Antioxidant enzymes and antioxidants in children with Pneumonia

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Abstract: In children pneumonia is a leading cause of death which is characterized by inflammation of the parenchyma of the lung tissue due to infection. Additional risk factors being continuous exposure of lung epithelium to environmental pollutants, microorganisms, resulting into inflammation and increased oxidative stress. Lung tissue are protected from environmental oxidants by the endogenous antioxidants. A disruption in the fine balance between the antioxidants and oxidants leads to oxidative stress. The present study was conducted to study the oxidative stress in pneumonia by measuring the levels of lipid peroxidation in terms of malondialdehyde, enzymatic and non-enzymatic antioxidants as compared to control group. The study observed significantly lowered levels of antioxidant enzymes glutathione peroxidase, glutathione reductase and paraoxonase 1 along with lowered levels of non enzymatic antioxidants Vitamin C, vitamin E, and β carotene (p < 0.001) and an increased malondialdehyde levels among patients diagnosed with pneumonia when compared with the control group(p <0.001). Our observations are suggestive of an increased oxidative stress in cases of pneumonia in the pediatric age group.

Key words: Antioxidants, Nrf2, Oxidative stress, Pneumonia, Reactive oxygen species.

I. Introduction

Pneumonia is an acute respiratory disease affecting all age group persons characterized by inflammation of the parenchyma of the lung tissue due to infection by viruses, bacteria or fungi. Among the respiratory diseases, pneumonia ranks first for the mortality and morbidity and kills an estimated 1.2 million children under the age of five years every year (WHO data) [1]. Various risk factors such as environmental pollutants/irritants, compromised immune system, malnutrition play an important role in disease development.

Microorganisms after infection produce edema in lung tissue proliferate there and invade adjacent tissue. This blocks the ciliary action of epithelial tissue resulting into increased cellular destruction and inflammation [2].

The childhood pulmonary susceptibility may be related to immature lung tissue. Lung development takes place in first 6 – 8 years of post natal period. During this period the lung undergoes alveolarization and continued morphogenesis with cell differentiation such as respiratory epithelial and critical immune effector cell populations, specific cell-cell interaction. These developmental events are disrupted if the lungs are exposed to vast array of materials of undefined toxicity, and results into drastic long-term consequences. Early evaluation and management in the predisposed infants is necessary to avoid the irreversible loss of lung function and high morbidity [3]. Lungs are exposed to many unwanted events such as infection, exposure to toxicants, irritants which triggers the synthesis of cytokines and interleukins by initiating the inflammatory process in lung tissue; a primary defense process against the pathogens. The inflammatory cells activates polymorphonuclear leucocytes, macrophages, monocytes, platelets and mast cells. All these inflammatory reactions are responsible for generation of free radicals and reactive oxygen species (ROS) such as hydroxyl, superoxide and peroxynitrite radicals [4]. Oxidative stress results from an imbalance between formation and neutralization of ROS which in excess, damage cell membranes and lipoproteins by a process called lipid peroxidation. Malondialdehyde (MDA) is a major aldehyde product of lipid peroxidation, believed to be responsible for cytopathological effects observed during lipid peroxidation induced oxidative stress [5]. Al-Abdulla et al have found reduced ascorbic acid and alpha-tocopherol concentrations and increased MDA levels in the respiratory lining fluid of adults with asthma [6]. An imbalance between antioxidants and oxidants in the epithelial lining fluid of the lung is thought to contribute to oxidative stress in respiratory disease.

Lipid peroxidation occurs by a radical chain reaction, i.e. once started it spreads rapidly and affects a great number of lipid molecules generating excess free radicals and lipid hydroperoxides that can seriously alter the cell membranes, membrane fluidity and permeability. These hydroperoxides and ROS attack other cell structures such as proteins, lipids, lipoproteins, and deoxyribonucleic acid (DNA) [7]. Oxidative damage to DNA leads to the formation of oxidative DNA lesions which can cause mutations and cytotoxicity. The body has several mechanisms to counteract these attacks by using endogenous antioxidant system consisting of antioxidant enzymes such as glutathione reductase, glutathione peroxidase, paraoxonase, super oxide dismutase, catalase, and non enzymatic antioxidant molecules like ascorbic acid, alpha tocopherol, beta...
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carotene, glutathione, uric acid. During infection and inflammatory processes, glutathione (GSH) is mobilized from the liver to the pathological site. Glutathione reductase catalyzes and regulates cellular levels of GSH and replenishes the reduced Glutathione (GSH). The biochemical function of glutathione peroxidase is to reduce lipid hydroperoxides and free hydrogen peroxide to their corresponding alcohols and water [8].

Paraoxonase (PON1) is a 43- to 45-kDa glycoprotein. It is a calcium dependent esterase, present in liver and Clara cells of lung epithelium. PON1 hydrolyzes a broad spectrum of substrates including organophosphates, arylesters and lactones. It functions as an antioxidant enzyme in serum and prevents the oxidation of LDL cholesterol. Its serum concentration is influenced by inflammatory changes and levels of oxidized-LDL [9][10][11]. Increased tolerance to chronic oxidant stress may be associated with increased secretory capacity of conducting airways, arising from both increased Clara cell numbers and altered Clara cell secretion.

