EVALUATION OF OXIDATIVE STRESS IN RESPIRATORY DISEASES IN PEDIATRIC PATIENTS

THESIS SUBMITTED FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN

MEDICAL BIOCHEMISTRY

UNDER

THE FACULTY OF MEDICAL SCIENCES

BHARATI VIDYAPEETH DEEMED UNIVERSITY, PUNE 43.

BY

NEELA VAIDYA

UNDER THE GUIDENCE OF

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DEPARTMENT OF BIOCHEMISTRY

BHARATI VIDYAPEETH DEEMED UNIVERSITY,

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Neela Anil Vaidya

CERTIFICATE

This is to certify that the work incorporated in the thesis entitled **"Evaluation of oxidative stress in respiratory diseases in paediatric patients"** for the degree of 'Doctor of Philosophy' in the subject of Medical Biochemistry under the faculty of Medical Sciences has been carried out by **Mrs. Neela Anil Vaidya** in the Department of Biochemistry at Bharati Vidyapeeth Deemed University, Medical College, Pune during the period from July, 2007 to March 2014 under the guidance of Dr. P. M. Bulakh, Professor of Biochemistry.

Place : Pune

Date :

Principal,

BVDU Medical College,

Pune.

Declaration by the Candidate

I declare that the thesis entitled **"Evaluation of oxidative stress in respiratory diseases in paediatric patients"** submitted by me under the guidance of Dr. P. M. Bulakh for the degree of Doctor of Philosophy, is the record of work carried out by me during the period July 2007 to March 2014 and has not formed the basis for the award of any degree, diploma, associateship, fellowship, titles in this or any other University or other institution of Higher learning.

I further declare that the material obtained from other sources has been duly acknowledged in the thesis.

Date : Signature of the Candidate

Place : Pune

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ABBREVIATIONS

3OC12-HSL	P. aeruginosa quorum-sensing molecule,.
AIDS	Acquired Immunodeficiency Syndrome
ALA	Linolenic acid
AOS,	Antioxidant system
ATT	Anti tuberculosis chemotherapy
CAP	Community-acquired pneumonia
CAT	Catalase
DHA	Docosahexaenoic acid
EFA	Essential fatty acids
EPA	Eicosapentaenoic acid
GPX	Glutathione peroxidase
GRx	Glutathione reductase
H_2O_2	Hydrogen peroxide
ICD	International Classification of Diseases
LA	α-lipoic acid
MDA	Malondialdehyde
NF-κB	Nuclear factor kappa beta
NO	Nitric oxide

Nrf2	Nuclear factor –like 2
PON1	Paraoxonase 1
PUFA	Poly unsaturated Fatty acids
ROS	Reactive oxygen species
RSV	Respiratory Syncytial virus
Se	Selenium
SOD	Superoxide dismutase
TB	Tuberculosis
TBRS	Thiobarbituric acid reacting substances
WHO	World Health Organisation

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Introduction

INTRODUCTION

Oxygen toxicity was first described in laboratory animals in late 18thcentury.The first experiment regarding a free radical reaction was reported in 1894 and in early 1970s that role of free radicals in newborn broncho-pulmonary dysplasia and adult respiratory distress syndrome were appreciated by the medical community. The presence of free radicals in biological systems was not generally considered until the discovery of superoxide dismutase in 1969, although in the 1950s the basis of oxygen toxicity and x ray radiation was proposed by a common free radical mechanism and the radical theory of aging was hypothesized. Oxy-radicals are now widely accepted as being very important, not only in the aging process but also in numerous human diseases/disorders.[1]

Although oxygen is a prerequisite to life, at concentrations beyond the physiological limits, it may be hazardous to the cells. Since the lungs are directly exposed to very high amounts of oxygen, it is imperative for the organ to possess defenses against possible oxidative challenge. The lungs are therefore endowed with a battery of endogenous agents called antioxidants. The antioxidant species help the lungs ward off the deleterious consequences of a wide variety of oxidants/reactive oxygen species such as superoxide anion, hydroxyl radical, hydrogen peroxide

and reactive nitrogen species such as nitric oxide, peroxynitrite and nitrite.[2]

Reactive oxygen species (ROS) are produced by living organisms as a result of normal cellular metabolism. At low to moderate concentrations, they are essential for physiological cell processes, but at high concentrations, they produce adverse modifications to cell components, such as lipids, proteins, DNA etc.[3]

Currently, there is extensive global basic research to define more clearly the role of free radicals and oxidative stress in pathological conditions. Continuing clinical research will lead to more reliable management and preventive measures for many of them Knight et al (1998)[4]. The shift in balance between oxidant/antioxidants in favour of oxidants is termed as "oxidative stress". Oxidative stress contributes to many pathological conditions, including cancer, diabetes, acute respiratory distress syndrome, idiopathic pulmonary fibrosis, chronic obstructive pulmonary diseases, and asthma.[5]

Respiratory system

Lungs play most vital functions in our body. They provide us with over 400 liters of oxygen everyday and get rid of over 300 liters of the waste carbon dioxide. Its unique architecture allows the organ to pull in 10,000 liters of air daily, which gets distributed to around 6 million

alveoli spread across a surface area of $100m^2$ via the airways. The primary function of lungs is gaseous exchange through the alveolar vascular tissue bed but it also plays important role in maintaining acid base balance, activation deactivation of various biologically active peptides, like angiotensin I, vasoactive substances like bradykinin and prostaglandins.

The lung development takes place in the age of first 6 – 8 years of childhood. During this period the lung undergoes alveolization and continued morphogenesis with cell differentiation and development in respiratory epithelial cell, critical immune effecter cell populations, specific cell-cell interaction etc. [6]

In childhood, pulmonary susceptibility to infection is mostly related to immature lung tissue, innate immunity and age specific respiratory pathogens.

Ames et al (1993)[7] reported chronic exposure of lungs to viral, bacterial or parasite infection results in chronic inflammation, which is a main risk factor for development of several diseases and increased oxidative damage. Phagocytosis is the normal host defense in response to infection against pathogenic micro-organisms and foreign body particles bigger than 0.1 μm. Continuous exposure of lung tissue to environmental factors leads to inflammatory or infectious conditions leading to

disruption in the function of respiratory system, termed as acute respiratory failure. Neutrophils and other phagocytes attack pathogens by mixture of reactive oxygen species: singlet oxygen (O_2^-), nitric oxide (NO), hydrogen peroxide (H_2O_2), hypochlorous acid. These free radicals or reactive oxygen species gives rise to oxidation of membrane PUFA. It is represented with common clinical signs such as respiratory tachypnea, altered depth and pattern of respiration, tachycardia, restlessness, headache, irritability, and wheezing and in untreated cases results into cyanosis, seizures, cardiac arrest or coma. The commonest laboratory findings are hypoxemia, hypercapnia & acidosis. Finkelstein et al (2004)[8] observed adverse long-term consequences in lung development in untreated paediatric patients with respiratory infections. Behraman et al (2004)[9] emphasized that early evaluation and management in the predisposed infants and school going children is necessary to avoid high morbidity in the adult life.

Respiratory diseases:

Respiratory diseases can be categorized in many ways, by the organ or tissue involved, by the type and pattern of associated signs and symptoms, by the cause or etiology of the disease. All respiratory diseases are classified according to the ICD classification.

Asthma:

Asthma is one of the major noncommunicable, chronic inflammatory conditions of the lung airways resulting into episodic airflow obstruction. Wheezing, shortness of breath, chest tightness is the most common symptoms of asthma in children. 20% of all children have had at least one wheezing illness by 1 year of age, almost 33% by 3 year of age and nearly 50% by 6 year of age.[10] Physical exertion, airways irritants and infection are some triggering factors for broncho-constriction of the bronchiolar muscular bands. A cellular inflammatory infiltrate of eosinophils, mast cells, lymphocytes release pro-allergic, pro-inflammatory cytokines and chemokines thereby filling up the airways. This leads to epithelial damage and desquamation of the airways lumen, affecting lung function.

Bronchiolitis:

Bronchiolitis is a common viral infection of the lower respiratory tract in infants and children resulting in to inflammatory obstruction of the small airways. In 50% of the affected children Respiratory Syncytial virus (RSV) is responsible while in others influenza virus, adenovirus, mycoplasma etc. are causative organisms. By age of two years nearly all infants are infected with this virus. Acute bronchiolitis is seasonal. Highest activity of RSV infection is during winter and early spring.[11]

Pneumonia:

It is an inflammation of the parenchyma of the lung tissue due to infection. Among the respiratory diseases, pneumonia ranks first for the mortality and morbidity. The incidence of pneumonia in developing countries ranges between 20% - 30%. Pneumonia is the single largest cause of death in children worldwide. Every year, it kills an estimated 1.1 million children under the age of five years, which is more than AIDS, malaria and tuberculosis combined, accounting for 18% of all deaths of children under five years worldwide.[12] Pneumonia is caused mainly by Streptococcus pneumoniae, Haemophilus influenza or Mycoplasma pneumoniae, and Respiratory Syncytial Virus. After invading, an organism produce edema in lung tissue, proliferate there and invades adjacent tissue. This blocks the ciliary action of epithelial tissue, increases bronchial obstruction and constriction resulting in to increased cellular destruction and mucus debris, viral infection and inflammation [13]

Pulmonary Tuberculosis:

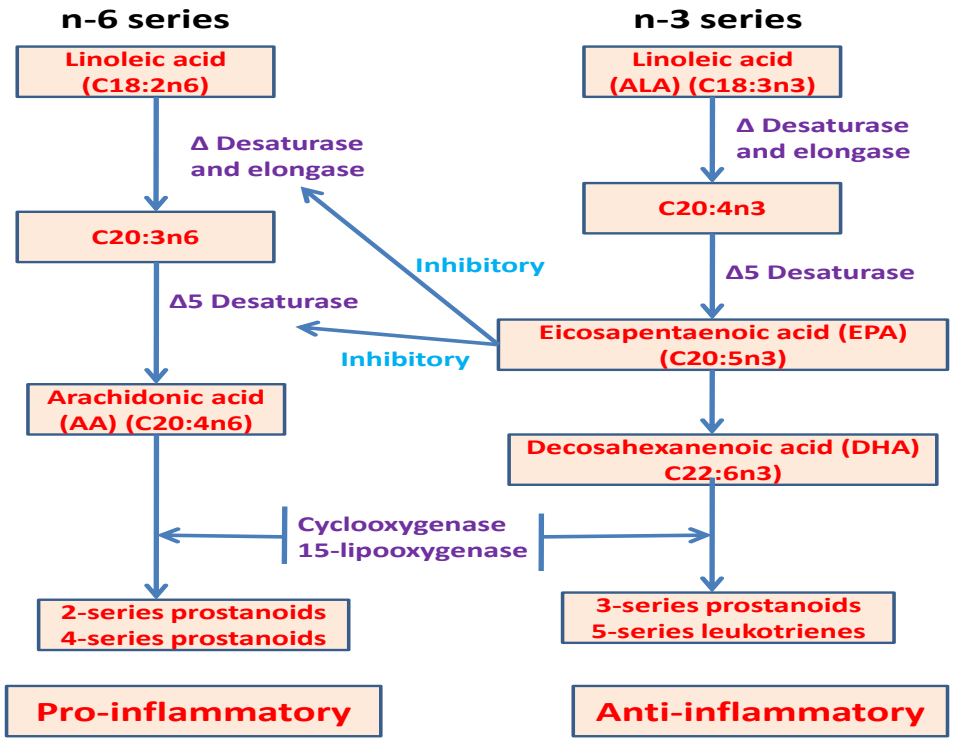
According to WHO the TB mortality rate has decreased 45% since 1990, and the 2015 global target of a 50% reduction in mortality is now within reach, still an estimated 53, 0000 children became ill with TB and 74, 000 children who were HIV-negative died of TB in 2012 and still at

least half a million children become ill with tuberculosis every year (WHO 2013).[14] Tuberculosis is one of the chronic infectious conditions, caused by Mycobacterium tuberculosis which is totally depending upon the host immunity. The tubercle bacilli gain entry into the body usually by inhalation or ingestion. The bacilli reach the bronchioles and alveoli where they are taken up by the macrophages. There they multiply or are carried to lymphatics. After 3-8 weeks macrophages rupture, releasing tubercle bacilli. Granulomatous tubercle may form, which consist of epithelial cells, langhans cells derived from macrophages lymphocytes and central caseation due to necrosis forms a primary complex. This primary complex remains dormant or actively progresses into pulmonary tuberculosis depending upon the host immune system.

The major non-enzymatic antioxidants of the lungs are glutathione, vitamins C and E, beta-carotene, uric acid and the enzymatic antioxidants are superoxide dismutases, catalase and peroxidases, paraoxonase1 etc.[15] These antioxidants are the first line of defense against the oxidants and usually act at a gross level. Therefore, when oxidative stress arises as a consequence of a pathologic event, to counterbalance it non-enzymatic and enzymatic antioxidant activities exist. A defense system promotes the regulation and expression of these enzymes.

Reactive oxygen species attack membrane PUFA from omega 3 and omega 6 series fatty acids The omega-3 fatty acid ALA can be metabolized into longer and more desaturated eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) while the omega-6 fatty acid LA can be synthesized into long-chain arachidonic acid.[16] Animal experiments and clinical intervention studies indicate that omega 3 fatty acids have anti-inflammatory properties while omega 6 fatty acids are responsible for proinflammatory leukotrienes[17, 18] as depicted in **figure 1**.

Figure 1: Role of n3 and n6 PUFA in inflammation



Therefore, comprehensive study of oxidative stress by measuring lipid peroxidation products such as malondialdehyde (MDA) and polyunsaturated fatty acids (PUFAs) along with evaluation of specific activities of antioxidant enzymes superoxide dismutase(SOD), catalase (CAT), paraoxonase 1 (PON1), glutathione peroxidase (GPx), glutathione reductase (GRx) and antioxidants vitamin C (ascorbic acid), Vitamin E and β (beta) carotene in respiratory diseases will probably help us to know their role in pathogenesis of lung diseases in paediatric patients. This basic knowledge may enlighten us to prevent disease progress and prevention of further episodes of respiratory disorders in child. In view of this, the present study was undertaken.

Review of Literature

REVIEW OF LITERATURE

Respiratory system gets continuously exposed to a variety of infectious pathogens and foreign substances. Airway epithelial cells are uniquely designed for monitoring and clearing invading microbes through a number of mechanisms including mucociliary clearance, proinflammatory cytokine and chemokine production, and release of antimicrobial peptides such as defensins, lactoferrin, and lysozyme. In the absence of impaired innate immune function or underlying co-morbid medical conditions, these mechanisms are generally effective at preventing the development of pulmonary infections. [20]

Changes in oxidant/antioxidant balance towards oxidants can lead to a variety of airway diseases, such as asthma, chronic obstructive pulmonary disease and idiopathic pulmonary fibrosis. During hypoxia, superoxide generated gets degraded into the mitochondria or cytosol by SOD. H_2O_2 produced by enzymes crosses cell membranes and reacts with Fe^{2+} or Cu^{+} to form hydroxyl radicals via Fenton reaction. [21]

Reactive oxygen species (ROS) are products of normal cellular metabolism and are known to act as second messengers. Under physiological conditions, ROS participate in maintenance of cellular ‘redox homeostasis in order to protect cells against oxidative stress

through various redox-regulatory mechanisms. Overproduction of ROS, most frequently due to excessive stimulation of reduced nicotinamide adenine dinucleotide phosphate by cytokines or the mitochondrial electron transport chain and xanthine oxidase, results in oxidative stress. Lung damage was observed to be due to oxidative stress and consequently leads to various disease states and knowledge of the mechanisms of ROS regulation could lead to the pharmacological manipulation of antioxidants in lung inflammation and injury.

Bowler et al (2002)[22] stated that oxidative stress in lung epithelium occurs due to exposure to air pollution and cigarette smoke, which results into inflammation. The specific localization of antioxidant enzymes in the lung and the rapid reaction of nitric oxide with reactive oxygen species, such as superoxide, suggested that antioxidant enzymes might also function as cell-signaling agents or regulators of cell signaling. The study suggested that therapeutic interventions that decrease exposure to environmental reactive oxygen species or augmentation with endogenous antioxidants might be beneficial as adjunctive therapies for allergic respiratory disorders.

Marcal et al (2004)[23] studied superoxide release and cellular glutathione peroxidase (GPx) activity in peripheral blood granulocytes

and monocytes in children and adolescents with intermittent or persistent asthma. Study with persistent asthma showed a higher respiratory burst activity compared to healthy individuals. These findings indicated a risk of oxidative stress, phagocyte auto-oxidation, and the subsequent release of intracellular toxic oxidants and enzymes, responsible for additional inflammation and lung damage in asthmatic children.

Rahman et al (2006)[24] revealed the presence of certain specialized proteins such as peroxiredoxins, thioredoxins, glutaredoxins, hemeoxygenases and reductases, which are involved in cellular adaptation and protection against an oxidative assault. These molecules were shown to exert their action at the level of cellular signaling processes.

Protection against the damaging effects of free radicals is carried out by nonenzymatic and enzymatic antioxidant systems important being glutathione system. In this system, glutathione peroxidase provides detoxification of organic and inorganic peroxides by using reduced glutathione (GSH) with regeneration of oxidized glutathione (GSSG) by glutathione reductase which uses NADPH as reduced equivalents.

PON1 EC.3.1.8.1, an antioxidant enzyme, is able to hydrolyze paraoxon, which is a potent inhibitor of cholinesterase.PON1 is localized

in Clara cells, endothelial cells and type 1 cells of the alveolar epithelium. Clara cell is one of the oxidant resistant airway cells in lungs of all species. PON1 and secretions of Clara cells have a role in protection from oxidative stress in the lungs. However, smoking, as well as chemicals causing vital damage to the airspace epithelium, cause reduction in levels of PON1. 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[25,26]

Birgul et al (2007)[27] showed that PON1 levels were lowered in patients with COPD compared to healthy smoker subject sand healthy nonsmokers. They have found a negative correlation between plasma MDA and PON1 levels in smokers and also in COPD patients.

Mango et al (2003)[28] showed a protective role for Clara cells and their secretions in mice, effective in their susceptibility to oxidant stress. Clara cells continuously get replaced by mucosal cells in smokers and a reduction in Clara cells in COPD patients and in smokers were observed.

Draganov et al **(**2004)[29] described three members of the PON family PON1, PON2,and PON3.The three genes encoding the enzymes

are located next to each other on the long arm of human chromosome.

Human PON1 and PON3 are primarily produced in the liver and found circulating in serum bound to HDL particles Ng et al (2001).[30] The majority of research related to PONs has been directed at the proposed protective role of PON1 in cardiovascular disease.[31]

PON2 is more ubiquitously expressed and is found in many tissues including placenta, brain, lung, and kidney. Stoltz et al (2006)[32] showed that murine tracheal epithelial cells are capable of degrading the P. aeruginosa quorum-sensing molecule, 3OC12-HSL. In addition, these cells express all three PON enzymes. This study emphasized the importance of PON1, PON2, and PON3 in airway epithelial cell 3OC12-HSL degradation.

Pneumonia was regarded by William Osler in the 19th century as "the captain of the men of death". The advent of antibiotic therapy and vaccines in the 20th century has seen improvements in survival but still morbidity and mortality in pneumonia is very high. [12]

Pneumonia is an inflammatory condition of the lung—affecting primarily the microscopic air sacs known as alveoli. Pneumonia can cause respiratory failure by triggering acute respiratory distress syndrome

(ARDS), which results from a combination of infection and inflammatory response.

Novozhenov et al (1989)[33] demonstrated changes in lipid per-oxidation (LPO), antioxidant system (AOS), immune system, and in the pituitary-adrenocortical system (PAS), in the patients with acute pneumonia. The most pronounced changes were seen in the patients with acute pneumonias eventuating in pneumo fibrosis. The high level of LPO was combined with AOS depletion, immunodeficiency formation, and with dysfunction of the PAS. Antibacterial treatment did not exert any appreciable effect on the characteristics under study. Thus, the level of LPO and AOS status were observed to be important determining components in the pathogenesis of acute pneumonias.

Katsoulis et al (2005)[34] assessed serum total antioxidant status (TAS) in patients with community-acquired pneumonia (CAP) and the probable correlation with the severity of the disease. Clinical, laboratory and radiological findings were recorded on the day of admission and after seven day. They have found good positive correlation in between TAS and CAP along with severity of the disease. They have also noted that the knowledge regarding levels of total antioxidants may be useful in administration of antioxidant therapy and management of disease.

During lower respiratory tract infections, massive influx and activation of phagocytes takes place. Reactive oxygen species (ROS) are released by macrophages and polymorpho nuclear neutrophils kill microorganisms and cause damage to host tissues by inducing enhanced lipid peroxidation.

Nowak et al (1996)[35] estimated the lipid peroxidation products malondialdehyde in combination with clinical and biochemical indicators of inflammation in patients with pneumonia. Serum concentration of lipid peroxides (CLP) and malondialdehyde (MDA) was measured at different intervals of Day 1 and 14 during a 2 week treatment of lower airway infection. Decrease in severity of symptoms with decrease in plasma MDA was observed. Study concluded that an enhanced process of lipid peroxidation during pneumonia and serum levels of lipid hydroperoxides returns to normal values quicker than the concentration of malondialdehyde during recovery.

Oxidant antioxidant balance is essential for the normal lung function. Both an increased oxidants and decreased antioxidants may reverse the physiologic oxidant balance in favor of oxidant leading to lung injury.

