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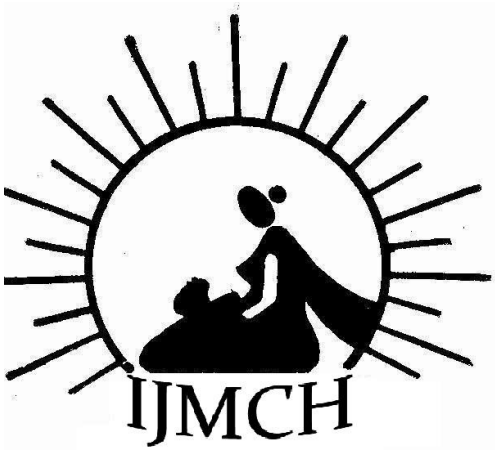

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The role of free radicals in the pathogenesis of enteric fever in humans, especially in children, are lacking.

Plasma oxidative stress in children with enteric fever

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Abstract:

Research Question: The role of free radicals in the pathogenesis of enteric fever in humans, especially in children, are lacking. This study was done with the objective of assessing the plasma oxidant and antioxidant status of children with enteric fever. **Settings:** This study was done in Mayo Institute of Medical Sciences, a tertiary care centre**. Study Design**: A prospective institution-based study **Material and Methods**: 60 patients of confirmed enteric fever in the age group of 0–15 years and other 60 age- and gender-matched healthy children (controls) were included in our study. Plasma MDA was estimated by thiobarbituric acid test. Protein carbonyl, Ascorbic acid, Ceruloplasmin and Superoxide Dismutase (SOD) were analyzed by the methods of Reznick and Packer, Roe, Ravin and Marklund and Marklund respectively. b-carotene was estimated by the method of Tietz. **Results:** The average plasma level of oxidants was significantly higher in cases as compared to control group ($p < 0.05$). The plasma MDA level in the cases was significantly higher ($p = 0.03$) than in control group. The plasma level of protein carbonyl in cases was significantly higher ($p = 0.02$) as compared to control group. The level of antioxidants was significantly lower in the cases as compared to the control group.

Key Words: ***Oxidative stress, Enteric fever, Ceruloplasmin, Ascorbic acid***

Introduction:

Enteric fever also known as typhoid is a potentially fatal multisystemic illness caused primarily by Salmonella enterica serovar typhi and to a lesser extent by related serovars *paratyphi* A, B, and C. In 2010 there were 27 million cases reported.(1) The disease is most common in India, and children are most commonly affected.(1,2) *Salmonella* usually enters into the body via ingestion of food or water contaminated with excreta from typhoid fever cases or asymptomatic carriers of the bacterium. There are two types of salmonella-derived food-borne infections causing serious medical problems worldwide: i) Intestinal or non-typhoid form (salmonellosis) caused by hundreds of different serovariants of S. enterica , including serovar Typhimurium and ii) Generalized or typhoid form (enteric fever) caused by S. enterica serovar Typhi.(3) Enteric fever is characterized by high grade fever, colicky pain abdomen, inflammation, hepatic injury and diarrhea or constipation with bacterial invasion and multiplication within the mononuclear phagocytic cells of the liver, spleen, lymph nodes