Non enzymatic antioxidant defenses includes compounds such as, Vitamin E (alpha tocopherol), Vitamin C (ascorbic acid), beta carotene which react rapidly with the ROS, inactivate them and control the damage by breaking the lipid peroxidation chain reaction or acts themselves as scavengers which are many times less toxic than the original free radical. Vitamin E, Vitamin C are antioxidants while beta carotene acts as scavenger. Ascorbic acid is water soluble antioxidant which is responsible for maintaining Vitamin E in reduced state. Alpha-tocopherol is located in the membrane bilayer of the cells and its distribution depends on the presence of extracellular lipids such as surfactant or lipoproteins. Indeed, alpha -tocopherol is secreted by alveolar type II cells together with surfactant lipids [12]. The antioxidants analyzed in the present study Vitamin E, Vitamin C, beta carotene, together represent the main antioxidants in the epithelial lining fluid of the lungs and provide a first line of defense against inhaled and endogenous oxidants.

II. Material and Methods

The present cross-sectional study comprised of 139 children, 79 presented with pneumonia and 60 normal healthy age and sex matched children as control group during June 2011 to December 2012. All these cases recruited in this study were aged between 3 months to 12 years and were clinically diagnosed by pediatricians. Ethical clearance was taken from institutional ethics committee and written consent was taken from the parents of the study group children. Blood samples were collected in EDTA and plain vacutainer from the control as well as from patients either admitted in the ward or visiting pediatric OPD in Bharati hospital, Pune.

Analysis of Glutathione peroxidase (GPx) and Glutathione reductase (GR) was done on RBC hemolysate. Lipid peroxidation was done on plasma samples. All non enzymatic antioxidants and PON1 estimations were done on serum samples. All the above parameters were analyzed using Perkin Elmer UV-VISIBLE spectrophotometer.

2.1 Estimation Lipid peroxidation

Estimation of Malondialdehyde (MDA) was done by K. Satoh method. To 0.5 ml volume of plasma, 0.5 ml Trichloro acetic acid was added. To this addition of TBA mixture (thiobarbituric acid in 2 molar sodium sulphate) was done followed by heating in boiling water bath for 30 minutes. The resulting chromogen was extracted in n- butyl alcohol and the absorbance of organic phase was determined at 532 nm. The MDA values expressed in terms of nmol/ml.

2.2 Antioxidant enzymes

Estimation of Paraoxonase-1 (PON1) was done by Hagen and Brock. The enzyme PON1 hydrolyzes p-nitro phenyl acetate into p-nitro phenol. The rate of formation of p-nitro phenol was determined at 405nm, 25°C for 225s after a 100s lag time. The amount of p-nitro phenol produced is directly proportional to the concentration of PON1. The activity, expressed in IU/L, was based on the molar absorptivity (14000) of p-nitrophenol at 405 nm, at pH 7.4

Estimation of Glutathione Peroxidase (GPx)Activity was done by Paglia and Valentine method using Randox kit. GPX catalyses the oxidation of Glutathione (GSH) by Cumene hydroperoxide. In the presence of Glutathione Reductase (GR) and NADPH, the oxidized Glutathione (GSSG) immediately converted to the reduced form with a concomitant oxidation of NADPH to NADP⁺. The decrease in absorbance at 340 nm was measured and expressed in terms of Glutathione peroxidase (U/gm of Hb)

Estimation of Glutathione Reductase (GR) was done by Goldberg and Spooner method using Randox kit. GR catalyses the reduction of Glutathione (GSSG) in the presence of NADPH, which in turn is oxidized to NADP⁺. The decrease in absorbance at 340 nm is measured and expressed in terms of Glutathione reductase (U/gm of Hb)

2.3 Non-Enzymatic Antioxidants
Vitamin E (alpha-tocopherol) and beta Carotene was done by Baker and Frank and Quaife et al method. Serum proteins were precipitated by ethanol, then subjected to extraction by n-heptane. The 2, 2 - dipyridyl was then added to an aliquot of the upper layer to estimate the principal interfering substance, beta-carotene at 460 nm. By Emmeri Engel reaction the extracted tocopherol reduce ferric to ferrous ions. Reduced ferrous ions then forms a red coloured complex with 2, 2 dipyridyl measured at 520 nm.

Estimation of vitamin C (ascorbic acid) was done by Aye Kyaw method. Ascorbic acid in serum reduces phosphotungstic acid reagent in acidic medium to blue colour phosphotungstase, which has absorption maxima at 700nm

III. Result

All the values obtained were expressed as mean ± SD. Student’s’ test was applied to compare the difference in means between control and study groups. The difference was considered as highly significant if p value was <0.01.

