Effect of Occupational Exposure on Cardiovascular and Hematological Parameters of Individuals working in Rice Mills Around Raichur Urban Area

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Certificate

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Dedicated to my parents

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Annexure-I

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Abbreviations

a-PM	ambient- Particulate matter
BMI	Body Mass Index
CAS	Chemical Abstracts Service
CBC	Complete Blood Count
CI	Confidence Interval
COPD	Chronic Obstructive Pulmonary Disease
CRP	C- Reactive Protein
CVD	Cardiovascular Disease
DBP	Diastolic Blood Pressure
DNA	Deooxyribo Nucleic Acid
DNPH	Dinitrophenyl Hydrazine
DTCS	Dinitrophenyl Hydrazine-Thiouracil-Copper Sulphate solution
ECG	Electrocardiography
EDTA	Ethylene Diamine Tetra Acetic acid
ETC	Electron Transport Chain
ETS	Electron Transport System
FEV_1	Forced Expiratory Volume
FVC	Forced Vital Capacity
GSSG	Glutathione disulfide
GSSH	Reduced Glutathione
Hb	Hemoglobin
HF	Heart Failure
HR	Heart Rate
IHD	Ischemic Heart Disease
IL	Interleukin
IUPAC	International Union of Pure and Applied Chemistry
LDL	Low Density Lipoprotien
LV	Left Ventricle
MAP	Mean Arterial Pressure
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
MCV	Mean Corpuscular Volume
MDA	Malondialdehyde
MI	Myocardial Infarction
mmHg	Millimeter of Mercury
NADP	Nicotinamide Dinucleotide Phosphate
NF-ßB	Nuclear Factor B
NIOSH	National Institute of Occupational Safety & Health
NO	Nitric Oxide
NOS	Nitric Oxide Synthase
OCD	Obsessive Compulsory Disorder
PCV	Packed Cell Volume
PEFR	Peak Expiratory Flow Rate
PP	Pulse Pressure
PUFA	Poly Unsaturated Fatty Acid
RBC	Red Blood Cell

RNS	Ribonucleic Acid
ROS	Reactive Oxygen Species
RONS	Reactive Oxygen Nonradical Species
RR	Relative Risk
SBP	Systolic Blood Pressure
SD	Standard Deviation
SEM	Standard Error of Mean
SOD	Super Oxide Desmutase
TAS	Total Antioxidant Status
TBA	Thiobarbituric Acid
TCA	Trichloroacetic Acid
TLC	Total Leucocyte Count
TNF-α	Tissue Necrosis Factor- α

Abstract

Title: Effect of Occupational Exposure on Cardiovascular and Hematological Parameters of Individuals working in Rice Mills Around Raichur Urban Area

Aim: To study the effect of occupational exposure on cardiovascular and hematological parameters of individuals working in rice mills

Materials & Methods: It was cross sectional study. 134 individuals were selected in each group.Hematological,PFTs,ECG, Blood Pressure and Oxidative stress markers were recorded by standard procedures. Statistical analysis was done by using one way ANOVA, unpaired student's 't' test and regression analysis.

Results: values were presented as Mean±SD at $P<0.05$ and 95% CI. There were no significant differences observed between exposed and control group. Higher normal level of NO & MDA was recorded and Lower normal level of Vitamin C was recorded and also lower normal level of FVC and FEV_1/FVC is seen among individuals exposed to dust for longer term than compared to short term exposed individuals. Similar values were seen among elderly age group individuals.

Conclusion: Though individuals are working in rice mills in dusty area, their blood counts, electrocardiographic recordings, blood pressure are not varied very significantly. Variations are within normal limits. Lung parameters, stressors and antistressors are also found in normal range. But, when individuals are grouped according to age and exposure duration, elderly age group and long term exposure groups have shown mild decrease in PFTs. These groups have also shown increased stressor level to upper normal values and decreased antistressor to lower normal values. As the age advances adverse effect of dust on serum NO level is enhanced. This is an alarming message for individuals working in rice mills that continued and longstanding exposure may lead to increased oxidative stress and oxidative damage.

Key words: Rice dust,PFTs,ECG, Serum NO, Serum MDA,Vitamin C and Oxidative stress.

INTRODUCTION

1. INTRODUCTION:

Rice is an important food for the world. The largest rice growing contries are China is in the first position and India is being the second. In India since ancient times rice processing is largest agro processing industry in India. Due to exposure to dust at workplace, morbidity is common among the individuals working in rice mills. Respiratory and cardiovascular health of rice mill workers is at risk. Occupational health hazard is- ill effect on health experienced at workplaces. Occupational health hazard can be chemical hazard(organic or inorganic substances), biological hazard(microorganisms) or Physical hazard (Radiation).According to the "epidemiological studies, the increase of respiratory and cardiovascular mortality and morbidity are associated with increased air pollution and increased particulate matter [PM]" [1][2]. At cellular levels PM can cuase oxidative stress by causing production of free radicals, or damage due to endotoxin release, inflammation may also be mediated by cytokine release and may also stimulation of capsacian receptors. Several interactions between PM and biological molecules trigger initiation or progression of diseases. The most important mechanism are generation of ROS[reactive oxygen species], oxidative stress and inflammation. *Pope CA III et al* [3] and Brook RD [1] have shown that increased level of air pollution may lead to cardiopulmonary diseases and consequently to death. The chemical and mechanical stimulation of re eptors cause change in autonomous nervous system activity in turn can also lead to change in blood pressure. The studies were conducted on carpenters & non carpenters[4] and sand stone mine workers[5] to know the blood pressure changes. According to these studies, due to dust exposure there were statistically significant variations in blood pressure. There are evidences which show that alterations in blood cell counts and increase in oxidative stress caused by exposure to dust. "This can be due to the mechanisms of allergic reactions and inflammation which are evoked by dust entering the lungs. These mechanisms may also cause dermatitis and hypersensitivity reactions" [3],[4]. Occupational dust exposure predominantly affects the pulmonary system and airways. Dust inhalation at work place causes airway irritation and allergic reactions. [6],[7].

The studies also have found there is a decrease in RBC count, PCV, MCV,Hb content and MCHC. Also there was an increase in lymphocyte and eosinophil counts[4],[8],[9],[10],[11],[12],[13],[14]. Further studies on rice mill workers for rice dust exposure have reported “non-specific irritative effects like kerato-conjunctival irritation, corneal scars, chronic conjunctival inflammation, pterygium, pruritis, asthma, chronic bronchitis, allergic bronchitis, backache and knee joint pain etc. The rice husk dust is also found to contain mechanical irritants and biological activators for inflammatory cells. The decrease in Hb content, RBC count and Eosinophila in rice dust exposed individuals was reported” [15],[16],[17],[18],[19],[20],[21].

A wood dust exposure also showed the effect similar to that of rice husk exposure. The researchers also revealed that wood dust exposure causes variation in blood pressure. The organic substances present in wood dust causes allergic hypersensitivity reactions like pneumonitis, contact dermatitis, eczema, irritation of eyes and nose, conjunctival inflammation etc.[4],[21],[22].

Occupational dust exposure predominantly affects the Respiratory system. Dust inhalation at work place causes airway irritation and allergic reactions. “The pulmonary defense mechanisms for inhaled dust particles consisting of three interrelated system and work together mechanical air filtration, liquid mucus which serves as a physical and chemical defense contains material which has bactericidal and detoxifying properties and specific defense by lungs are divided into two systems the primary humoral immunity (antibody production) and cellular immunity (T lymphocytes). *Macrophages* act as a cellular defense system that cleans all the small particles. *Alveolar macrophages* to clean particles deposited by phagocytosis mechanism” [23]. “Dust exposure can lead to Obstructive or Restrictive type of lung diseases. Basic tools for evaluating the effect of exposure on respiratory system include pulmonary function tests(PFT)” [24],[25]. Therefore, we have to determine PFTs for supporting the diagnosis of obstructive and restrictive lung diseases. In addition, pulmonary function tests are useful in distinguishing between obstructive and restrictive ventilatory disorder. Obstructive ventilatory disorders affect the ability to exhale, and restrictive ventilatory disorder affecting the ability to inspire air [26]. Risk factors other than tobacco smoking, most notably the sequelae of lung infections (including tuberculosis), as well as occupational, environmental and domestic air

pollution, are important in many parts of Asia and Africa and must be evaluated more thoroughly to enable effective action.[27]

Many researchers have conducted studies on exposure to ambient air pollution on body systems. The exposure may lead oxidative stress . which can cause damage to many components of the cell . Oxidative stress markers mainly increase in lipid peroxide and free radical level. Predominant oxidative markers are MDA and NO.

The source of MDA is lipid peroxidation which damages the cellular component by releasing free radicals. NO on the other hand, found to have adverse effect on myocardial cells and endothelial cells.

These oxidants result in atherosclerotic hypertension,endothelial dysfunction, preeclampsia, cerebrovascular and cardiovascular shocks. These oxidants also cause CVDs like myocardial infarction, coronary syndrome, IHD etc.[28,29,30,31,32,33]

The increase in oxidant levels cause damaging effect on genetic component of cells and also cellular functions.[34]

Cotton dust exposure also has been shown to cause adverse effect on body systems. The exposure caused increased lipid peroxidation, increased NO levels. The increase in oxidative stress and they result in exhaustion of antioxidants which in turn increase the oxidative stress and tissue damage [35,36].

A cement dust exposure is having deleterious effect on blood, cardiovascular, respiratory and liver functions. There is decrease in erythrocyte count and PCV. The exposure to cement dust causes increase in inflammatory activity. The inflammatory reactions cause tissue damage. In support of this, there are studies conducted on cement dust exposure and lipid peroxidation. They found that exposure causes increase in serum levels of NO and MDA. Also few studies revealed, there is decrease in plasma Vit-C level associated with increased oxidative stress. These studies strained on role of vit-C in preventing vascular dysfunctioning and regulation of NO. [37,38,39,40]

The cardiovascular and hematological abnormalities are related with decreased Vit-C antioxidant level.[41,8,37].

AIMS AND OBJECTIVES OF THE STUDY

2. AIMS AND OBJECTIVES OF THE STUDY

2.1- Aims and Objectives

- To compare the Pulmonary, Hematological, Cardiovascular and Biochemical parameters of Rice mill workers with those of control group.
- To determine the effect of duration of exposure to rice dust on Pulmonary, Hematological, Cardiovascular and Biochemical parameters of Rice mill workers.
- To study the changes in PFTs, BP, ECG and Absolute counts of WBCs as well as Oxidative Stress related changes in levels of MDA, NO and Ascorbic Acid in Rice Mill workers.

2.2- Hypothesis:

- **Null Hypothesis:**
  Occupational exposure to dust may not have significant effect on Cardiovascular, Hematological and Pulmonary function parameters and exposure to dust does not affect the levels of oxidants and antioxidants in blood.
- **Alternate Hypothesis:**
  Occupational exposure to dust cause significant variation in Cardiovascular, Hematological and Pulmonary function parameters and exposure to dust increases serum oxidative stress markers like MDA, NO and Vitamin-C.

REVIEW OF LITERATURE

3. REVIEW OF LITERATURE:

Many studies have found that “increased cardiovascular morbidity and mortality is associated with exposure to ambient air pollution, particularly particulate matter” [(*Hoek et al.* 2001)[42]; (*Peters et al.* 2001)[43];(*Pope et al.* 2002)[44];(Pope & Dockery, 2006)[45] and (*Mills et al.* 2008)[46]]. Till now, there is limited knowledge of the underlying mechanisms in pathogenesis. But it has been proposed that, “there is an association between increased blood levels of inflammatory cytokines such as Tumot Necrosis Factor-α [TNF-α]and its receptors, interleukins-6 [IL-6], Adhesion molecules and acute phase proteins such as C-reactive Protiens(CRP) and Fibrinogen in the risk of cardiovascular events including myocardial infarction due to inhalation of fine particles. Another expression of this response is, increased number of neutrophils in the peripheral blood which is an indicator of ischemic heart disease (IHD)” [*Sjogren,* 1997,[47],*Seaton et al.,* 1999[48], *Pai et al.,* 2004[49] and *Mills et al.,* 2008][46]. “There is often high exposure to particulate air pollution such as silica dust, asbestos or welding fumes, wood dust in occupationally exposed groups. But there is no clear evidence whether such dust exposures increase the risk for ischemic heart disease or not” [*Tore´n et al.,* 2007,[50] and *Delfino et al.,* 2008][51]. A study on carpenters was carried out to look into the role of probable mechanism behind increasing risk of Ischemic Heart Disease(IHD) by long term occupational exposure to airborne wood dust particulate emitted during carpentry work. They observed a significant increase in serum malondialdehyde with significant decrease in antioxidant level among carpenters than control group.

Stavros A et al have observed that “chronic release of reactive oxygen species (ROS) is linked to the development and progression of HF” [52]. Experimental and clinical HF trials have shown “an increased production of ROS, such as superoxide, hydrogen peroxide and hydroxyl radicals. Other different pathways are also implicated in the increased ROS production in the failing heart, including NADPH and xanthine oxidase, dysfunctional nitric oxide synthase and the mitochondrial electron transport chain” [53]. “Abnormal activation of the phagocytic NAD(P)H oxidase in response to neurohormonal activation can contribute to cardiomyocyte apoptosis and matrix metalloproteinase activation, leading to remodelling of the

failing LV. Nonetheless, the major consequences of high ROS levels in HF may relate to endothelial dysfunction”[54].

3.1- Air particulate matter- Size, Constituents, Sources and Role in Oxidative stress:

“Air particulate matter (a-PM) otherwise known as aerosol is a major atmospheric pollutant [considering the vast sources of emission (Table 1)] with a composition mixture of particles (solid, liquid or both) suspended in air (*Seaton et al.,* 1995)” [55].

Table 1 : “Summary of PM size, constituents and possible sources

Particulate matter size	Aerodynamic diameter (μm)	Constituents	Sources
coarse fraction (PM2.5-10)	2.5 – 10	Dust, Endotoxin, pollen, fungi debris, ground materials, metals.	Agriculture, soil, road dust, sea spray, suspension in air from grinding and erosion
fine particles PM2.5	< 2.5	Organic/elemental carbon, organic compounds, hydrocarbons	Primary from all combustion sources including coal, oil, wood and gas
Ultra-fine particles PM0.1	< 0.1	Primary combustion-hydrocarbons, metals, organic carbon	Fresh automobile and combustion emissions. Volatile and Semi-volatile organic carbons Secondary photochemical formation from gases

Source: *Brook*, 2008”.

“Depending on emission sources (natural or anthropogenic), a-PM contains complex mixture of chemical and/or biological components [*Alfaro-Moreno et al.,* 2002, *Soukup & Becker*, 2001]” [56][57]. “This composition represents there is a complex mixture of organic, inorganic and biological components including viable or non-viable microorganisms and fragments of microorganisms which could include toxic components like endotoxin and mycotoxins (*Gangamma*, 2012)[58] varying in size, composition and origin with properties summarized based on their aerodynamic

diameter” (Table 1). Particulate matter (PM) is mostly composed of sodium chloride, nitrates, sulphates, mineral dust, carbon, ammonia and water. “Depending on the mechanism of formation these particles are classified as primary and secondary. Although, primary particles are emitted into the atmosphere by natural processes, the greater sources of primary particles emitted into the atmosphere are, anthropogenic processes such as combustion from solid fuel; car engines; combustion in households and industrial activities” [*Hammond et al.,* 2008[59]; *Watson & Chow*, 2001][60]. According to the field measurements conducted in the milling section of several rice mills by central pollution control board “the particulate matter emission measured in the stack is quite high in the range of 5050 mg/Nm^3. This indicates inadequate control system for controlling the particulates. Ambient suspended particulate matter concentration is 255 to 810µg/m^3 and PM<10 is 180 to 626 µg/m^3”. It shows that in some areas the fugitive emissions lead to higher suspended particulate matter concentration in the ambient air.

Several studies have been carried out on lung function parameters to know the effect of dust exposure at workplace. It has been proved that there are several adverse effects of dust exposure on lung functions. A study carried out by *Purushottam Pramanik and Archana Chaudhury* proved that “Occupational exposure to wood dust in carpentry harmfully affects lung function. This mutilation is associated with duration of exposure to wood dust”. “Occupational exposure to wood dust in carpentry for more than 10 years increases the risk of asthma” [61]. Many studies have found “association between increased cardiorespiratory hospital admissions, along with cardiorespiratory mortality and daily variations in ambient particulate air pollution (*Nyberg and Pershagen*, 2000)” [62]. An approach to assess the “potential chronic effects of air pollution is to determine changes in lung functions by measuring lung volumes and capacities. Spirometry is the most commonly used among all the pulmonary function tests to diagnose and monitor respiratory problems. Moreover, to produce norms or policies related to preventive measures against occupational air pollutants, the measurement and monitoring of lung volumes and capacities are done in order to rate occupational hazard is used (*NIOSH*, 2012)” [63].

According to a recent review of chronic obstructive pulmonary disease (COPD), “ 25% to 45% of patients diagnosed with the disease have never smoked, which makes occupational exposure as an attributing cause of COPD more likely

(*Salvi and Barnes*, 2009)[64]. In developed countries, an estimated 15% of all COPD is attributable to occupational exposure (*Hnizdo and Vallyathan*, 2003[65]". A Malaysian study has shown "total dust exposure is associated to respiratory symptoms such as chest tightness, cough, phlegm and also with lung function indices (*Noor et al.,* 2000)" [66]. Similarly, in panel studies it is stated that "acute episodes of particulate air pollution have been associated with increased incidence of respiratory symptoms and decreased lung functions (*Pope et al.,* 1995)" [67].

The study carried out by *Bushra Iftikar et al* revealed that "majority of respondents who were exposed to silica dust for ten years or more had respiratory problems. The severity of the problem was directly proportional to the duration of exposure to silica dust, density of dust (maximum in stone crushing), hours of daily exposure and other contributory factors like tobacco smoking and increasing age" [68].

A study conducted by *Bhat MR and Ramaswamy C* [69] and *Erdinc osman* [70] , in saw mill workers showed that significant reduction in FEV_1, PEFR and FVC of saw mill workers as compared to controls.

"The pathophysiological aspects of a drop in the values of the aforesaid lung function parameters, FVC is decreased in pulmonary obstruction, emphysema, pleural effusion, pneumothorax, pulmonary edema and poliomyelitis" [71] . Similarly, "the FEV_1 value is low in obstructive lung diseases and in reduced lung volume" [72]. "The decline in FEV_1 is a convenient standard against which we can measure marked declines in subjects with the history of chronic obstructive pulmonary disease (COPD) or in subject exposed to environmental pollutants" [73].

According to the reputed biological mechanisms, "air pollution to heart disease linkage involve the direct effects of pollutants on the CVS, blood/lung receptors and/or indirect effects also mediated through inflammatory responses and pulmonary oxidative stress (*Brook et al.,* 2004)" [74]. "The direct effects may possibly mediated through many variety of agents which can readily cross the pulmonary epithelium into the systemic circulation. Within the systemic circulation, these direct effects stand for a probable explanation for the mechanism of rapid cardiovascular responses such as increased myocardial infarctions (*Peters et al.,* 2001)" [43]. "The intermediary and chronic indirect effects possibly occur via pulmonary oxidative

stress and/or inflammation induced by inhaled pollutants and result in activation of haemostatic pathways causing vascular dysfunction and acceleration of atherosclerosis [*Mutlu et al.,* 2007, *Nemmar et al.,* 2003]" [75] [76].

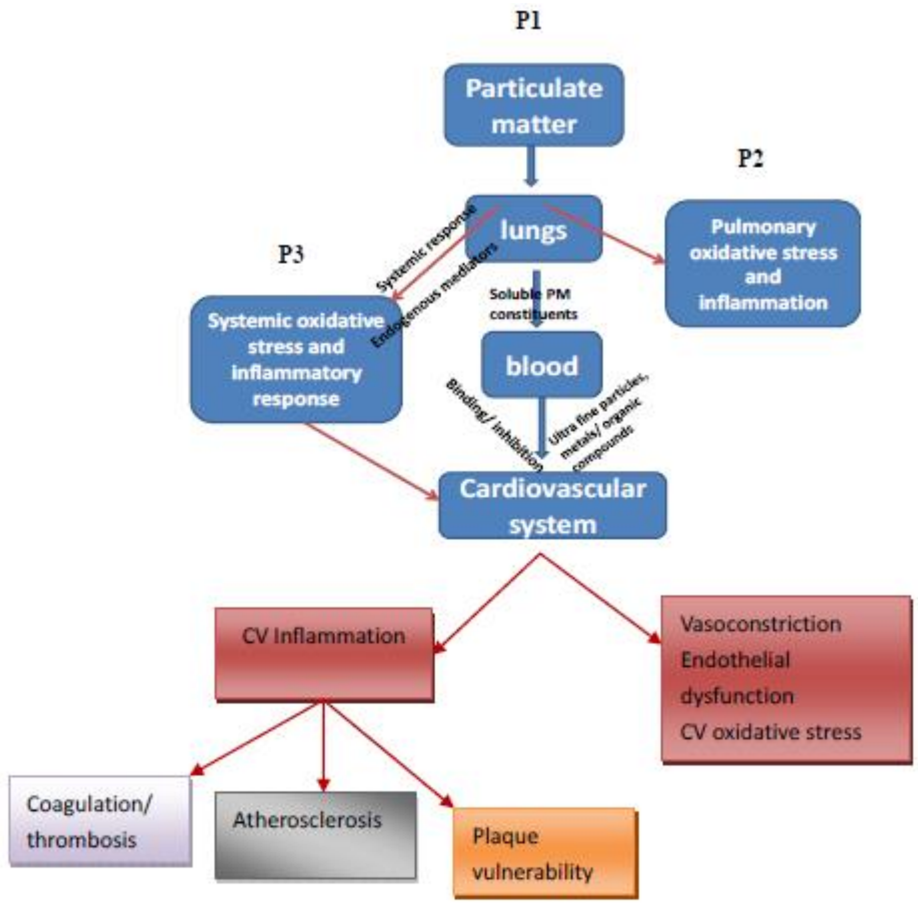


Figure -1: "The possible pathways through which particulate matter exerts toxicity leading to initiation of atherosclerosis and progression (P1: pathway 1, P2: pathway 2, P3: pathway 3)" [85]

"The lung is an important target for pro-oxidant compounds mediated genotoxicity because the bronchial epithelium being a physicochemical barrier plays a crucial role in initiating and augmenting defence as well as signaling systemic responses (*Vineis et al.,* 2004; *Mills et al.,* 1999; *Ollikainen et al.,* 1998)" [77] [78] [79]. "Mechanisms of PM at cellular level involve free radical production (by transition metals and organic compounds), oxidative stress, cytokine release, inflammation, endotoxin-mediated damage, stimulation of capsaicin receptors, autonomic nervous system activity, covalent modification of key cellular molecules and increased pro-

coagulant activity (*Araujo & Nel*, 2009; *Brook*, 2008; *Mills et al.,* 2008; *Bhatnagar*, 2006; *Nel et al.,* 1998)" [80] [81] [82] [83] [84].