and Peyer patches.[4] Although typhoid fever is largely considered an endemic disease, epidemics also occur due to the breakdown in water supplies and sanitation systems. It is increasingly being realized now that majority of the diseases like cancer[5], atherosclerosis, myocardial infarction[6,7] and various infections[8] including enteric fever are mainly due to the imbalance between pro-oxidant and anti-oxidant homeostatic mechanisms in the body. The localized inflammation following salmonella invasion generates many activating signals for phagocytes resulting in killing of the pathogens. In addition to the beneficial effects of inflammation and priming of polymorphonuclear leukocytes for phagocytosis, the production of reactive oxygen species (ROS) can also occur and create collateral tissue injury.[9] Proxidant condition dominates either due to increased generation of free radicals and/or their poor quenching/scavenging into the body.[10] On the other hand antioxidants form the defense mechanism of the body which readily detoxify the reactive intermediates or repair the resulting damage. Disturbances in the normal redox state of cells can cause toxic effects through the production of peroxides and free radicals that damage all components of the cell, including proteins, lipids, and DNA.[11] Free radicals which originate from the per oxidation of lipids presumably from plasma membrane are short-lived and cannot be measured directly, but their activity can be measured by estimating the by products and substances involved in defense against the oxidant injury. Markers of oxidant stress can be malondialdehyde (MDA),[12] a byproduct of lipid peroxidation; protein carbonyl; nitrite; and trace metals such as copper . Defenses against free-radical injury can be substances such as ascorbic acid, ceruloplasmin, glutathione peroxidase,superoxide dismutase and zinc. The role of free radicals in the pathogenesis of enteric fever has been mainly shown in experimental and animal studies ,[10,13] but studies in humans, especially in children, are lacking. Morever, so many parameters have never been studied together. Therefore, our study was undertaken with the objective of assessing the plasma oxidant and antioxidant status of children with enteric fever by estimating the levels of MDA, protein carbonyl, ascorbic acid, ceruloplasmin, superoxide dismutase and b-carotene in cases confirmed to be infected with Salmonella typhi with a view to assess the status of oxidative stress during the progress of typhoid fever.

Materials and Methods

This was a prospective, hospital-based study that included 60 patients of confirmed enteric fever in the age group of 0–15 years admitted to the pediatric ward of our hospital between Sep 2012 to Sep 2014. This study protocol was approved by the Institute Ethics Committee. The inclusion criteria for our study were children of 0 to 15 years age group having clinical features suggestive of enteric fever with one of the positive diagnostic tests (positive blood culture and/or positive widal and/or positive typhidot IgM assay) depending upon the time of presentation. The clinical history, examination and relevant investigations aforementioned were recorded. Another 60 age- and gender-matched healthy children were included in our study to serve as controls. They had normal nutritional status and no abnormality on general physical and systemic examinations. Informed consent regarding inclusion in the study was given by the parents in each of the included cases.

Blood sample collection and estimation:

Taking all aseptic precautions, about 10 ml of blood was drawn by venipuncture from a peripheral vein, with a heparinized disposable syringe and transferred to sterile, heparinized, de-ionized poly-ethylene vials. Plasma was separated from the blood samples immediately by centrifugation at 2000 rpm for 5 minutes and was stored in separate de-ionized vials in a deep freezer. Plasma MDA was estimated by thiobarbituric acid test. Protein carbonyl, ascorbic acid, ceruloplasmin and Superoxide Dismutase (SOD) were analyzed by the methods of Reznick and Packer, Roe, Ravin, and Marklund and Marklund respectively. b-carotene was estimated by the method of Tietz .

Statistical analysis:

Data were analyzed using SPSS 16.0 software. Student's t test was applied to the parameters with normal (Gaussian) distribution, and Mann–Whitney U test was used to the parameters with distribution different from normal. $\chi 2$ tests were used to find the association of age group with study variables. Statistical significance was taken as 0.05 ($p < 0.05$).

Observations:

There was no significant difference found between both the groups in terms of demographic profile ($p > 0.05$). The average age of children was 8.3 ± 3.9 years in cases which was comparable ($p = 0.12$) to the control group (7.9 ± 2.8 years). There was no significant difference found in the male : female ratio between case and control group ($p=0.09$). Blood culture was positive in 20 out of 25 cases and Typhidot IgM was positive in 18 out of 25 cases presenting in the first week of illness and both the tests were positive in 13 cases. Blood culture was positive in 22 out of 35 cases and Widal was positive in 28 out of 35 cases presenting in the second week of illness and both the tests were positive in 15 cases.

