payer's patches in the terminal ileum. Typhoid perforation of the ileum is a serious complication of typhoid fever and the resulting peritonitis in such a seriously ill patient may be rapidly fatal, unless treated promptly and vigorously. Under this condition, surgery, although associated with a high morbidity and mortality offers the greatest hope of survival (Ansari *et al.* 2009).

The ability of *Salmonella* to replicate within the macrophages allows this enteric pathogen to cause this disseminated disease. The immune system responses of humans to this disease via inflammation of the bowel and subsequent activation of phagocytic leucocytes such as neutrophlic polymorphonuclear cells, eosinophils, monocytes and macrophages into the lamina propria of mucosal interstitium leads to OS-mediated production of ROS, through respiratory burst (Eziegbo and Nwaehujor 2010). In addition, the superoxide radicals produced react together with nitric oxide to form peroxynitrite, a strong biological oxidant (Rastaldo *et al.* 2007; Haque 2011). The observed elevation in MDA during the pre-surgical period can be adduced to the synergistic effects of both superoxide free radicals and peroxynitrite, and oxidative burst arising as consequence of the inflammatory response associated with this disease. This finding is in consonance with earlier reports (Rastaldo *et al.* 2007; Khan 2009a; Eziegbo and Nwaehujor 2010; Haque 2011).

Under normal conditions, this increased state of cellular oxidative stress can be ameliorated by the body's antioxidant system. However, serum SOD first in the line of providing antioxidant protection is found to decrease in pre-surgical typhoid perforation. Similarly, serum CAT activity is also decreased. The combination of low SOD and CAT leads to a compromised ability of the body to initiate an effective defense against the resulting oxidative stress during typhoid perforation. Following surgical intervention however, there was a significant decrease in the serum concentration of MDA, which indicates the beneficial effect of this procedure in typhoid perforation in both disease management and particularly reduction of ROS-mediated oxidative stress.

Furthermore the observed decrease in serum concentration of both iron and zinc can have a negative effect on the free-radical scavenging activities of both CAT and SOD since iron and zinc are crucial to the effective antioxidant functions of these metalloenzymes. In a study on the effect of copper on infectivity of *Salmonella* using a mice model Sharan *et al.* (2011) reported that copper has the ability to decrease the pathogenicity of the bacterium. The elevated serum levels seen in our study during the pre-surgical period may not be unrelated with the onset of a similar copper-mediated protective mechanism in human *salmonellosis*. More confirmatory work is however required on this aspect. The relatively non-perturbed state of serum manganese is a potential indicator of the stability of the mitochondrial SOD, which is manganese-dependent on its action. The assessment of the variation in Zn/Cu ratio from normal through to pre- and post-surgical periods can be applied potentially as a diagnostic / prognostic marker in the surgical management of typhoid perforation.

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