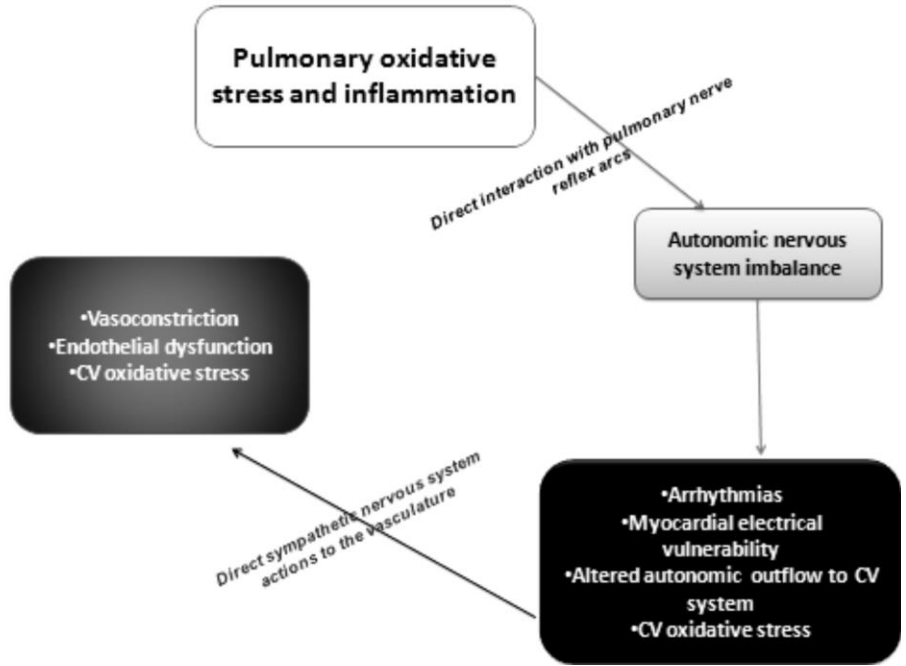


Figure -2: "The possible pathways through which particulate matter enhances atherosclerosis progression via the pulmonary oxidative stress pathway"

Obinaju proposed that "Particulate matter constantly keps changing and continuously interacts with gaseous, semi-volatile and volatile compounds. A broad variety of these vapour phase compounds attach to the surfaces of PM and form secondary aerosol particles. The cascade events which initiate or aid the progression of disease conditions through cellular responses are triggered by the various interactions between particulate matter and biological molecules which could give rise to oxidized and mutagenic lesions such as are found within the atherosclerotic plaque and cancers with the most important mechanisms possibly being ROS generation, oxidative stress and inflammation" [85].

Attention has focused on the level of PM_{10} in cities where pollution is routinely monitored and hence the association between most deaths occurring due to cardiovascular and respiratory illness are best evaluated. "Typical urban PM_{10} is comprised of up to 50% by mass of combustion derived, ultrafine carbon centered particles with associated metals and transition metals. Along with major components

like ammonium salts of nitrogen, sulphur and chlorine plus geological dust and organic matter" [86]. "There has been extensive discussion as to which composition of the PM_{10} particle might be causing its associated adverse health effects. There are number of the components of PM_{10} have been hypothesised to drive the toxicological effects, one such component being the ultrafine fraction" [87,88]. There is a suspicion on "the combustion derived ultrafine particles because of the general perception that most of other components are relatively harmless at the measured exposure levels and also toxicological evidence that ultrafine particles and metals have potential toxicity. It has also been hypothesized that endotoxin drives the inflammation. Endotoxin is a component of some PM samples, especially those collected in rural locations where wind blown soil is a major component of PM_{10}. Research has shown adverse effects of environmental particles at the levels found in European and UK cities, but there is heterogeneity in magnitude of the health effects estimates in different cities" [89].

Ken Donaldson et al suggested following "mechanisms of adverse effects of ultrafine particles:

- There is good toxicological evidence that ultrafine particles cause inflammation in the lungs even when composed of relatively low toxicity materials".

- The mechanism of the induction of inflammation appears to be via oxidative stress and Ca2+ signaling perturbations".

- Ultrafine particles can also inhibit phagocytosis more than the same mass of fine particles" [90].

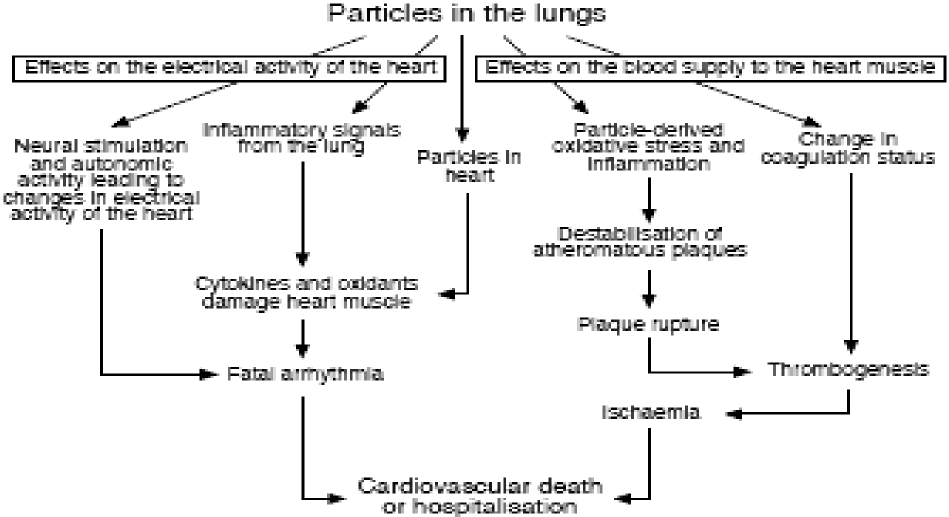


Figure -3: "Hypothetical events that link particles exposure via the lungs to adverse cardiovascular effects". (Ken Donaldson et al[90])

"Ambient PM does consist of complex and various mixtures of particles suspended in the breathing air" [91]. "Major sources of PM are factories, power plants, refuse incinerators, motor vehicles, building activity, fires and natural windblown dust. The size of the particles vary. There is a strong evidence supporting that ultrafine and fine particles are more hazardous than larger ones in terms of mortality and cardiovascular and respiratory effects" [92].

Polymorphonuclear neutrophils are very important in the body's acute inflammatory process. Lymphocytes, macrophages and monocytes are mononuclear phagocytic cells, which are indispensable in the body immune system and chronic inflammatory response. Eosinophils respond to chemotactic substances produced by mast cell when introduced by the presence of persistent antigen-antibody complexes such as chronic parasitic, dermatological and allergic conditions while Basophils, whose granules contain a number of preformed mediators of the inflammatory response including Histamine and Chondroitin sulphate also stimulate leukotriene and other mediators upon stimulations.

K. Rudrama Devi et al have found that peripheral blood neutrophil,monocyte,eosinophil,lymphocyte and basophil counts of Bidi factory workers were significantly increased in comparison with controls[93].

B.P. Chattopadhyay has noticed the allergic responses like itching, redness, watering of eyes,running nose,sneezing,coughing,breathlessness etc with increased eosinophil count in storage house grain dust exposed individuals. They have not found significant variation in total leucocyte count, as well as absolute counts of monocyte, neutrophil and lymphocyte[94].

"Monocytes play a principal role in immune defence, inflammation and also in tissue remodeling. The mechanism behind these roles are phagocytosis, antigen processing and presentation and also by cytokine secretion" [95]. Monocytes are heterogenous cellular populations which are multifunctional. *Metchnikoff* described "an evolution of the infiltering cells in inflammatory exudates. He has first observed and proved the phenomenon of plasticity of monocytes" [96].

Under several stimuli, macrophages are formed by the differentiation of circulating monocytes. "Macrophages are phagocytic in nature, specialized in removing unwanted cellular and extracellular debris, invading microorganisms and other foreign matter by various forms of endocytosis mechanisms" [97].

"Monocytes have been involved in the pathophysiological processes underlying coronary heart diseases and subsequent ischemic cardiomyopathy and also involved in atherosclerosis" [98].

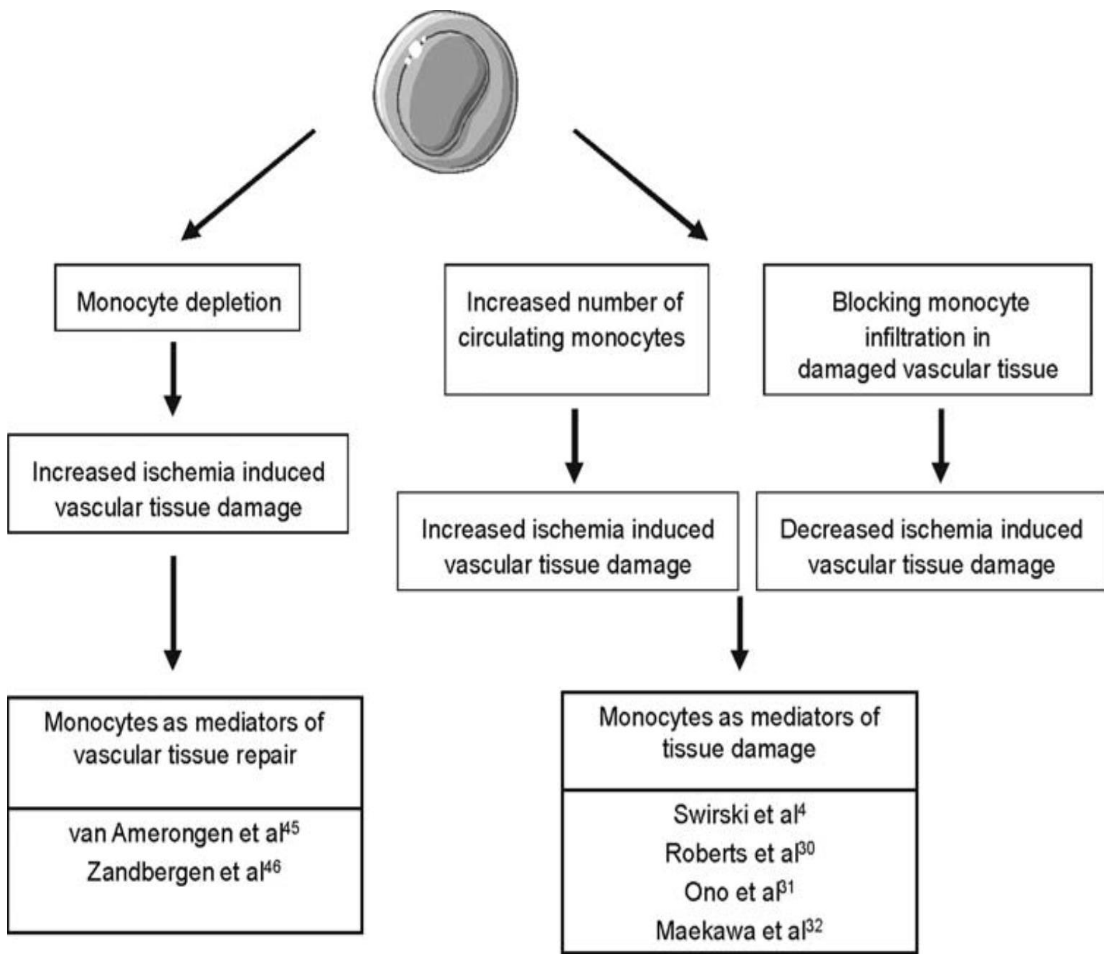


Figure -4: "The role of monocytes either as mediators of vascular tissue injury or as mediators of vascular tissue repair". Starvos A [52]

There is also decisive evidence found in support of a "significant role of monocytes in the process of angiogenesis which is a significant step in the process of tissue repair following ischemic myocardial injury. There is increased monocyte activation in post ischemic injury phase which is more likely an attempt for tissue repair rather than a deteriorating immune hyperreaction. In this fashion initiation of monocyte activation is a potential therapeutic target rather than inhibition of monocytes activation" (Figure 5) [52].

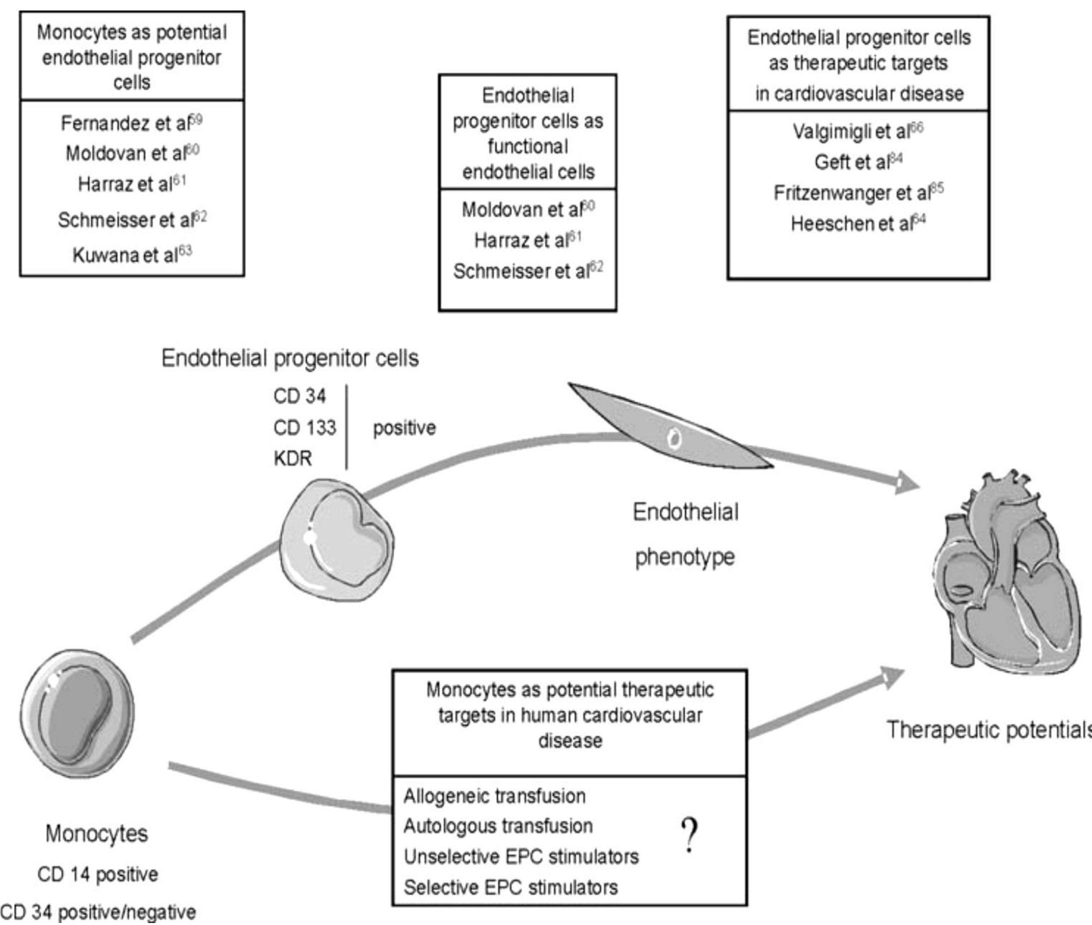


Figure -5: "Representative bibliographic data suggesting the role of monocytes in vascular tissue repair"

"All animals have got protective mechanisms against invasion of microorganisms, parasites, fungi, viruses and any foreign molecules. Phagocytosis in immunity is the major mechanism by which microbes are removed from the body and is especially important for defense against extracellular microbes. The mechanism of the phagocytic process is the killing of microbes by attacking microbes with oxidants (superoxide and hydroxyl radicals, hydrogen peroxide, nitric oxide, etc.). With the defiecient antioxidant reserves, cellular machinery will be damaged by the action of free radicals, thereby decreasing the effectiveness of the immune cell. The life span of immune cells is reduced with limited antioxidant capacity and an infection can become established or severity of an infection can also increase (*Weiss*, 2005)" [99].

3.2- Oxidative stress

"A free radical is any species capable of existence, containing one or more unpaired electrons" [100]. This unpaired electron is thus free to react with adjacent molecules in an effort to achieve stability, effectively modifying the function/structure of the once non radical species, potentially resulting in a chain reaction sequence of radical formation. "Although large number of free radicals exist [transition metal ions, carbon centered radicals (e.g., trichloromethyl), hydrogen atoms, sulfur centered radicals (e.g., thiyl)]" [100], those formed within living systems are in primary initiation by the molecular oxygen consumption. Moreover, "RONS(Reactive Oxygen Nonradical Species) are, both oxygen and nitrogen centered radicals, as well as the non radical species created via interaction with such radicals. RONS are regarded as the most important class of radicals generated in biological systems" [101]. "The body's antioxidant defense system serves to protect the cells from excess RONS production and is comprised of both enzymatic (superoxide dismutases, catalase, glutathione peroxidase, etc.) and non enzymatic (bilirubin, uric acid) endogenous compounds, as well as exogenous nutrients consumed within the diet (carotenoids, tocopherols, vitamin C, bioflavonoids, etc.)" [102]. "The production or formation of RONS in vivo and their subsequent removal via the antioxidant defense system is a continuous and delicately balanced process present within living systems which will in turn elicitates both positive and negative effects on physiological function. This delicate balance (RONS production vs. antioxidant defense) serves to determine the intracellular redox state" [103], which plays a role in optimizing cellular function (initiating cell signaling, aiding antibacterial immune responses and apoptosis) as well as gene expression [104]. However, "disruption of redox balance in favor of free radical expression resulting from either exacerbated RONS production and/or decreased antioxidant defenses is referred to as oxidative stress. This oxidative stress occurs secondary to the exposure to certain environmental (radiation, cigarette smoke, ozone) and/or physiological (consumption of dietary nutrients, physical exercise) stressors" [105]. "Over expression of RONS can then result in oxidative damage to various biological components, including nucleic acids, lipids and proteins, which over time has the potential to contribute to the development of disease" [106].

3.3- Types of Reactive Oxygen Species

"Most reactive oxygen species are by-products generated during mitochondrial electron transport. In addition ROS are formed as necessary intermediates of metal catalyzed oxidation reactions. There are two unpaired electrons in separate orbits in outer electron shell of an atomic oxygen. This structure of electron makes oxygen susceptible to radical formation. The process of sequential reduction of oxygen by the addition of electrons leads to the formation of a number of ROS including: superoxide; hydrogen peroxide; hydroxyl radical; hydroxyl ion; and nitric oxide" (Figure 6) [107].

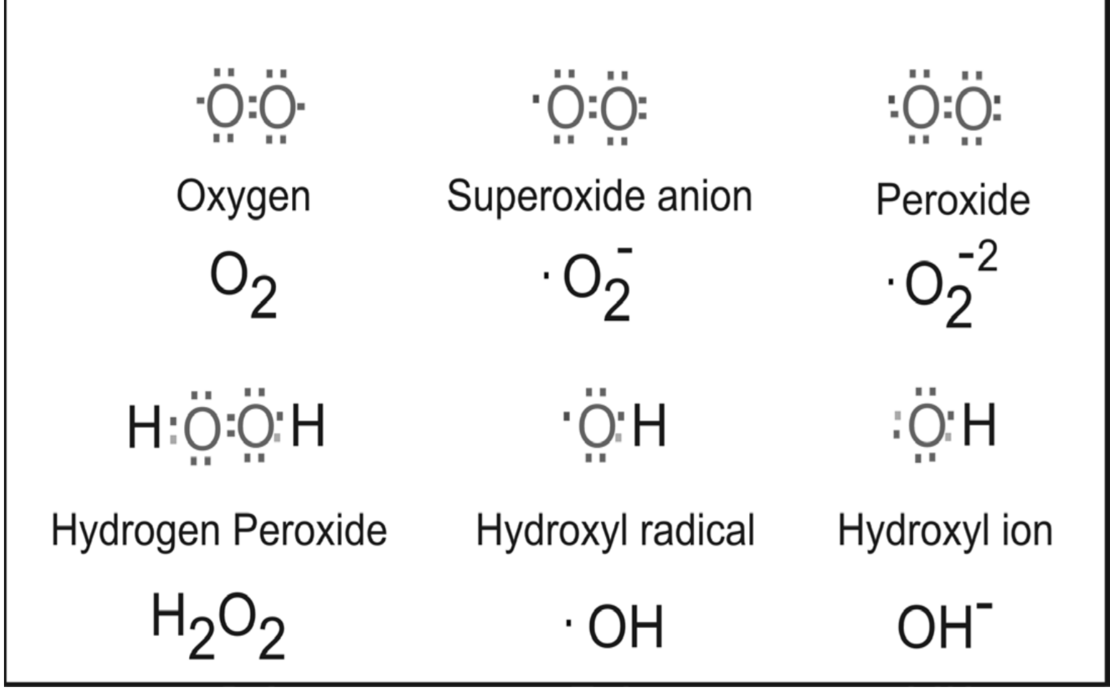


Figure -6: "Electron structures of common reactive oxygen species. Each structure is provided with its name and chemical formula. The • designates an unpaired electron". [107]

3.4- Free Radicals

Biological life is a combustion process which needs oxygen. But it is also endangered by reactive oxygen metabolites, so called reactive oxygen species. *Behnke* in 1936 carried out the first experiment in humans demonstrating the effects of oxygen toxicity[108].