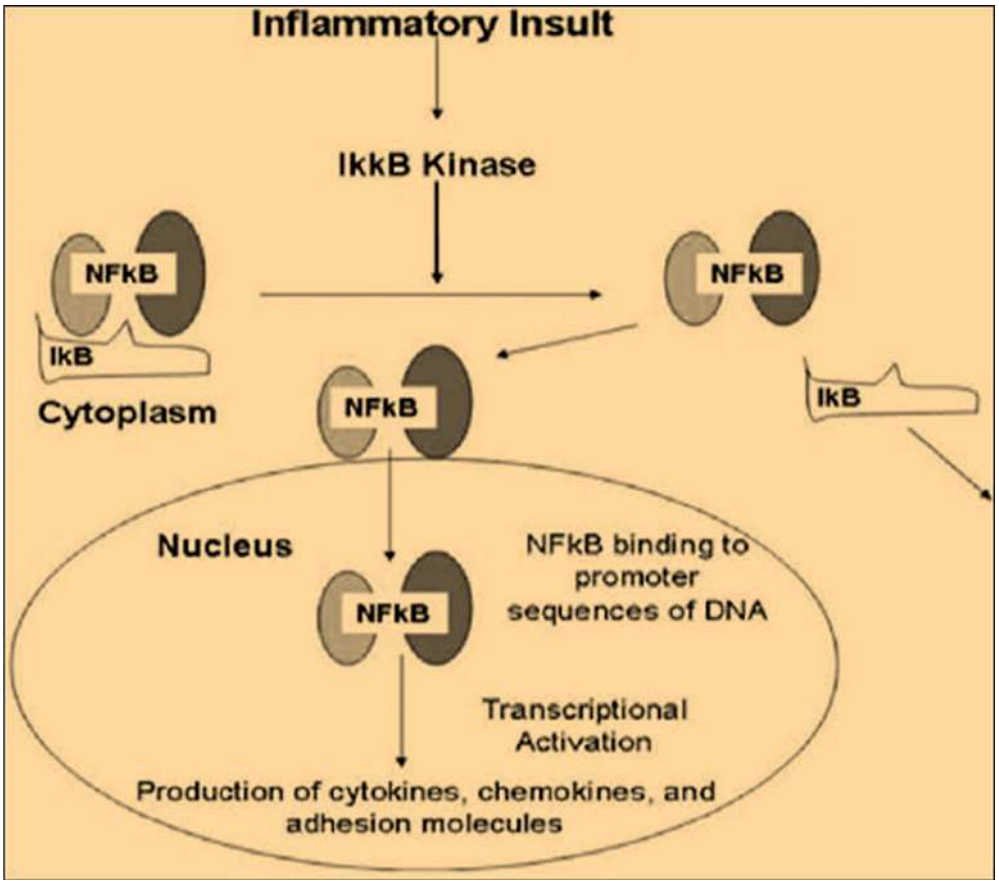
Bhoite et al (2011)[36] examined the levels of vitamin E & C in children with pneumonia. They observed that highly significant decrease in the concentrations of vitamin E & vitamin C in children with pneumonia compared to controls.

Harini et al (2005)[37] reviewed possible role of PUFAs in various diseases with increased oxidative stress. PUFA being an important macronutrient affect gene expression through various mechanisms including changes in membrane composition, intracellular calcium levels, and eicosanoid production.

PUFAs and their various metabolites can act at the level of the nucleus, in conjunction with nuclear receptors and transcription factors, to affect the transcription of a variety of genes. Several of these transcription mediators have been identified as nuclear receptors peroxisome proliferators-activated receptor (PPAR), hepatocyte nuclear factor (HNF)-4α, liver X receptor (LXR), the transcription factors sterol-regulatory element binding protein (SREBP) and nuclear factor-κB (NFκB).

Their interaction with PUFAs has been shown to be critical to the regulation of several key genes of lipid metabolism. Thereby play an important role in disease management and prevention.

Figure 2: Role of NfkB pathway in lungs



Iskusnykh et al (2013)[38] observed the expression of GRx and GPx in their in vitro studies on rat liver. They observed that microsomal membrane lipid peroxidation leads to calcium release and uncontrolled activation of calcium-dependent proteases and lipases whereas attack on mitochondrial membranes alters permeability and induce a disruption of cellular energetic.

Similar observations were noted by Rahman et al (2000)[39].In their study, Trolox equivalent antioxidant capacity (TEAC) was measured as an index of overall systemic oxidative stress, and protein thiol levels in

patients with stable COPD, and in healthy smokers. The data confirmed decreased antioxidant capacity in smokers and patients with COPD, indicating the presence of systemic oxidative stress. Study proved that an oxidant/antioxidant imbalance occurs in the distal air spaces of smokers and in patients with COPD which is reflected systemically in the plasma. Supplementation of various antioxidants during diseased condition was also done by various scientists in their studies.

Seyedrezazadeh et al (2008)[40] conducted double-blind, placebo-controlled trial on newly diagnosed TB patients. Assessment of micronutrient levels, oxidative markers and total antioxidant capacity were carried out at baseline and 2 months after the intervention. MDA levels were significantly reduced in the intervention group while minimal reduction in the control group was observed which supports increased production of reactive oxygen species secondary to phagocyte respiratory burst occurs in pulmonary tuberculosis .The mean plasma level of total antioxidants was increased significantly with a two month intervention with vitamin E and selenium supplementation in both the intervention and the control groups.

Nadeem et al (2008)[41] noted that airway inflammation was responsible for repeated episodes of airway obstructions in asthmatics.

Reactive oxygen species (ROS) play a key role in initiation as well as amplification of inflammation in asthmatic airways. Excessive ROS production in asthma leads to alteration in enzymatic as well as nonenzymatic antioxidants such as glutathione, vitamins C and E, beta-carotene, uric acid, thioredoxin, superoxide dismutases, catalase, and glutathione peroxidases leading to oxidant-antioxidant imbalance in airways. Oxidant-antioxidant imbalance leads to path physiological effects associated with asthma the supplementation of antioxidants to boost the endogenous antioxidants or scavenge excessive ROS production could be utilized to dampen/prevent the inflammatory response in asthma by restoring oxidant-antioxidant balance.

Dietary interventions may reduce oxidant stress and prevent or minimize asthmatic symptoms. Such interventions may provide a cost-effective approach to asthma management that may supplement current pharmacological strategies. Riccioni et al (2007)[42] studied the antioxidant vitamins modulation in the development of asthma by counteracting oxidants and reducing external attacks (bacteria, virus, toxins, xenobiotics) in the lung. The influence of nutrition on chronic bronchial asthma has an important place in the management of this disease. Evidence suggested that specific inflammatory abnormalities exist in the

airways of subjects suffering from mild-to-moderate persistent asthma, in association with increased generation of reactive oxygen species and the damaging effects of free radicals. For this reason oxidant stress may be an important pathogenic factor in the progress of the disease. The role of nutrition in bronchial asthma is related to antioxidant vitamins A, C, and E. Dietary studies suggest relations between oxidative stress, bronchial inflammation, development of asthmatic symptoms, and reduction of cellular functions.

This conclusion was not supported by many researchers regarding the relations between antioxidant vitamins and the treatment of bronchial asthma.

Cakmak et al (2009)[43] studied changes in oxidative status by determining serum paraoxonase activity and total oxidative status, total antioxidant capacity and lipid hydroperoxidation in asthmatic children and healthy children. In asthmatic children, when total oxidant status, total antioxidant capacity and lipid hydro-peroxidation levels increased, paraoxonase activity was decreased.

Al-Abdulla et al (2010)[44] conducted study on oxidant- antioxidant imbalance in asthmatic children, by measuring the levels of malondialdehyde (MDA) as an oxidant marker of lipid peroxidation as

well as antioxidant compounds like vitamin C, vitamin E and their association with severity of asthma. It was observed that, there was inverse correlation of MDA levels to that of vitamin C and vitamin E. In similar studies by Birben et al (2012)[45] significantly lowered serum levels of vitamin C, vitamin E and significantly high malondialdehyde as compared with the controls during asthma exacerbation in children was observed. Significantly elevated MDA levels and significantly decreased antioxidants levels correlated with increased severity of asthmatic attack and a high degree of reactive oxygen species and oxidative stress formation.

Singh et al **(**2010)[46] studied role of free radicals, oxidative stress and ascorbic acid levels in pulmonary tuberculosis cases. Plasma levels of lipid peroxidation (MDA), vitamin c were estimated. Oxidative stress was found to be increased in advanced pulmonary tuberculosis and was observed to be the indicative of damage caused to the lung tissue which was shown by the significantly decreased levels of the antioxidants (vitamin c) with an increase in lipid peroxidation levels. Similar results were noted by Wiid et al (2004)[47] in tuberculosis patients.

Ramesh et al (2011)[48] used protein carbonyl content and advanced oxidation protein product (AOPP) as a marker of oxidative modification

of proteins, reduced glutathione (GSH), and total thiol as an antioxidants in evaluating oxidative stress in inflammatory diseases and in TB patients. Lower concentration of antioxidants observed was due to their increased utilization by reactive oxygen species (ROS) and as an important contributing factor to the increased oxidative stress. Reduced glutathione levels indicated the potential of oxidative damage to erythrocyte and erythrocyte membrane of pulmonary TB patients.

GPx/GSH/GRx system was found to be more important in the protection of phagocytic leukocytes from H_2O_2 .Sudha et al (2002)[49] have found the relation of oxidative stress and GPx/GSH/GRx system antioxidants in tubercular meningitis. Erythrocyte glutathione level was decreased in Tubercular meningitis (TBM) patients as compared to normal whereas Erythrocyte GRx activity was observed to be low in TBM patients.

Dalvi et al (2012)[50] postulated that, increased defense mechanism observed in tuberculosis might be due to increased oxidative stress .SOD and CAT scavenge free oxygen radicals, interrupt inflammatory cascades and thereby limit further disease progression. In their study MDA levels were significantly increased and the activity of SOD, CAT was found to be significantly decreased in subjects of all categories of pulmonary and

extra pulmonary tuberculosis. The changes were reversed after six month anti-tubercular treatment in patients with good recovery but increased oxidative stress was not completely reversed.

Madebo et al (2003)[51] studied antioxidant profile and its relation to lipid peroxidation in tuberculosis patients with or without accompanying HIV infection and their relations with markers of oxidative stress in a large population of Ethiopians. Concentrations of the antioxidant vitamins C and E and of vitamin A were significantly lowered in tuberculosis patients than in healthy Ethiopians. Ethiopian control subjects had lower concentrations of vitamin E and higher concentrations of malondialdehyde than did Norwegian control subjects.

Vijayamalini and Manoharan (2004)[52] studied the relationship between lipid peroxidation and vitamin C, vitamin E and reduced glutathione levels in plasma, erythrocytes and erythrocyte membranes of pulmonary tuberculosis patients and an equal number of age-and sex-matched healthy subjects. Enhanced plasma, erythrocytes and erythrocyte membrane lipid peroxidation with concomitant decline in vitamin C, vitamin E and reduced glutathione levels were found in pulmonary tuberculosis patients. The elevated lipid peroxidation and decreased vitamin C, vitamin E and reduced glutathione levels indicated the

potential of oxidative damage to erythrocytes and erythrocyte membranes of pulmonary tuberculosis patients.

Lamsal et al (2007) [53] evaluated the level of MDA and nitrite and the levels of antioxidants vitamin C and vitamin E in pulmonary tuberculosis and followed with standard anti tuberculosis chemotherapy (ATT) for two months. Elevated MDA and nitrite levels with concomitant depressed vitamin C and E levels were indicative of increased lipid peroxidation and oxidative stress. The decrease in levels of MDA and nitrite with subsequent increase in vitamin C levels after two months of follow-up indicated a good response to treatment with standard ATT no significant change in mean plasma vitamin E level before and after 2 months on ATT was found.

Some researchers reported not only the blood levels but changes in the lung tissue and pleural fluid were also observed by various scientists. Reddy et al (2009)[54] analyzed pleural fluid from the tuberculosis patients both untreated and under treatment with three months anti-tuberculosis therapy. The amount of malondialdehyde, lactate dehydrogenase, and total protein content in pleural fluid of untreated tuberculosis patients were found to be significantly higher when compared with under treatment group. The pleural fluid total antioxidant levels were

significantly lower in untreated cases in comparison to under treatment patients. Decrease in the total antioxidant status was more pronounced in untreated cases, proved that antioxidants were nearly completely utilized to scavenge the free radicals. Importance of supplementation of antioxidants in the treatment of tuberculosis patients was also suggested.

Jack et al **(**1994)[55] toxic free radicals have been implicated in the development of lung fibrosis which may be a long-term sequela of pulmonary tuberculosis. Active pulmonary TB patients shown elevated levels of circulating free radical activity, and these levels correlated with disease activity as determined by other blood markers of inflammation. All serum markers of free radical activity were elevated in patients with active pulmonary tuberculosis. During serial measurements in 8 patients the % molar ratio of 9,11 linoleic acid /9,12 linoleic acid fell progressively with treatment. Thiobarbituric acid reactive substances (TBARS) were initially elevated in patients and remained elevated despite treatment. In 2 patients TBARS were in the normal range at presentation but subsequently increased with treatment. These results suggested that increased circulating levels of free radical activity found in active pulmonary tuberculosis may play a role in the resultant fibrosis and

measuring these indicators may be useful in different stages of the disease process.

Hashmi et al (2012)[56] reported severe oxidative stress in pulmonary tuberculosis(TB) patients because of increased production of reactive oxygen species (ROS) secondary to phagocyte respiratory burst, malnutrition and poor immunity. Superoxide Dismutase (SOD) activity in terms of percent inhibition was low and MDA, as lipid peroxidation products were significantly high in above patients.

Exposure of the lung epithelium to reactive oxygen species without adequate antioxidant defenses leads to airway inflammation, and may contribute to lung injury. Glutathione peroxidase catalyzes the reduction of peroxides by oxidation of glutathione (GSH) to glutathione disulfide (GSSG), which can in turn be reduced by glutathione reductase. Heyob et al (2008)[57] in their in vitro studies observed increased levels of GSSG which correlated negatively with outcome after oxidant exposure, and increased GRx activity has been protective against hyperoxia in lung epithelial cells.

Ascorbate can regenerate vitamin E from the tocopheroxyl radical and act synergistically with vitamin E to inhibit lipid peroxidation therefore, both nutrients appear to be important. In view of this, Romieu

et al (2002)[58] studied the role of vitamin E and vitamin C in asthmatic children who are exposed to high levels of pollutants. In these patients moderate benefit was observed in children with low intake of vitamin E and provided some protection against the acute effects of ozone on their lungs. Supplementation of vitamins remains to be determined with optimal dose of antioxidant for adequate protection from ozone-induced lung injury and in subjects already considered to have a normal diet.

Oxidative stress, enzymatic and non-enzymatic antioxidants status were investigated in children with acute pneumonia by Cemek et al (2006).[59] Whole blood MDA, total bilirubin levels were higher in the study group than those of the control group. However, SOD, GPx, beta-carotene, retinol, vitamin C, vitamin E and GSH levels were lower in the pneumonia compared with the control group with no change in CAT levels. Study demonstrated that oxidative stress was increased whereas enzymic and non-enzymic antioxidant activities were significantly decreased in children with acute pneumonia.

Comhair et al (2001)[60] considered extracellular glutathione peroxidase (eGPx) as critical first-line antioxidant defense in the airway epithelial surface against reactive oxygen and nitrogen species and studied the eGPx role in regulation of ROS-mediated lung diseases such

as asthma. They showed that eGPx increased in the asthmatic airway in comparison to healthy controls. Higher levels of eGPx mRNA in asthmatic airway epithelium verified bronchial epithelial cells as the source for the increased eGPx. The eGPx mRNA in bronchial epithelial cells in vitro increased eightfold after exposure to ROS and glutathione, an essential cofactor for eGPx activity. Alterations in intracellular and extracellular oxidized and reduced glutathione were temporally associated with eGPx induction, further supporting redox mechanisms in gene expression. The eGPx mRNA half-life was not affected by ROS, suggesting a transcriptional mechanism for eGPx regulation. Fusion genes of deletion fragments of the eGPx gene 5*flanking region driving a reporter gene conclusively identified the ROS-responsive region, which contained the consensus DNA binding site for the redox-regulated transcription factor, activator protein.

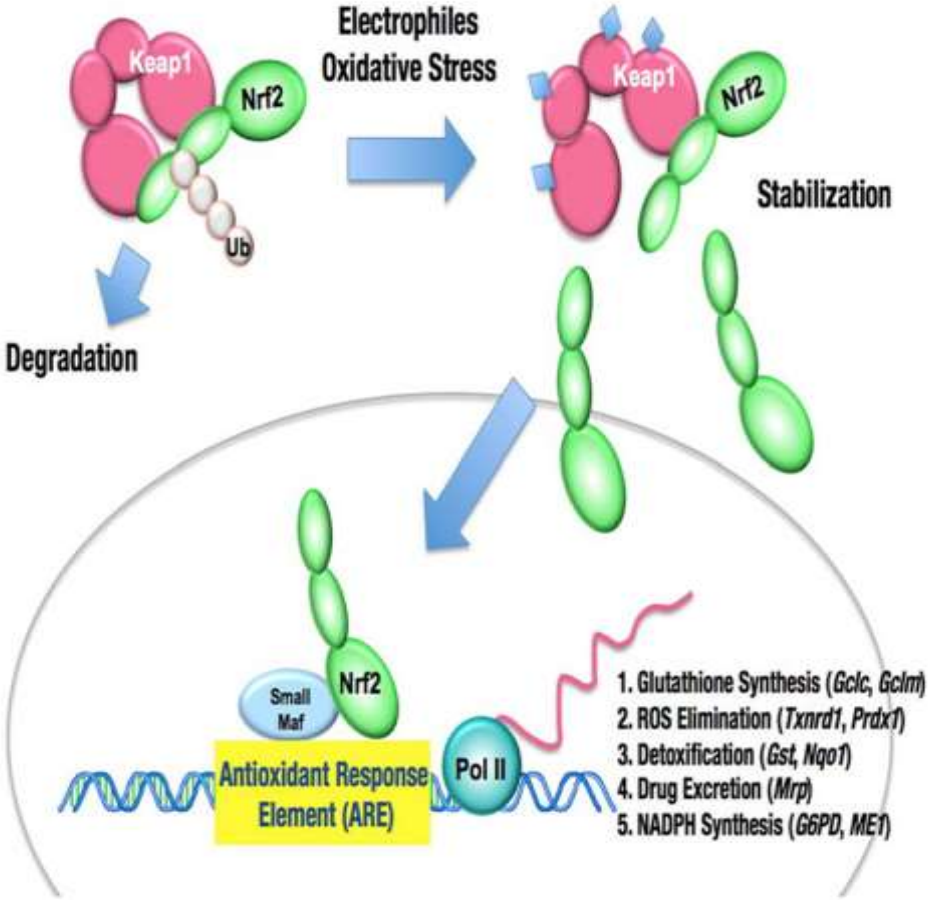
Simopoulos et al (2002)[61] observed that, among the fatty acids, omega-3 polyunsaturated fatty acids (PUFA) possess the most potent immune-modulatory activities, and among the omega-3 PUFA, those from fish oil—eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)—are more biologically potent than α-linolenic acid (ALA). Some of the effects of omega-3 PUFA are brought about by modulation of the

amount and types of eicosanoids made, another effects are elicited by eicosanoid-independent mechanisms, including actions upon intracellular signaling pathways, transcription factor activity and gene expression Patients with autoimmune diseases, such as rheumatoid arthritis, inflammatory bowel disease and asthma, usually respond to eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) supplementation by decreasing the elevated levels of cytokines.

Park et al (2012)[62] studied the effect of antioxidants on airway remodeling and signaling pathways in chronic asthma. Experiments were conducted with long-term exposure murine model of allergic airway disease to evaluate the effects of an antioxidant, L-2-oxothiazolidine-4-carboxylic acid (OTC) or α-lipoic acid (LA) on airway remodeling, focusing on the ROS-related hypoxia-inducible signaling. LA reduced these features of asthma, including airway remodeling, which was accompanied by suppression of transforming growth factor-β1, vascular endothelial growth factor, and T-helper 2 cytokines. In addition, activation of nuclear factor-κB (NF-κB), nuclear factor erythroid 2 related factor-2 (Nrf2), hypoxia-inducible factor (HIF)-1α, and HIF-2α was reduced by OTC or LA. Their results also showed that OTC or LA down-regulated phosphoinositide 3-kinase activity and decreased

phosphorylation of p38 mitogen-activated protein kinase but not extracellular signal-regulated kinase 1/2 or c-Jun **N**-terminal kinase. These findings demonstrated that OTC and LA can inhibit activation of NF-κB, Nrf2, and HIF, leading to attenuate allergen-induced airway remodeling.

Figure 3: Role of oxidative stress and Nrf2 activation/deactivation



Naderi et al (2011)[63] aimed their study to determine serum paraoxonase and arylesterase activities in tubercular, non tubercular

pulmonary diseases. The results showed that serum paraoxonase (PON) activity was significantly lowered in patients with pulmonary tuberculosis and non-tubercular pulmonary disease than healthy controls. There were no significant differences regarding PON activity between patients with pulmonary tuberculosis and non-tubercular pulmonary disease. The arylesterase activity was significantly lower in patients with pulmonary tuberculosis than non-tuberculosis pulmonary disease and normal subjects. The study concluded with lowered paraoxonase and aryesterase activities in pulmonary tuberculosis patients compared to healthy subjects due to imbalance of oxidant/antioxidant systems in pulmonary tuberculosis patients.