The average plasma level of oxidants was significantly higher in cases as compared to control group ($p < 0.05$). The plasma MDA level in the cases (0.84 ± 0.18 μmol/l) was significantly higher ($p = 0.03$) than in control group (0.52 ± 0.12 μmol/l). The plasma level of protein carbonyl in cases (38.8 ± 10.6 nmol/mg) was significantly higher ($p = 0.02$) as compared to control group (19.7 ± 2.09 nmol/mg). The level of antioxidants was significantly lower in the cases as compared to the control group (for SOD, $p =0.028$; for b-carotene, $p=0.03$; for ceruloplasmin, $p=0.019$ and for ascorbic acid, $p=0.031$) as shown in Table 1

Table 1: Serum MDA, Protein Carbonyl , SOD, Glutathione, Ascorbic acid, b-carotene levels in cases of Enteric fever and Control

Parameters	Mean ± 1S.D.		t/U value
	Cases (n=60)	Control (n=60)	
MDA (µmol/l)	0.84 ± 0.18	0.52 ± 0.12	5.32**
Protein carbonyl (nmol/mg)	38.8 ± 10.6	19.7 ± 2.09	378.8**
SOD (µmol/ml)	0.09 ± 0.05	0.21 ± 0.05	436.0**
b- carotene ((µgm/ml)	6.92 ± 1.18	15.82 ± 3.22	8.22**
ceruloplasmin (mg/ml)	0.06 ± 0.02	0.14 ± 0.03	6.19**
Ascorbic acid (mg/l)	11.4 ± 9.8	92.3 ± 42.4	4.18**

**** p > 0.05 Non-significant ** p < 0.05 Significant***

Discussion

Oxidative stress is a situation in which there is an imbalance between the production of reactive oxygen-species (ROS) that can damage cell structures and the body's ability to detoxify these molecules or repair the resulting damage [(14)] and it has been defined as a disturbance in the equilibrium status of pro-oxidant/anti-oxidant system in intact cells. Production of reactive oxygen species is a particularly destructive aspect of oxidative stress. Such species include free radicals and peroxides. Some of the less reactive of these species (such as superoxide) can be converted by oxidoreduction reactions with transition metals or other redox cycling compounds (including quinones) into more aggressive radical species that can cause extensive cellular damage [(15)].The increased lipid peroxidation as depicted by the high concentration of MDA and protein carbonyl, 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).] In this study the results indicates that serum concentrations of MDA in the typhoid patients was significantly higher than ($p<0.05$) that of the control group.

Ascorbic acid acts as the first line of defence against oxidative stress during the destructive cycle of typhoid disease.It is the only antioxidant in serum or plasma capable of completely

inhibiting oxidative modification of the low density lipoprotein by aqueous peroxyl radical [17]. Ascorbate administration exerts a protective role against peroxidative damage of lipid [18]. The serum concentrations of ascorbate in the typhoid patients was significantly lower than ($p<0.05$) that of the control group in this study. Significantly lowered levels of ascorbic acid concentration 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.

b- carotene reacts with lipid peroxidation products to terminate chain reactions [19] and also directly scavenge singlet oxygen and dissipate the energy as heat [20]. This study reveals significant diminution ($P<0.05$) in the mean plasma b- carotene concentrations in the cases of typhoid fever patients when compared with the control group.

Superoxide dismutase: The mean plasma concentrations of SOD and ceruloplasmin were significantly lower ($p<0.001$ each) in the cases as compared to controls. This supports the role they play as an antioxidant in cases of enteric fever, where levels decrease in an attempt to counteract the oxidant stress.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 .

Conclusion:

The plasma MDA, protein carbonyl were significantly raised and the plasma ascorbate, ceruloplasmin, b-carotene and SOD level were significantly decreased in cases with enteric fever as compared to controls ($p <0.05$) reflecting the increased oxidative stress with the simultaneous utilization of the anti-oxidants to counteract the oxidative stress. Therefore, it appears that these biochemical alterations are indicative of oxidative damage during enteric fever. However, further studies are needed to determine the cause-and-effect relationship and its prognostic value in patients with enteric fever.

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