A free radical is a molecule or molecular fragment that contains one or more unpaired electrons in its outer orbital. The unpaired electron and the radical nature of a species have conventionally indicated by writing it with a heavy superscript dot. Most of the oxidation reactions in aerobic organisms ensure that molecular oxygen has completely reduced to water. However, sometimes partial reduction of the oxygen occurs in the tissue. "Free radicals can be formed by three ways-

1. By the hemolytic cleavage of the covalent bond of a normal molecule with each fragment retaining one of the paired electrons.
2. By the loss of a single electron from a normal molecule.
3. By the addition of a single electron to a normal molecule.

Single electron transfer is the most common process in biological system, whereas hemolytic cleavage requires high energy input from high temperature, UV radiation or ionizing radiation . Free radical conversion of oxygen in the body is about 1-4%" [108].

During normal oxidation of food stuffs, due to leak in ETC in mitochondria, free radicals are constantly formed. This is the major source of generating oxygen reactive species under normal conditions These free radicals can be positively charged, negatively charged or can be neutral.[109]

There are several enzymes such as xanthine oxidase, aldehyde oxidase, peroxidases etc. which form superoxide anion radicals[110]. The free radicals are characteristically have short life span, generate new ROS by chain reactions, extremely reactive and can damage various molecules[111].

These ROS can react with variety of biological molecules like carbohydrates, proteins, lipids, nucleic acids etc and can also lead to various pathological conditions like cancer, atherosclerosis, vascular dysfunctions, myocardial infarction etc.

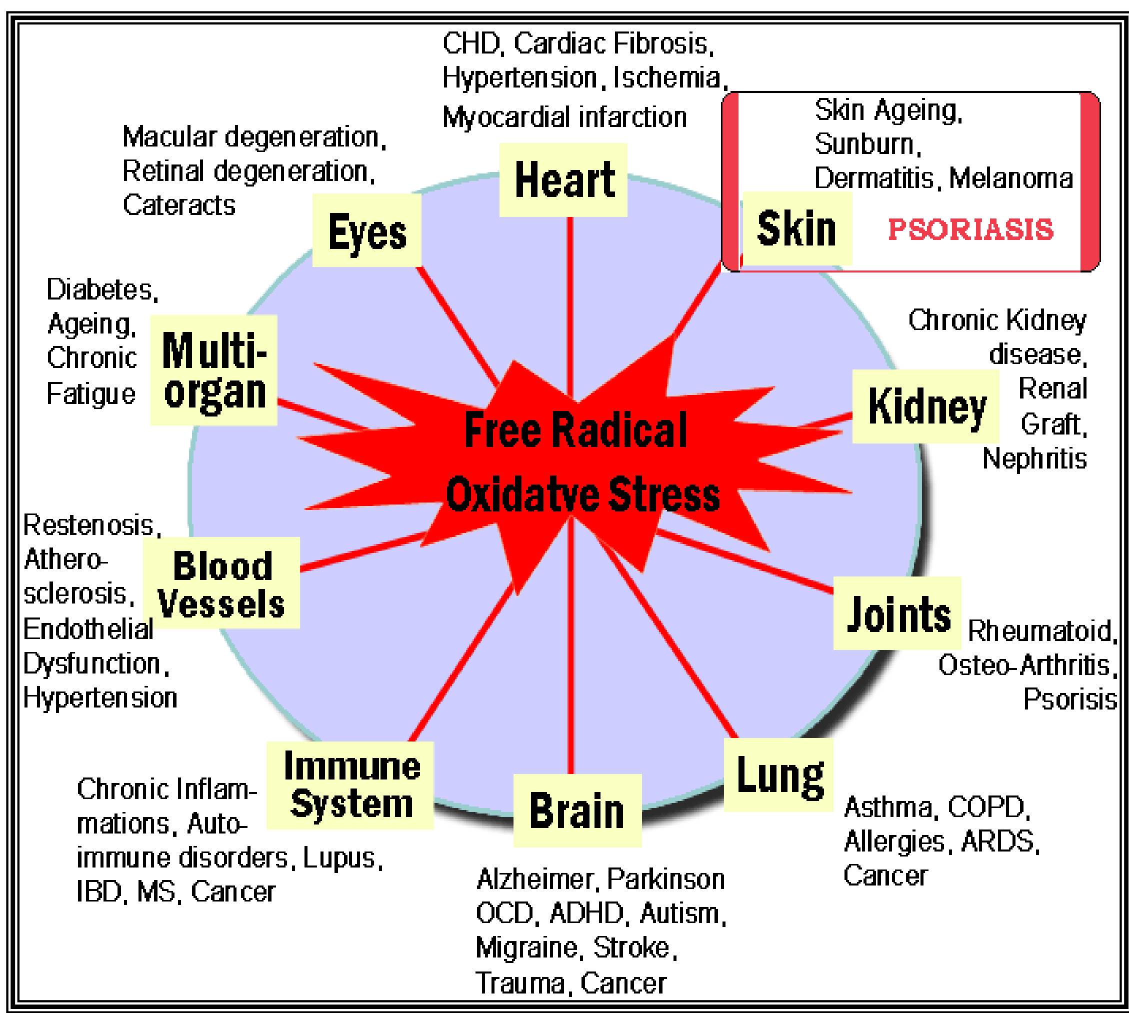


Figure -7: Sources of free radicals can be exogenous or endogenous.

Exogenous sources of free radicals constitute electromagnetic radiation, UV rays, Vehicular Exhaust, smoke, organic or inorganic dust exposure.Endogenous sources for free radicals are mitochondrial ETS[112,113,114,115], respiratory burst by phagocytes, microsomes[116] and peroxysomes[117], β oxidation of fatty acids [110], other leucocytes infiltered during infection[118]

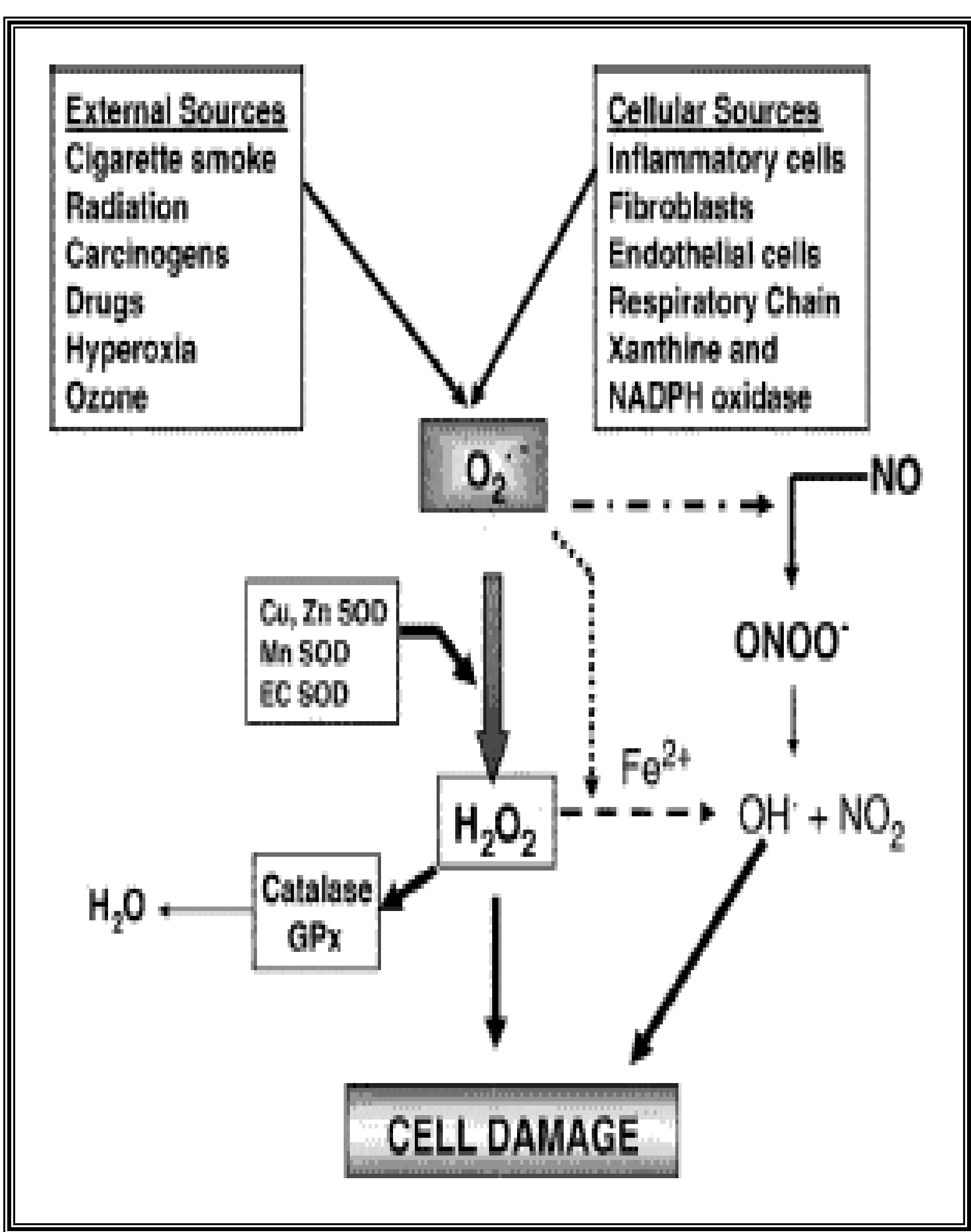


Figure -8: Showing sources of free radical

3.5- Oxidants

In the mitochondria, by using cytochrome oxidase, the molecular oxygen is completely reduced to form water. However, partial reduction of oxygen causes formation of reactive oxygen metabolites. These metabolites are free radicals themselves or may be non radical compounds which can potentially induce radicals and radical chain reactions in biological system[119].

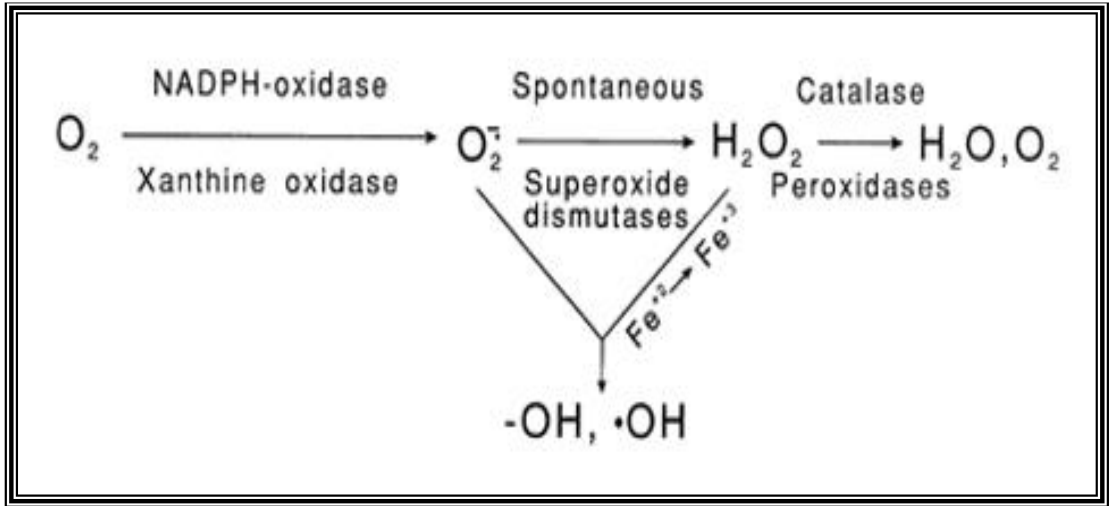
The different reactive species are Superoxide radical $O_2^{\bullet}$, Hydroperoxyl radical ($HO_2^{\bullet}$), Hydrogen peroxide (H_2O_2), Hydroxy radical ($OH^{\bullet}$) [120], [121]. There are carbon, sulfer and nitrogen centered radicals also(Fig-6). There are carbon centered radicals which are formed during lipid peroxidation[108].

Reactive oxidants in the body are H_2O_2, singlet oxygen, Ozone, hydroperoxides, hypobromic and hypochloric acid, excited carbonyls. These reactive oxidants may cause 'oxidative injury' [108]

Due to high reactivity, these free radical species are highly unstable. "Free radicals of intermediate reactivity are able to diffuse over significant distances and may then react with some specificity and selectivity with target molecules. They are the most likely species to lead to direct biological damage" [108].

Superoxide anion radicals ($HO_2^{\bullet}$)→ source of superoxide anion radicals in mammals are mitochondria. It is generally formed by auto oxidation of endogenous metabolites and also altered ETC activity in mitochondria[112,113]. Superoxide anion radical ($O_2^{\bullet}$) is generally not a highly reactive with lipids, proteins, polysaccharides and nucleic acids. Superoxide anion are charged species, poorly reactive in aqueous solution and highly reactive in hydrophobic environment. Superoxide anion forms hydroperoxy radical which is a stronger oxidant. It is more lipid soluble and have longer half life[108].

Hydrogen Peroxide: it is a most stable intermediate of oxygen reduction product. It is considered the major cytotoxic product formed by the xanthine/ xanthine oxidase system[121].



Hydroxyl radical: It is a strong oxidizing species and it readily reacts with any kind of biological molecule in the body system. Hydroxyl radicals may presumably generate during the respiratory burst of the neutrophils and macrophages[118].

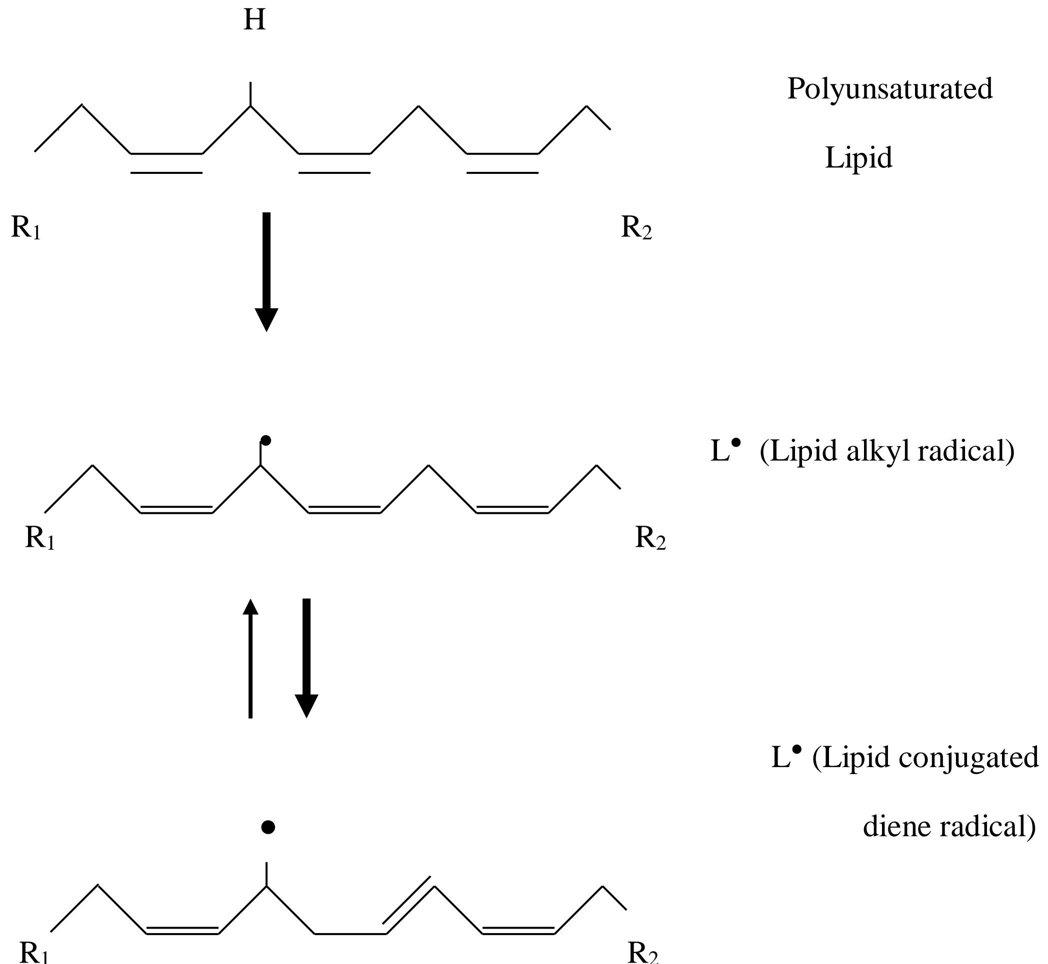
Singlet oxygen: Singlet oxygen arises if one of the two unpaired electrons of the molecular oxygen absorbs energy and transformed into an orbital position of higher energy with inversion of spin[122]. "Singlet oxygen is not a free radical but an electrically excited form of oxygen. It is a strongly electrophilic molecule. Singlet electron has high reactivity with variety of biomolecules and readily combines chemically. It can also transfer its electronic energy and form the excited state of the donor molecule and then returning to ground state(quenching). Singlet oxygen has a longer life time than superoxide anion radical" [108]. It is capable of diffusing across the membrane and causing damage away from its origin[123].

Hydroperoxide and lipid radicals:

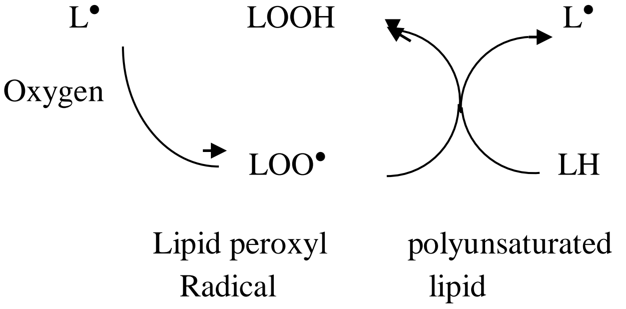
Polyunsaturated fatty acids [PUFA] may readily be oxidized in vivo and vitro. Lipid oxidation may occur slowly in vivo and is strictly controlled process. however,an uncontrolled and rapid peroxidation may occur if lipid alkyl radicals have formed. the mechanism of lipid peroxidation involves 3 distinct steps- initiation, prolonged and termination.

Initiation : The initiation step occurs when radicals like hydroxyl radical, thiyl radical, abstract an allelic proton from a polyunsaturated lipid or a carbon centered radical and subsequently lipid alkyl radicals are formed. The lipid alkyl

radical stabilizes by forming a conjugate diene radical, which is subject to further reaction.



Propagation : The propagation step follows, in which the lipid alkyl radical reacts with oxygen to form a lipid peroxyl radical. This can abstract a second allylic hydrogen atom from a polyunsaturated lipid resulting in a free radical chain reaction. Finally, lipid hydroperoxides are formed.



Termination: The final step is termination of the free radical chain reaction by bond rearrangement to form conjugated dienes or by degradation of peroxyl and alkoxyl radicals, yielding alkenals, hydroxylalkenals and alkanes. Radical-radical addition reaction, resulting in the formation of di or polymers, is a further possibility. Primary antioxidants, which inhibit the radical chain reaction, scavenge the propagating species.

$$2\,LOO^{\bullet} \longrightarrow LOOL + \text{oxygen}$$

Lipid-peroxyl radical → Lipid peroxide

$$LOO^{\bullet} + XH \longrightarrow LOOH + X^{\bullet}$$

Lipid peroxy radical + Antioxidant → Lipid hydroperoxide

$$2\,L^{\bullet} \longrightarrow L - L$$

Lipid alkyl radical → Lipid dimmer

$$L^{\bullet} + XH \longrightarrow LH + X^{\bullet}$$

Lipid alkyl radical → Regenerated Lipid

The highly reactive products of lipid peroxidation such as lipid alkyl radicals ($R^{\bullet}$), alkylperoxyl radical ($R\text{-}O\text{-}O^{\bullet}$) and alkoxyl radicals ($R\text{-}O^{\bullet}$) may damage all basic biomolecules in their ultimate vicinity [124]. Lipid hydroperoxyde R-O-O-H and their degradation products e.g. 2-alkenals and 4- hydroxylalkenals can damage cells and tissues at more distant sites not directly exposed to lipid peroxidation. The stable degradation products of the lipid peroxidation can be readily analyzed by different methods. Alkenals and hydroxyalkenals (Malondialdehyde and 4-hydroxynonenal) are analyzed by colorimetric methods and chromatography.

3.5.1- Lipid Peroxide (MDA)

"The detection and measurement of lipid peroxidation products such Malondialdehyde (MDA) provides the evidence to support the involvement of free radical reaction in toxicology and disease" [125].

Kari Punnonen et al. (1991) described that UV irradiation causes an inflammatory reaction, which is accompanied by changes in membrane lipid metabolism. The most remarkable changes were detected in the amounts of thiobarbituric acid reactive material i.e. MDA, arises predominantly from the oxidation of PUFA with 3 or more double bonds [126].

"There is convincing evidence that oxidative stress enhances the production of oxidation products, such as 4-hydroxy-2 nonenal or malondialdehyde that can denature proteins, alter apoptosis, and influence the release of proinflammatory mediators such as cytokines, which may be critical for the induction of some inflammatory diseases" [127].

Lipid peroxidation may be induced by physiological and pathological reactions. Heat, UV and ionization irradiation, redox action metal ions, redox cycling drugs and free radical initiators can trigger nonenzymatic peroxidation of the lipids. Enzymatic peroxidation may involve the neutrophil plasma membrane associated NADPH dehydrogenase and microsomal NADPH dependent cytochrome P-450 reductase and cyclooxigenase/ lipoxigenase.

Lipid peroxidation has suggested playing significant role in basic pathological processes in humans such as carcinogenesis, inflammation, xenobiotic toxicity [128].

It is hypothesized that "naturally existing anti-inflammatory protein participate in the regulation of endothelial cell contraction. It is readily inactivated by reactive oxygen species. The oxidative damage of this protein may leads to increased vascular permeability and thus contribute to the inflammatory tissue response" [129].