The paraoxonase (PON) gene family includes three members, PON1, PON2 and PON3, aligned in tandem on Chromosome 7 in humans and on chromosome 6 in mice. All PON proteins share considerable structural homology and have the capacity to protect cells from oxidative stress; therefore, they have been implicated in the pathogenesis of several inflammatory diseases, particularly atherosclerosis. Farid et al (2012)[64] hypothesized that, the modulation of each of the PONs by infective (bacterial, viral and parasitic) agents, is possible and decreases in the

interaction between infectious diseases and PONs activities in order to effectively reduce the risk of developing atherosclerosis.

Schock et al (2001)[65]analyzed antioxidant-oxidant imbalances in BAL fluid. The study showed that age, gender, and exposure to environmental tobacco smoke did not affect the concentration of ascorbate, urate, α-tocopherol, and protein carbonyls in BAL fluid. There was no significant difference between uric acid and α-tocopherol concentrations in BAL fluid and serum but serum and BAL ascorbate concentrations were significantly correlated which could be basis for supplementing the diet with vitamin C for improvement in asthma symptoms and in children with inflammatory respiratory diseases.

Kondaveeti et al (2012)[66] the oxidative stress index significantly increased in untreated TB patients and decreased in TB patients on ATT with antioxidant supplementation. Hence, oxidative stress index can be considered as a novel marker in TB patients. Also, the antioxidant supplementation in adjunct with ATT showed better improvement in outcome of TB. The development of granulomatous inflammation with caseous necrosis is an important but poorly understood manifestation of tuberculosis in humans and some animal models.

Palanisamy et al(2011)[67] measured the byproducts of oxidative stress in granulomatous lesions as well as the systemic antioxidant capacity of BCG vaccinated and non-vaccinated guinea pigs experimentally infected with Mycobacterium tuberculosis. In non-vaccinated guinea pigs, oxidative stress was evident within 2 weeks of infection as measured by a decrease in the serum total antioxidant capacity and blood glutathione levels accompanied by an increase in malondialdehyde, a byproduct of lipid peroxidation, within lesions. Despite a decrease in total and reduced blood glutathione concentrations, an increase in lesion glutathione by immunohistochemistry in response to localized oxidative stress was observed. In addition there was an increase in the expression of the host transcription factor nuclear erythroid 2 p45-related factor 2 (Nrf2), which regulated several protein and non-proteins antioxidants, including glutathione. Despite the increase in cytoplasmic expression of Nrf2, immune histochemical staining revealed a defect in Nrf2 nuclear translocation within granulomatous lesions as well as a decrease in the expression of the Nrf2-regulated antioxidant protein NQO1. Treating M. tuberculosis–infected guinea pigs with the antioxidant drug N-acetyl cysteine (NAC) partially restored blood glutathione concentrations and the serum total antioxidant capacity.

These data suggest that the therapeutic strategies that reduce oxidant-mediated tissue damage may be beneficial as an adjunct therapy in the treatment and prevention of tuberculosis in humans.

Cynamon and Isenberg (1987)[68] studied Cystic fibrosis patients. The functional in vitro malondialdehyde formation test proved to be a better measure of vitamin E status than static plasma vitamin E levels and plasma vitamin E to total plasma lipid ratios.

Colin et al (1994)[69] this study examined the antioxidant status in children with asthma compared with a control group in a cross-sectional analysis. Red blood cell GSH-Px, superoxide dismutase (SOD), and plasma concentrations of retinol, vitamin C, alpha tocopherol, and cholesterol were measured in stable controlled asthma and in control group. Children with asthma had significantly reduced red blood cell GSH-Px activity compared with controls. There were no significant differences in activity of SOD or vitamin C, retinol, or alpha tocopherol/cholesterol ratio. Study suggested reduction in GSH-Px activity could have therapeutic and etiological implications for asthma with enhanced study on the effects of disease activity and treatment on antioxidant status

Rubin et al (2004)[70] The relationship of serum vitamin E, β-carotene, vitamin C, and selenium to asthma was investigated among 7,505 youth (4–16 years old) in the Third National Health and Nutrition Examination Survey. Serum vitamin E had little or no association with asthma. In separate models, a increase in β-carotene, vitamin C, and selenium, was associated with a 10–20% reduction in asthma prevalence. The findings support an association of antioxidants with prevalent asthma, which for some antioxidants is stronger among children exposed to cigarette smoke

Hubbard et al (2004)[71] suggested that number of long term randomized controlled trials of dietary interventions are essential in patients with asthma, with those using antioxidants such as vitamin C and vitamin E, and using fish oil supplements.

Brigitte et al (1997)[72] studied seasonal variations in plasma concentrations of tocopherols, and β-carotene (cis and trans isomers), lycopene, and retinol in Swiss individuals. Seasonal variations affected tocopherols and retinol (higher in winter) and gamma-tocopherol and cholesterol concentrations higher in winter and spring than in the other seasons.

Measles is a highly contagious viral disease of childhood associated with serious complications and significant morbidity and mortality. Respiratory symptoms are always observed along with this Cemek et al (2006)[73] investigated whole blood MDA (as a marker of lipid peroxidation) and GSH, and serum ß-carotene, retinol, alpha-tocopherol and ascorbic acid levels in children with measles to evaluate the oxidative stress in measles..Non-enzymatic antioxidant status was found to be decreased but, lipid peroxidation was increased in the study group. As a conclusion, these finding suggested that oxidant and antioxidant defense system were altered in children with measles.

Rai and Phadke (2006)[74] studied status of plasma oxidants and antioxidants in different respiratory disorders. The status of oxidants in plasma as represented by malondialdehyde (MDA) levels observed as increased significantly in the conditions of chronic obstructive pulmonary disease (COPD), emphysema, bronchiectasis and bronchial asthma. The two vitamin antioxidants vitamin C and vitamin E showed decreased levels than in controls. In patients with COPD the endogenous antioxidant viz. reduced glutathione (GSH) estimated from whole blood was comparable to that of control group, whereas in patients with emphysema, bronchiectasis and bronchial asthma, GSH concentration

was increased to that of control group. The activity of enzyme superoxide dismutase (SOD) was significantly decreased in all study groups. Pulmonary function tests were found to have no correlation with MDA and antioxidants.

Mohod and Kumar (2012)[75] analyzed treated and untreated patients of pulmonary tuberculosis for oxidative stress markers MDA nitric oxide (NO) and SOD, reduced glutathione(GSH) and vitamin C. MDA and NO levels were found to be elevated in cases with pulmonary tuberculosis and in untreated tuberculosis cases compared to healthy controls. SOD, GSH and vitamin C levels were found to be decreased in tubercular patients compared to controls. Study concluded with association of oxidative stress with the disease pathology, malnutrition and poor immunity.

Similar result was noted by Parchwani et al (2011)[76] in their study on patients with pulmonary tuberculosis MDA and TAS (Total Antioxidant status)was calculated in patients with pulmonary tuberculosis before and after the anti tubercular drugs . Results of this study indicated a definite relation between the levels of lipid peroxides (measured as MDA) and severity of disease which represent an increased lipid peroxidation, as a general mechanism of tissue damage by free radicals.

Increase in MDA levels were correlated with advanced disease and with small radio graphical changes. Elevated MDA concentration decreased significantly in both the groups, after treatment whereas TAS does not show any significant difference before and after the treatment. The study concluded with direct relationship of decreased antioxidants and increased lipid peroxidation with pulmonary inflammation. Increased amounts of reactive oxygen species and reactive oxygen nitrogen intermediates are observed to be involved as a consequence of phagocyte respiratory burst. Thus, toxic free radicals were found to be implicated in the development of lung fibrosis.

Trefler et al (2014)[77] measured TBARS level as an index of oxidative injury in community acquired pneumonia (CAP) patients. SOD, CAT and redox glutathione system (GSH, GSSG, GRx, GPx) activities were measured as reflecting antioxidant capacity. Plasma TBARS levels and the glutathione redox system were higher in CAP patients with no change in oxidative stress levels in between survivors and non-survivors. They have suggested the occurrence of higher OS in sCAP patients with an increase in antioxidant activity related to the redox glutathione system.

Madebo et al (2003)[78] studied circulating antioxidants and lipid peroxidation products in untreated tuberculosis patients and with or

without immune compromised patients in Ethiopia. In a cross-sectional study, antioxidants vitamins C and E and vitamin A were significantly lower in tuberculosis patients than in healthy Ethiopians and lowered thiol concentrations as markers of oxidative stress. Study reported high MDA concentrations were associated with clinical severity and supports link between oxidative stress, tuberculosis, and HIV infection.

Association of severe oxidative stress because of malnutrition and poor immunity was studied by Reddy et al (2004).[79] Serum lipid peroxidation products, superoxide dismutase (SOD), catalase, antioxidant glutathione levels and total antioxidant status in TB patients including untreated ,under treatment and after treatment with anti-tuberculosis therapy (ATT)were estimated. The levels of lipid peroxidation products MDA was increased significantly in all patients. These levels gradually decreased with clinical improvement with treatment with anti-tubercular therapy patients.

Sequeira et al(2012)[80] estimated erythrocyte lipid peroxidation, erythrocyte antioxidants viz., glutathione, glutathione reductase, superoxide dismutase, catalase and plasma antioxidants viz., ceruloplasmin, glutathione-S-transferase, vitamin C, total antioxidant activity in patient's chronic allergic rhinitis. Erythrocyte lipid

peroxidation and superoxide dismutase were significantly higher, whereas plasma vitamin C and total antioxidant activity were significantly lower in chronic allergic rhinitis patients when compared to controls. Plasma Glutathione S transferase and erythrocyte catalase, glutathione, and glutathione reductase remained unchanged.

Shermatov et al (2012)[81] observed significantly higher TOS levels, OS index and DNA damage in peripheral blood lymphocytes in children exposed to secondhand cigarette smoke than in those not exposed to secondhand cigarette smoke.

Emin et al (2012)[82] measured plasma TAS and TOS levels in children with allergic rhinitis. The high level of TOS indicated that these patients are exposed to severe oxidative stress which may have a role in the pathogenesis of allergic rhinitis

Cantin et al (1987)[83] Observed that total glutathione (the reduced form GSH and the disulfide GSSG concentration) of normal epithelial lining fluid (ELF) was 140-fold higher than that in plasma of the same individuals, and 96% of the glutathione in ELF was in the reduced form. Compared with nonsmokers, cigarette smokers had 80% higher levels of total glutathione in ELF, 98% of which was in the reduced form. Although these lung cells have intracellular antioxidants, these defenses

were insufficient to protect the epithelial surface against oxidants present at the alveolar surface. This study showed that the ELF of the lower respiratory tract contains large amounts of the sulfhydryl- containing antioxidant glutathione suggesting that the glutathione present in the alveolar ELF of normal individuals likely contributes to the protective screen against oxidants in the extracellular milieu of the lower respiratory tract.

Glutathione (GSH)-dependent antioxidant systems protect against ROS, and regenerating GSH from GSH disulfide (GSSG) by the flavoenzyme GSH reductase (GRx) is essential for the optimal function of this system. Donough et al (1999)[84] investigated adenovirus-mediated gene transfer of leader sequence GR (LGR) to H441 cells and resistance of such cells to t-BuOOH. Adenovirus-mediated transfection of H441 cells with LGR increased total GRx activities more than 11-fold (mitochondria more than 10-fold and cytosolic more than 7-fold) and protected against t-BuOOH cytotoxicity, was observed in wild-type untransfected cells (CON) or in cells transfected with a control gene.

The study concluded that adenovirus-mediated gene transfer of LGR enhanced cellular GR activities and protected H441 cells from oxidant stresses.

Recent studies suggest pivotal roles of reactive oxygen species not only in pathogenesis under oxidative insult but also in intracellular signal transduction. Fujii et al (2011)[85] observed that, redox status affects various cellular activities as proliferation, differentiation, and cell death. Glutathione present in several millimolar concentrations in the cytoplasm has multiple roles in the regulation of cellular homeostasis. Two enzymes, glutamyl cysteinesynthetase and glutathione synthetase, constitute the de novo synthesis machinery, differentiation, and cell death.

Dittrich et al (2010)[86] observed involvement of new genes/proteins in the development of allergic airway disease in a murine asthma model. Increased expression of two antioxidant enzymes, glutathione peroxidase-2 (GPX2) and glutathione S transferase omega (GSTO) 1-1 indicated that genes encoding the antioxidants GPX2 and GSTO 1-1 are common inflammatory genes expressed upon induction of allergic airway inflammation, and independently of allergic susceptibility. GPX2 is upregulated by nuclear factor erythroid 2-related factor 2 (Nrf2), and increased levels in hyperoxia-induced lung injury in Nrf2-null mice points towards a role of GPX2 in protection against oxidative stress.

Scott et al (1989)[87] conducted experiments to prove role of superoxide dismutase (SOD) in red blood cell (RBC) oxidant defense. Normal human erythrocytes were osmotically lysed and resealed in the presence of varying concentrations of exogenous SOD. It was observed that elevated SOD activity may imbalance cellular oxidant defense resulting in enhanced oxidation due to the accelerated generation of H_2O_2, the product of O_2 dismutation.

Respiratory syncytial virus (RSV) is major cause of lower respiratory tract infection in children. No definite treatment has been shown to significantly improve the clinical outcome of patients with this infection. Recent evidence suggests that oxidative stress could play an important role in the pathogenesis of acute and chronic lung inflammatory diseases.

In view of this, Castro et al (2006)[88] studied Experiments done by the effect of antioxidant administration on RSV-induced lung inflammation, MDA and 4- hydroxynonenal in BAL fluid . Antioxidant treatments inhibited neutrophil recruitment to the lung and significantly reduced pulmonary cytokine and chemokine production after RSV infection.

Kelly et al (1999)[89] stated that, oxidative stress is implicated in the pathology of numerous diseases of the lung. These include cystic fibrosis,

chronic obstructive airway disease and asthma. The lungs, like many other tissues, has a range of antioxidant defenses which help to maintain a balanced redox status. These antioxidants are present in the intracellular, the vascular and extracellular respiratory tract lining fluid (RTLF) compartments. The reduced glutathione (GSH) content of RTLF is particularly high and recent findings reveal the important role of the RTLF GSH pool plays in defending the lung.

Cystic fibrosis (CF) is characterized by accumulation of activated neutrophils and macrophages on the respiratory epithelial surface (RES); toxic oxidants released by these cells, contribute to the marked epithelial derangements seen in CF. These deleterious consequences are magnified since reduced glutathione (GSH), an antioxidant present in high concentrations in normal respiratory epithelial lining fluid (ELF), is deficient in CF ELF. To evaluate the feasibility of increasing ELF GSH levels and enhancing RES antioxidant protection, GSH aerosol was delivered by Roum et al (1993)[90] to individuals with CF. Increase in ELF total, reduced and oxidized glutathione was observed suggesting adequate RES delivery and utilization of GSH. Particulate matter-stimulated superoxide anion (O_2.) release by ELF inflammatory cells decreased after GSH therapy. This paralleled addition of GSH to ELF inflammatory cells

suppressed O_2 release. No adverse effects were noted during treatment. Together, these observations demonstrated the feasibility of using GSH aerosol to restore RES oxidant-antioxidant balance in CF, and support the rationale for further clinical evaluation.

Lands et al (1999)[91] cystic fibrosis (CF) is characterized by a neutrophilic inflammatory response with the production of oxidants, and to oxidative stress in the lungs. Glutathione (GSH) represents the primary intracellular antioxidant, and provides an important defense in the epithelial lining fluid. Lymphocyte GSH reflects lung GSH concentrations, and the inverse correlation between lymphocyte GSH concentration Red blood cell GSH concentration may provide a biologic marker for disease severity and a rationale for antioxidant manipulation in respiratory diseases.

Mangione et al (1994)[92] selected patients with cystic fibrosis characterized by severe bronchial inflammation. Marked oxidant-antioxidant imbalance with no significant change in the GSH concentration was observed.

Rahman et al (2000)[93] described the redox control and involvement of nuclear factor-kappa B and activator protein-1 in the regulation of

cellular glutathione and gamma-glutamylcysteinesynthetase under conditions of oxidative stress and inflammation.

Glutathione (GSH) plays a major role in pulmonary antioxidant protection. As an alternative or complement to anti-inflammatory therapy, augmenting antioxidant protection could diminish the effects of inflammation.

Lothian et al (2000)[94] reported case study of obstructive lung disease responsive to corticosteroids, and low whole blood GSH levels. After 1 month of supplementation of whey-based oral supplement as GSH precursors, pulmonary function increased significantly and dramatically.

Tager et al (2000)[95] observed that significant thiol deficiency in alveolar macrophages obtained from bronchoalveolar lavage (BAL) of smokers and patients with chronic obstructive pulmonary disease (COPD) as compared to a nonsmoker/non-COPD group. This intracellular thiol deficiency significantly correlated with reduced lung function. Normal alveolar macrophages show tightly regulated thiol metabolism of immune cells but in highly compromised thiol deficient alveolar macrophages increased oxidative stress showed functional disturbances in cellular function and defense capacity of the cell.

Kelly (1999) noted similar observations of antioxidant glutathione as major and important molecule in respiratory lining fluid of patient's lung diseases.

Misso et al (1996)[97]observed mean platelet GPx activity was lowered in asthmatic than in non-asthmatic subjects and in atopic compared with non-atopic subjects. Mean whole blood GPx activity was also lowered in atopic than in non-atopic subjects. In non-asthmatic subjects, the mean whole blood GPx activity was lower in men than in women and was positively correlated with age. Mean serum Se was lower in asthmatic than in non-asthmatic subjects. The decrease in GPx and selenium was found to be responsible in increased oxygen free radical generation and asthmatic inflammation.

Oxygen metabolites play a direct or indirect role in the modulation of airway inflammation. Excessive superoxide and hydroxyl radical production are better markers for susceptibility to asthma and for monitoring therapeutic measures. Superoxide dismutase and free radical scavengers in blood were observed to be significantly lower in asthma, even during resting condition.

In support of this**,** Shanmugasundaram et al (2001)[98] conducted prospective cross sectional study in children with asthma aged 5-18 years

matched healthy controls. Superoxide and hydroxyl radical assays were used as a measure of free radical formation. SOD, CAT and LPO were assayed and was shown to be correlated well with Serum Ig E concentrations and peak expiratory flow rate (PEFR) Excessive production of superoxide and hydroxyl radicals were noted in the blood cells in asthmatics and were correlated to the severity of disease and PEFR.

Raida et al (2004)[99] conducted epidemiological study on Dietary intake, of antioxidant vitamins A, C, E, carotenoids, and severity of asthma. From the Third National Health and Nutrition Examination Survey (NHANESIII), conducted in the United States between 1988 and 1994, Bivariate analyses showed that asthma diagnosis was associated with lower levels of serum vitamin C, α-carotene, β-carotene, and β-cryptoxanthin. The authors concluded that low vitamin C and α-carotene intakes are associated with asthma risk in children.

Lang et al (2002)[100] showed acute respiratory disease syndrome (ARDS) is characterized by diffuse inflammation in the lung parenchyma. The involvement of inflammatory mediators in ARDS has been the subject of intense investigation, and oxidant-mediated tissue injury is likely to be important in the pathogenesis of ARDS. In response to

various inflammatory stimuli, lung endothelial cells, alveolar cells, and airway epithelial cells, as well as activated alveolar macrophages, produce both nitric oxide and superoxide, which may react to form peroxynitrite, which can nitrate and oxidize key amino acids in various lung proteins, such as surfactant protein A, and inhibit their functions. The nitration and oxidation of a variety of crucial proteins present in the alveolar space have been shown to be associated with diminished function in vitro and also have been identified ex vivo in proteins sampled from patients with acute lung injury (ALI)/ARDS. Various enzymes and low-molecular-weight scavengers that are present in the lung tissue and alveolar lining fluid decreased the concentration of these toxic species.

Birch et al (2010)[101] observed dietary supplementation of DHA and arachidonic acid is associated with delayed onset and reduced risk of upper respiratory infection and asthma, allergic rhinitis, allergic conjunctivitis, atopic dermatitis up to three years of age.

Oxidative stress has been implicated in the pathogenesis of pulmonary emphysema by many researchers. Possible protective role of Nuclear factor erythroid-2-related factor 2 (Nrf2) a major antioxidant transcription factor in pulmonary emphysema was studied by Boutten et

al (2010)[102] experiments with Nrf2-deficient mice Nrf2 and several Nrf2 downstream genes revealed an essential protective role of Nrf2 in the lungs against oxidative stress from environmental pollutants and toxicants. Study also suggested Nrf2 gene could be the next therapeutic target for intervention and prevention of pulmonary emphysema.