**Table 1** Comparison of Vitamin C, Vitamin E, and beta carotene in pneumonia and control group

<table>
<thead>
<tr>
<th>Group</th>
<th>Vitamin C (mg/dl)</th>
<th>Vitamin E (mg/dl)</th>
<th>beta carotene (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.70 ± 0.52</td>
<td>2.03 ± 0.38</td>
<td>44.32 ± 3.60</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>0.88±0.1049*</td>
<td>1.446±0.3743*</td>
<td>20.018±1.8166*</td>
</tr>
</tbody>
</table>

*p- value < 0.001

**Table 2** Comparison of Lipid peroxidation in pneumonia and control group

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Pneumonia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean± Std Deviation</td>
<td>Mean± Std Deviation</td>
</tr>
<tr>
<td>LPO(MDA)</td>
<td>2.608±0.4804</td>
<td>10.922±0.8333</td>
</tr>
</tbody>
</table>

*p value < 0.001

**Figure 1**: Comparison of antioxidant enzymes PON1, GPx, GR, in pneumonia and control group

Levels of Paraoxonase1, (93.26 ± 8.85) Glutathione peroxidase(47.05 ± 8.32) and glutathione reductase (9.27 ± 2.57) were found to be significantly lower in cases diagnosed with pneumonia as compared to control group (PON1 232.54 ± 34.69, GPx 67.90 ± 12.37 and GR 12.05 ± 2.57).

IV. Discussion

In the present study as shown in **Table 1** levels of non-enzymatic antioxidants, Vitamin C, vitamin E and beta carotene are significantly low in cases diagnosed with pneumonia as compared to control group along with increase in MDA level as shown in **Table 2**. Similar results were shown by Cemek M et al[8]. Rai RR, Phadke MS [13]. These antioxidants are acting as lipid peroxidation chain breaking molecules and scavengers to reduce the oxidative stress. It might be possible that there is a depletion of these vitamins due to reduced dietary intake. It is possible to increase the ascorbate alpha tocopherol and beta carotene concentration in the epithelial lining fluid by increasing the ascorbate intake through the children’s diet. Wood et al vitamins [14]observed dietary supplementation with vitamins (ascorbic acid, alpha tocopherol, and beta-carotene) has some positive effect on symptoms and lung function in asthma patients. However, in our study no data was collected about the dietary intake of these vitamins.
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The results of this study indicates lowered activity of enzymatic antioxidants and GR/GPx system in cases diagnosed with pneumonia as compared to control group as shown in FIGURE 1. This strongly suggests increased oxidative stress and over expression of these enzymes is important for increased resistance to oxidative stress and pathophysiology of disease. Glutathione (GSH) provides protection to the lungs from oxidative injury induced by different endogenous or exogenous pulmonary toxicants. The redox system of GSH depends on non enzymatic antioxidants and antioxidants, which includes glutathione peroxidase (GPx), glutathione reductase (GR), glutathione S-transferase (GST) [15][16]. Serum PON1 expression is down regulated by oxidative stress [11]. Additionally, it has been demonstrated that serum paraoxonase-1 (PON1) deficiency is related to increased susceptibility to low density lipoprotein oxidation and development of atherosclerosis [17]. In recent years, results of some studies have revealed the presumptive role of several infectious agents in the inflammatory mechanism of atherosclerosis, so decreased activity of PON1 in this study may be suggestive of early initiation of atherosclerosis [18][19].

Petri et al. showed that Nrf2 (nuclear erythroid 2-related factor 2) is a basic region leucine-zipper transcription factor which binds to the antioxidant response element (ARE) and thereby regulates the expression of a large battery of genes involved in the cellular antioxidant and anti-inflammatory defenses as well as mitochondrial protection[20]. Beata Kosmider et al observed that Nrf2 is involved in the cellular antioxidant defense system. Nrf2 gets activated upon infection with PR8 virus, and protects host cells from the cytopathic effects of oxidative stress induced by influenza A virus in interferon-independent manner and can modify PR8 virus response [21].

Malhotra et al. observed activity of Sulforaphane, a phytochemical present in broccoli and cruciferous vegetables, which activates Nrf2-regulated increase in antioxidants in COPD patients. Alveolar macrophages and attenuates oxidative stress and inflammation [22]. In present study it is possible that, antioxidant imbalance might be the effect of inactivation or suppression of Nrf2 gene.

V. Conclusion

Infection causes imbalance in the antioxidant system and increased oxidative stress. In the present study data shows strong correlation of enhanced lipid peroxidation with decrease in antioxidant enzymes as well as low levels of non-enzymatic antioxidants in systemic circulation that is imbalance in antioxidant system with increased oxidative stress. This might be a key factor for inflammatory changes and disease progress in pneumonia patients. To counterbalance increased oxidative stress dietary supplementation of non enzymatic antioxidants and molecules with active principle directed to activate Nrf2 can also improve the pneumonia outcome in children, but further study in this aspect is necessary.

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References

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