3.5.2- Nitric Oxide :

"The synthesis and regulation of Nitric Oxide (NO) by mammalian cells has been the focus of many reviews and has many of its physiological and pathological actions" [130,131,132,133,134,135]. Nitric Oxide is locally synthesized messenger. "There are isoforms of Nitric Oxide synthase (NOS) enzymes responsible for synthesis of NO. Neurotransmission relies on constitutive neuronal isoform (NOS 1). NOS 1 recently has been identified in human keratinocytes and melanocytes. The *N*-methyl-D-aspartate receptors in keratinocytes activate NOS1 in neurons providing a possible mechanism for the controlled release of NO from Keratinocytes" [136]. "Smooth muscle tone in blood vessels is regulated by calcium dependent constitutive endothelial isoform (NOS 3). The inducible isoform (NOS 2) has been first identified in macrophages" [137]. However, "there is evidence of NOS2 production from keratinocytes" [138,139].

"NO is reported to have potential toxic effects. Most of the toxic effects of NO are mediated by its oxidation product peroxynitrite rather than NO itself.peroxynitrite is formed by spontaneous combination of Nitric Oxide and superoxide which are synthesized within a closely located cell diameters. NO and Superoxide do not even have to be produced within the same cell to form peroxynitrite because NO can so readily move through membrane and between cells. Although peroxynitrite is a strong oxidant, it reacts at a relatively slow rate with most biological molecules. Peroxynitrite require anion channels to traverse cell membrane" [140,141].

"Thought half life of peroxynitrite is short it is sufficient to cross biological membrane and diffuse one to two cell diameters [141] and allows adequate interactions with most critical biomolecules" [142,143].

It is indicated by kinetic studies that there are two mechanisms by which peroxynitrite oxidizes target molecules. First, "direct oxidative modifications through one or two electron oxidation processes by peroxynitrite and its protonated form peroxynitrous acid (ONOOH). Thiols, iron sulfur centers and zinc fingers are only a few chemical groups directly react with peroxynitrite, which favors selective reactions with key moieties in proteins" [144,143].

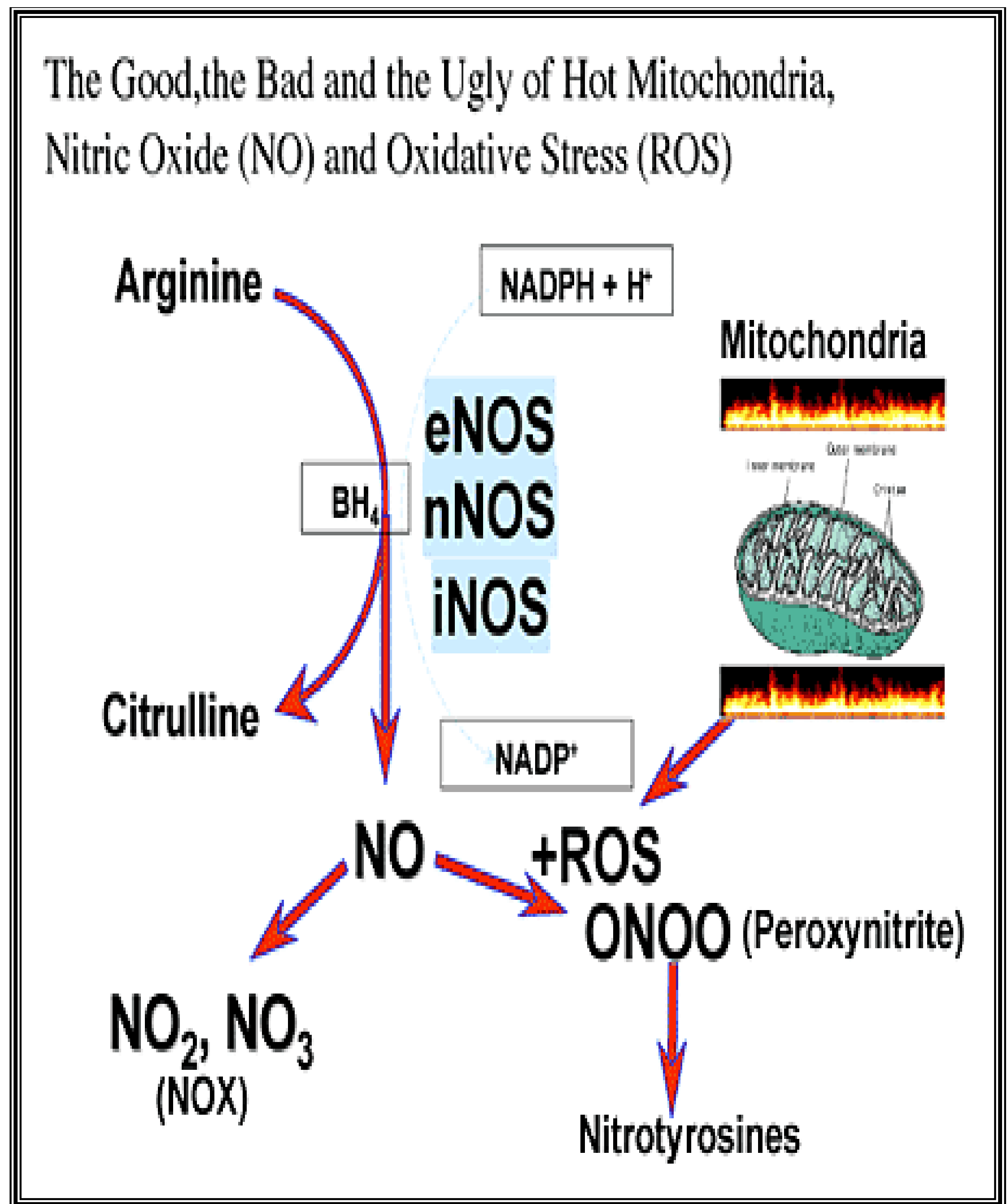


Figure No -9: Showing synthesis of NO and formation of peroxinitrite radical.

"The second mechanism involves peroxynitrite indirectly mediating oxidation of target molecules by decomposing into highly reactive radicals" [145]. In proteins, peroxynitrite reacts with transition metal centers, oxidizes cysteine, and nitrates tyrosine as a target reaction [143].

"A major aspect of the peroxynitrite dependent cytotoxicity relies on its ability of abstracting a hydrogen atom from polyunsaturated fatty acids (PUFA) to trigger lipid peroxidation in membranes, liposomes, and lipoproteins" [132]. Resulting products are lipid hydroperoxyradical and aldehydes [146] [147]. "These radicals in turn

attack neighboring PUFAs, to generate additional radicals which propagate free radical reactions and these free radicals degenerate membrane lipids causing membrane permeability and changes in fluidity with significant biological consequences” [148].

“Cells in the central and peripheral nervous system, lungs, liver, gastrointestinal tract, blood vessels, immune system and skin are known to generate this molecule” [149,150,151].

According to *Robert M Clancy* (1998) NO was having dualistic role according to the enzyme producing it as well as its concentration. Whenever NO was produced by cNOS, it is a part of immunity, relaxing smooth muscles around endothelial cells, preventing adhesion of leukocytes and platelets. Secondly when NO is produced in large concentration & by iNOS due to stimuli by cytokines, leads to inflammation [152].

3.6- Formation of RONS (Reactive Oxygen Nonradical Species): Non-Exercise Conditions

Free radicals are produced by a variety of mechanisms in biological systems. However, all reactions involve either the initial reduction (addition) or oxidation (removal) of an electron via enzymatic or nonenzymatic action [153]. In short, radical species are simply a consequence of chemical reactions occurring within the body. Moreover, RONS are resultant products of normal cellular metabolism and consist of both radical (e.g., hydroxyl radical, superoxide, nitrogen monoxide) and nonradical (e.g., peroxynitrite, hydrogen peroxide) species all with various physiological functions and/or consequences [101]. “Superoxide radical is the single electron reduction of molecular oxygen and is a byproduct of normal cellular metabolism. Superoxide radical, hydrogen peroxide, hydroxyl radical and finally water molecules are produced when oxygen undergoes a series of one electron reductions with cytochrome oxidase in complex IV of the mitochondrial electron transport chain(ETC)” [154]. This is due to the preference of molecular oxygen to accept its electrons one at a time [153]. Although, the reduction of oxygen is a very efficient process, it seems that some of the electrons transferred may lead to oxygen prematurely resulting in the production of superoxide [101]. The process of energy production via oxidative phosphorylation and subsequent formation (leakage) of

superoxide intracellularly is a constant process. It is believed that approximately 1-3% of all electrons in the transport chain may lead to generate superoxide, rather than contributing to the reduction of oxygen to water [101]. As such, it could be inferred that an acute form of oxidative stress occurs due to any situation that results in increased transfer of electrons by the electron transport chain could potentially result in increased formation of superoxide anion. One situation in which electron transfer is increased includes the performance of physical exercise. This issue is addressed in detail in a later section. "Superoxide can also be produced enzymatically by way of several oxidase enzymes, such as NAD(P)H oxidase or xanthine oxidase. Xanthine oxidase generates superoxide, primarily in response to conditions of ischemia followed by reperfusion, by catalyzing the oxidation of hypoxanthine to xanthine and xanthine to uric acid" [155]. Intentional production of superoxide also occurs as a component of the respiratory burst (accelerated oxygen uptake) of phagocytic cells like neutrophils, macrophages, and lymphocytes [153]. This mechanism serves to protect the body against invading bacteria and is mediated by the enzyme NAD(P)H oxidase, this mechanism is present in the plasma membrane of these cells [156]. This protective mechanism can become problematic. However, if phagocytic cells become activated in the wrong location or to excessive extent, releasing excess superoxide radical and potentially resulting in cellular damage or disease progression [153]. In either case, superoxide, arising either metabolically or during respiratory burst is considered to be the primary RONS produced in biological systems, which can then interact with other molecules to form secondary RONS via enzyme or metal catalyzed processes [101]. In fact, most of the damage caused by superoxide is done by initiating secondary sources of RONS formation, as well as by acting as a potent pro inflammatory and pro atherogenic signaling molecule [156]. Combination of superoxide with nitric oxide forms the harmful radical species- peroxynitrite, which is a long-lived oxidant with the potential to damage DNA [157],and also leads to the formation of hydroxyl radical [105]. Moreover, dismutation of superoxide and subsequent formation of hydrogen peroxide may also lay the foundation for the formation of a highly reactive and more harmful radical (hydroxyl radical) by the Fenton reaction, further illustrating the primary role of superoxide formation in initiating a series of downstream reactions that result in secondary RONS production and cellular damage[105].

3.7- Association of Free Radicals with Health and Disease

It is clearly indicated that "a basal level of RONS production and removal is a constant process, it in turn elicits both positive and negative effects on physiological function. Oxidation/ Reduction potential within the cell is represented by intracellular redox state and/or redox balance. Like pH regulation in the body redox balance is also tightly regulated and is commonly assessed by the ratio between reduced (GSH) and oxidized (GSSG) glutathione (the major non-enzymatic antioxidant) or other thiol/disulfide compounds" [101]. "Mammalian cells are provided with signaling pathways which are sensitive to the intracellular redox environment and hence can be activated by oxidative stress" [158]. Hence, "transient disturbances a redox balance shift towards a more oxidizing environment can occur via increased RONS production and/or decreased antioxidant defense and it serves as a stimulus for the activation of several cell signaling mechanisms important for optimal physiological function" [104]. Examples of specific redox-sensitive regulated functions are - regulation of vascular tone, [159] immune response amplification and apoptosis,[160,161] modulation of insulin receptor kinase activity, [162] and "increased expression of antioxidant enzymes and/or glutathione in response to MAPK and NF-κB activation in an attempt to restore redox balance [158]. The latter example is especially applicable to exercise. There is an increase in RONS during and after acute exercise which is believed to serve as the necessary signal for the hermetic associated upregulation in antioxidant defense and also associated redox shift (favoring more reducing conditions) commonly observed with chronic exercise training" [158]. In general, "a more reducing environment is consistent with optimal physiological function and health enhancement while chronic deregulation of this balance in favor of a more oxidizing environment is linked with the development of numerous diseased states, along with the aging process. Therefore, capacity of the antioxidant defense system in place is overwhelmed by the conditions that favor accelerated and/or chronic production of RONS, thereby damaging normal redox-sensitive signaling and causing a permanent shift in redox balance" [163][104]. Furthermore, this permanent shift of redox environment may then induce damaging effects via direct RONS-mediated oxidative damage to nucleic acids, Protiens and lipids [106], as well as causing apoptosis within healthy cells through changes in gene expression, along with systemic inflammation [163]. It has been suggested that "both moderate and excessive

shift in redox potential, resulting from chronic oxidative stress play a role in the functional decline commonly observed with aging as well as in the pathophysiology of several diseased states” [104,163]. In fact, “oxidative stress has been propsed to play a primary or secondary role in the progression of multiple (>100) acute and chronic human diseases” [106] including but not limited to cardiovascular [atherosclerosis, heart failure] [164], neurodegenerative [multiple sclerosis and Parkinson‘s] [165] [166], inflammatory [rheumatoid arthritis] [167], metabolic [diabetes, obesity] [101] and cancerous diseases [168].

3.8- Antioxidants:

Each tissue has an “antioxidant potential” which is determined by the balance between factors promoting auto oxidation and those exerting an antioxidant action. Multiple lines of antioxidant defense have evolved and serve to protect human body from oxidative stress including prevention, interception and repair [108], [169].

Primary defense mechanism prevents oxidative damage by scavenging reactive species directly. Secondary defense mechanism combat processes elicited by reactive oxygen species such as lipid peroxidation.

3.9- Nomenclature and Classification of Antioxidants

“Characterization of various antioxidants is based on their structure, coexistence and mechanism of action, solubility and kinetics. Kinetically antioxidants can be classified into six categories as follows: (1) Antioxidants that break chains by reacting with peroxyl radicals having weak O-H or N-H bonds e.g., phenol, napthol, hydro- quinone, aromatic amines and aminophenols. (2) Antioxidants that break chains by reacting with alkyl radicals e.g., quinones, nitrones, iminoquinones. (3) Hydro peroxide decomposing antioxidants e.g., sulphide, phosphide, thiophosphate. (4) Metal deactivating antioxidants e.g., diamines, hydroxyl acids and bifunctional compounds. (5) Cyclic chain termination by antioxidants e.g., aromatic amines, nitroxyl radical, variable valence metal compounds. (6) Synergism of action of several antioxidants e.g., phenol sulphide in which phenolic group reacts with peroxyl radical and sulphide group with hydro peroxide” [170].

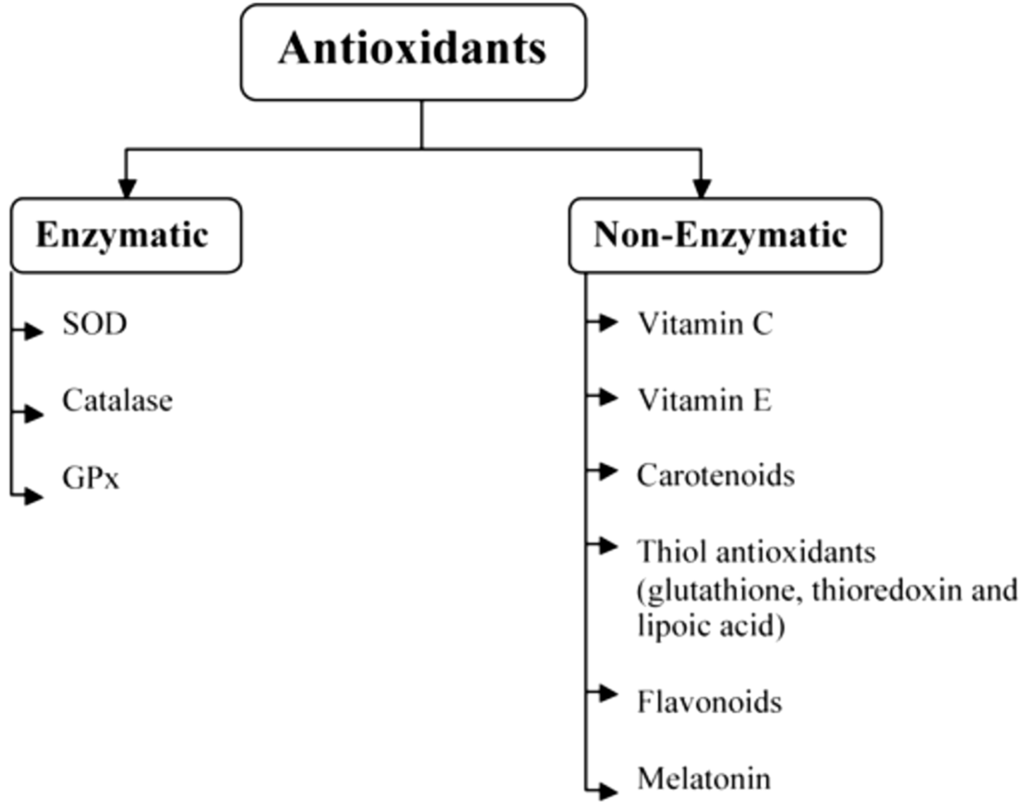


Figure -10: "Enzymatic and non enzymatic classification of antioxidants with few [170] antioxidants as chelating agents and their mechanism of action".

Both non-enzymatic antioxidants[171] and antioxidant enzymes[172] in (Fig- 10) are generally known to counteract the effect of ROS and RNS. These antioxidants are also recognized to diffuse free radicals leading to limited risk of oxidative stress. At molecular and celular level these antioxidants inactivate ROS and under specific low concentration they interrupt the radical chain reaction and hence inhibit or delay oxidative processes by interrupting the radical chain reaction.

The enzyme catalase, Vitamin C and SOD scavenge hydrogen peroxide and superoxide anion directly. Tocopherol, β-carotene, Vitamin A and Bilirubin are chain breaking antioxidant that reacts with peroxyl and alkoxyl radicals in the phospholipids membranes. However, they also quench singlet oxygen directly [173]. Antioxidants are effective in protecting biological tissues below a critical threshold of reactive oxygen species. If steady state free radical concentrations exceed this threshold, then autocatalytic cell injury will result.

3.9.1- Superoxide Desmutase

SOD is ubiquitous in mammalian tissues. It is metal protein complex (metalloprotien) and it causes formation of hydrogen peroxide and oxygen by catalizing the dismuation of the superoxide anion radical. In mammals, two types of the enzyme are reported: 1.)Cu, Zn-SOD is mainly localized within the cytosol, 2.) Mn-SOD is predominantly present within mitochondria but is also found in the cytosol of higher primates. In the blood most of the SOD is located in the blood cells and minor fraction is found in the serum [174].

3.9.2- Catalase

Catalase is a ubiquitous heme enzyme, which functions in two ways. First, it causes catalytic decomposition of two molecules of hydrogen peroxide to water and oxygen. Second, it causes oxidization of a substrate using hydroperoxide as a hydrogen donor.

Catalytic and peroxidic activity of catalase

$$2\,H_2O_2 \xrightarrow{\text{Catalase}} 2\,H_2O + O_2$$

(Catalytic activity)

$$ROOH + AH_2 \xrightarrow{\text{Catalase}} H_2O + ROH + A$$

(peroxidic activity)

The enzyme is largely located within the peroxidesomes where it constitutes about 50% of the protein in this organelle. It has a high turnover and is probably synthesized by rough endoplasmic reticulum, conveyed and concentrated in the Golgi apparatus, and finally released in the small-coated vesicles. “Catalase has relatively low affinity for its substrate but a high capacity for the reaction. A genetic deficiency in humans dose not usually results in the clinical manifestations of organ pathology indicating the presence of compensating mechanism or still effective residual amount of catalase” [175]. In severe oxidative stress catalase may have a role in detoxification of the hydrogen peroxide. During mild oxidative stress, other antioxidant have more important role than catalase. [176,177,178].

3.9.3- Vitamin E: (Lipophilic Antioxidant)

Vitamin-E was an essential nutrient that is receiving growing attention because of its antioxidant function in human biological system. In 1922, an unknown substance was discovered in lettuce by Evans and Bishop, which prevented fetal death in animals fed a rancid food. Subsequently, in 1924, Sure designated this unknown compound a fertility vitamin. Olcott and Emerson first studied the antioxidant property of the new vitamin. In 1936, Evans isolated the fertility vitamin from wheat germ oil. *Fernholtz et al.* revealed the chemical structure of the vitamin E in 1938 and is shortly thereafter Karrer synthesized it.

There are 8 natural substances have vitamin E activity, namely- α, β, γ and δ-tocopherol as well as α, β, γ and δ-tocotrienol.Vitamin E is a generic term that includes all entities that exhibit the biological activity of natural vitamin E (α – Tocopherol).

Vitamin E is among the oldest recognized biological antioxidants [179]. Direct and indirect evidence that α-tocopherol is a powerful antioxidant in sub cellular structures, in isolated cells and organs, in animals and in humans is convincing. Vit E is major lipophilic antioxidant in the tissues [180]. It is primary and secondary antioxidant. It reacts directly with reactive oxygen species in biological membranes and inhibits already initiated lipid peroxidation chain reaction by capturing the propagating species (peroxyl radical). The superoxide anion radical readily oxidizes tocopherol [181,182]. Tocopherol quenches triplet carbonyls and reacts with singlet oxygen, either in reaction without formation of products or in an oxidation reaction [183,184].

The initial oxidation product of tocopherol is the metastable tocopheroxyl radical, which can be further oxidized to tocopherolquinone. Other oxidation product includes epoxides as well as oligomers. The tocopheroxyl radical is the central to the antioxidant potency.