Pike et al (2012)[103] observed that variation in exposure to n-6 and n-3 fatty acids during pregnancy influence the development of risk of childhood wheeze and atopy. The study also evidenced that maternal arachidonic acid was positively associated with increased nitric acid production and inflammation while higher ratio of linoleic acid to its unsaturated metabolic products was found to be associated with reduced risk of skin sensitization

Janice et al (2011)[104] conducted clinical trial with supplementation of n-3 PUFA in their data .The data suggested that, lower omega-3 (n-3) polyunsaturated fatty acids (PUFAs) and higher omega-6 (n-6) PUFAs decrease proinflammatory cytokine production which are linked with inflammation and depression, in healthy young adults, receiving n-3 diet showed a 14% decrease in lipid polysaccharide (LPS) stimulated interleukin 6 (IL-6) production and a 20% reduction in anxiety symptoms, without significant change in depressive symptoms. Data also

showed that decreasing n-6: n-3 ratios led to lower anxiety and reductions in stimulated IL-6 and tumor necrosis factor alpha (TNF-α) production, as well as marginal differences in serum TNF-α.

Shek et al (2012)[105] examined the role of PUFAs consumption during pregnancy and early childhood and its influence on allergy and respiratory diseases. PUFAs act via several mechanisms to modulate immune function. Omega-3 PUFAs may alter the T helper (Th) cell balance by inhibiting cytokine production which in turn inhibits immunoglobulin E synthesis and Th type 2 cell differentiations. PUFAs may further modify cellular membrane, induce eicosanoid metabolism, and alter gene expression. The study also indicated the benefits of supplementation of omega-3 PUFAs to assess the long-term effects of omega-3 PUFAs in preventing other immune mediated diseases, as well as its effects on the later immune defense and health status during early growth and development.

Radhakrishnan et al (2013)[106]correlated high serum IgE levels and strong genetic component with the clinical expression of allergy and asthma. It has been observed that in asthmatic patients significantly higher IL-4and IL-13, are produced by T-helper-2(TH2) cells in response to antigen presentation. The binding of IL-4 and IL-13 to IL-4Rα triggers

stimulatory activity to multiple cell types that are involved in asthma, including B cells, mast cells, eosinophils, pulmonary epithelial cells, fibroblasts and airway smooth muscle cells.

Sharma et al (2013)[107] observed beneficial effect of dietary n-3 PUFA supplementation in acute experimental pneumonia. Supplementation of n3 PUFA resulted in to upregulation of nonspecific and specific immune defenses of the host and significant increase in the lung levels of pro-inflammatory cytokines (tumor necrosis factor alpha (TNF-α) and interleukin-1 beta (IL-1β)) with no significant change in the levels of interleukin-10 (IL-10).

Aim and Objectives

AIMS AND OBJECTIVES

The respiratory system gets continually exposed to relatively-high O2 tensions, and, they represent a unique tissue for the damaging effects of oxidant attack, in comparison with other organs.

Various extraneous sources like environmental pollutants, various infectious agents such as viruses, bacteria, and particulate matter gain entry into lung tissue and trigger various reactions through activation of macrophages, neutrophils and eosinophils. These activated macrophages induce number of inflammatory reactions and formation of ROS. ROS produced by phagocytes at sites of inflammation are a major cause of cell and tissue damage associated with various acute and chronic inflammatory lung diseases.[108] In the alveolar tissue, this increased ROS get neutralized by enzymatic and non enzymatic antioxidant system to prevent the disease progress.

In view of this, present study was conducted with following aims and objectives

AIM :

To study status of oxidative stress and role of antioxidants and antioxidant enzymes in respiratory disorders in pediatric patients.

OBJECTIVES

Following objectives are defined

- To estimate serum malonaldehyde, as an index of lipid peroxidation.
- To estimate plasma PUFA concentration as a degree of oxidative injury reflected by lipid peroxidation.

Enzymatic antioxidants:

- To estimate serum paraoxoanase 1level as one of the antioxidant enzyme in the alveolar epithelium.
- To estimate erythrocyte superoxide dismutase (SOD) as an inducible enzyme directed against the superoxide radical.
- To estimate erythrocyte catalase (CAT) as an important enzyme for detoxification of hydrogen peroxide to water and oxygen.
- To estimate erythrocyte glutathione peroxidase (GPx) as defensive enzyme against hydroperoxide radicals.
- To estimate erythrocyte glutathione reductase (GRx) as an important enzyme for glutathione recycling.

Non enzymatic antioxidants:

- To estimate plasma vitamin E (alpha-tocopherol) as lipid peroxidation chain breaking molecule.
- To estimate plasma beta-carotene as a free radical scavenging molecule in epithelial tissue.
- To estimate plasma vitamin C (ascorbic acid) as potential antioxidant in aqueous phase of blood.

Materials and Methods

MATERIAL AND METHODS

Present study was conducted in the department of Biochemistry of BVDU Medical College and hospital, Pune.

Study group: comprised of clinically established new untreated cases of Respiratory diseases of both the sex and attending pediatric OPD and IPD. Distribution of cases is shown in **Table no.1**

Age of the children was in the range of 3 months to 12 yrs of both the sex.

Table no 1: Distribution of number of cases in Respiratory diseases and controls in present study.

Group		**No of cases (407)**
Control Healthy children		60
Infective	a) Bronchiolitis	108
	b) Pneumonia	119
	c) Pulmonary Tuberculosis	42
Non infective	d) Asthma	78

Age and sex matched 60 healthy children served as Controls.

Total 60 cases were selected for follow up study, 30 each from bronchiolitis and pneumonia on their day of admission and on day of discharge from Bharati Hospital, Pune.

The subjects were chosen based on following inclusion and exclusion criteria.

Inclusion Criteria: Clinically established cases of respiratory disorders were included in this study.

Exclusion criteria:

1) Patients suffering from any other diseases other than respiratory disease e.g. liver disorders, autoimmune diseases, psychiatric disorders, and endocrinal disorders etc.
2) Patients who are already on anti tuberculosis treatment.

Sample collection:

Total 3 ml volume of blood was collected in EDTA and 1 ml in plain vacutainer aseptically. Serum and plasma was separated by centrifugation at 3200- 3500rpm for 15 min by taking necessary precautions to avoid haemolysis.

Serum: Following investigations were carried out on seum samples

1) Estimation of Malondialdehyde, by method of Kei Satoh 1978

2) Estimation of paraoxonase 1 by method of Hagen & Brock 1992

Plasma: Following investigations were carried out on plasma samples

1) Estimation of vitamin C, by method of Aye kyaw 1978.

2) Estimation of vitamin E and β carotene by method of Baker and Frank for Vitamin E 1968 and Modified Quaife method 1949 for Vitamin A

3) Estimstion of PUFA by method of Manku and Horrobin 1983

RBC hemolysate : RBC hemolysate : RBCs were washed with 0.9% saline for three times. Then cold distilled water was added to haemolyse RBCs Following investigations were carried out on plasma samples.

1) Estimation of superoxide dismutase, Marklund and Marklund method 1974
2) Estimation of catalase by the method of Hugo Aebi 1963
3) Estimation of glutathione peroxidase by Paglia and Valentine 1967 using the RANDOX kit.

4) Glutathione reductase by Goldberg and Spooner 1983 using the RANDOX kit.

1) Estimation of serum malondialdehyde (MDA)

Kei Satoh method 1978.

Principle:

Serum sample was treated with trichloroacetic acid (TCA) for protein precipitation and then heated with thiobarbituric acid (TBA) in acidic medium in a boiling water-bath for 30 minutes. The resulting chromogen was extracted in n- butyl alcohol and the absorbance was measured at the wavelength of 532 nm. The MDA values were expressed in terms of nmol/ml with malondialdehyde used as reference standard.

Reagents:

1) Trichloro acetic acid (TCA) - 20%.

20 gm of trichloro acetic acid was dissolved in 100 ml of distilled water.

2) Sulphuric acid – 0.05 M

4.9 ml of concentrated H_2S0_4 was diluted to one liter with distilled water.

3) Sodium sulphate solution (2 M)

In about 90 ml of distilled water, 28.4 gms of anhydrous sodium sulphate was dissolved by heating and stirring and diluted to 100ml with distilled water.

4) Thiobarbituric acid (TBA) reagent - 0.67 % in sodium sulphate .

0.67 gm TBA was dissolved in 2 molar sodium sulphate by heating and the volume was made up to 100ml.

5) n - butyl alcohol.

6) Standard solution of malondialdehyde (Fluka) (1mMol/L)

(1,1,3,3 - tetra methoxypropane with molecular weight -164.2.)

Malondialdehyde (MDA) 164.2ml was dissolved in distilled water and final volume was made to 1000 ml used as a standard for calibration.

Procedure:

1) To the stopper test tube 0.5 ml serum, 2.5 ml 20% TCA was added and was left to stand for 10 minutes at room temperature.
2) After centrifugation at 3,500 rpm for 10 minutes, the supernatant was decanted and the precipitate was washed once with 0.5M sulphuric acid.
3) Then 2.5 ml of 0.05M sulphuric acid and 1 ml of 0.67% TBA in 2M sodium sulfates were added to this precipitate.
4) Coupling of lipid peroxide with TBA reagent was carried out by heating in boiling water bath for 30 minutes.
5) After cooling in cold water the resulting chromogen was extracted with 4 ml of n - butyl alcohol by vortex mixer.
6) Separation of organic phase was facilitated by centrifugation at

3000 rpm and its absorbance was determined at wavelength 532 nm.

7) Similar procedure was carried out for a standard.

8) The results obtained for standards were plotted on the graph. A linear graph was obtained. From this graph, concentrations of samples were determined and expressed in terms of nmole/ml of serum.

2) Estimation of Superoxide Dismutase Activity (SOD)

Marklund and Marklund method: (1974)

The enzyme superoxide dismutase (SOD) (E.C.I. 15.1.1.) promotes the conversion of superoxide free radical anion into hydrogen peroxide.

$$0^{-}_{2} + 0^{-}_{2} + 2H^{+} \xrightarrow{[SOD]} H_20_2 + 0_2$$

In normal metabolism, SOD enzyme prevents, the formation of highly cytotoxic oxygen-derived free radicals (OH).SOD is estimated by indirect method, because its substrate i.e, superoxide ions are highly unstable.

Principle:

Pyrogallol autooxidises rapidly in aqueous alkaline solution. Superoxide dismutase (SOD) inhibits the autooxidation of pyrogallol and

this principle has been employed for the estimation of superoxide dismutase.

A product of pyrogallol autooxidation is purpurogallin which is formed from two pyrogallol molecules in which one carbon has been oxidized to carbon dioxide. The degree of inhibition of autooxidation of pyragallolat an alkaline pH by SOD was used as a measure of the enzyme activity.

Reagents:

a) 50mM Tris-HCI buffer /3.3 ml.

0.667 gm. Of Tris is dissolved in 75 ml. Distilled water. Check the pH. Adjust the pH to 8.2 with HCI.

b) 0.2 mM Pyrogallol / 3.3ml.

Everyday freshly prepared.

Procedure:

Take following quantities in a cuvette to start the reaction.

Reagents	Control	Test
Tris-HCI Buffer	3.0 ml	2.7 ml
Pyrogallol	0.3 ml	0.3 ml
Hemolysate	--	0.3 ml

Absorbance was measured continuously on semi-autoanalyzer for 4 minutes at 420nm.

Calculations:

$$\frac{A-B}{A\times 50} \times \frac{100}{0.3}$$

Where A - Absorbance of control (blank)

B - Absorbance of sample

One unit of SOD is described as the amount of enzyme required to cause 50% inhibition of pyrogallol by autoxidation per 3.0 ml. of assay mixture.

Results were expressed by converting into Units/gm of Hb.

3. Estimation of Specific activity of catalase

Hugo and Aebi Method:1963

Principle:

In the UV range H_2O_2shows a continual increase in absorption with decreasing wavelength. The decomposition of H_2O_2 noted directly by the decrease in extinction at 240 nm.

Reagents

1. Phosphate buffer (50mM, pH 7.0)

A. Dissolved 6.81 gm of potassium dihydrogen phosphate (KH_2PO_4) in glass distilled water and volume made to 1 liter.

B. Dissolve 8.90 gm of disodium hydrogen phosphate (Na_2HP0_4) in glass distilled water and volume made to 1 liter. Mix solution A and B in the proportion of 1: 1:55

2. Hydrogen Peroxide (30mM)

Dilute 0.34 ml of 30% H_2O_2 solution with phosphate buffer to 100 ml just before use.

Assay system

Wavelength 240 nm, light path 1 cm, final volume 3.0 ml.. Read against a reference cuvette containing enzyme solution but no substrate.

Pipette Successively	**Reference Cuvette**	**Test Cuvette**
Phosphate buffer	2.9 ml	1.9 ml
Haemolysate	0.1 ml	0.1ml
H_20_2 Solution		1.0 ml

The reaction was started by addition of H_2O_2.The solutions were mixed well and the decrease in extinction was read on Perkin Elmer UV Visible Spectrophotometer for 180 seconds. The readings were taken at 10 seconds interval.

A unit of Catalase activity is the amount of enzymes which liberates half the peroxide Oxygen from H_2O_2 solution of concentration (10mM) in 100 seconds. The unit is related to half life time λ. of a first order kinetic reaction

Calculation:

$$1 \text{ unit} = \frac{2.3}{0.693} \times \frac{\text{Log E1}}{\text{Log E 2}} \times \frac{1000}{6.93} \times \frac{1}{\text{C0}} \times 10$$

Co = concentration of the original enzyme sample in assay system. Results were expressed by converting in K unit / gm of Hb.

4. Estimation of Paraoxonase-1(PON-1) as arylesterase activity:

Hagen and Brock method:1992

Principle:

The enzyme paraoxonase 1 (PON1) hydrolyzes p-nitro phenyl acetate into p-nitrophenol. The rate of formation of p-nitrophenol was determined at 405nm at 25·C over 225s after a 100s lag time. The amount of p-nitrophenol produced is directly proportional to the concentration of paraoxonase 1

Reagents:

Working buffer: 25mmol/L triethanolamine-hydrochlorine buffer with 1.0 mmol/L $CaCl_2$, the pH 7.4 was adjusted by o.1N HCL. (Triethanolamine (M.W.-149.19)

1. Start reagent: 2.5mM/L p-nitrophenyl acetate. (M.W. 181.15)

Procedure:

Reagents	**Test**	**Control**
Serum (dilution 1:20)	20µl	-
Working reagent	288µl	288µl
Start reagent	72µl	72µl

The rate of formation of p-nitrophenol was determined at 405nm at 25^0c over 225s with 100 s lag time.

The activity, expressed in IU/L, was based on the molar absorption of p-nitrophenol (14000) at 405 nm, at pH 7.4

5. Estimstion of PUFA by Gas chromatography

Method : Manku and Horrobin 1983

Preparation of methyl esters of the plasma

1ml of plasma was taken in 15-ml screw cap vial and methanolic HCI containing of 0.5% BHT added. The vials were sealed and incubated

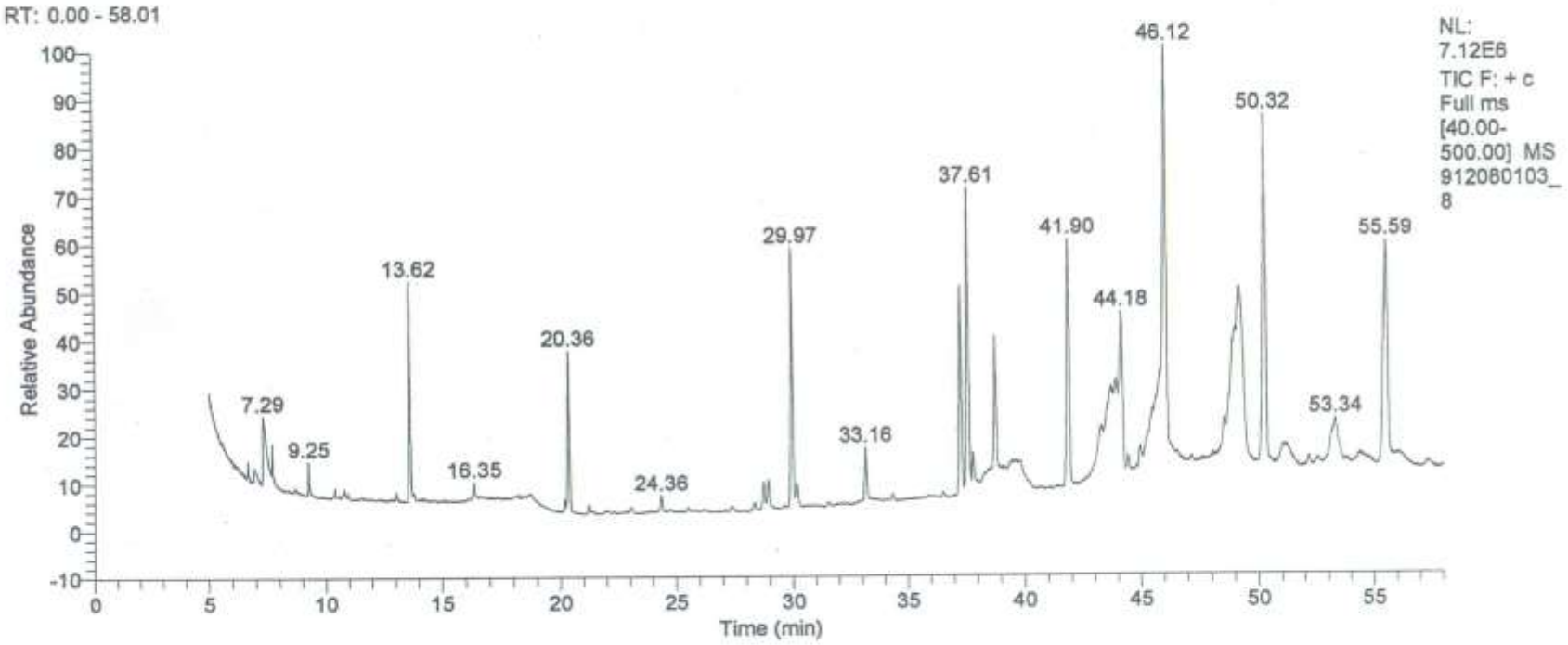
at 80° C for 2 hours. After incubation, methylated fatty acids were extracted with hexane and separated by centrifugation at room temperature. The hexane layer was carefully removed and collected in a separate vial. The hexane extract was completely dried by passing argon gas and stored at - 20° C until analyzed.

Fatty acid analysis:

The methylated fatty acids were resuspended in 50 µl of chloroform and 1 µl was analyzed by gas chromatography performed on a Shimadzu gas chromatograh, model GC 17 A Ver 3 using a capillary column of 30 m X 0.32 mm x 0.20 µm dimensions (Supelco, USA). A flame ionization detector (FID) was used with a column oven temperature at 175 ^{0}C for 15 min, programmed at 10 °C rise/min up to 220 ^{o} C and finally held at 220 ^{0}C for 10 min. The temperature of the injector was 240 °C and the detector temperature was set at 275 °C. The column was calibrated by injecting the standard fatty acid mixture in approximately equal proportion. The data was recorded and the peaks were identified as per the retention time of the standard fatty acids run under the identical conditions. The data is presented as percent of the total polyunsaturated fatty acids per 100 gms of total fatty acids. From the chromatogram the values for total PUFA% n6 PUFA and n3 PUFA levels were calculated.

Current Data Path: C:\DALPL\DATA\sept2012\110912

Sample Type: Unknown Bl.
Sample ID: 1
Acquisition Date: 09/11/12 01:13:41 AM
Injection Volume(µl): 5.00
Scans: 6234



Component Name	RT	Area	Area %
cis11_Eicosenoic_Acid_ME	46.11	192917.49	2.23
cis10_Heptadacanoic_Acid_ME	33.22	8166.93	0.09
Capric_Acid_ME	8.80	24588.50	0.28
Linoleic_Acid_ME	37.29	411201.39	4.75
Oleic_Acid_ME	37.82	127863.87	1.48
cis10_Pentadacanoic_Acid_ME	25.44	987.80	0.01
Undacanoic_Acid_ME	10.78	65549.49	0.76
cis5,8,11,14,17_Eicosapenaenoic_Acid_ME	43.91	47716.04	0.55
cis11,14,17_Eicosatrienoic_Acid_ME_	44.97	1226888.27	14.16
Stearic_Acid_ME	38.76	1954980.01	22.56
cis11,14_Eicosadienoic_Acid_ME	45.61	52077.67	0.60
Arachidic_Acid_ME	47.17	123534.62	1.43
Behenic_Acid_ME	57.38	87367.52	1.01
Linolelaidic_Acid_ME	37.29	300561.13	3.47
Palmitic_Acid_ME	29.97	3406212.74	39.31
Tridacanoic_Acid_ME	17.35	15024.50	0.17
Heptadacanoic_Acid_ME	34.34	53738.49	0.62
Pentadacanoic_Acid_ME	N/A	N/A	N/A
cis13,16_Docosadienoic_Acid_ME	55.61	228780.28	2.64
Heneicosanoic_Acid_ME	51.42	29041.52	0.34
Lauric_Acid_ME	13.80	34840.82	0.40
gama_Linolenic_acid	36.19	6537.58	0.08
Palmitoleic_Acid_ME	28.94	146199.35	1.69
myristic_Acid_ME	21.22	120552.69	1.39

6. Estimation of Glutathione Peroxidase Activity

Paglia and Valentine 1967 Kit used: Randox (Ransel)

Principle:

Glutathione Peroxidase (GPX) catalyses the oxidation of Glutathione (GSH) by Cumene Hydroperoxide. In the presence of Glutathione Reductase (GRx) and NADPH, the oxidized Glutathione (GSSG) is immediately converted to the reduced form with a concomitant oxidation of NADPH to $NADP^+$. The decrease in absorbance at 340 nm is measured.

$$2\ GSH + ROOH \xrightarrow{GPx} ROH + GSSG + H_2O$$

$$GSSG + NADPH + H^+ \xrightarrow{GRx} NADP^+ + 2GSH$$

Reagents:

1) Reagent 1a (R1a): Glutathione, Glutathione Reductase, NADPH

   Reagent 1b (R1b): Phosphate buffer, EDTA

   One vial of R1a was reconstituted with 10 ml of buffer R1b.

2) Reagent 2 (R2): Cumene Hydroperoxide

   10 µl of cumenehydroperoxide was freshly diluted with 10 ml saline by shaking vigorously. (cumene hydro peroxide is difficult to dissolve).

3) Reagent 3 (R3): Dilute1 volume of Hemoglobin reagent with 4

volumes of redistilled water. Stable for six months. Protect from sunlight

Procedure:

Dilute 0.05ml hemolysate with 1ml diluting agent (R3) incubate for 5 min and add 1ml of hemoglobin reagent

Hemolysate	10µl
Reagent R1	500 µl
Cumene R2	20 µl

Mix sample, R1 and R2. The initial absorbance of sample and reagent blank after 1 minute 2 and 3 min was recorded. Reagent blank value was subtracted from that of test.

Calculation:

$$\text{Glutathione Peroxidase (U/L Hemolysate)} = 8412 \times \triangle A \text{ 340 nm/min} \times \text{Dilution factor}$$

This was converted to U/gm of Hb

7. Estimation of Glutathione Reductase Activity

Goldberg and Spooner 1983 Kit used: Randox

Principle:

Glutathione Reductase GR catalyses the reduction of Glutathione (GSSG) in the presence of NADPH, which in turn is oxidised to $NADP^+$. The decrease in absorbance at 340 nm is measured.

$GSSG + NADPH + H^+ \xrightarrow{GRx} NADP^+ + 2GSH$ (reduced glutathione)

Reagents:

1) Reagent 1a (R1a): Phosphate buffer, EDTA

2) Reagent 1b (R1b): Substrate (GSSG)

One vial of substrate R1b was reconstituted with 5 ml of buffer R1a.

3) Reagent 2 (R2): NADPH

One vial of NADPH R2 was reconstituted with 3 ml of redistilled water.

Procedure:

Hemolysate	20µl	
Substrate R1	500 µl	Mix well
NADPH R2	200 µl	

Mix. The initial absorbance of sample and reagent blank was recorded for 5 minutes. Reagent blank value was subtracted from that of test.

Calculation:

Glutathione Reductase (U/L Hemolysate) = △A 340 nm/min X 4983 x Dilution factor. This was converted to U/gm of Hb

8. Estimation of vitamin C(ascorbic acid)

Method – Aye kyaw 1978

Principle – Ascorbic acid reduces phosphotungstic acid in acidic medium to blue phosphotungstate chromogen, which has absorption maximum at 700nm.

Reagents.

1. Phosphotungstic acid –Colour developing solution.

   Solution A – 20 gm of sodium tungstate Na_2HPO . $2H_2O$ – 10 gm taken in 300ml distilled water and warmed to dissolve.

   Solution B – 15ml distilled water and 5ml was then cooled to room temperature by allowing it to stand on the table and mixture was diluted to 500ml with distilled water

2. 0.5% Oxalic acid solution – 0.5 gm of Oxalic acid dissolved in distilled water and volume made up to 100ml

3. Stock std. ascorbic acid – This solution was prepared by dissolving 50 mg of L-ascorbic acid in 100ml of 0.5% (W/V) oxalic acid solution.

4. Working standard solution (1mg/100ml) – Dilute stock standard solution 50 times with 0.5% Oxalic acid.

   Procedure – 1ml of plasma sample was taken in a test tube marked test ‘T’ and 2ml of distilled water in a test tube marked blank ‘B’

		T	B
1)	Plasma	1.0ml	-
2)	Distilled water	-	2.0 ml
3)	Colored reagent	2.0ml	2.0 ml

Mix thoroughly and allowed to stand for 30 minutes at room temperature. Then tubes were centrifuged at 3000 rpm for 10 minutes. The clear supernatant was taken in cuvette without disturbing precipitate and absorbance was measured at 700nm against blank. Serial dilutions of standard solution were prepared. Standard Graph was plotted using absorbance (optical density) against concentration .Values expressed in terms of mg/dl.

9. Estimation of serum vitamin E (alpha-tocopherol) and β-carotene (vitamin A)

Baker and Frank method for Vitamin E: (1968)

Modified Quaife et al method for Vitamin A: (1949)

Principle:

Proteins in the serum were precipitated by absolute ethanol. Then the whole mixture was subjected to extraction by n-heptane. The 2,2 - dipyridyl was then added to an extracted aliquot to estimate the principle interfering substance, β-carotene at 460 nm. Then ferric chloride added

reacts with tocopherol which reduces ferric ions to ferrous ion (by Emmeri Engel reaction). The reduced ferrous ions then forms a red coloured complex with 2,2 dipyridyl. The red colour developed was read at 520 nm.

Reagents:

1) Absolute ethanol
2) N- heptane.
3) 2,2'- Dipyridyl in absolute ethanol. (0.120 gm/ 100ml).
4) Ferric chloride hydrated in absolute ethanol (0.120/100 ml)
5) Std. solution of α-tocopherol (100 mg % in absolute ethanol)
6) Working standard (2mg%): 2 ml of stock standard dilute up to 100 ml of absolute ethanol.

Now, with this working standard various dilutions were prepared and standard graph was plotted using absorbance against concentration of standard.

Test Procedure:

1) In stopper centrifuge tube add 1.5 ml of plasma and in another test tube add distilled water (Blank).
2) Then in each tube add 1.5 ml of absolute ethanol.

3) Add 1.5 ml of heptane to each tube. Mix well for 10 min on vortex mixer and centrifuge for 10 min at 2500 rpm.
4) Transfer 1 ml of heptane layer into a clean stoppered tube, carefully excluding any protein or ethanol.
5) Add 1 ml of dipyridyl reagent to each tube, and mix.
6) Take absorbance of the test against the blank at 460 nm.
7) Then beginning with blank add 0.33 ml of ferric chloride solution, mix, set the wavelength at 520 nm and read the absorbance of test against blank after 90s.
8) A correction for the carotenes was made after taking readings at 520 nm.

Calculation:

For vitamin E, the results were calculated from standard graph in mg/dl.

Final absorbance = Absorbance at 520 - (0.29 x Absorbance at 460 nm).

β Carotene was determined in µgm/dl by multiplying absorbance at 460 nm by 856 factor.

β carotene (Vitamin A) (µgm/dl)= 856 x absorbance at 460 nm.

Observations and Results

OBSERVATIONS AND RESULTS

In the present study , 407 pediatric cases were selected including control and study group subjects as depicted in Table 1 and in Graph 1.The study group subjects were further sub-grouped as Infective and non-infective . Age and sex matched 60 healthy children were selected as controls.

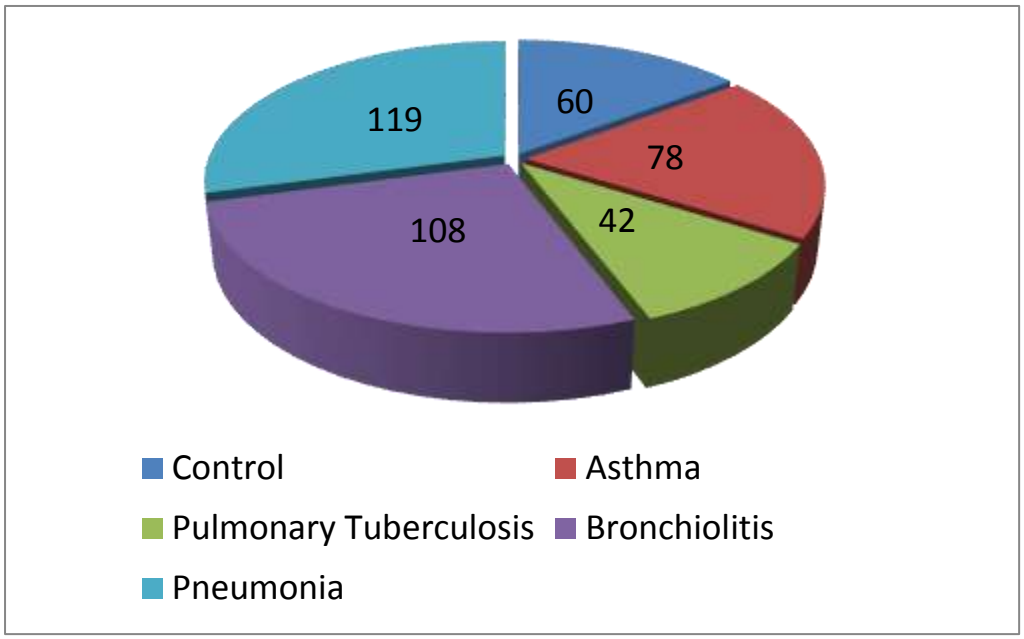
Infective group was further divided as bronchiolitis with 119 patients, pneumonia with 108 patients, and pulmonary tuberculosis with 42 patients. The non-infective asthma group had 78 patients. Blood samples were collected from these patients on day of visit to the hospital.

Total 60 cases were selected for follow up study 30 each from bronchiolitis and pneumonia group. Blood samples were collected from these patients on day of admission and at the time of discharge from Bharati Hospital, Pune Distribution of cases is depicted in **Table no.1**

Table 1: Distribution of number of cases

Sr. No	Group	Total cases
1	Control	60
2	Bronchiolitis	108
3	Pneumonia	119
4	Pulmonary Tuberculosis	42
5	Asthma	78

Graph 1 :Distribution of number of cases



Staistical analysis was done by using SPSS software.

- Unpaired 't' test, was used to compare the significance of each parameter as compared to control as well as in between the study groups.
- Post Hoc Tukey-test was applied to compare the parameters in between all the groups.
- paired 't' test was applied to compare the results in follow up patients.

Table 2: Serum MDA Levels in nmole /ml in control and in bronchiolitis, pneumonia, pulmonary tuberculosis, and asthma

Sr. No	Group	Mean ±SD (nmoles/ml)
1	Control	2.60±0.48
2	Bronchiolitis	6.77±0.99
3	Pneumonia	10.92±0.83
4	Pulmonary Tuberculosis	11.89±1.16
5	Asthma	7.71±0.74

P-value <0.001

Graph 2: Serum MDA Levels in nmole /ml in control and in bronchiolitis, pneumonia, pulmonary tuberculosis, and asthma

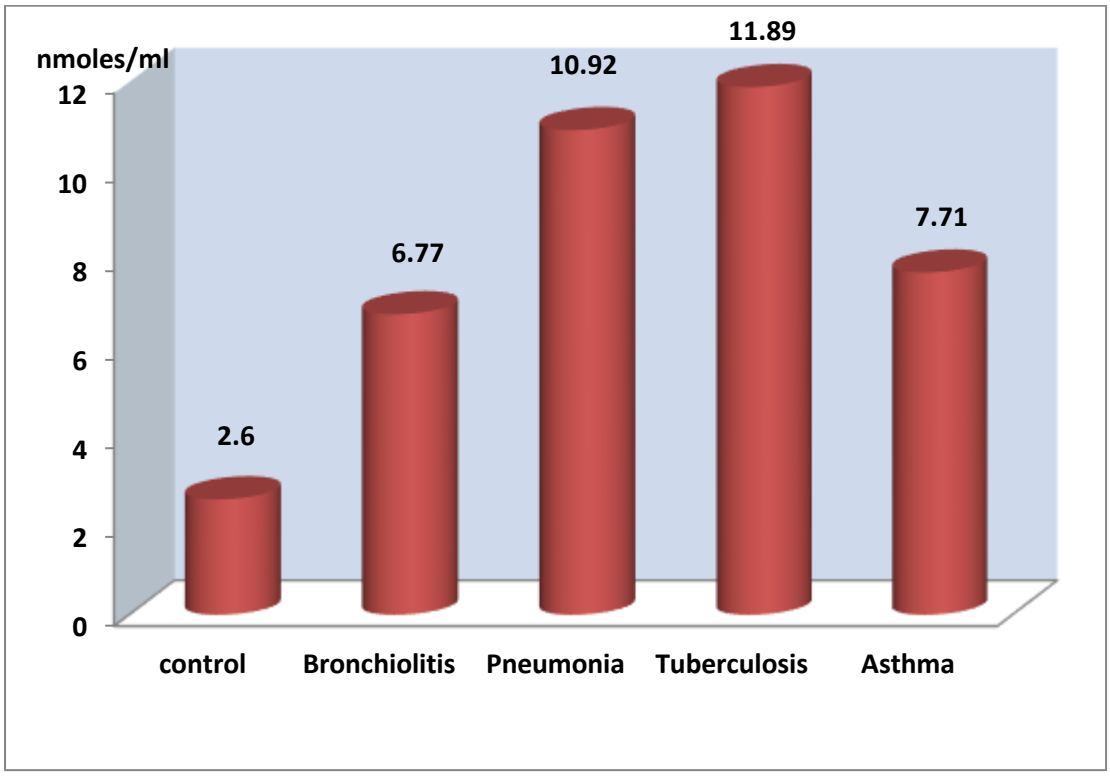


Table 2 and Graph 2 shows comparison of MDA levels in study groups and controls

MDA levels were found significantly increased in all groups suggestive of increased lipid peroxidation in bronchiolitis, pneumonia, pulmonary tuberculosis, and asthma when compared to control group. In case of pulmonary tuberculosis patients, serum MDA level was observed to be highest amongst all study group subjects followed by pneumonia, asthma, and bronchiolitis .This difference was statistically significant.

Table 3: Plasma levels of antioxidants vitamin C, Vitamin E and β carotene in control and bronchiolitis , pneumonia, pulmonary Tuberculosis, and Asthma

Groups	**Control n(60)**	**Bronchiolitis n(78)**	**Pneumonia n(89)**	**Tuberculosis n(42)**	**Asthma n(78)**
Parameters	**Mean ±SD**	**Mean ±SD**	**Mean ±SD**	**Mean ±SD**	**Mean ±SD**
Vit C mg/dl	2.70±0.51	0.82±0.11	0.88±0.104	0.56±0.18	1.25±0.20
Vit E mg/dl	2.04±0.38	1.47±0.27	1.44±0.374	1.45±0.44	1.76±0.46
β Carotene µg/dl	44.33±3.61	28.86±3.81	20.01±1.81	14.57±3.46	33.31±1.97

P-value <0.001 statistically significant

Graph 3: Plasma levels of antioxidants vitamin C, Vitamin E and β carotene in control and bronchiolitis , pneumonia, pulmonary Tuberculosis, and Asthma

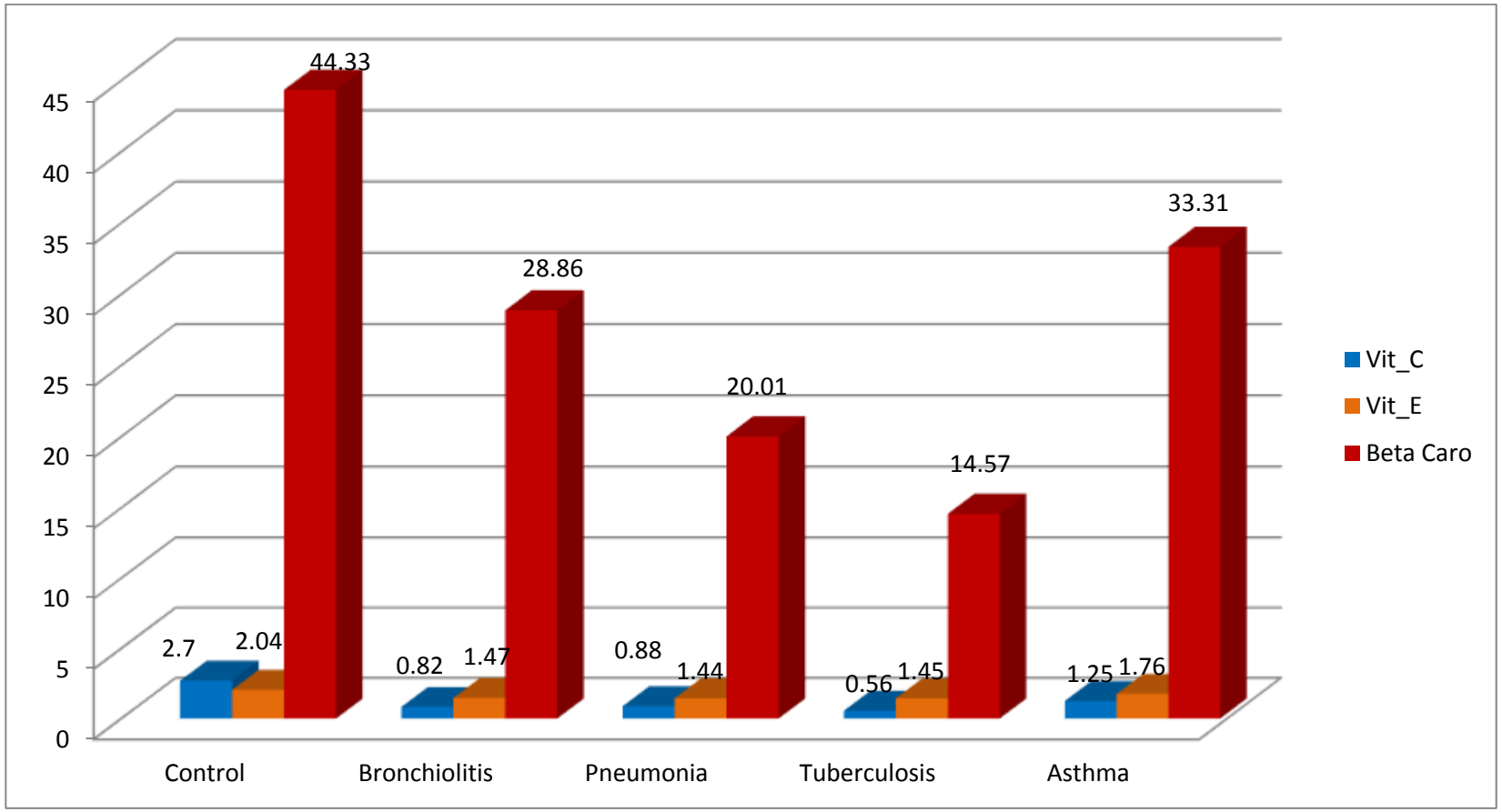


Table 3 and Graph 3 show plasma levels of antioxidant molecules, vit C, vit E, and β carotene in study groups and controls.

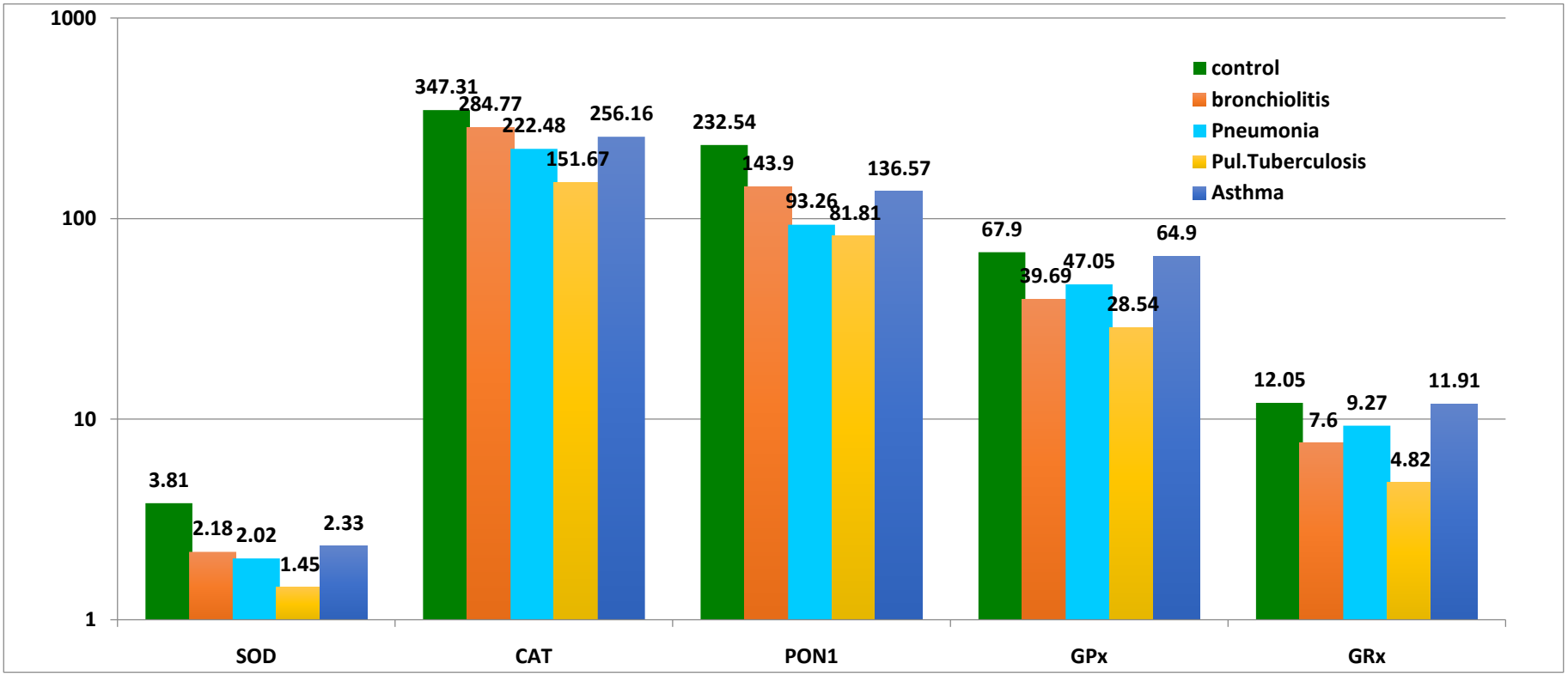
All antioxidant molecules levels were found significantly decreased (p value-<0.01) in Bronchiolitis, Pneumonia, Pulmonary Tuberculosis, and Asthma when compared to control group which is suggestive of complete utilization of antioxidants in counterattacking ROS molecules during lipid peroxidation in disease progress.