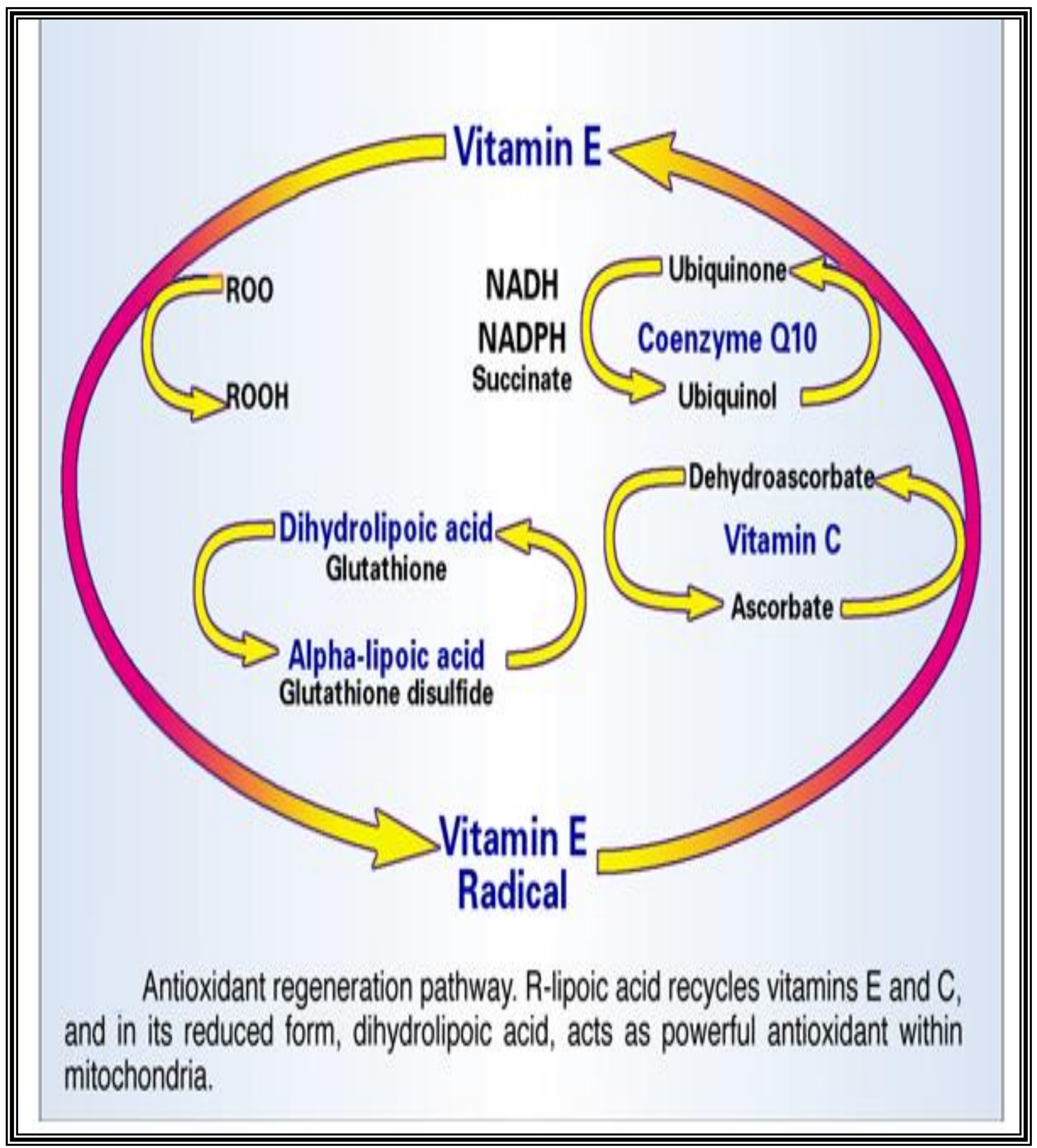
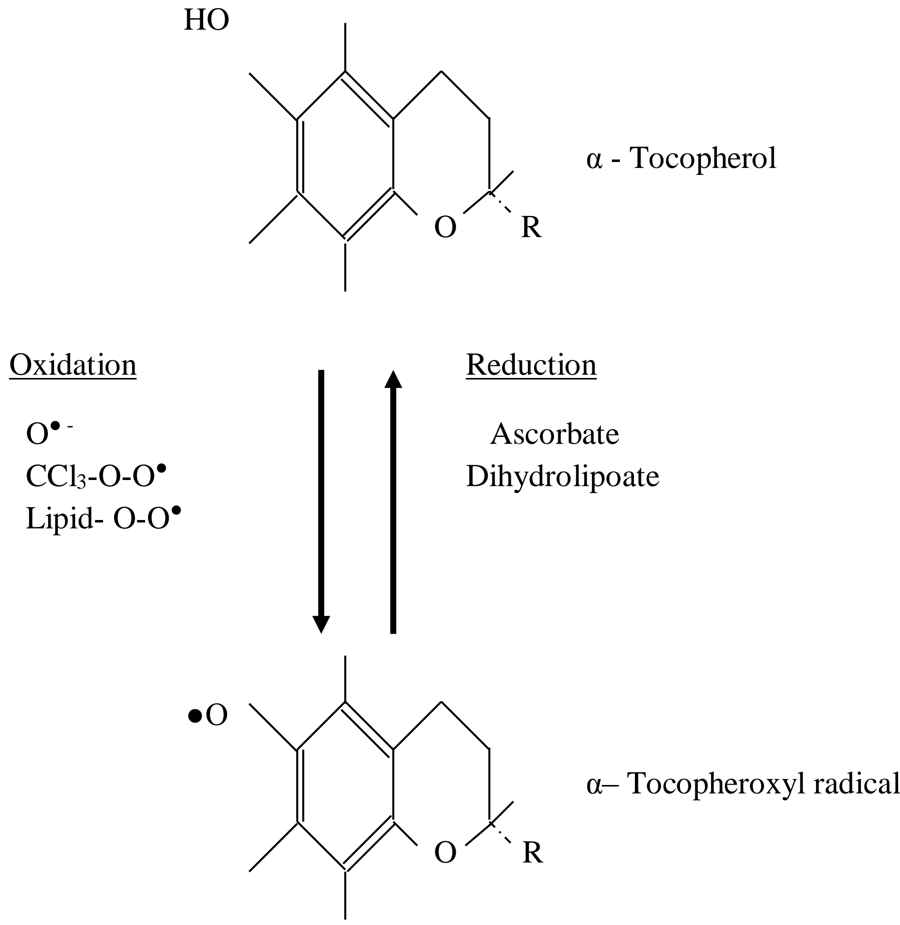


Figure No -11: Showing recycling of Vitamin E as antioxidant

It can reduce under physiological conditions to tocopherol, thus generating antioxidant. "Since the physiological molar ratio of tocopherol to polyunsaturated phospholipids is less than about 1:1000 in most biological membranes, regeneration of tocopherol is essential for its high antioxidant efficiency in vivo. Small physiological molecules such as ascorbate, glutathione and ubiquinole contributes to tocopherol regeneration.(Fig No- 11) Dysfunction of regeneration process may cause tocopherol deficiency in cellular microcampartments and result in prooxidative environment" [184].

The effect of α- tocopherol on membrane fluidity and permeability is quite complex and depends upon the composition of the phospholipid and the molar tocopherol /phospholipids ratio [185]. α-Tocopherol decreases the membrane fluidity of phospholipidcholine liposomes and reduces the permeability of the membrane to uncharged molecules like Glucose [186]. In high concentration however, tocopherol increases membrane fluidity of human erythrocytes and thrombocytes [187] by contrast it has been reported that low concentrations of tocopherol increase the fluidity of mitochondrial membranes and high concentrations produce a decrease in lipid movement [119]

Antioxidative reaction of Vit-E



3.9.4- Vitamin A:

"Vitamin A is largely found as an ester and is highly soluble in organic solvents but not in aqueous solutions. The major provitamin carotenoids have similar solvent property. Beta-carotene is a precursor of Vit A and both can function as effective radical trapping antioxidant. These two are vary effective quenchers of singlet oxygen"[188,189].

3.9.5- Transition Metals

Activation of the molecular oxygen in biological systems can be achieved by its interaction with a paramagnetic transition metal ions and exchange coupling, which overcomes the spin restriction. Transition metals have an unpaired electron in an inner electron orbital. Enzymatic activation of oxygen by oxidases or oxygenases depends in general on transition metal ions associated with the enzyme. Transition metal ions are indicated to play a vital role in the manifestation of the oxygen toxicity. [153] In particular, iron and copper may be involved in oxidative stress by a) accelerating auto oxidation of various small molecular substances such as phenols & thiols, b) participating as a catalysts for Haber-Weiss and Fanton reactions and c) decomposing lipid hydroperoxides to peroxyl radicals [190,191](*Davis & Slater* 1987). Copper (I) salts react with hydrogen peroxide to yield hydroxyl radicals with much greater rate constant than do iron (II) salts.

"In biological systems, actual concentrations of redox active transition metals capable of catalyzing the formation of reactive oxygen species are usually relatively low. However, under certain pathological conditions concentration of redox active metal ions can be increased (*Aust* 1985)" [171]. Transition metals of biological importance were Iron, Copper, Zinc, Selenium, Manganese, Vanadium, Cobalt, Nickel, Chromium, and Molybdenum.

Superoxide itself can release iron from ferritin in vivo (*Mazur* 1958) [192] and in vitro (*Thomas* 1985) [193]. This mechanism was proposed to potentiate the reactive oxygen species formation in inflammatory sites via iron mobilization from ferritin by superoxide radicals derived from activated neutrophils (*Biemond* 1984) [194].

3.9.5.1- Dualistic Mode of Action

Transition metal complexes may protect against oxygen damage or sensitize the effects of oxygen. Metal chelates (Cu, Zn- superoxide dismutase SOD) can either serve as catalysts of superoxide anion radical dismutation or as catalysts of the reaction between hydrogen peroxide and superoxide anion radical to form hydroxyl radical (Haber Weiss reaction) The reaction type depends on many factors, one of them being chelator composition. Chelator may alter the redox potential of the metal ion and their by control the reaction thermodynamics[108].

3.9.5.2- Selenium

Selenium is involved in reoxidation of reduced glutathione and has a close metabolic relationship with Vitamin E. it is an essential cofactor for the enzyme glutathione peroxidase, which destroys peroxides derived from unsaturated fatty acids. Selenium protects against heavy metal toxicity and plays an important role in platelet aggregation [195]. The key role played by selenium along with other antioxidants, in protecting against free oxygen radical damage is well recognized.

The enzymic glutathione system comprises glutathione peroxide, reductase and transferase. Selenium dependant glutathione peroxidases reduce both hydrogen peroxide and free organic hydroperoxides and were found in the cytosol and within mitochondria.

Nonselenium dependent glutathione peroxidase reduces only free organic hydroperoxides and do not metabolize hydrogen peroxide [196]. These processes are dependent on an adequate supply of glutathione and NADPH and are therefore glutathione reductase dependent. Glutathione reductase also reduces various mixed disulfides.

“Rapid oxidation of glutathione to glutathione disulfide (GSSG) is caused by free radicals, hydroperoxides and also reactive oxygen species. Glutathione reductase catalyzes the reduction of GSSG using NADPH generated for example, by glucose-6-phoshate dehydrogenase” [197].

3.9.5.3- Zinc

Zinc has an atomic number 30 and atomic weight 65.4. This metal has good reducing power and has melting point of 419^0C. Zinc has several biological functions. Although present in all body tissues, its concentration is high in epidermis of the skin. It is immediately concerned with cell division and growth, protection against free radicals etc. Zinc acts as a cofactor for the activity of SOD isoform.

3.9.6- Vitamin C

" Vitamin C (ascorbic acid) is a principal and powerful antioxidant. It works in aqueous environment of the body. In membranes and lipoproteins, vitamin C cooperates with Vitamin E to regenerate α-tocopherol by utilizing α-tocopherol radicals" [198]. "Ascorbic acid behaves as a vinylogous carboxylic acid, wherein the double bond ("vinyl") helps in transmission of electron pairs between the hydroxyl and the carbonyl. Ascorbate being as an antioxidant is available for energetically favorable oxidation. Reactive oxygen species first cause oxidation of ascobate to (take electrons from) monodehydroascorbate and then to dehydroascorbate. The reactive oxygen species are reduced to water, since the oxidized forms of ascorbate are relatively stable and unreactive hence they do not cause cellular damage.

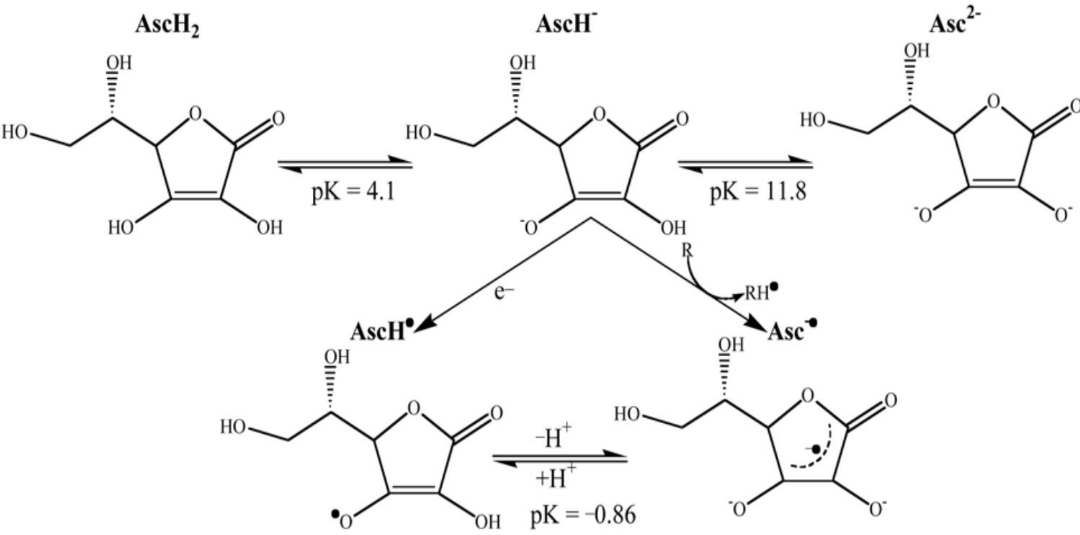


Figure -12: Various forms of Ascorbic Acid and its reaction with radicals (R•)" [199,200]

"Vitamin C scavenges the aqueous reactive oxygen species (ROS) by very rapid electron transfer that inhibits lipid peroxidation" [201,202]. "Animal studies have suggested an antagonistic effect of ascorbic acid on lead absorption with its excellent chelating ability towards lead which is in good comparison to standard chelator ethylenediamine tetra acetic acid (EDTA)" [203,204].

3.9.6.1- Vitamin C as an Antioxidant

"Both vitamin C and vitamin E are powerful antioxidants found in the lungs where they protect cells from oxidative damage" [205]. "Although vitamin E is predominantly membrane bound, there is a very close interaction between vitamins C and E, as Vitamin C not only functions directly as an antioxidant, but it also helps in the recycling of the antioxidant capacity of oxidized vitamin E" [206][207].

Like vitamin C, "Retinol (vitamin A) and carotenoids are also having anti-oxidant activity and anti-inflamatory activity as well, which has been seen in pulmonary diseases" [208-213].

Definition (*José Luis et al*) [214]

"Vitamin C is defined as hexuronic acid, cevitáminic acid or xiloascórbic acid. The term vita- min C is generally used to describe all these compounds, although the representative of which is ascorbic acid" [214].

Structure, formula and chemical characteristics

Enolic form of one α-ketolactone Ascorbic acid. Dehydroascorbic acid is a diketo form, obtained easily by oxidation of Ascorbic acid solution. Dehydroascorbic acid can easily be converted into oxalic acid , diketogulonic acid or threonic acid.

Chemical behavior of ascorbic acid is contributed by several structural elements like physical and chemical properties of ascorbic acid. A primary and secondary alcohol groups namely lactones and two enolic hydroxyl groups are structural contents of the ascorbic acid. The antioxidant properties of ascorbic acid is motivated by enediol. Enediols can be easily oxidized to diketones. For this reason, the carbonyl groups of endiole neighbors are also called reductive in nature. Intermolecularhydrogen bonds are formed by ascorbic acid. These bonds contribute to the stability and the chemical qualities of the structure endiol with it.

Ascorbic acid undergoes for rapid interconversion into two unstable diketone tautomers by proton transfer, however it is most stable in the enol form. Loss of the proton from the enol and reacquiring by the electrons from the double bond produce a diketone. There are two possible ways: 1, 2-diketone and 1, 3-diketone (figure-13).

Figure -13: "Nucleophilic attack on the proton ascorbic enol to give 1,3 diketone".

Vitamers or vitameric forms

"The vitamer is any chemical compound in a particular vitamin that generally has same molecular structure but shows a different vitamin activity in a biological system which is deficient of the vitamin. The vitamin activity of multiple vitamers is depend upon the ability (sometimes limited) of the body to convert one or many vitamers in another vitameric form for the same enzymatic cofactor which is active in the body as the most important form of the vitamin. As part of the definition of the vitamin, the body can not completely synthesize an optimal amount of vitamin activity of foodstuffs, without a certain minimum amount of vitamer as base. By mass and weight not all vitamers have the same vitamin power it is due to difference in the absorption and the variability in interconversion of several vitamers in the vitamin. For ascorbic acid essentially, following vitamers can be mentioned: dehydroascorbic acid, erythorbic acid (figure-14) and also the following salts like calcium ascorbate, sodium ascorbate and others. (*Rogur*, 2010)" [215].

Figure -14: "Structure of erythorbic acid, isoascorbic or Arabian-ascorbic"

"Chemical data: IUPAC Name: D-isoascorbic acid, CAS Number: [89-65-6] Molecular formula: C6H8O6, molar mass: 176.13 g/mol; Melting point: 169-172 ° C"

3.9.6.2- Interaction with ROS

"Vitamin C quickly identify and remove the reactive species of oxygen and nitrogen just as singlet oxygen,superoxide, aqueous peroxyl radicals, hydroperoxile radicals, ozone, peroxynitrite, nitroxide radicals, nitrogen dioxide, and hypochlorous acid thereby protecting the oxidative damage other substrate in fact" [216].

3.10- Total Antioxidant Status

Free radicals generated in the body, which may be due to variable reasons and or due to normal metabolic reactions, its adverse effects are nullified by the antioxidants in the body. Different antioxidants are having their role at various stages of free radical generating reactions to counteract the free radicals.

Antioxidants in the body acts simultaneously and always it may not be possible to determine the antioxidant potential of individuals. This made researchers to think on determining total antioxidants potential of the samples, which will measure all the antioxidants in it. So Total antioxidant potential is the test giving general idea about body's defense status against ROS by all known and unknown antioxidants. It may be impendent of any single antioxidant to be increased or decreased.

The results of the *Gavan et al*(1997) showed increase in the plasma Total Antioxidant status of the psoriatic patients than controls, which was decreased by the antipsoriatic topical treatment of fluocinolone Acetonide for 24 hrs [217].

Severein et al (1999) studied values of TAS Bilirubin, Tocopherol and Urate in the sera of 33 psoriasis patients and 36 healthy controls. According to their observations, serum total Antioxidant Status of psoriasis patients was unchanged as compared to controls. The authors got unchanged serum TAS, at the same time when results of serum Bilirubin, Uric Acid and Vitamin E were found to be increased significantly than controls. The Authors mentioned for explanation that TAS may not measuring Vitamin and other antioxidants in the serum[218].

3.11- Effects and role of antioxidants in cardiovascular disease

"It has been proposed that antioxidants such as vitamin C, A (β-carotene) and E plus selenium have a protective effect in cardiovascular disease. Many prospective studies so far have observed an inverse relationship between Vitamin C intake and cardiovascular diseases and also a strong protective effect of vitamin E supplementation on coronary patients. Finnish and Swiss studies indicated that myocardial infarction is predicted in diminished nutritional status of vitamin and low blood levels of ascorbate. Low levels of vitamin C increases 2.7 times the risk of myocardial infarction and this is independent of other risk factors. Mediterranean studies showed a 70% reduction in mortality and risk of myocardial infarction on supplementation of vitamin C independent of the effect on blood pressure and lipids. Any protective effect of these antioxidants in heart disease is mediated by the oxidation of LDL cholesterol, and also there may be other mechanisms of homeostasis and inflammation(*Simon J.A*)" [219].

"Protection of LDL oxidation by Vitamin C is concentration dependent showing maximum effect of Vitamin C at 150mM concentration. At a dose of 400mg Vitamin C/day, plasma is completely saturated and steady state plasma Vitamin C concentration will approximately 80mM, this might be maximally improved the lag time of the LDL oxidation reaction in vivo and it is useful in decreasing atherosclerosis" [220,221].

In relation to antioxidant role of vitamin C it is regarded as an ideal free radical scavenger acting within the plasma [222], where it serves to quench aqueous peroxyl radicals, oxidants leaking from activated neutrophils and macrophages, as well as other lipid peroxidation products [223,224]. Additionally, Vitamin C is easily accessible (multiple food sources and over the counter supplements) and appears to

possess high bioavailability. "The ability for Vitamin C to compartmentalize and/or translocate to specific sites of attack by reactive oxygen and nitrogen species (RONS), also commonly referred to as prooxidants or free radicals" [223]. This is represented by the Vitamin C mobilization and subsequent increased release into circulation to combat the increased production of RONS as a response to stimulation by an acute exercise [223].

"Vitamin C also helps in prevention and treatment of Non Scurvy Diseases in human.Clinical trials of Vitamin C supplementation have demonstrated a protective effect of Vitamin C in cardiovascular diseases. In fact, no other antioxidant Vitamin supplementation has been shown to affect the risk of cardiovascular diseases" [225-227].

The susceptibility of lipoproteins to mononuclear cell-mediated oxidationis decreased to 78% as a result of Vitamin C and E supplementation(*Rifici and Khachadurian*, 1993) [228]. It is reported that ascorbic acid has a stimulating effect on function of the reticuloendothelial system, on phagocytic activity of leukocytes and on formation of antibodies. Reason for Phagocytic cells having a very high concentration of ascorbic acid, is to protect the cell from oxidative damage during phagocytosis of invaded pathogens by using highly reactive free radicals and oxygen containing molecules.

MATERIALS AND METHODS

4. MATERIALS AND METHODS:

4.1- Study Design: It was cross sectional study.

1. Exposed 2.Unexposed.

Risk factor included was – exposure to dust.

4.2- Sample size: 134 individuals were selected in each group.

Control – Appropriate number of non exposed subjects of same age group were selected as control.

4.3- Inclusion criteria:

- Volunteered Rice mill workers aged above 20 and below 50 were included.
- Only male individuals were selected.
- Individuals working in rice mills having experience of 5 years or more were included.
- Only rice mill workers who were working in dust and exposed to dusty environment were included in the study.