Table 4: Specific activities of SOD,CAT,PON1,GPx,GRx enzymes in control and in study groups

Groups →	Control n(60)	Bronchiolitis n(78)	Pneumonia n(89)	Tuberculosis n(42)	Asthma n(78)
Parameters ↓	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD
SOD U/mg of Hb	3.81±0.49	2.18±0.29	2.02±0.47	1.45±0.17	2.33±0.28
Catalase K/mg of Hb	347.31±51.20	284.77±27.7	222.48±16.98	151.67±13.43	256.16±24.34
PON1 IU/L	232.54±34.69	143.90±21.30	93.26±8.85	81.81±11.25	136.57±13.54
GPX U/g of Hb	67.90±12.37	39.69±7.94	47.05±8.32	28.54±5.50	64.9±8.69*
GRx U/g of Hb	12.05±2.57	7.60±2.48	9.27±2.24	4.82±1.09	11.91±2.46*

p value <0.001 statistically significant *p value >0.5 statistically not significant

Graph 4: Specific activities of SOD, CAT, PON1, GPx, GRx antioxidant enzymes in control and in bronchiolitis, pneumonia, pulmonary tuberculosis and asthma subjects



Specific activity of all antioxidant enzymes SOD, CAT, PON1, GRX, GPx were found significantly decreased (p value-<0.01) in Bronchiolitis, Pneumonia, Pulmonary Tuberculosis, when compared to control group which is suggestive of decreased synthesis or repression of antioxidants enzymes. This is suggestive of lowered activity in counterattacking ROS molecules formed during lipid peroxidation and basis of disease progress.

In case of Asthma group, levels of SOD, CAT, PON-1 activity observed statistically decreased when compared to pneumonia, bronchiolitis and pulmonary tuberculosis and in controls. But GPx and GRx activity not shown significant difference (p>0.5) when compared to pneumonia, bronchiolitis and pulmonary tuberculosis and in controls. This shows that functioning capacity of GPx/GRX system was not repressed and enough supply of reduced glutathione was maintained at the alveolar site in spite of reduced activity of other antioxidant enzymes .

Table 5: Total %PUFA, n6PUFA, n3PUFA and n6:n3 PUFA ratio in in bronchiolitis, pneumonia, pulmonary tuberculosis and asthma subjects as well as in controls.

Groups →	Control n(60)	Bronchiolitis n(78)	Pneumonia n(89)	Pulmonary Tuberculosis n(42)	Asthma n(78)
Parameters↓	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD
%PUFA	37.36±4.89	20.10±4.80	22.36±6.21	10.83±3.50	34.23±4.90
n6 fatty acids	34.2±3.2	18.9±2.34	21.6±4.2	10.1±2.2	32.4±2.9
n3 fatty acids	2.6±1.2	1.1±0.42	0.98±0.21	0.34±0.094	1.5±0.076
n6:n3 ratio	13.15:1	17.18:1	22.04:1	29.7:1	21.6:1

$p<0.01$ statistically significant

Graph 5: Total PUFA, n6 PUFA, n3 PUFA and n6:n3 PUFA ratio in bronchiolitis, pneumonia, pulmonary tuberculosis and asthma subjects as well as in controls.

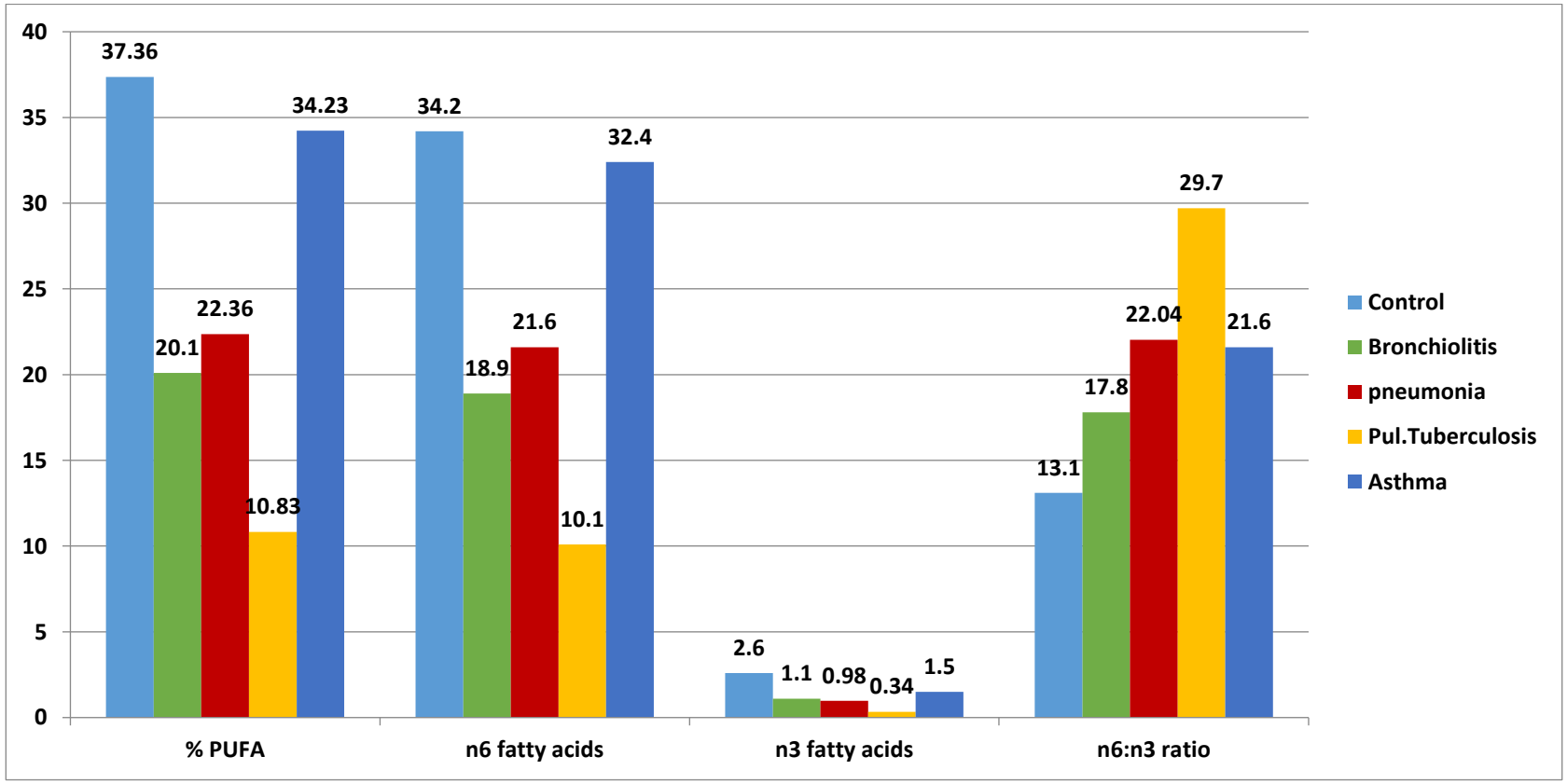


Table 5 and Graph 5 shows % PUFA, n6 PUFA, n3 PUFA and n6:n3 PUFA ratio in bronchiolitis, pneumonia, pulmonary tuberculosis and asthma subjects as well as in controls.% PUFA, n6 fatty acids and n3 fatty acids levels were observed to be decreased in bronchiolitis, pneumonia, pulmonary tuberculosis and asthma subjects as compared to controls. But n6:n3 ratio was observed to be increased.

Follow up study was done by selecting 30 IPD patients each from Bronchiolitis and Pneumonia.

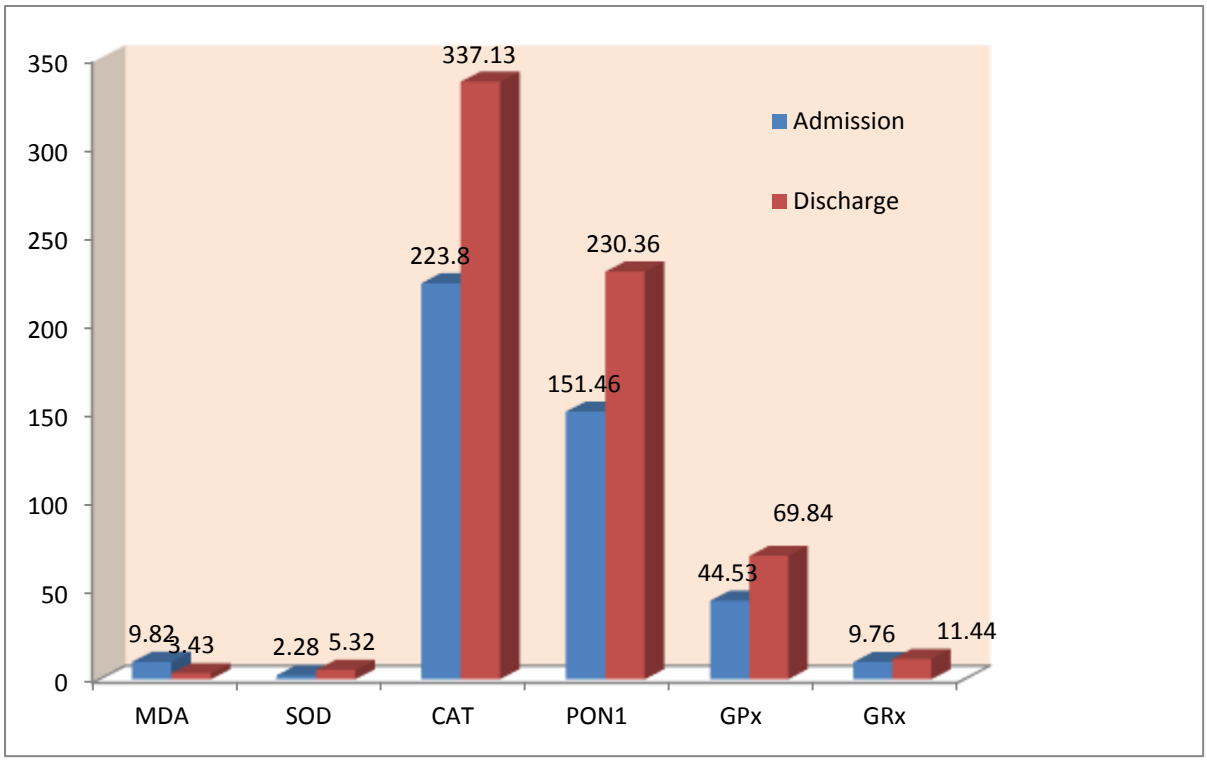
Average Hospital stay in Pneumonia patients was 5 -7 days and that of Bronchiolitis was 7 -9 days.

Table 6 Serum MDA levels and Specific activities of SOD, CAT, PON1, and GPx, GRx antioxidant enzymes in pneumonia patients on admission and on the day of discharge from hospital

Pneumonia Parameters	**On Admission n(30) Mean ±SD**	**On Discharge n(30) Mean ± SD**
MDA	9.82±1.14	3.43±.83*
SOD	2.28±.41	5.32±.92*
CAT	223.80±65.65	337.13±52.66*
PON1	151.46±25.70	230.36±33.98*
GPx	44.53±0.60	69.84±9.52*
GRx	9.76±2.26	11.44±2.29*

*P-value <0.001 statistically significant

Graph 6 serum MDA levels and Specific activities of SOD, CAT, PON1, GPx, GRx antioxidant enzymes in pneumonia patients on admission and on the day of discharge from hospital.



MDA levels were significantly increased in pneumonia patient's shows evidence of increased lipid peroxidation at the time of admission. Antioxidant enzymes SOD, CAT, PON-1, GRx, GPx. were significantly decreased at the time of admission as compared to discharge from hospital.

At the time of discharge, significant increase was observed in SOD, CAT, PON-1, GPx, GRx antioxidant enzymes when compared with the

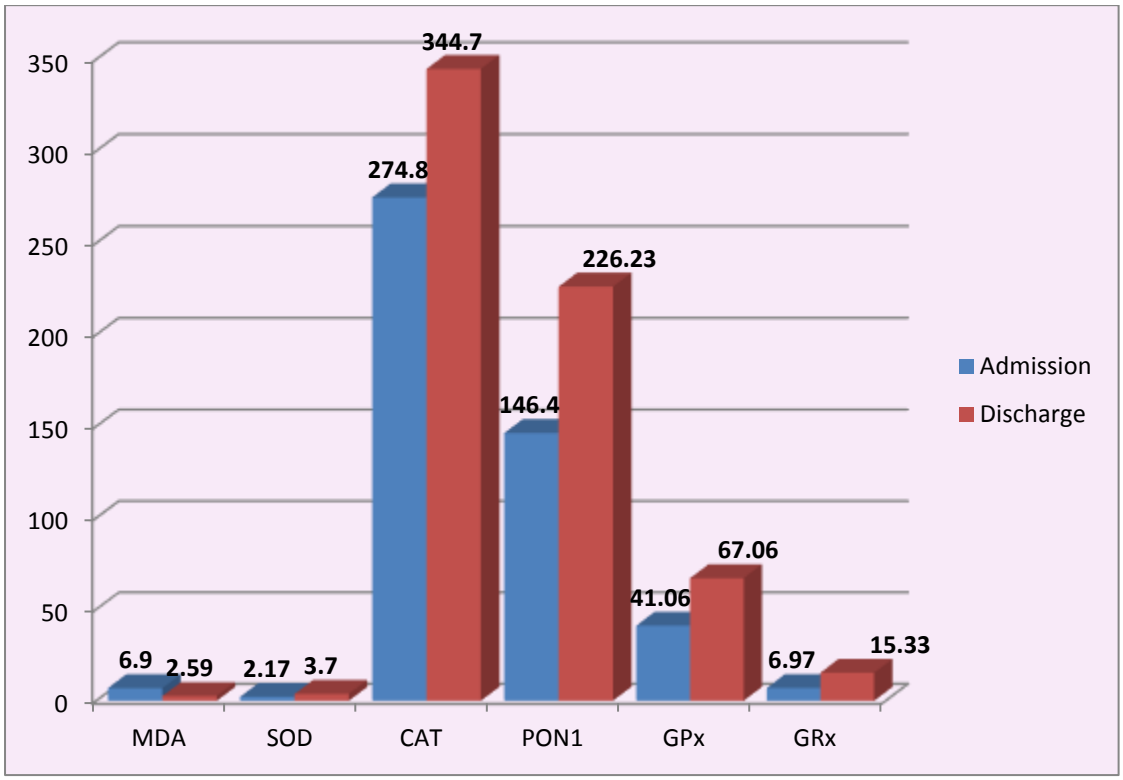
time of admission in hospital. MDA levels were significantly decreased shows evidence of decreased lipid peroxidation

Table 7 serum MDA levels and Specific activities of SOD,CAT, PON-1,GPx,GRx antioxidant enzymes in Bronchiolitis patients on admission and on the day of discharge from hospital.

Bronchiolitis Parameters	**On Admission n(30) Mean ±SD**	**On Discharge n(30) Mean ± SD**
MDA	6.9680±1.19	2.59±.42*
SOD	2.17±.31	3.7±.52*
CAT	274.80±57.99	344.70±52.66*
PON-1	146.40±20.41	226.23±32.98*
GPx	41.06.53±6.98	67.06±11.75*
GRx	6.97±1.86	15.33±2.60*

*P< 0.01 statistically significant

Graph7: serum MDA levels and Specific activities of SOD,CAT, PON-1,GPx,GRx antioxidant enzymes in Bronchiolitis patients on admission and on the day of discharge from hospital.



MDA levels were significantly increased in bronchiolitis patient's shows evidence of increased lipid peroxidation at the time of admission. Antioxidant enzymes SOD, CAT, PON-1, GRx, GPx. were significantly decreased at the time of admission as compared to discharge from hospital.

At the time of discharge, significant increase was observed in SOD, CAT, PON-1, GPx, GRx antioxidant enzymes when compared with the time of admission in hospital. MDA levels were significantly decreased shows evidence of decreased lipid peroxidation.

Discussion

DISCUSSION

Reactive oxygen species (ROS) includes a large variety of free oxygen radicals e.g. superoxide anion $O2^{\bullet-}$ and hydroxyl radicals OH, as well as derivatives of oxygen hydrogen peroxide(H_2O_2), hypochlorous acid, peroxynitrite, which are capable of initiating oxidation. Respiratory system gets continuously exposed to environmental, oxidants, infectious agents or pollutants. This triggers the ROS production and activation of inflammatory pathways like activation of phagocytes in alveolar tissue which together with its large surface area and blood supply make it susceptible to injury mediated by ROS. In alveolar tissue an oxidant/antioxidant imbalance in favour of oxidants, results into oxidative stress and induces a variety of cellular responses through the generation of secondary metabolic ROS and lipid peroxidation phenomenon responsible for oxidation of proteins, DNA and lipids, as well as pathogenesis of the disease process.

Considering all these metabolic events present study was undertaken to evaluate the facts about the role of oxidative stress and number of factors involved in creating imbalance in oxidant/antioxidants, and its correlation to pathogenesis of bronchiolitis, pneumonia, pulmonary tuberculosis and asthma.

In the present study , 407 pediatric cases were selected including control and study group subjects as depicted in **Table 1** and **Graph 1**.The study group subjects were further sub-grouped as infective and non-infective . Age and sex matched 60 healthy children were selected as controls.

Infective group was further divided as bronchiolitis with 119 patients, pneumonia with 108 patients, and pulmonary tuberculosis with 42 patients. The non-infective asthma group had 78 patients.

For follow up study, 60 patients from bronchiolitis and pneumonia group were selected. Blood samples were collected from these patients on day of admission and at the time of discharge from Bharati Hospital, Pune.

Statistical analysis was done using SPSS software.

In the present study increased lipid peroxidation in terms of MDA nmole/ml was observed in bronchiolitis 6.77±0.99,pneumonia10.92 ± 0.83, pulmonary tuberculosis 11.89±1.16 and asthma 7.71±0.74 as compared to control 2.6± 0.48.

In case of bronchiolitis and asthma more than two fold increases was observed in serum MDA levels as compared to control while this increase was more than four fold in case of pneumonia and pulmonary

tuberculosis as shown in **Table no 2 and Graph no 2.** These values indicated increased lipid peroxidation in all the respiratory subjects.

In case of the follow up study of the patients from bronchiolitis, serum MDA levels(nmoles/ml) were 6.96 ±1.19 at the time of admission and found decreased at the time of discharge 2.59±.42, with average stay 7-9 days.

In follow up study of the patients from Pneumonia, serum MDA levels (nmole/ml) were 9.82 ± 1.14 at the time of admission and found decreased after treatment at the time of discharge 3.43±.83 with average stay in hospital 5-7 days.

In case of respiratory diseases inflammatory response to the infection or allergen gives rise to generation of hydroxyl, peroxyl, and various ROS in the lung epithelium.

The hydroxyl radical react with PUFA resulting in the formation of lipid peroxyl radical. If this resulting lipid peroxyl radical is not reduced by antioxidants, lipid peroxidation occurs. Products of lipid peroxidation are generally stable, diffuse within the tissue, and in the blood. In the present study these increased lipid peroxidation products were measured in terms of malondialdehyde (MDA) as thiobarbituric acid reacting substances (TBRS) in the serum of patients as an index of lipid peroxidation.

Supporting findings were shown by Gurkan et al (2004)[119], in their study on infants with acute bronchiolitis. They have evaluated the role between serum malondialdehyde (MDA) and selenium (Se) levels in patients on admission and two months after discharge from the hospital. Mean serum MDA levels were significantly higher in patients with acute bronchiolitis than at the post bronchiolitis stage and the infants with bronchiolitis had lowered mean serum Se levels at the acute stage than after two months. They have found a negative correlation between serum MDA and Se levels in the patient with pathogenesis of acute bronchiolitis.

From this study, it is evident that, in case of hospitalized patients, during acute symptoms of the disease, increased lipid peroxidation could have resulted into disease state. Similar results were reported by Shokry et al (2013)[120] in asthmatic patients ,they have reported MDA levels 19.8 ± 6.6 and after treatment reduced to 11.1 ± 5.2 as compared to control group 7.6 ± 2.3 Supporting values were also reported by Liao M-F et al(2004)[121] in children and Mate´s, et al(1999)[122] on adult asthma population.

Romieu et al (2002)[58] studied children exposed to pollutants. They have observed rapid and significant rise in malondialdehyde levels

(1.12nmol/ml) within 8 hrs exposure to environmental pollutants which also correlated with simultaneous changes in pulmonary function.