4.4- Exclusion criteria:

- Individuals having the history of chronic respiratory illness or cardiovascular illness before working in rice mills were excluded.
- Alcoholics and smokers were excluded from the study.
- Other staff members of rice mill like clerical staff, secretarial staff and management staff were excluded from the study.
- Female individuals were excluded due to inadequate number.

Informed consent was taken from all the individuals participated in the study. While the collection of data, structured questionnaire was used. It has helped to determine information in regard of general health, disease history, duration of exposure, details of habits like smoking and alcohol consumption.

4.5- Estimation of Hematological Parameters: For hematological parameters- 2ml of intravenous blood was drawn and collected in EDTA tubes and it processed in CBC Counter Machine.

4.6- Recording of Heart Rate and ECG: For the determination of cardiac electrical activity and heart rate- ECG was recorded.

4.7- Recording of Blood Pressure: Blood pressure was recorded by mercurial sphygmomanometer.

4.8- Recording of Lung Function Parameters: For the recording of lung function parameters, a computerized digital spirometer(SPIRO EXCEL 1.3)was used.

Before recording of pulmonary function tests, individual participants were thoroughly explained regarding the procedure in their own language along with demonstration of the procedure.

For estimation of serum MDA and serum NO- 6ml of intravenous blood was collected in plane tubes. and 2ml of blood was collected in EDTA tube for estimation of Ascorbic acid in plasma.

4.9- Estimation of Serum Lipid Peroxide(Malondialdehyde) (Method: - Kei Satoh. Clinica Chemica Acta,1978;90:37-43)[229]

PRINCIPLE:-

Lipoproteins were precipitated from the serum by addition of 20 mg% TCA. Then, serum was heated in boiling waterbath with Thiobarbituric Acid (TBA) in Sodium sulfate to form the chromogen. The resulting chromogen was extracted in butanol and the absorbance of the organic phase was measured at 530nm wavelength.

REAGENT:-

1) Trichloroacetic acid (20 mg %)

   20 mg of TCA was dissolved in 75 ml of distilled water and total volume was adjusted to 100 ml with distilled water.

2) Thiobarbituric acid [TBA] (0.67%)

   670 mg of TBA was dissolved in 75 ml of 0.5 M Sodium Sulfate and final volume was adjusted to 100 ml by 0.5 M Sodium sulfate

3) n- Butanol

4) Malondialdehyde (MDA-1,1,3,3 tetra ethoxy propane)

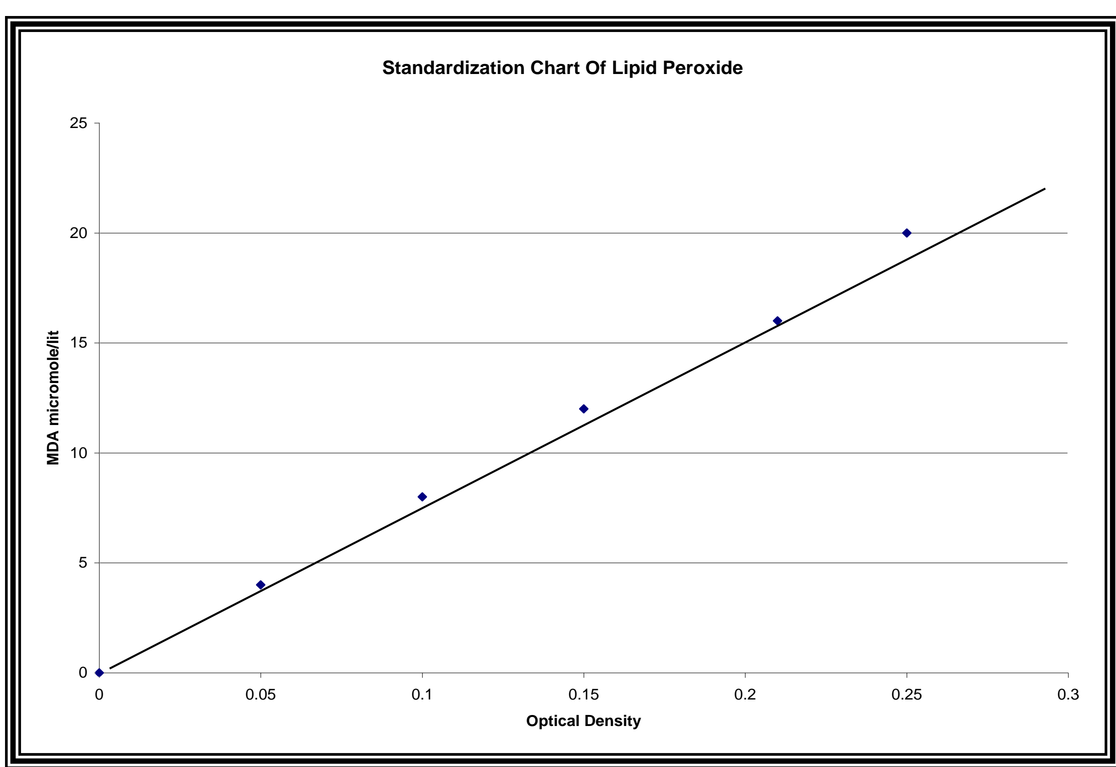
MW-164.2, 1ml=0.99 gm: Purity – 98%

Standard (20µ mol/Lit) was prepared and used to prepare standard curve.

PREPARATION OF STANDARD CURVE:-

Primary Std of concentration 20µm/L was serially diluted in 5 test tubes and used for the assay to get the optical density (O.D.) & The graph was obtained showing plot of OD against concentration.

Test tubes →	1	2	3	4	5
Primary std (ml)	1	2	3	4	5
D/W (ml)	4	3	2	1	0
Conc. (µM/lit)	04.0	08.0	12.0	16.0	20.0
Optical density	0.05	0.10	0.15	0.21	0.25



PROCEDURE:-

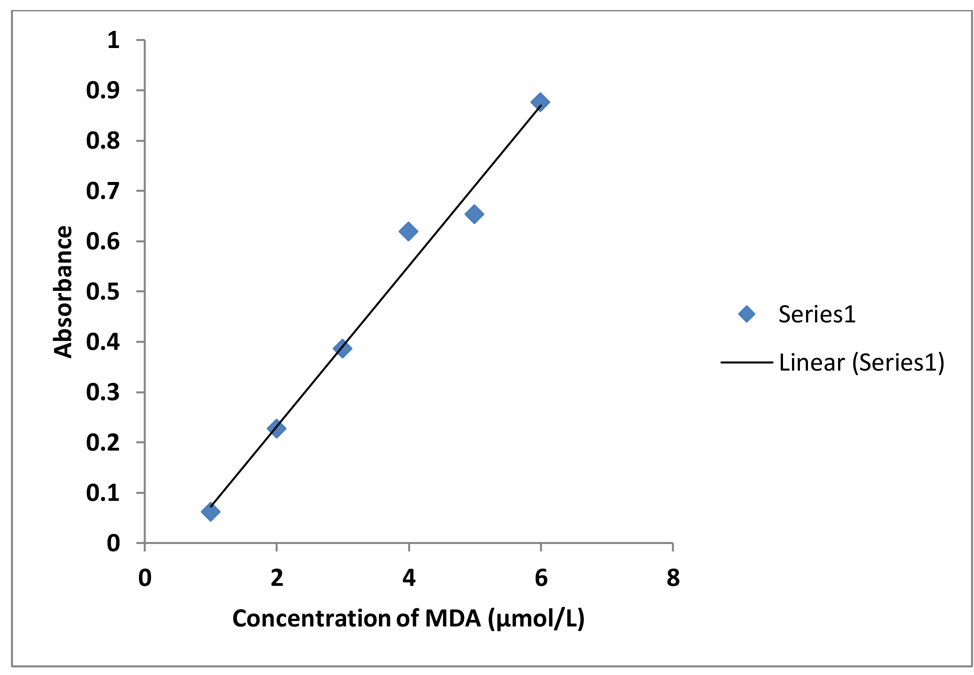
Two clean and dry test tubes were taken and labeled as Test & Blank and reagents were added as follows

Reagents	Test	Blank
Serum	250 µl	--
Distilled Water	--	250µl
TCA (20mg %)	1.25 ml	1.25 ml
TBA	500µl	500µl
Tubes were kept in boiling waterbath fro 30 mins & Cooled under tap water		
n-Butanol	2 ml	2 ml
Mixed well and centrifuged at 1500rpm for 10 mins Butanol layer was read at 530 nm		

The concentration of serum Lipid peroxide was determined by using the graph of standard.

Standardized Graphs for the Estimation of Malondialdehyde.

Concentrations(µmol/L)	Absorbances
0	0.062
4	0.228
8	0.386
12	0.62
16	0.654
20	0.876



4.10- Estimation of Serum Nitric Oxide as Nitrite(NO) (Method:-N.K. Cortas, Clin Chem.1990; 36/8, 1440-1443)[230]

PRINCIPLE:-

Nitrate, the stable product of Nitric Oxide was reduced to nitrite by Cadmium reduction method after deproteinization of sample by somogyi reagent. The nitrite produced was determined by diazotization of sulfanil amide and coupling to napthylethyline diamine.

REAGENTS:-

1) Glycine-NaOH Buffer (pH- 9.7):-

   7.5 gm of glycine was dissolved in 200ml of deionized distilled water, and then pH of solution was adjusted to 9.7 with 2 M NaOH. Finally the volume was adjusted to 500 ml by deionised distilled water.

2) Sulfanilamide:-

   2.5 gm of sulfanilamide was dissolved in 250 ml of warm 3 mol/Lit HCL solution & allowed to cool (Stable for 01 yr at Room temperature.)

3) N- Naphthylethylene diamine:-

   50 mg of Naphthylethylene diamine was dissolved in 200 ml of deionised distilled water and final volume was adjusted to 250 ml

5) $ZnSO_4$ Solution (75 mMol/Lit)
6) H_2SO_4 Solution (0.1 Mol/Lit)
7) NaOH Solution (55 mMol/Lit)
8) $CuSO_4$ Solution (5 mMol/Lit)

   79.8 mg of $CuSO_4$ (M.W.-159.6) was dissolved in Glycine NaOH buffer (pH- 9.7) to make final volume of 100 ml.

9) Cadmium Granules.

10) $NaNO_2$ Standard:

i) Stock Standard (0.1 mol/Lit):- 690 mg $NaNO_2$ was dissolved in 100 ml of 10 mMol/Lit Sodium Borate

ii) Working Standard (10 µMol/Lit):- 100 µl of stock standard was diluted to 1000 ml with 10 mMol/Lit Sodium Borate.

PROCEDURE:-

I] Deproteinization:-

A centrifuge was taken and following additions were done.

Solutions	'T'
Serum	0.5 ml.
$ZnSO_4$(75 mMol/Lit)	2.0 ml.
NaOH (55 mMol/Lit)	2.5 ml.

Solutions were mixed well & centrifuged at 1500 rpm for 10 mins, supernatant was used for further assay

II] Activation of cadmium Granules:-

Cadmium granules were stored in 0.1 mol/Lit H_2SO_4

Solution. At the time of assay the cadmium granules were removed from acid and rinsed 3 times by deionised distilled water. Then granules were swirled in 5 mMol/Lit $CuSO_4$ solution for 1 to 2 min. These copper coated granules were drained and washed by Glycine-NaOH buffer. Thus activated granules were used within 10 mins after activation. The granules after use were washed by deionised distilled water and stored in 0.1mol/Lit H_2SO_4.

III] Nitrite Assay

A set of three test tubes was arranged and additions were done as follows.

Reagents	Test	Standard	Blank
Glycine- NaOH Buffer	0.5 ml	0.5 ml	0.5 ml
Serum	0.5 ml	--	--
$NaNO_2$ Std (10µmol/Lit)	--	0.5 ml	--
Distilled Water	1.0 ml	1.0 ml	1.5 ml
2.5 to 3 gm of freshly activated Cadmium-Granules added in each tube & stirred once by swirling. Exactly after 90 mins 1.0 ml of the solutions was transferred in other respective three test tubes			
Sulfanilamide	0.5 ml	0.5 ml	0.5 ml
Napthylethylenediamine	0.5 ml	0.5 ml	0.5 ml

Mixed and kept for 20 mins, then read at 540 nm.

CALCULATIONS:-

Serum nitrite = (OD of Test- OD of Blank/OD of Std - OD of Blank) X 100 (µmol/Lit)

4.11- Estimation of Serum Ascorbic Acid by phenyl-hydrazine [231]spectrophotometry method:

Preparation of 2g% Dinitrophenylhydrazine (DNPH) Reagent:

2 grams of DNPH was dissolved in 100 ml of 4.5mol/L H_2SO_4 . Kept in refrigerator overnight, and then filtered.

1. **5% Thiourea solution:**

   5 grams of thiourea was dissolved in 100 ml of deionized distilled water and preserved at 4 ^{0}C

2. **6% Copper Sulphate Solution:**
   0.6 grams of $CuSO_4$ was dissolved in 100 ml of deionized distilled water.

3. **Preparation of DNPH-Thiourea-Copper Sulphate Reagent:**
   5ml of thiourea, 5ml of copper sulphate and 100ml of DNPH reagent were mixed and. stored at 4 ^{0}C in refrigerator
4. **Preparation of 12M H_2SO_4:** 65 ml of H_2SO_4 was dissolved in 35ml of distilled water (65% H_2SO_4).
5. **Preparation of 5% Trichloro acetic Acid(TCA)**: 5gm of TCA was dissolved in 100ml of distilled water.
6. 50mg/dL Ascorbic Acid Stock Calibrator: Dissolve 50mg of Ascorbic Acid was dissolved in 100ml of 6% metaphophoric acid.

   5mg/dL Intermediate Calibrator: 5ml of stock calibrator was made upto 100ml with 6% metaphophoric acid.

   Working Calibrator: In a series of 25ml volumetric flasks, pipette the following amounts of intermediate calibrators were pipetted: 0.5, 2.0, 4.0, 6.0, 10.0, 15.0 and 20.0ml. Final volume was brought upto 25ml with 6% metaphophoric acid to yield working calibrators of concentrations 0.1, 0.4, 0.8, 1.2, 2.0, 3.0and 4.0 mg/dL.

Standardized Protocol:

Reagents	Blank	S_1	S_2	S_3	S_4	S_5
Intermediate Calibrators		0.5	2.0	4.0	6.0	10.0
6% metaphophoric acid	1.2	24.5	23.0	21.0	19.0	15.0
Working Calibrators	-	1.2	1.2	1.2	1.2	1.2
Vit C in mg/dL	-	0.1	0.4	0.8	1.2	2.0
DTCS	0.4	0.4	0.4	0.4	0.4	0.4
Mixed and incubated at 37 ^{0}C for 3 hours, then were cooled the tubes in ice bath for 10 minutes.						
12 M H_2SO_4 (ml)	2.0	2.0	2.0	2.0	2.0	2.0

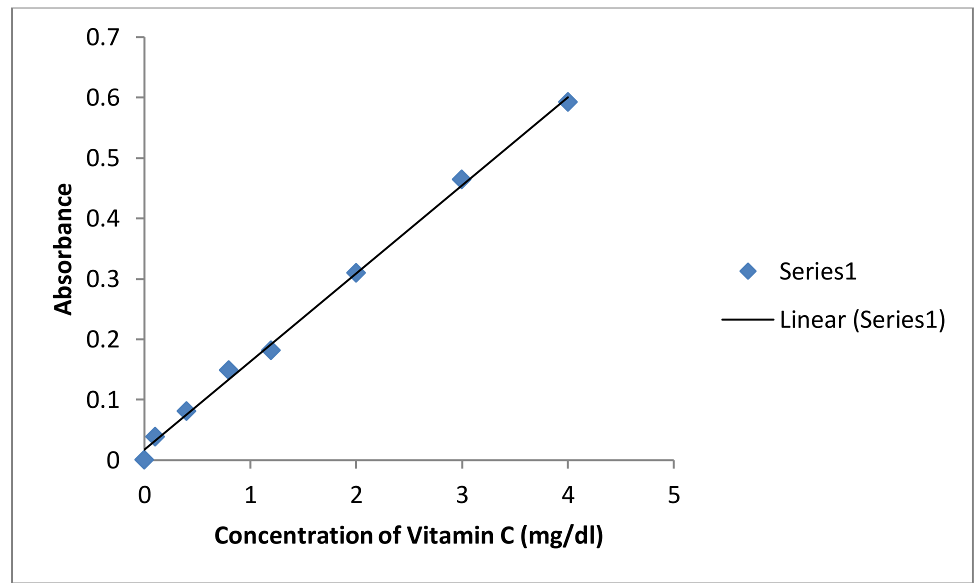
Procedure:

0.3 ml of plasma + 1.2ml of 5% TCA→mixed well and centrifuged at 3000rpm for 10 mins.→0.96ml of supernatant was taken +0.4ml of DTCS is added→kept for incubation for 3hrs→Chilled in Ice cold water for 10 mins→2.0ml of 12 M H_2SO_4 is added gradually.

Absorbance was read at 520nm wavelength.

Standardized Graphs for the Estimation of Vitamin C

Concentrations(mg/dl)	Absorbances
0	0
0.1	0.0381
0.4	0.0809
0.8	0.1485
1.2	0.1817
2	0.3094
3	0.4645
4	0.5924



Data obtained was expressed in terms of Mean±SD. Statistical significance was established at $P<0.05$ with 95% confidence interval. Unpaired't' test, One way analysis of variance (ANOVA), linear regression were used to determine the significant differences between the groups. Data was analyzed by using GraphPad Prism System (version 5.0)

RESULTS

5. RESULTS:

Table 2 : Anthropometric Parameters of the Study and Control Groups.

Parameters	Mean ± SD of Exposed(N= 134)	Mean ± SD of Control(N= 134)	P value
Age (years)	29.24± 5.899	32.81± 8.705	-
Height (cms)	165.0±4.799	164.6±6.168	0.562
Weight (kgs)	59.21±8.777	60.87±6.830	0.084
BMI (kg/m^2)	21.75±3.019	22.43± 1.541	0.022*
Years of Exposure (years)	9.776±6.476	-	-

Note: *-P<0.05, **-P<0.01, ***-P<0.001

Table -2, shows the Mean±SD of exposed and control groups. Students 't' test was applied to know the significance between both the groups, P<0.05 is considered as significant difference at CI of 95%. There were no significant differences in age, height and weight between individuals of both the groups. Body Mass Index of both the groups have found in normal limits though groups show statistically significant difference(P<0.05).

Table 3 : Shows comparison of Mean ± SD of Pulmonary Function Test Parameters of study and Control groups.

Parameters	Mean ± SD of Exposed (N=134)	Mean ± SD of Control (N=134)	P- value
FVC (Forced Vital Capacity)(in liters)	2.890±0.465	3.233±0.322	P<0.0001***
FEV_1 (Forced Expiratory Volume in first second) (in liters)	2.232±0.472	2.630±0.361	P<0.0001***
FEV_1/FVC Ratio(%)	76.88± 8.937	81.16±5.914	P<0.0001***

Note: *-P<0.05, **-P<0.01, ***-P<0.001

Table-3, shows the Mean ± SD of Pulmonary Function Test parameters of study and Control groups. Forced Vital Capacity(FVC) of exposed individuals have found significantly lower than control group individuals (P<0.0001). Forced Expiratory Volume in first second(FEV_1) of exposed group individuals is also found significantly low compared to control group. Hence, FEV_1/FVC ratio is exposed group individuals is also found significantly lower in comparison with control group individuals. Obtained values of PFT parameters of both the groups were in the lower normal limit.

Table 4 : Shows comparison of Mean ± SD of Haematological Parameters of Study and Control groups.

Parameters	Mean ± SD of Exposed (N=134)	Mean ± SD of Control(N=134)	P- value
RBC count (millions/cumm)	4.826±0.510	4.792±0.548	0.603
TLC (cells×10^3/cumm)	7.765±2.145	7.771 ±2.169	0.981
Hb(gms/dl) concentration	13.66±1.830	13.85 ±1.510	0.345
PCV (%)	40.94±5.114	40.99±4.043	0.940
MCV (fl)	85.09±8.879	85.88±6.520	0.404
MCH (pg)	28.40±3.489	29.05 ±2.660	0.088
MCHC (%)	33.36±2.296	33.80 ±1.580	0.070
Neutrophils (cells/cumm)	4034±1726.603	4560 ±1889.319	0.018*
Lymphocytes (cells/cumm)	2713±886.611	2464±844.432	0.020*
Eosinophils (cells/cumm)	467.0±419.470	261.4±240.992	P<0.0001*
Basophils (cells/cumm)	43.06±18.917	34.55±20.697	0.0005*
Monocytes (cells/cumm)	508.7±208.719	450.0±170.608	0.012*

Note: *-P<0.05,**-P<0.01,***-P<0.001. (Hb-Haemoglobin concentration, RBC-Red Blood Cell count, TLC-Total Leucocyte Count, PCV-Packed Cell Volume, MCV-Mean Corpuscular Volume, MCH-Mean Corpuscular Haemoglobin, MCHC-Mean Corpuscular Haemoglobin Concentration).

Table-4 represents the Mean ± SD of hematological parameters of exposed and control groups. On comparison of mean values with standard deviation by considering significance of P<0.05, no significant difference in Counts of RBC,WBC and Platelets, Hemoglobin content, MCV,MCH,MCHC values. Absolute counts of Lymphocytes, Eosinophils, Monocyte and Basophils in exposed group were comparatively higher than control group. Though values of exposed were significantly differed from control group, are found in higher normal ranges. This depicts, exposure to dust in rice mills has less adverse effect on hematological values.

Table 5 : Comparison Mean ± SD of Blood Pressure and ECG parameters of Study and Control groups.