Guney et al (2004)[123] studied patients with pulmonary tuberculosis for MDA and SOD levels. MDA levels increased in TB group 28.43±9.83 than control group 15.95 ±3, and lowered activities of SOD were found in TB group 17.64±0.93 when compared with control group 21.52±1.49

Present study illustrates decrease in %PUFA in bronchiolitis 20.10±4.80, pneumonia 22.36±6.21, pulmonary tuberculosis 10.83±3.50, and asthma 34.23±4.90, as compared to controls 37.36±4.89.

Present study shows decrease in n6PUFA% in bronchiolitis 18.9±2.34, pneumonia 21.6±4.2, pulmonary tuberculosis10.1±2.2, and asthma 32.4±2.9, as compared to controls 34.2±3.2.

Decrease in n3 PUFA% in bronchiolitis1.1±0.42, pneumonia 0.98±0.21, pulmonary tuberculosis 0.34±0.094, and asthma 1.5±0.076, was observed as compared to controls 2.6±1.2.

This significant decrease in %PUFA, n6 and n6 PUFA% could be due to increased oxidation of PUFA by lipid peroxidation. These lipid hydroperoxides initiates the peroxidation chain propagation in lung epithelium and further destruction of membrane PUFA.

Present study also shows increased n6:n3 PUFA ratio in bronchiolitis17.18:1 pneumonia 22.04:1, pulmonary tuberculosis 29.7:1,

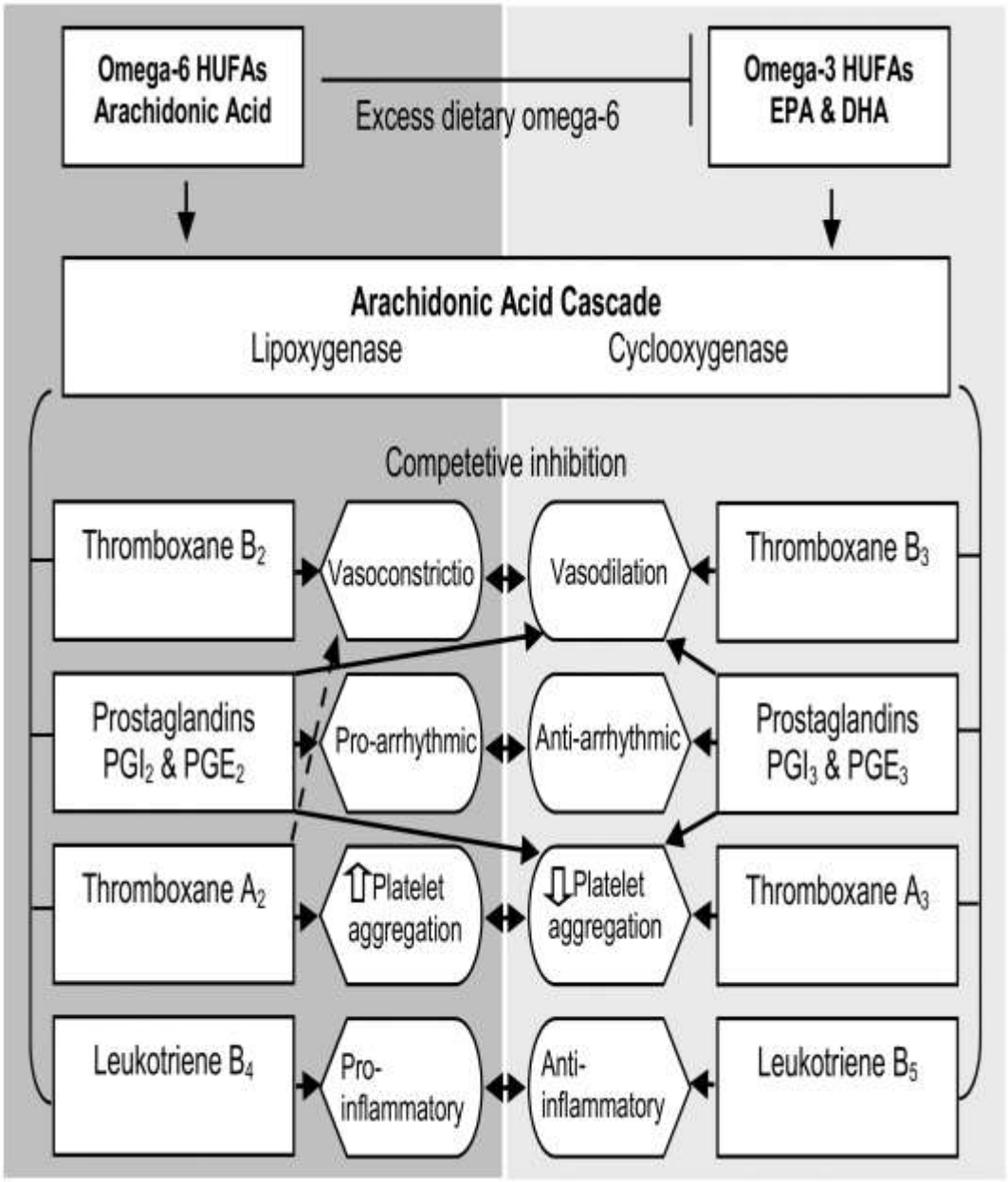
and asthma 21.6:1, as compared to control 13.15:1. In all study groups subjects moderate to severe change in n6:n3 ratio was observed which may have triggered the inflammatory pathways and cell signaling.

Several researchers suggested that human beings are evolved on a diet with a ratio of omega-6 to omega-3 essential fatty acids (EFA) of approximately 1. Traditional Indian diet supplies n6 and n3 PUFA in sufficient amount with high EFA [124], [125] whereas in Western diet, which is based on seed oil and storage fat; the ratio is 15/1-16.7/1. Excessive amounts of omega-6 polyunsaturated fatty acids (PUFA) and a very high omega-6/omega-3 ratio, as is found in today's Western diet promotes pathogenesis of many diseases, including cardiovascular disease, cancer, and inflammatory and autoimmune diseases,. However, increased levels of omega-3 PUFA (a low omega-6/omega-3 ratio) exert suppressive effects. A ratio of 2-3/1 suppressed inflammation in patients with rheumatoid arthritis, and a ratio of 5/1 had a beneficial effect on patients with asthma, whereas a ratio of 10/1 had adverse consequences.[61]

Duan et al (2013)[126] studied animal experiments to investigate the optimal dietary n-6:n-3 PUFA ratios and their relation with inflammation. Ratio of 5:1 was found to be the best, which significantly suppressed the expression levels of the inflammatory cytokines IL-1β, TNF-α and IL-6.

The results indicated that the optimal n-6:n-3 PUFA ratios of 1:1 and 5:1 exerted beneficial effects on lipid metabolism and inflammatory system, leading to the availability of more energy and nutrients for high performance and homeostatic pathways.

Figure 5: Role of n6, and n3 PUFA in lung epithelium



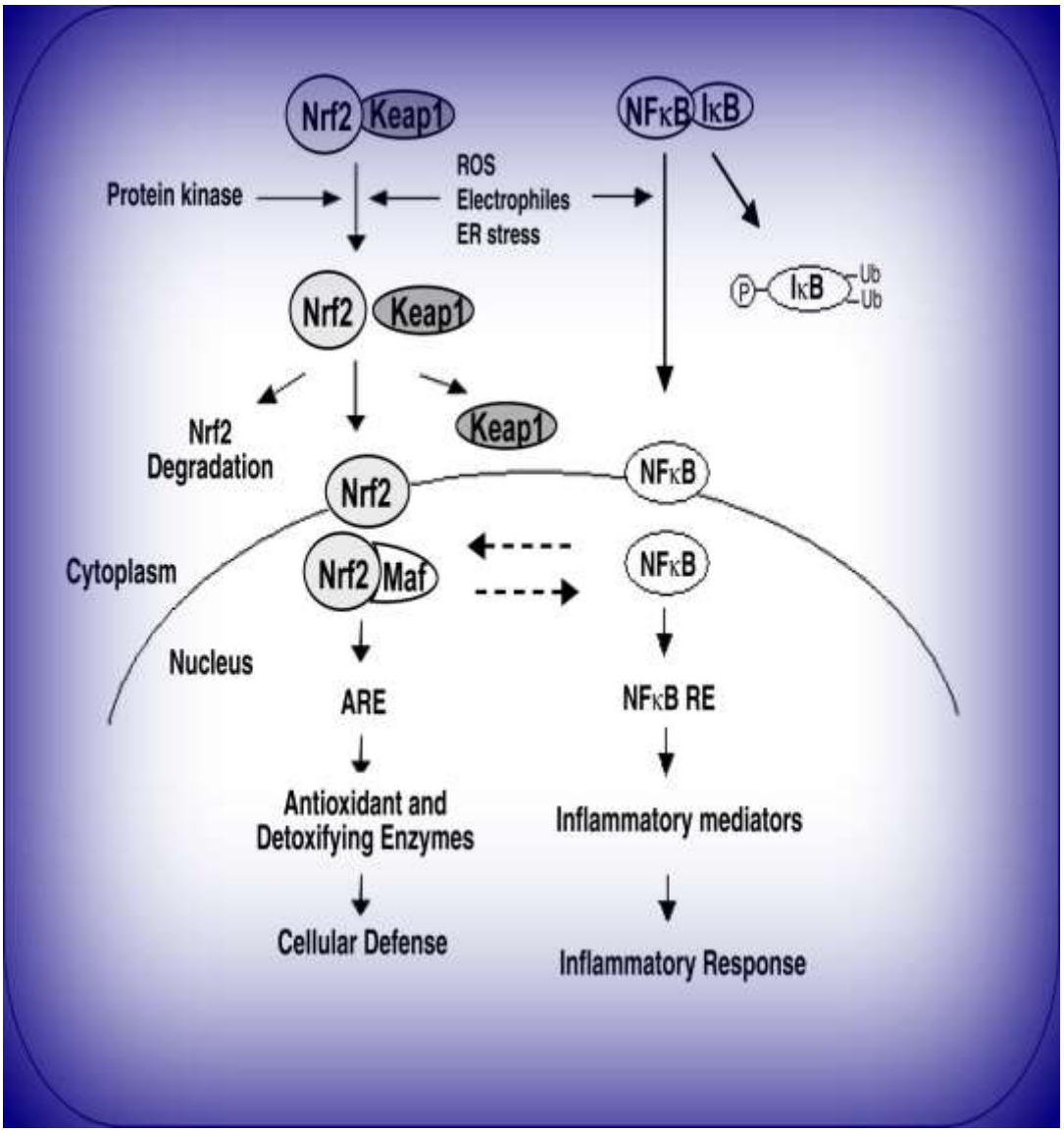
In physiological conditions it was also noted that n3 PUFA inhibits the activation of nuclear factor kappa beta (NF-κB) and thereby transcription of number of inflammatory cytokines and chemokines.[127]

This change in MDA levels with decrease in n3 PUFA and association of disease process could be explained by Nrf2pathway. Hosakote et al (2011)[128] observed changes in RSV infected BAL fluid of children with bronchiolitis for oxidative markers and did experiments on mice to investigate role of Nrf2 and antioxidant enzymes. It was observed that, RSV infection induced a significant decrease in the expression and activity of SOD, catalase, glutathione S-transferase, and glutathione peroxidase in murine lungs and in the airways of children with severe bronchiolitis. All these oxidative damage markers well correlated with severity of clinical illness in RSV infected infants. RSV infection of airway epithelial cells induced ROS production, which was involved in transcription factor activation and chemokine gene expression which was observed as significant reduction in Nrf2 mRNA expression and significant decrease in AOE gene expression in the lungs of virus-infected mice.

These pathological events will further increase the ROS generation and imbalance in the redox status leading to increased oxidative stress.

This increased oxidative stress could have resulted into activation /deactivation of signaling pathways as shown in **figure no 6**.

Figure 6 :Combined pathway for Nfkb and Nrf2 signaling



ARE: Antioxidant Responsive element

In present study, similarly we observed increased serum MDA levels, increased lipid peroxidation and low levels of n3 PUFA which could have triggered inflammatory pathway by increasing gene

transcription through activation of NF-κB. Activated NF-κB can translocate into the nucleus, binds with the promotor site on DNA to commence gene transcription for cytokines and chemokines. This might have attracted phagocytes, granulocytes , eosinophils and mast cells at the site of alveolar tissue. This increased inflammatory reactions and increase in ROS are suggestive of the observed pulmonary tissue damage which induce symptoms like bronchoconstriction, elevated mucus secretion, and cause microvascular leakage, which leads to edema formation and pathogenesis in cases of respiratory disorders.

Antioxidant enzymes :

Specific activity of SOD(U/mg of Hb) an antioxidant enzyme which was found to be decreased in bronchiolitis 2.18 ±0.29, pneumonia 2.02± 0.47, pulmonary tuberculosis1.45 ±0.47, and asthma 2.33 ±0.28 patients as compared to control group 3.81± 0.49.

In follow up study, at the time of admission in pneumonia patients, specific activity for SOD (U/mg of Hb) was observed as,2.28±.41 and in bronchiolitis patients, 2.17±.31 and at the time of discharge from hospital in pneumonia patients, 5.32±.92 and in bronchiolitis patients, was 3.7±.52.

Suzy et al **(**2005)[129] observed reduced SOD activity in the oxidant-rich environment of the asthmatic airways and during asthma

exacerbation. Further loss of SOD activity occurs with enhanced production of oxygen radicals by inflammatory cells which would be reflected systemically in loss of circulating SOD activity and clinically by development of severe asthma and/or worsening airflow limitation.

Activity of CAT (K/mg of Hb) was found to be decreased in bronchiolitis 284.77 ± 27.47, pneumonia 222.48 ± 16.98, pulmonary tuberculosis151.67 ± 13.43, and asthma 256.16 ± 24.34 patients as compared to control group 347.31 ± 51.20.

However, CAT activity for pneumonia on admission was 223.80±65.65 and in bronchiolitis, 274.80±57.99. During the recovery phase in disease condition with significant increase in the CAT activity was observed on discharge in pneumonia, 337.13±52.66 and bronchiolitis, 344.70±52.66

Mate´s et al (1999)[122] observed enhanced, SODs and CAT activities and a decrease in GPx activity in mononuclear cells from allergic patients compared to controls. Conversely, in erythrocytes, higher values for GPx and SODs and similar CAT activities were found in allergic patients and controls.

PON1 antioxidant enzyme which was found to be decreased in bronchiolitis143.90 ±21.30, pneumonia 93.26 ±8.85, pulmonary

tuberculosis 81.81 ±11.25, and asthma patients136.5±7 13.54 as compared to control group 232.54± 34.69.

However, PO1 activity was significantly low for pneumonia on admission 151.46±25.70 and in bronchiolitis, 146.40±20.41.During the recovery phase from disease condition with significant increase in the PON1 activity was observed in pneumonia, 230.36±33.98 and bronchiolitis, 226.23±32.98 on discharge.

Clara cells are one of the oxidant resistant airway cells in lungs of all species PON1 is mainly localized in clara cells, endothelial cells and in type 1 pneumocytes in the lungs.

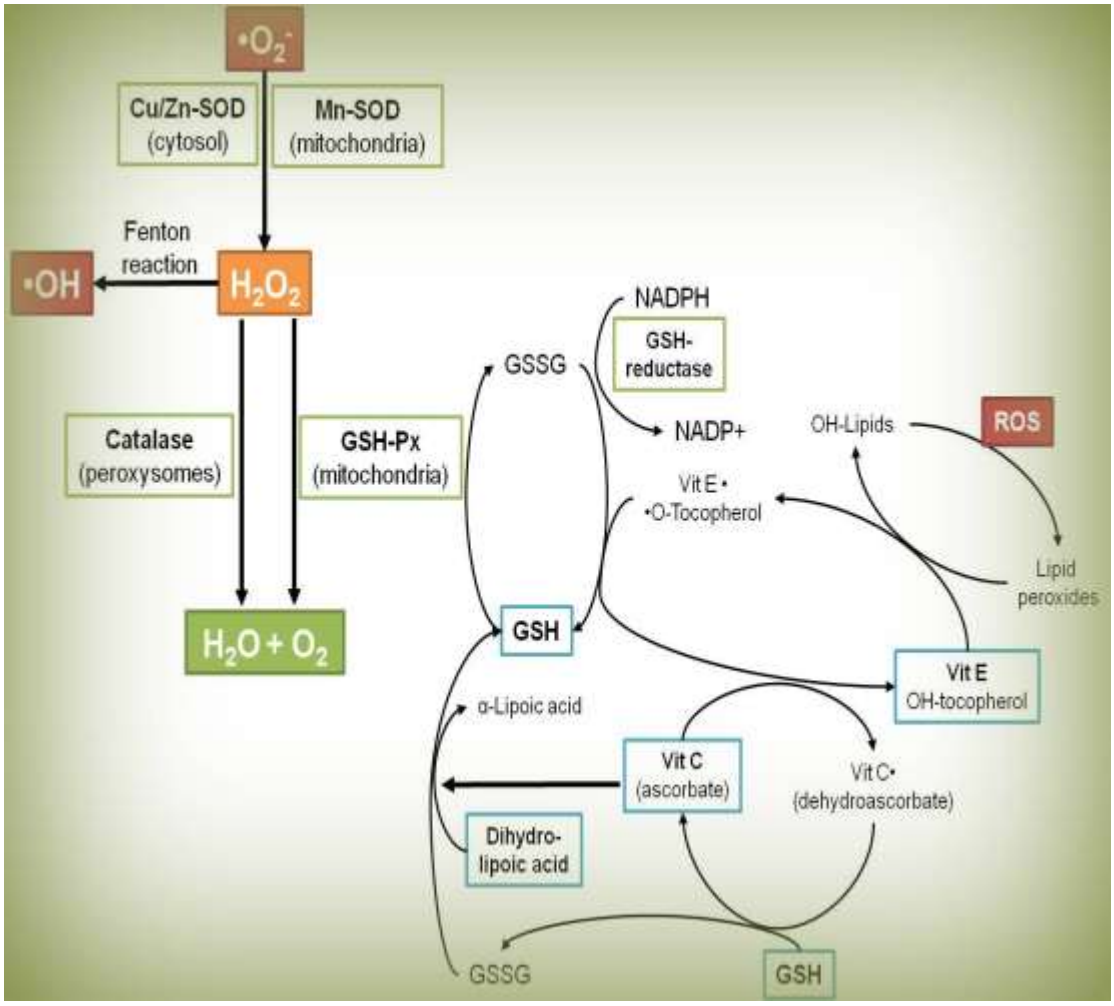
In the present study significantly lowered activity of PON 1 was observed in bronchiolitis, pneumonia, pulmonary tuberculosis, asthma patients as compared to control group. This could be due to shedding off and continuous replacement of clara cells by mucosal cells due to increased lipid peroxidation and increased oxidative stress.[28] (Mango et al 1998). 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.[130]

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. [131,132]

In physiological conditions GPX/GRx enzyme system continuously replenishes reduced glutathione in lung tissue to scavenge the increased load of ROS and lipid peroxidation with the help of various antioxidants like Vitamin C, vitamin E and PUFAs.

Figure 7 : Pathway showing role of SOD,CAT,GRx/GPx ,vitamin E vitamin C and PUFA



In present study GPx(U/gm of Hb) , an antioxidant enzyme was found to be decreased in bronchiolitis 39.69 ±7.94, pneumonia 47.05 ±8.32, pulmonary tuberculosis 28.54±5.5 as compared to control group 67.90 ±12.37.GRx was also found to be decreased in bronchiolitis7.60± 2.48 ,pneumonia 9.27 ±2.24, pulmonary tuberculosis 4.82 ±1.09 as compared to control group12.05 ±2.57.

This clearly indicates that in bronchiolitis, pneumonia and pulmonary tuberculosis, GRx/GPX system was repressed due to infectious condition and increased lipid peroxidation. At the same time it could not replenished reduced glutathione for scavenging increased ROS production in alveolar tissue and has resulted into further increase in disease process.

In follow up study, at the time of admission in pneumonia patients, specific activity for GPx (U/gm of Hb) was 44.53±0.60 and GRx (U/gm of Hb) was 9.76±2.26, and in bronchiolitis patients, GPx was 41.06.53±6.98 and GRx was 6.97±1.86 and at the time of discharge from hospital in pneumonia patients, Gpx 69.84±9.52 and GRx 11.44 ± 2.29,and in bronchiolitis patients, GPx 67.06±11.75 and GRx was 15.33±2.60 .

This significant increase in SOD, CAT, PON1, GPx, and GRx activity at the time of discharge clearly indicates efficacy of antibacterial

and supporting treatment in pneumonia and bronchiolitis. This has resulted into decreased bacterial and viral load in epithelial tissue, decrease in inflammatory reactions and decreased ROS generation, which could have triggered upregulation of ARE gene responsible for antioxidant enzyme synthesis.

In our study, in case of asthma patients GPx 64.9 ±8.69 and GRx 11.91 ±2.46 was not significantly changed as compared to control group12.05 ±2.57 which is suggestive of sustained activity of GPx/GRx for counterbalancing lipid peroxidation. Iskusnykh et al (2013)[38] observed the increased expression of GRx and GPx in their in vitro studies on rat liver. They observed that microsomal membrane lipid peroxidation leads to calcium release and uncontrolled activation of calcium-dependent proteases and lipases whereas ROS attack on mitochondrial membranes alters permeability and induce a disruption of cellular energetics. In addition, an accumulation of lipid peroxidation products under pathological conditions indicated the involvement of oxygen radicals in these disorders.