Parameters	Mean±SD of Exposed(N=134)	Mean±SD of Control(N=134)	P value
ECG Parameters			
P Wave amplitude (mV)	0.155± 0.064	0.145± 0.057	0.166
P Wave duration (sec)	0.091± 0.021	0.089 ± 0.017	0.307
QRS amplitude (mV)	1.230± 0.415	0.962± 0.331	P<0.0001*
QRS duration (sec)	0.086± 0.016	0.085± 0.015	0.630
T Wave amplitude (mV)	0.319± 0.130	0.253± 0.114	P<0.0001*
T Wave duration (sec)	0.176± 0.037	0.162± 0.030	0.0006*
PR Interval (sec)	0.144± 0.033	0.101± 0.048	P<0.0001*
QT Interval (sec)	0.365± 0.032	0.364± 0.033	0.737
ST Segment (sec)	0.100± 0.031	0.114± 0.036	0.0005*
Heart Rate (bpm)	75.62± 9.646	76.42± 9.147	0.487
Blood Pressure Parameters			
Systolic BP (mmHg)	123.9±11.452	121.4±8.342	0.045*
Diastolic BP (mmHg)	80.19±9.590	77.99±6.298	0.027*
Pulse Pressure (mmHg)	43.60±10.981	43.42±7.422	0.876
Mean Arterial BP (mmHg)	94.83±8.833	92.53±6.137	0.014*

Note: *-P<0.05, **-P<0.01, ***-P<0.001. BP-Blood Pressure.

Table-5 represents Mean±SD of the electrocardiographic Blood Pressure parameters of exposed and control groups. There was no significant difference obtained among ECG parameters of exposed and control group. There were statistical significant differences obtained in amplitude of QRS complex and T wave, also significant differences in duration of T wave, PR interval and ST segment between exposed and control group.

Similarly, there was significant difference in Systolic Blood Pressure(SBP), Diastolic Blood Pressure(DBP) and Mean Arterial Blood Pressure(MABP) between Exposed and Control group.

All the values obtained by ECG and Blood Pressure measurement were found to be in normal ranges.

Table 6 : Comparison Mean ± SD of Biochemical parameters of Study and Control groups.

Parameters	Mean ± SD of Exposed (N=134)	Mean ± SD of Control (N=134)	P- value
Serum MDA (µmol/l)	7.009±1.892	5.639±2.091	P<0.0001*
Serum Nitric Oxide (µmol/l)	51.03±16.812	36.73±14.157	P<0.0001*
Plasma Vitamin C (mg/l)	0.863±0.324	1.173±0.262	P<0.0001*

Note: *-P<0.05,**-P<0.01,***-P<0.001

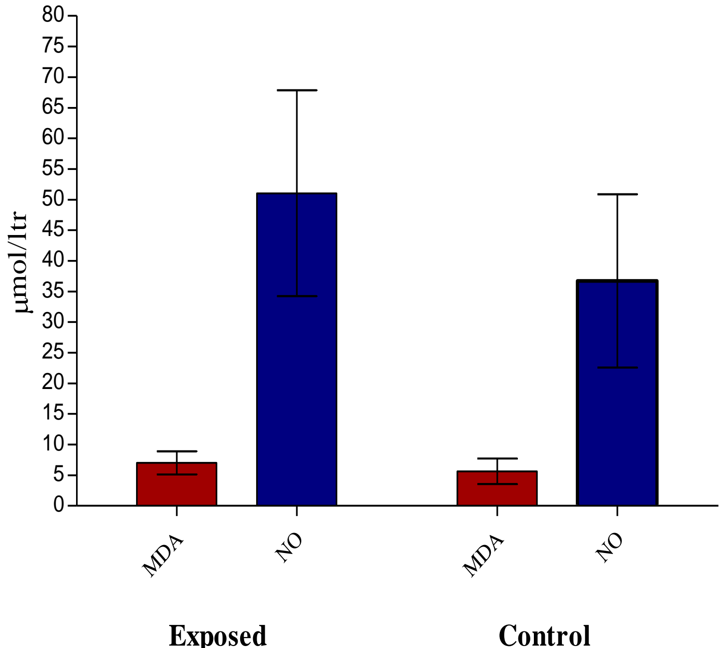


Figure-15

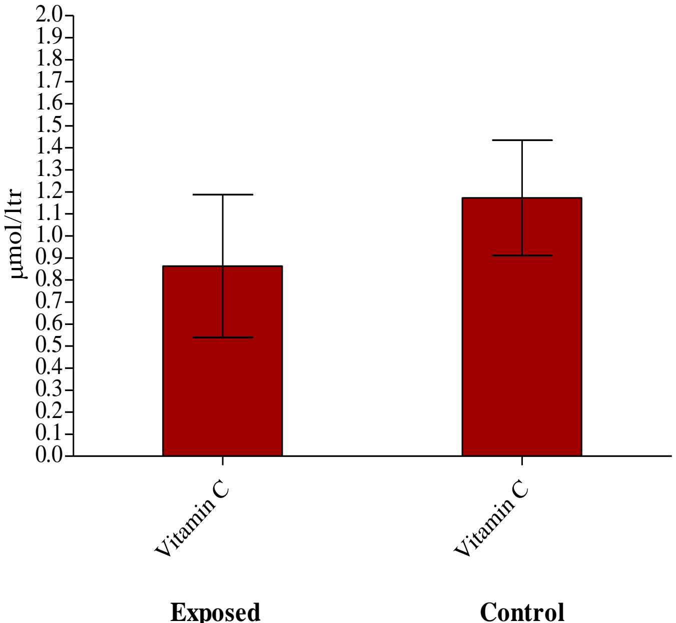


Figure-16

Table-6, figure- 15 depicts the Mean±SD values of serum levels of MDA, NO and also Plasma level of vitamin-C of exposed and control group. Oxidants Level(MDA and NO) in exposed group were highly significant($P<0.0001$) compared to control group.

Exposed group was divided into three subgroups based on exposure duration to know the effect of duration of exposure. 5-9 years as exposed-I (N=82) ,10-18 years as exposed-II(N=31) and 19-27 years as exposed –III(N=21).

Table 7 : ANOVA followed by post hoc 't' test showing Mean ± SD of Pulmonary Function Test parameters of Study group with Duration of Exposure.

Parameters	Control (Mean±SD) N=134	Exposed –I (5-9 years) (Mean±SD) N=82	Exposed-II (10-18 years) (Mean±SD) N=31	Exposed-III (19-27 years) (Mean±SD) N=21	F Value	P Value
FVC (Forced Vital Capacity)(in liters)	3.233±0.322	2.924±0.374	2.801±0.643	2.891±0.485	17.133	0.000***
FEV_1 (Forced Expiratory Volume in first second) (in liters)	2.629±0.361	2.335±0.369	2.091±0.665	2.037±0.389	25.370	0.000***
FEV_1/FVC Ratio(%)	81.161±5.914	79.816±6.855	73.339±11.478	70.018±7.863	20.763	0.000***
Post hoc 't' test						
Parameters	Control vs Exposed-I		Control vs Exposed-II		Control vs Exposed-III	
	P-value		P-value		P-value	
FVC (Forced Vital Capacity)(in liters)	0.000***		0.000***		0.001**	
FEV_1 (Forced Expiratory Volume in first second) (in liters)	0.000***		0.000***		0.000***	
FEV_1/FVC Ratio(%)	0.434		0.000***		0.000***	

Note: *-P<0.05, **-P<0.01, ***-P<0.001

Table- 7 represents Mean±SD of different groups and analysis of effect of duration of exposure to dust in rice mills on Pulmonary Function Tests by using ANOVA followed by post hoc 't' test. There was significant effect of exposure duration on FVC, FEV_1 and FEV_1/FVC of rice mill workers, Exposed-III group showed below normal level of FEV_1/FVC ratio than exposed-I and exposed-II groups. post hoc 't' test was performed to know the significantly affected group. There was significant decrease in FVC, FEV_1 and FEV_1/FVC ratio in all the groups of comparison.

Table 8 : ANOVA showing Mean ± SD of Haematological parameters of Study group with Duration of Exposure.

Parameters	Control (Mean±SD) N=134	Exposed –I (5-9 years) (Mean±SD) N=82	Exposed-II (10-18 years) (Mean±SD) N=31	Exposed-III (19-27 years) (Mean±SD) N=21	F Value	P Value
RBC count(millions/mm^3)	4.792±0.548	4.899±0.493	4.766±0.577	4.629±0.423	1.720	0.163
TLC (cells×10^3/cumm)	7.771±2.169	7.259±2.051	8.806±2.191	8.200±1.852	4.367	0.005**
Hb(gms/dl) concentration	13.849±1.509	13.707±1.868	13.826±1.877	13.200±1.611	0.947	0.418
Platelets count(lakhs/ mm^3)	2.624±0.769	2.686±0.714	2.874±0.897	2.574±0.808	0.999	0.394
PCV (%)	40.985±4.043	41.309±4.889	40.861±6.035	39.628±4.484	0.748	0.524
MCV (fl)	85.884±6.519	84.57±8.655	85.784±7.876	86.067±11.178	0.541	0.655
MCH (pg)	29.051±2.659	28.068±3.605	29.084±2.757	28.695±3.953	1.861	0.137
MCHC (%)	33.802±1.580	33.140±2.252	33.955±2.152	33.362±2.608	2.405	0.068
Neutrophils (cells/ mm^3)	4560±1889.3	3592±1370.4	5049.42±2193.8	4261±1595.4	7.235	0.000***
Lymphocytes (cells/ mm^3)	2664.10±844.4	2779.27±1059.5	2570.97±485.9	2661.90±523.9	2.298	0.078
Eosinophils (cells/ mm^3)	261.42±240.9	371.34±387.2	564.52±406.5	696.43±455.1	15.129	0.000***
Monocytes (cells/ mm^3)	450.00±170.6	480.39±216.5	572.19±199.4	525.48±455.1	3.972	0.009**
Basophils (cells/ mm^3)	34.55±20.6	37.80±14.9	49.35±20.6	54.29±22.8	9.824	0.000***

Note: *-P<0.05, **-P<0.01, ***-P<0.001

Continued…

Post hoc 't' test			
Parameters	Control vs Exposed-I	Control vs Exposed-II	Control vs Exposed-III
	P-value	P-value	P-value
RBC count(millions/mm^3)	0.375	0.992	0.456
TLC (cells×10^3/cumm)	0.228	0.042*	0.761
Hb(gms/dl) concentration	0.901	1.000	0.264
Platelets count(lakhs/ mm^3)	0.914	0.276	0.989
PCV (%)	0.940	0.999	0.496
MCV (fl)	0.536	1.000	0.999
MCH (pg)	0.070	1.000	0.944
MCHC (%)	0.049*	0.970	0.701
Neutrophils (cells/ mm^3)	0.000***	0.407	0.845
Lymphocytes (cells/ mm^3)	0.029*	0.895	0.690
Eosinophils (cells/ mm^3)	0.054	0.000***	0.000***
Monocytes (cells/ mm^3)	0.571	0.004**	0.241
Basophils (cells/ mm^3)	0.532	0.000***	0.000***

Note: *-P<0.05, **-P<0.01, ***-P<0.001

Table-8, shows the Mean±SD by ANOVA and post hoc 't' test for hematological parameters in different exposure duration groups and control. There were significant variations obtained in TLC, Absolute counts of Neutrophils,Eosinophils, Monocytes and Basophils by ANOVA. Post hoc test revealed significant difference(P<0.05) in TLC of control vs exposed-II group, Absolute counts of Neutrophil and Lymphocytes were significantly varied in control vs exposed-I group, where neutrophils count in exposed-I was significantly low(P<0.001) compared to control and lymphocyte count was significantly higher(P<0.05) than control. Whereas Absolute counts of Eosinophils,Monocytes and Basophils were significantly increased(P<0.001) in control vs exposed-II group. Control vs exposed-III group showed significant increase(P<0.001) in absolute counts of Eosinophils and Basophils.

Table 9 : ANOVA showing Mean ± SD of ECG parameters of Study group with Duration of Exposure.

Parameters	Control (Mean±SD) N=134	Exposed –I(5-9 yrs) (Mean±SD) N=82	Exposed-II (10-18 yrs) (Mean±SD) N=31	Exposed-III (19-27 yrs) (Mean±SD) N=21	F Value	P Value
P Wave amplitude (mV)	0.145± 0.057	0.144± 0.052	0.164± 0.055	0.186± 0.101	3.709	0.012*
P Wave duration (sec)	0.089± 0.017	0.090± 0.019	0.094± 0.019	0.091± 0.026	0.669	0.572
QRS amplitude (mV)	0.962± 0.330	1.282± 0.450	1.187± 0.357	1.090± 0.318	13.155	0.000***
QRS duration (sec)	0.085± 0.015	0.086± 0.017	0.088± 0.016	0.082± 0.009	0.703	0.551
T Wave amplitude (mV)	0.253± 0.114	0.337± 0.134	0.316± 0.129	0.252± 0.093	9.300	0.000***
T Wave duration (sec)	0.161± 0.030	0.174± 0.036	0.174± 0.034	0.185± 0.045	4.616	0.004**
PR Interval (sec)	0.101± 0.048	0.142± 0.036	0.142± 0.025	0.152± 0.027	24.121	0.000***
QT Interval (sec)	0.364± 0.033	0.364± 0.030	0.369± 0.030	0.366± 0.041	0.222	0.881
ST Segment (sec)	0.114± 0.036	0.099± 0.033	0.107± 0.028	0.093± 0.023	4.875	0.003**
Heart Rate (bpm)	76.42± 9.147	75.07± 9.472	75.13± 8.958	78.48± 10.191	0.929	0.427

Continued…

Post hoc 't' test			
Parameters	Control vs Exposed-I	Control vs Exposed-II	Control vs Exposed-III
	P-value	P-value	P-value
P Wave amplitude(mV)	0.999	0.267	0.012*
P Wave duration (sec)	0.946	0.417	0.922
QRS amplitude (mV)	0.000***	0.008**	0.361
QRS duration (sec)	0.939	0.685	0.797
T Wave amplitude (mV)	0.000***	0.027*	1.000
T Wave duration (sec)	0.023*	0.164	0.011*
PR Interval (sec)	0.000***	0.000***	0.000***
QT Interval (sec)	1.000	0.820	0.994
ST Segment (sec)	0.004**	0.612	0.023*
Heart Rate (bpm)	0.657	0.861	0.716

Note: *-P<0.05, **-P<0.01, ***-P<0.001

Table-9, shows the Mean±SD by ANOVA and post hoc 't' test for ECG parameters in different exposure duration groups and control. There were significant variations obtained in amplitudes of P wave, QRS complex and T wave and also durations of T wave, PR interval and ST segments by ANOVA. Post hoc test showed significant increase in P wave amplitude(P<0.05) of exposed-III in control vs exposed-III group. QRS amplitude (P<0.001), T wave amplitude (P<0.001), duration of T wave (P<0.05), PR interval(P<0.001) and ST segment (P<0.001) was significantly increased in exposed-I of control vs exposed-I group. There was significant increase in QRS amplitude (P<0.001), T wave amplitude (P<0.05) and duration of PR interval in exposed-II of control vs exposed-II group. Significantly longer duration of T wave (P<0.05), PR interval (P<0.001) and ST segment (P<0.05) in exposed-III of control vs exposed-III group.

Table 10 : ANOVA showing Mean ± SD of Blood Pressure parameters of Study group with Duration of Exposure.

Parameters	Control (Mean±SD) N=134	Exposed –I (5-9 years) (Mean±SD) N=82	Exposed-II (10-18 years) (Mean±SD) N=31	Exposed-III (19-27 years) (Mean±SD) N=21	F Value	P Value
Systolic BP (mmHg)	121.40±8.342	123.37±11.478	124.84±12.678	124.38±9.729	1.525	0.208
Diastolic BP (mmHg)	77.99±6.298	79.93±9.770	78.97±10.222	83.05±7.553	2.802	0.040*
Pulse Pressure (mmHg)	43.42±7.422	43.32±10.700	45.87±12.596	41.33±9.302	1.054	0.369
Mean Arterial BP (mmHg)	92.53±6.137	94.41±9.058	94.52±9.298	96.90±7.176	2.658	0.049*

Post hoc 't' test

Parameters	Control vs Exposed-I	Control vs Exposed-II	Control vs Exposed-III
	P-value	P-value	P Value
Systolic BP (mmHg)	0.406	0.233	0.490
Diastolic BP (mmHg)	0.235	0.899	0.024*
Pulse Pressure (mmHg)	1.000	0.455	0.705
Mean Arterial BP (mmHg)	0.211	0.459	0.043*

Note: *-P<0.05, **-P<0.01, ***-P<0.001

Table-10, shows the Mean±SD by ANOVA and post hoc 't' test for Blood Pressure parameters in different exposure duration groups and control. There was a significant difference seen in Mean±SD of Diastolic Blood Pressure(DBP) and Mean Arterial Blood Pressure(MABP). Post hoc 't' test indicated significant increase in Diastolic Blood Pressure ($P<0.05$) and Mean Arterial Blood Pressure ($P<0.05$) of exposed-III in control vs exposed-III.

Table 11 : ANOVA showing Mean ± SD of Biochemical parameters of Study group with Duration of Exposure.

Parameters	Control (Mean±SD) N=134	Exposed –I (5-9 years) (Mean±SD) N=82	Exposed-II (10-18 years) (Mean±SD) N=31	Exposed-III (19-27 years) (Mean±SD) N=21	F Value	P Value
Serum MDA (μmol/l)	5.639±2.091	6.627±1.749	7.600±2.103	7.629±1.797	13.425	0.000***
Serum Nitric Oxide (μmol/l)	36.729±14.157	48.316±17.620	54.251±15.252	56.899±13.817	21.591	0.000***
Plasma Vitamin C (mg/l)	1.173±0.262	0.915±0.331	0.798±0.330	0.752±0.248	27.708	0.000***
Post hoc 't' test						
Parameters	Control vs Exposed-I		Control vs Exposed-II		Control vs Exposed-III	
	t-value	P-value	t-value	P-value	t-value	P Value
Serum MDA (μmol/l)		0.001**		0.000***		0.000***
Serum Nitric Oxide (μmol/l)		0.000***		0.000***		0.000***
Plasma Vitamin C (mg/l)		0.000***		0.000***		0.000***

Note: *-P<0.05,**-P<0.01,***-P<0.001

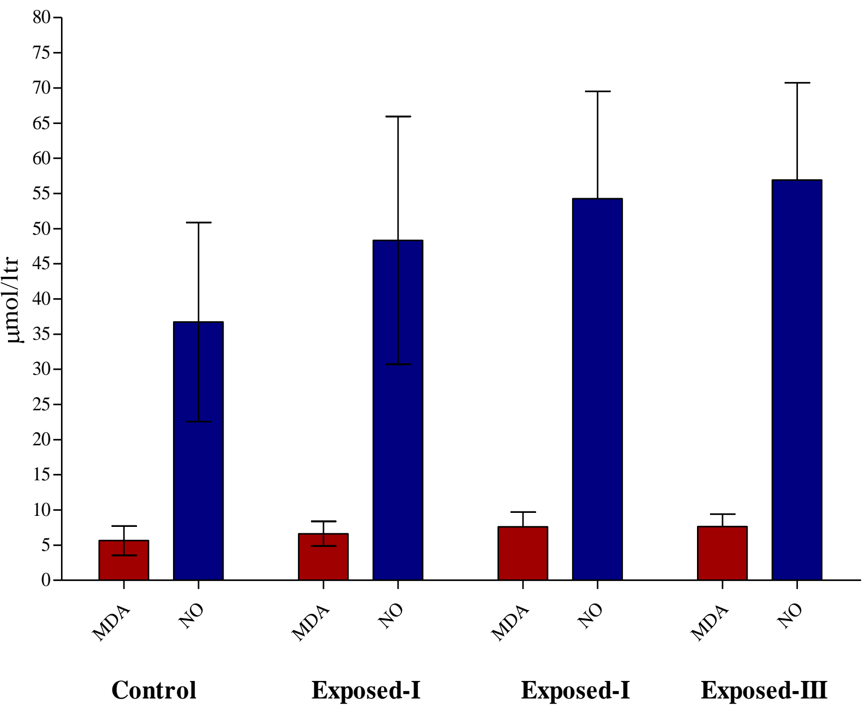


Figure-17

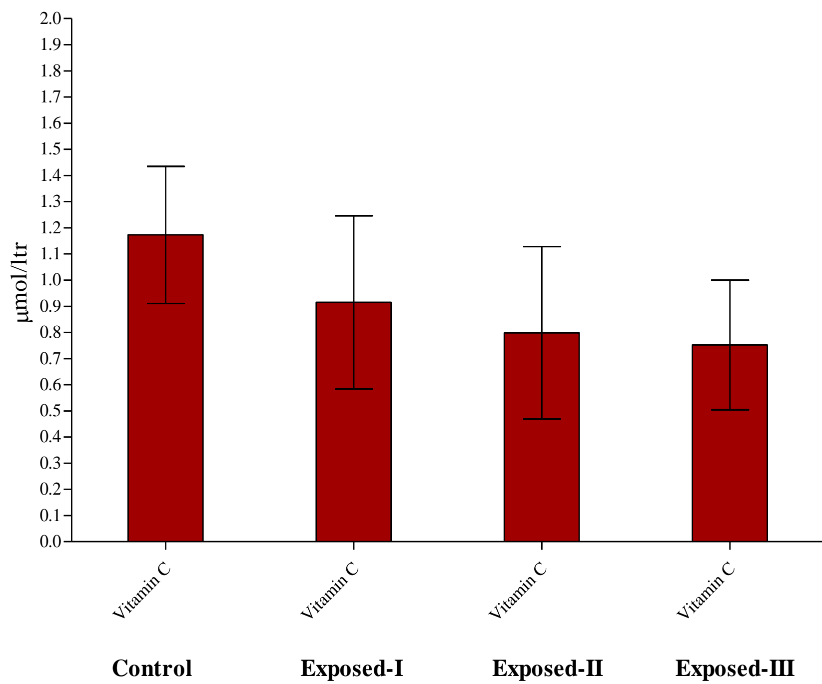


Figure-18

Table-11, Figure-17 and Figure-18 represent the effect of duration of exposure on serum levels of MDA, NO and Plasma level of Vitamin-C in exposed group individuals. There was significant increase in serum MDA($P<0.001$), serum NO($P<0.001$) and Significant decrease in plasma Vitamin-C($P<0.001$) of exposed group compared to control.

Post hoc 't' test revealed that serum levels of oxidants(MDA and NO) were significantly high and Plasma level of Anti-oxidant(Vitamin-C) was significantly low in all the exposed groups of comparison.