The results of this study indicated that induction of synthesis of these enzymes may be one of the factors promoting increased activity of the GRx/GPx system and over expression of these enzymes was found to be important for increased AREs function which is associated with GR

gene expression, and resistance to oxidative stress, the key pathogenic factor in various diseases.

Cemek et al (2006)[133] studied oxidative stress and enzymatic and non-enzymatic antioxidant status in children with acute pneumonia .The study reported high levels of blood MDA, in the study group than those of the control group. However, SOD, GPx, beta-carotene, retinol, vitamin C, vitamin E and GSH activities were lowered in children with acute pneumonia compared with the control group.

Similar observations were noted by Oluwole et al (2013)[134] in their study on exposure to household pollution from biomass fuel and its association with pulmonary dysfunction, reduced antioxidant defense and inflammation of the airways. It was observed that increased production of free radicals was secondary to pollutant exposure and inflammation which exceeded the capacity of the antioxidant defense system, resulted in increased serum concentrations of MDA in children (5.44±1.88nmol/ml) which was in positive correlation with inflammatory markers and in negative correlation with SOD, (2.39±0.99 U/L) vitamin C (1.04±0.30 mg/dl) and vitamin E (12.46±2.79 mg/ dl)

Non enzymatic antioxidants:

In present study, efficacy of antioxidant vitamin C, vitamin E and β carotene were analyzed against the increased lipid peroxidation and increased redox state.

Vitamin C is a potent water soluble antioxidant which is responsible for maintaining vitamin E in reduced state. In our study, vitamin C was found to be decreased (mg/dl) in bronchiolitis 0.82 ±0.11, pneumonia (0.88± 0.10), pulmonary tuberculosis (0.56 ±0.18) and asthma (1.25± 0.20) patients as compared to control group (2.7 ±0.51).

Birben et al (2012)[45] observed children during asthma exacerbation had significantly lowered serum levels of antioxidant compounds like vitamin C, vitamin E and significantly high MDA levels as compared with the controls. MDA was found significantly elevated, while that of vitamin C, vitamin E were significantly decreased with increasing severity of asthmatic attack. A significant negative correlation between MDA with vitamin C was observed in acute asthmatic attacks correlates with a high degree of reactive oxygen species and oxidative stress formation.

Antioxidant vitamin C and vitamin E supplementation and nasal inflammatory responses among young asthmatics exposed to high levels of ozone was studied by Sienra-Monge et al (2004)[135]. Antioxidant

supplementations were given randomly as a daily supplement of vitamins (50 mg of vitamin E and 250 mg of vitamin C) or a placebo. During a 4-month follow-up period nasal lavage analysis showed increase in IL-6, IL-8, with low levels glutathione in placebo group.

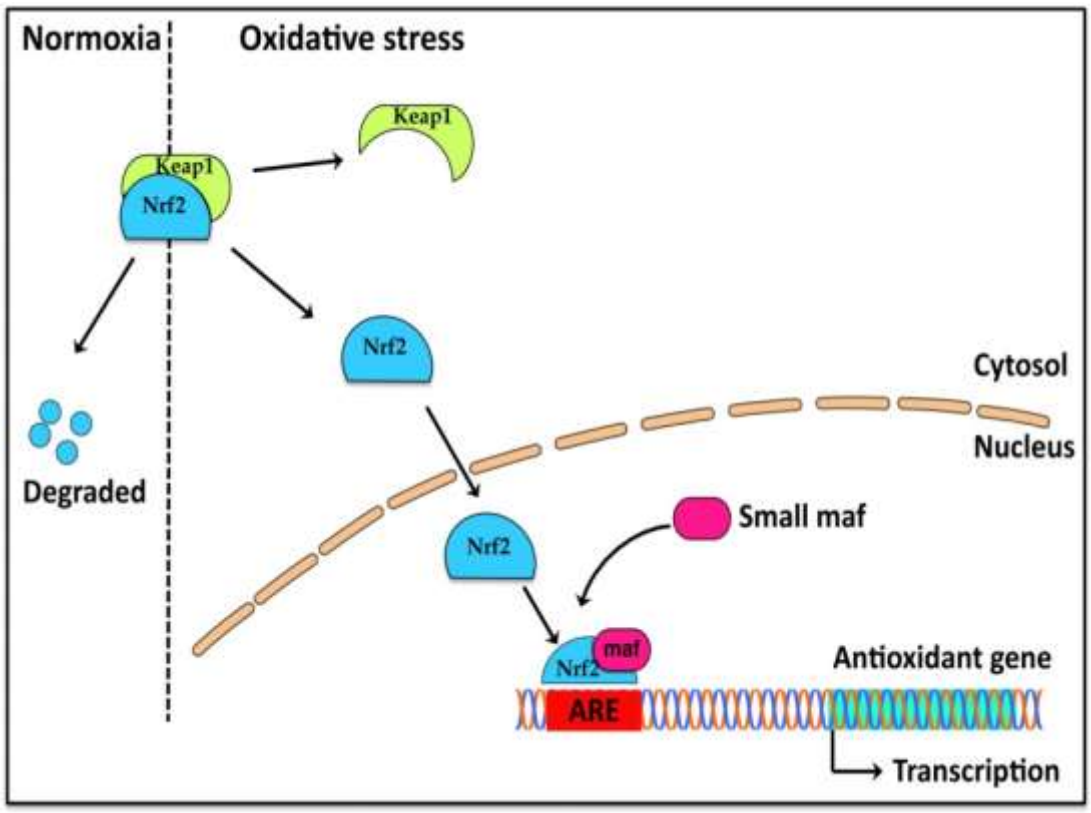
Vitamin E(mg/dl) is a lipid soluble antioxidant that represents the principal defense against oxidant induced membrane injury and protects membrane PUFAs.The levels reported as (mg/dl) in bronchiolitis 1.47 ± 0.27, pneumonia 1.44± 0.37, pulmonary tuberculosis 1.45±0.44 and asthma 1.76 ±0.46 patients as compared to control group 2.04 ±0.38.

A randomised controlled trials demonstrated that prophylactic vitamin E supplementation does not prevent chronic lung diseases. Ehrenkranz et al (39)[135] Similar studies were done by Kelly et al(2003)[136] and noted that, the amount of vitamin E that can enter cellular lipid membranes may be limited by the membrane lipid composition rather than its supply. After administration vitamin E accumulates slowly, taking weeks to achieve small increments in tissue concentrations.

In present study, β carotene (µgm/dl)is an potent antioxidant scavenger was found to be decreased in bronchiolitis 28.86 ±3.81, pneumonia 20.01 ±1.81, pulmonary tuberculosis 14.57 ±3.46 and asthma 33.31± 1.97 patients as compared to control group 44.33±3.61.

Kinnula(2005)[137] noted that, cigarette smoke causes significant oxidant stress which was further enhanced by recruitment and activation of inflammatory cells to the lung. Some antioxidant enzymes are inducted, but the extent of induction is insufficient to protect the lung/alveolar epithelium against cigarette smoke. Exogenous antioxidants such as vitamins did not seem to protect against cigarette smoke related lung injury. Synthetic compounds with superoxide dismutase and catalase activities have shown promising results in animal models against a variety of oxidant exposures including cigarette smoke in the lung.

Figure 8: Role of Nrf2 and antioxidant responsive gene activation



ARE-Antioxidant Responsive Element

Zolfaghari et al (2003)[138] observed that, vitamin A and its active metabolite, retinoic acid, regulate the expression of fatty acid desaturases including stearoyl-CoA desaturase catalyzes the formation of monounsaturated fatty acids from saturated fatty acids, and delta-5 desaturase involved in the desaturation of dietary essential fatty acids for production of polyunsaturated fatty acids.

Cho et al (2006)[139] conducted experiments on application of Nrf2 germ-line mutant mice and elucidated protective roles of Nrf2 in various models of human disorders in the liver, lung, kidney, brain, and circulation.

Nrf2-ARE binding regulates the expression of more than 200 genes involved in the cellular antioxidant and anti-inflammatory defense such as phase 2 detoxification enzymes (NAD(P)H quinone oxy reductase, glutathione), enzymes which are necessary for glutathione biosynthesis, extracellular superoxide dismutase, glutamate-6-phosphate dehydrogenase, heat shock proteins and ferritin, furthermore pro- and anti-inflammatory enzymes such as cyclooxygenase 2 (COX-2), Nrf2 has also been reported to regulate the expression of genes promoting mitochondrial biogenesis such as mitochondrial transcription factors (TFAM) and is therefore directly involved in mitochondrial preservation

role of NrF2 gene was also observed in viral mediated pneumonia by **Kosmider** et al(2011)[140]

Influenza A virus (IAV) infection primarily targets respiratory epithelial cells and produces clinical outcomes ranging from mild upper respiratory infection to severe pneumonia. Alveolar type II (ATII) cells and alveolar macrophages (AM) isolated from human lungs were infected with A/PR/8/34 (PR8) virus. Nrf2 translocation and Nrf2, HO-1 and caspase 1 and 3 cleavage was detected. It was noted that Nrf2 over expression followed by infection with PR8 virus decreased virus replication, influenza A nucleoprotein expression, antiviral response and while infection with PR8 virus inhibited release of keap1 and resulted into increase in oxidative stress.[141]

Summary and Conclusion

SUMMARY AND CONCLUSION

Reactive oxygen species are generated intracellularly from several sources, including mitochondrial respiration, cytochrome P-450, the NADPH oxidase system, xanthine/xanthine oxidase and metabolism of arachidonic acid.

In case of respiratory system, continuous exposure to environmental factors, pollutants and various infectious agents such as viruses, bacteria etc. make the system vulnerable to inflammatory reactions. Epithelial cells, resident macrophages, endothelial cells and recruited inflammatory cells, such as neutrophils, eosinophils, monocytes and lymphocytes, generate ROS in response to increased levels of secretory toxins. Activation of macrophages, neutrophils and eosinophils results first in the formation of O_2, which is rapidly converted to H_2O_2 by SOD, and OH is formed non enzymatically in the presence of Fe2+ as a secondary reaction. ROS produced by phagocytes at sites of inflammation are a major cause of cell and tissue damage associated with various acute and chronic inflammatory lung diseases.

The objective of the present study was to evaluate oxidative stress in respiratory disorders in pediatric patients by measuring MDA as an index of lipid peroxidation. PUFA% concentration and n6: n3 PUFA ratio

was measured as a degree of oxidative injury reflected by lipid peroxidation.

Efficacy of the antioxidant system was evaluated by measuring specific activities of antioxidant enzymes PON1,SOD,CAT,GPx,GRx and measuring antioxidants vitamin E as lipid peroxidation chain breaking molecule ,beta-carotene as a free radical scavenging molecule in epithelial tissue and ascorbic acid as potential antioxidant in aqueous phase of blood.

The study included 347 children which were clinically diagnosed and established cases of respiratory disorders grouped as infective and non-infective. Infective group was further divided into pneumonia, bronchiolitis, pulmonary tuberculosis and non infective group was asthma. These cases were compared with 60 normal healthy children as controls. Age of the study group as well as control group was 3 months to 12 yrs of both the sex**.**

In case of Pneumonia and Bronchiolitis 60 patients were studied on their day of admission and at the time of discharge from hospital, for lipid peroxidation with MDA estimation and for activity of antioxidant enzymes SOD, CAT, PON1, GPx, GRx.

In the present study, serum MDA levels were found to be increased in all respiratory groups i.e pneumonia, bronchiolitis, pulmonary

tuberculosis, and asthma as compared to control. This shows that there was increased lipid peroxidation in all respiratory disorders. This was also evident from the low levels of %PUFA and increased n6: n3 PUFA ratio as compared to controls.

In case of pneumonia, and pulmonary tuberculosis serum MDA levels are significantly high as compared to bronchiolitis and asthma group .This may be due to increased level of lipid peroxidation and are suggestive of severity of the disease.

In our study significant decrease in SOD, CAT, PON1, GPx, GRx, antioxidant enzymes in all the respiratory groups with the exception of moderate changes in GPx and GRx levels in asthma group as compared to control. This is indicative of decreased or suppressed activity of all antioxidant enzymes. This was in positive correlation with all antioxidants which were also decreased due to getting utilized completely to neutralize the ROS produced during lipid peroxidation.

Lipid Peroxidation:

Present study shows significant rise in the levels of MDA, as an index of lipid peroxidation in all study group patients of respiratory diseases as compared to control group which is suggestive of increased lipid peroxidation and oxidative stress.

It is also observed that in case of pneumonia and pulmonary tuberculosis, levels of lipid peroxidation are increased significantly as compared to asthma and bronchiolitis.

Increased levels of MDA are indicators of membrane damage, protein aggregation and inflammatory changes which is a basis of development of respiratory disorders. It also indicates decreased capacity of antioxidants and antioxidant enzymes to scavenge against superoxide radicals and these radicals initiate the peroxidation of unsaturated fatty acids resulting into increased oxidative stress

Poly Unsaturated Fatty Acids:

Present study shows decrease in %PUFA in all study group subjects as compared to control. This is due to the oxidation of PUFA by lipid peroxidation. These lipid hydroperoxides initiates the peroxidation chain propagation in lung epithelium.

In physiological conditions n3 PUFA inhibits the activation of nuclear factor kappa beta (NF-κB) and thereby transcription of number of inflammatory cytokines and chemokines.

Low levels of n3 PUFA trigger the inflammatory pathway by increasing gene transcription through activation of NF-κB. Activated NF-κB translocates into the nucleus, binds with the promotor site on DNA to commence gene transcription for cytokines and chemokines. This attracts

phagocytes, granulocytes eosinophils and mast cells at the site of alveolar tissue. This increases the inflammatory reactions and increase in ROS which are responsible for the observed pulmonary tissue damage. This induces symptoms like bronchoconstriction, elevated mucus secretion, and cause microvascular leakage, which leads to edema formation and pathogenesis in cases of respiratory disorders.

Paraoxonases:

(PONs) are enzymes which hydrolyze organophosphates. These enzymes show antioxidant as well as anti- atherogenic property.PON1 is mainly localized in clara cells, endothelial cells and in type1 pneumocytes in the lung. Clara cells are one of the oxidant resistant airway cells in lungs of all species and secretions of clara cells have a role in protection from oxidative stress.

In the present study significantly lowered activity of PON 1 was observed in all study group patients s as compared to control group. This might be due to shedding off and continuous replacement of clara cells by mucosal cells due to increased lipid peroxidation and increased oxidative stress.

Superoxide dismutases :

SOD is the first line of enzymatic defense against free radicals. These enzymes catalyze the dismutation of superoxide (O_2^-) into oxygen

and hydrogen peroxide. In the present study SOD levels are decreased in all respiratory disorders .No marked difference was observed among the study group. Decrease in SOD enzyme may be due to its extracellular secretion in lung tissue or due to its decreased synthesis. Thus decrease in SOD activity would expose the cell membrane and other components to oxidative damage.

Catalase:

(CAT) is an enzyme which catalyzes the decomposition of hydrogen peroxide to water and oxygen. It is a very important enzyme in protecting the cell from oxidative damage by reactive oxygen species (ROS). In the present study we found significant decrease in the activity of catalase in all study group patients of respiratory diseases as compared to control group, which indirectly shows increase in concentration of hydrogen peroxide in lung tissue.

Glutathione peroxidase and Glutathione reductase:

Glutathione reductase catalyses and regulates cellular levels of glutathione (GSH) and replenishes the reduced glutathione. Glutathione peroxidase reduces lipid hydroperoxides and hydrogen peroxide to alcohol and water in which GSH gets continuously utilized. It may result into decreased GSH/GSSG ratio. A high GSH/GSSG ratio is important for the protection of the cell from oxidative damage.

Disruption of this ratio induces activation of redox sensitive transcription factors, such as NF-kB, AP-1, nuclear factor of activated T cells and hypoxia-inducible factor 1, which are involved in the inflammatory response.

Present study shows decrease in glutathione enzyme system in all study group patients (except in Asthma group) which is suggestive of inability of glutathione enzyme system to protect the lung tissue from increased oxidative stress. In case of asthma GPx/GRx enzyme system was moderately working efficiently.

In case of prospective study group patients with post treatment all antioxidant levels were increased with decrease in serum MDA levels are suggestive of recovery of the patient with decrease in ROS production and lipid peroxidation. This could be due to the removal of the suppression of ARE gene by antibiotics or supporting therapy for pneumonia and/or bronchiolitis. If these patients were supplemented with antioxidant therapy the hospital stay could be reduced.

In case of asthma group patients, glutathione reductase and peroxidase levels are not significantly decreased which is suggestive of efficient working of glutathione enzyme system against the oxidative stress or reduced GSH was available in ample amount. There is also

possibility that there is no repression of glutathione enzyme system in Asthma patients.

Vitamin C (Ascorbic Acid):Water-soluble vitamin C shows its intracellular and extracellular antioxidant capacity primarily by scavenging oxygen free radicals. Vitamin C is an excellent reducing agent that prevents lipid peroxidation through its reaction with membrane α-tocopherol and thereby restores its scavenging activity.

Vitamin E (α-Tocopherol): Lipid-soluble vitamin E is concentrated in the cell membrane and is the principal defense against oxidant-induced membrane injury. Vitamin E donates electron to peroxyl radical, which is produced during lipid peroxidation. α-Tocopherol is the most active form of vitamin E and the major membrane-bound antioxidant in cell .α-Tocopherol (vitamin E) is secreted by type 2 in the respiratory epithelial lining fluid but at relatively low concentrations. It is a powerful antioxidant, both in terms of its direct free-radical scavenging activity and its ability to terminate lipid peroxidation.

Carotenoids (β-Carotene):

Carotenoids are pigments found in plants. Primarily β-carotene has been found to react with peroxyl (ROO), hydroxyl (OH), and superoxide (O_2^-) radicals. Carotenoids show their antioxidant effects in low oxygen partial pressure. Both carotenoids and retinoic acids (RAs) are capable of

regulating transcription factors. β carotene acts as scavenger as well as helps in growth of new alveolar tissue. β-Carotene inhibits the oxidant-induced NF-kB activation and interleukin (IL)-6 and tumor necrosis factor-Tα production. This effect of RA is mediated mainly by retinoic acid receptors.

Present study shows that all three antioxidants, vitamin C, vitamin E and β carotene are significantly decreased in all study group patients as compared to control group. It clearly indicates that they are utilized maximally to detoxify the flux of ROS but the concentration is not sufficient to withstand against increased oxidative stress. Dietary insufficiency of these antioxidants may be responsible for decrease in observed values.

Present study shows increased lipid peroxidation with decreased efficiency of antioxidant enzyme system and decreased antioxidants .This could be due to decreased synthesis of antioxidant enzymes at the molecular levels by repression of Nrf2 pathway.

Role of Nrf2 in regulating Antioxidant gene :

Nrf2, (nuclear erythroid 2–related factor 2) is responsible for activation of antioxidant response element (ARE). Nrf2 is present in cytosol attached to Keap1, causing inhibition of Nrf2 activity .

During an oxidative stress event, Nrf2 dissociates from Keap1, translocates to the nucleus then binds to the (ARE) in the promoter region. This activates transcription of number of antioxidant enzyme system genes.

It was observed that, in patients with respiratory disorders like, bronchiolitis, pneumonia, pulmonary tuberculosis, asthma and allergic conditions, dissociation of Nrf2 molecule from Keap1 could have altered either by viruses, bacteria, or some pollutants. This alteration in Nrf2 molecule may leads to suppression of ARE genes.

This leads to decrease in synthesis of antioxidant enzymes and effectiveness of antioxidant system which resulted into increased oxidative stress with disease progress.

CONCLUSION

The following conclusions are drawn from the present study

- In present study, oxidative stress was increased in bronchiolitis, pneumonia, pulmonary tuberculosis, and asthma subjects as evident from the increased MDA levels and thereby increased lipid peroxidation.
- In our study, decreased %PUFA and increased n6:n3 PUFA ratio was observed which could be the triggering factor for suppression of immune response and inflammatory pathways and could be leading cause for pathological changes in alveolar tissue for disease development.
- In case of infective disease group, antioxidant enzyme system was not significantly expressed as shown by decreased levels in all antioxidant enzymes.
- Decrease in levels of all antioxidant molecules signifies that all antioxidant molecules were getting exhausted to counterbalance the ROS load.
- In case of non infective disease group, i.e. asthma, glutathione enzyme system was slightly effective but all other antioxidant enzymes (SOD PON, CAT,) were significantly decreased. All

antioxidants were also exhausted showing significantly decreased concentration. This was noted as increased lipid peroxidation with increased oxidative stress.

- In our study, in case of follow up study, increased hospital stay along with increased oxidative stress was observed while lowered oxidative stress resulted into recovery from the disease and discharge from the hospital which signifies the importance of antioxidant enzymes in counterbalancing oxidative stress.

  Limitations: Dietary consumption of antioxidants and PUFA quantities were not taken into consideration.

Scope

- Evaluation of increased oxidative stress can be clubbed with pathogenesis of respiratory diseases
- In case of increased oxidative stress, controlled clinical trials by dietary supplementation of antioxidants such as vitamin C, vitamin A vitamin E and n3 PUFA and recovery of the patients could be evaluated.
- Genetic studies to evaluate role of Nrf2 in pediatric patients and supplementation of dietary factors to activate Nrf2 as therapeutic modalities can be considered.

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