Control and exposed groups were categorized into subgroups according to age and analyzed to know the effect of age. 21-30 years of age as Group-I(control, N=68 and Exposed, N=68), 31-40 years as Group-II(control, N=32 and exposed, N=33), 41-50 years as Group-III(control, N=34 and exposed, N=33)

Table 12 : Comparison of Mean ± SD of Pulmonary Function Test parameters of Exposed and Control individuals with different Age groups.

Parameters	Group I(21-30 years)			Group II(31-40 years)			Group III(41-50 years)		
	Control (N=68)	Exposed (N=68)	P Value	Control(N=32)	Exposed (N=33)	P Value	Control (N=34)	Exposed (N=33)	P Value
FVC (Forced Vital Capacity)(in liters)	3.272 ± 0.30	3.033 ± 0.346	P<0.0001***	3.280 ± 0.302	2.906 ± 0.439	0.0002***	3.111 ± 0.344	2.580 ± 0.560	P<0.0001***
FEV_1 (Forced Expiratory Volume in first second) (in liters)	2.644 ± 0.334	2.443 ± 0.349	0.0008***	2.712 ± 0.370	2.198 ± 0.387	P<0.0001***	2.522 ± 0.389	1.831 ± 0.510	P<0.0001***
FEV_1/FVC Ratio(%)	80.72 ± 5.946	80.55 ± 7.002	0.885	82.49 ± 6.485	75.70 ± 6.708	P<0.0001***	80.80 ± 5.236	70.47 ± 10.58	P<0.0001***

Note: *-P<0.05, **-P<0.01, ***-P<0.001

Table-12 represents Mean ± SD of Pulmonary Function Test parameters of Exposed individuals with different age groups. Students unpaired 't' test showed significant differences in FVC(P<0.001) and FEV_1(P<0.001) among all the age groups of comparison. Whereas FEV_1/ FVC ratio was significantly decreased in exposed individuals of Group-II and Group-III. There was no significance in FEV_1/ FVC ratio of Group-I.

Table 13 : Comparison of Mean ± SD of Haematological parameters of Exposed individuals with different Age groups.

Parameters	Group I(21-30 years)			Group II(31-40 years)			Group III(41-50 years)		
	Control (N=68)	Exposed (N=68)	P Value	Control (N=32)	Exposed (N=33)	P Value	Control (N=34)	Exposed (N=33)	P Value
RBC count(millions/ mm^3)	4.758±0.614	4.943±0.505	0.057	4.927±0.441	4.620±0.505	0.011*	4.733 ±0.490	4.789 ±0.468	0.633
TLC (cells×10^3/ mm^3)	8.087 ±2.147	7.582±2.126	0.171	7.473±2.215	8.300±2.438	0.157	7.421 ±2.140	7.606 ±1.825	0.704
Hb(gms/dl) concentration	13.89 ±1.616	13.94±1.774	0.868	14.04±1.204	13.22±2.173	0.065	13.59 ±1.556	13.51 ±1.490	0.833
Platelets count(lkhs/mm^3)	2.653 ±0.779	2.763±0.710	0.390	2.544±0.794	2.909±0.897	0.087	2.641±0.744	2.408 ±0.701	0.193
PCV (%)	40.55±4.313	41.81±4.744	0.108	42.29±3.037	40.02±6.201	0.067	40.62 ±4.155	40.08 ±4.460	0.607
MCV (fl)	85.68 ±7.091	84.86±8.118	0.529	86.15±5.707	86.64±10.52	0.817	86.04 ±6.202	84.02 ±8.679	0.276
MCH (pg)	29.41±2.936	28.32±3.460	0.049*	28.59±2.273	28.63±3.986	0.958	28.76 ±2.363	28.34 ±3.090	0.534
MCHC (%)	34.28 ±1.674	33.34±2.301	0.008*	33.19±1.603	33.01±2.530	0.741	33.43 ±0.997	33.76 ±2.033	0.407
Neutrophils (cells/cumm)	4911±1972	3867 ±1643	0.001***	4247±1881	4318±1976	0.882	4154 ±1627	4094 ±1640	0.881
Lymphocytes (cells/cumm)	2448 ±902.7	2763±881.0	0.042*	2506±761.2	2809±1122	0.209	2456 ±820.3	2512 ±571.6	0.747
Eosinophils (cells/cumm)	251.7±290.1	403.3±416.9	0.015*	226.3±158.3	612.9±427.8	P<0.0001***	314.0 ±188.2	452.3± 391.8	0.069
Monocytes (cells/cumm)	433.2±182.1	511.8± 219.9	0.025*	475.2±106.2	510.6±186.4	0.352	459.9±195.2	500.3±212.0	0.419
Basophils (cells/cumm)	39.78±21.95	38.09± 14.20	0.595	20.78±10.56	48.94±20.26	P<0.0001***	37.06± 19.93	47.42± 23.39	0.055

Note: *-P<0.05, **-P<0.01, ***-P<0.001

Table-13 represents the Mean ± SD of hematological parameters in control and exposed groups. Student 't' test revealed a significant decrease in RBC count (P<0.05) of exposed individuals compared to controls in Group-II. There were significant decrease in MCH (P<0.05),MCHC (P<0.05) and Absolute counts of Neutrophils (P<0.001) of exposed individuals in Group-I. Absolute counts of Lymphocytes, Eosinophils and Monocytes were significantly high (P< 0.05) in exposed individuals of Group-I. Exposed individuals of Group-II showed significant increase in Absolute counts of Eosinophils (P< 0.001) and Basophils (P< 0.001).

Table 14 : Comparison of Mean ± SD of ECG parameters of Exposed individuals with different Age groups.

Parameters	Group I(21-30 years)			Group II(31-40 years)			Group III(41-50 years)		
	Control (N=68)	Exposed (N=68)	P Value	Control (N=32)	Exposed (N=33)	P Value	Control (N=34)	Exposed (N=33)	P Value
P Wave amplitude (mV)	0.134±0.051	0.146±0.053	0.189	0.153± 0.057	0.176± 0.087	0.219	0.159±0.064	0.155±0.056	0.743
P Wave duration (sec)	0.086±0.016	0.094±0.020	0.027*	0.090± 0.018	0.092± 0.023	0.682	0.093±0.019	0.086±0.018	0.130
QRS amplitude (mV)	0.941±0.317	1.297±0.398	P<0.0001***	0.947± 0.385	1.203± 0.348	0.007**	1.018±0.304	1.118±0.490	0.315
QRS duration (sec)	0.086±0.016	0.087±0.018	0.843	0.079± 0.007	0.082± 0.010	0.087	0.087±0.015	0.086±0.0146	0.787
T Wave amplitude (mV)	0.221±0.110	0.328±0.144	P<0.0001***	0.288± 0.113	0.324± 0.103	0.175	0.285±0.108	0.294±0.122	0.760
T Wave duration (sec)	0.151±0.027	0.174±0.036	P<0.0001***	0.171± 0.031	0.170± 0.035	0.850	0.173±0.035	0.186±0.040	0.177
PR Interval (sec)	0.066±0.038	0.143±0.033	P<0.0001***	0.136± 0.027	0.150± 0.028	0.044*	0.139±0.028	0.139±0.035	0.941
QT Interval (sec)	0.365±0.037	0.365±0.030	0.959	0.358± 0.029	0.360± 0.026	0.716	0.368±0.029	0.372±0.041	0.654
ST Segment (sec)	0.125±0.032	0.099±0.034	P<0.0001***	0.106± 0.036	0.102± 0.025	0.564	0.100±0.037	0.099±0.030	0.942
Heart Rate (bpm)	78.10±7.109	75.37±9.643	0.062	74.47± 11.12	74.79± 10.12	0.904	74.88± 10.32	76.97± 9.326	0.389

Note: *-P<0.05, **-P<0.01, ***-P<0.001

Table-14 represents the Mean ± SD of ECG parameters of exposed and control individuals in different age groups. There was significant increase in the amplitude of P wave (P<0.05), QRS complex (P<0.001) and T wave (P<0.001) of exposed individuals in Group-I. Significantly longer duration of P wave (P<0.05), T wave (P<0.001), PR interval (P<0.001) and ST segment (P<0.001) were obtained in exposed individuals of Group-I. Also there was increase in the amplitude of QRS complex (P<0.01) and longer duration of PR interval (P<0.05) of exposed individuals in Group -II.

Table 15 : Comparison of Mean ± SD of Blood Pressure parameters of Exposed individuals with different Age groups.

Parameters	Group I(21-30 years)			Group II(31-40 years)			Group III(41-50 years)		
	Control (N=68)	Exposed (N=68)	P Value	Control (N=32)	Exposed (N=33)	P Value	Control (N=34)	Exposed (N=33)	P Value
Systolic BP (mmHg)	123.2±7.324	124.1±11.66	0.562	119.6± 8.662	119.2± 9.500	0.856	119.6±9.378	128.0±11.38	0.002**
Diastolic BP (mmHg)	78.71±6.003	80.18±9.277	0.274	75.81±6.018	78.30±9.684	0.219	78.59± 6.832	82.12± 10.04	0.096
Pulse Pressure (mmHg)	44.47±6.657	43.82±11.44	0.688	43.75± 8.219	40.85± 9.963	0.206	41.00±7.758	45.88±10.70	0.036*
Mean Arterial BP(mmHg)	93.53±5.682	94.85±8.599	0.292	90.41± 5.929	91.94± 8.370	0.399	92.53± 6.841	97.67± 9.082	0.011*

Note: *-P<0.05, **-P<0.01, ***-P<0.001

Table-15 represents the Mean ± SD of Blood Pressure parameters of exposed and control individuals in different age groups. There was significant increase in Systolic Blood Pressure (P<0.01), Pulse Pressure (P<0.05) and Mean Arterial Blood Pressure (P<0.05) of exposed individuals in Group-III. There were no significant differences obtained between exposed and control individuals in Group-I and Group-II.

Table 16 : Comparison of Mean ± SD of Biochemical parameters of Exposed individuals with different Age groups.

Parameters	Group I(21-30 years)			Group II(31-40 years)			Group III(41-50 years)		
	Control (N=68)	Exposed (N=68)	P Value	Control (N=32)	Exposed (N=33)	P Value	Control (N=34)	Exposed (N=33)	P Value
Serum MDA (µmol/l)	5.659±2.251	6.291±1.647	0.064	5.969± 1.981	7.503± 1.757	0.002**	5.288±1.848	7.994±1.941	P<0.0001***
Serum Nitric Oxide(µmol/l)	37.61±16.26	42.18±14.09	0.082	36.56± 9.591	59.63± 15.22	P<0.0001***	35.13±13.43	60.69±13.72	P<0.0001***
Plasma Vitamin C (mg/l)	1.176±0.265	1.018±0.308	0.002**	1.241± 0.201	0.709± 0.257	P<0.0001***	1.103±0.292	0.696±0.258	P<0.0001***

Note: *-P<0.05,**-P<0.01,***-P<0.001

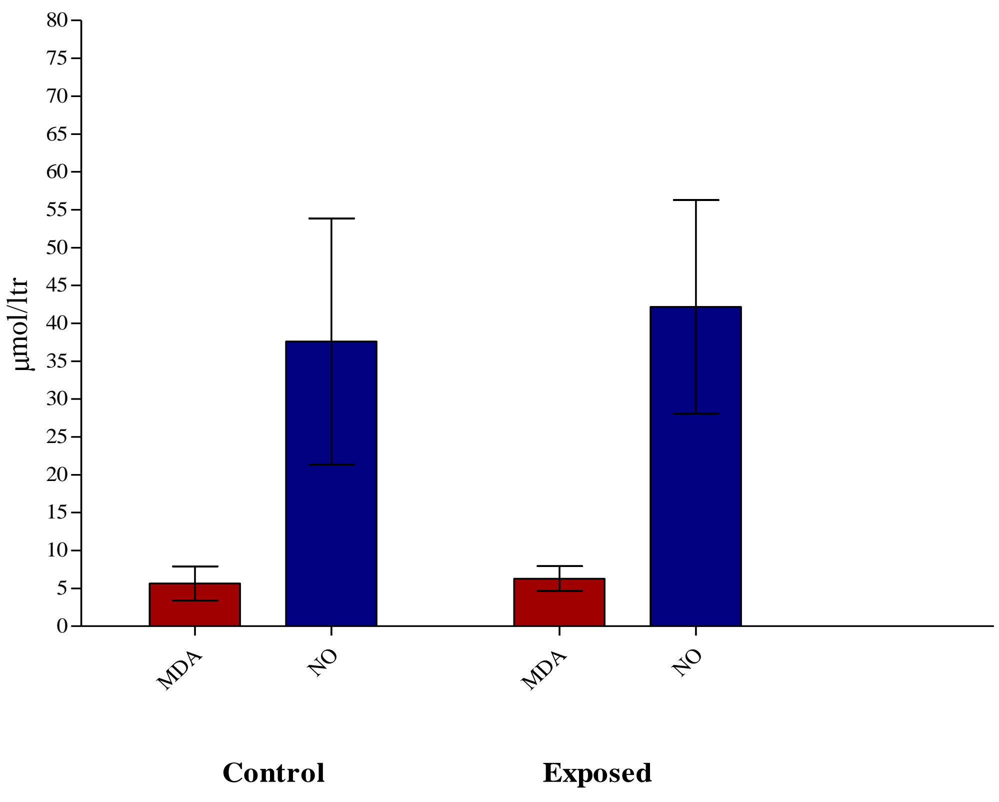


Figure-19

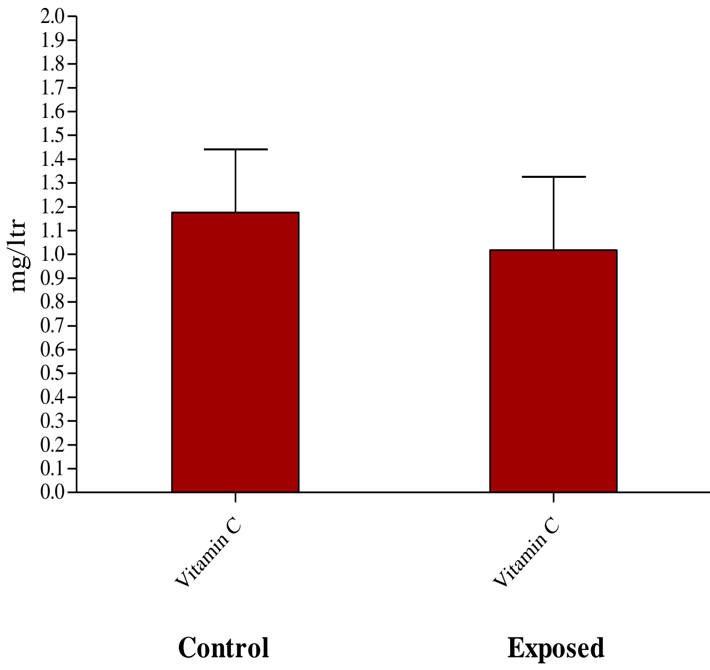


Figure-20

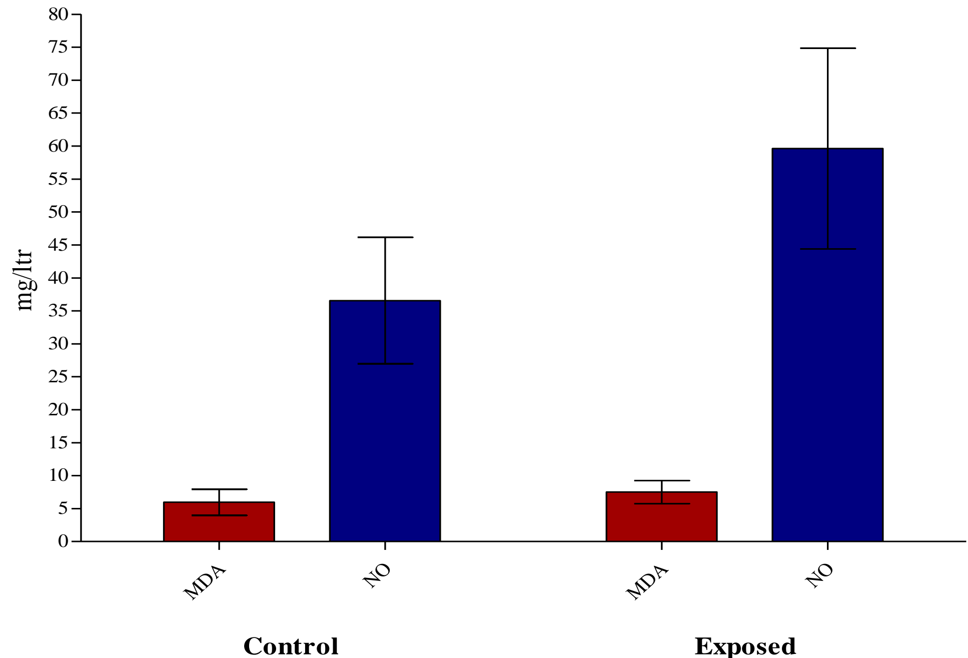
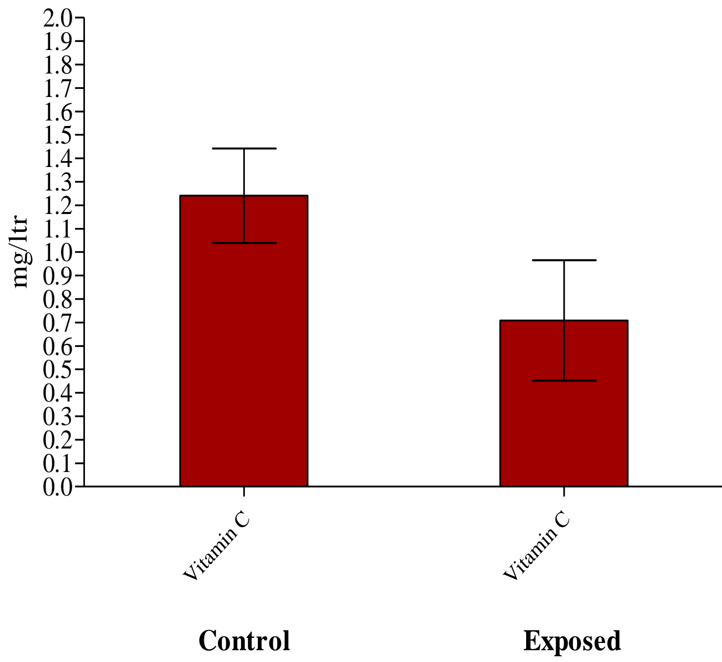


Figure-21



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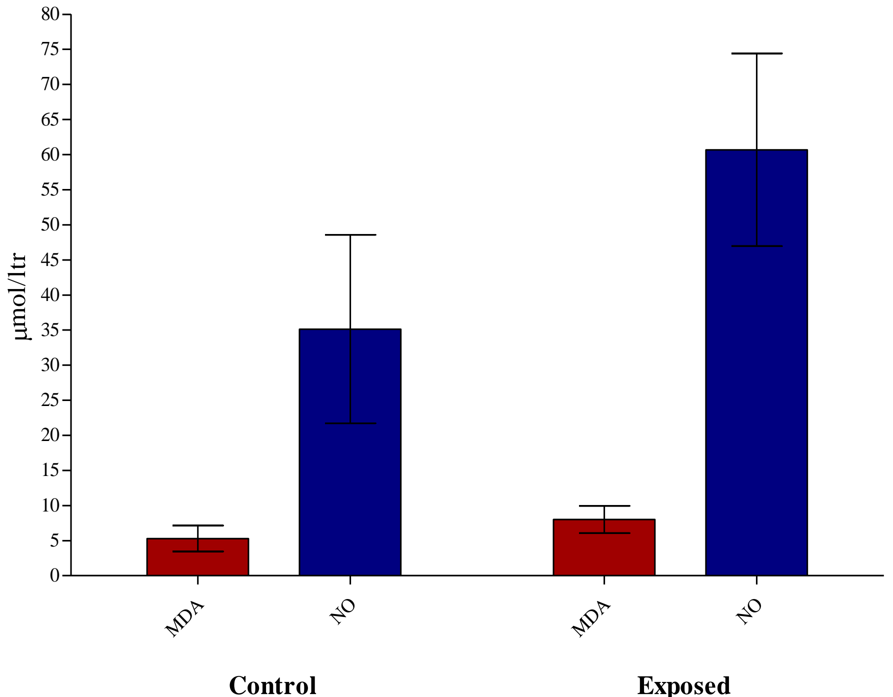

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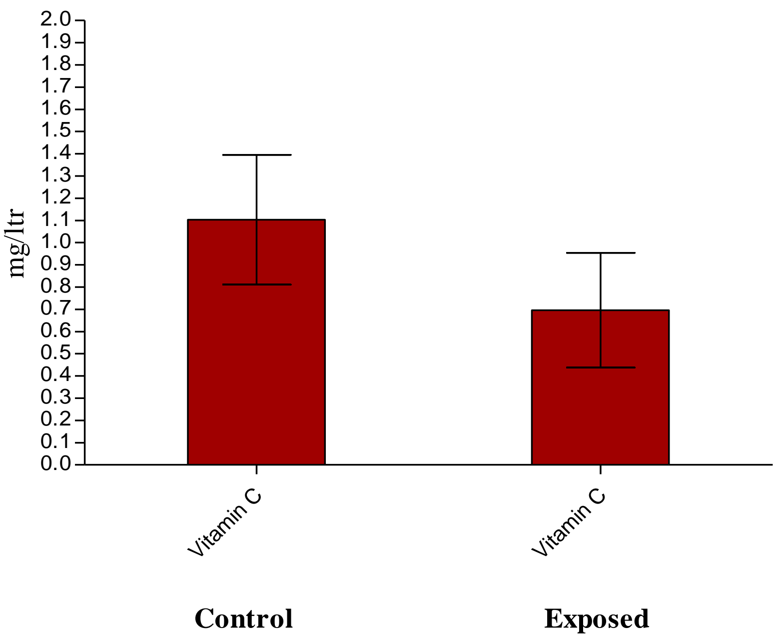


Figure-24

Table-16 , Figure-19 and Figure-20 depict the Mean ± SD of serum MDA, Serum NO and Plasma Vitamin-C of exposed and control individuals in different age groups(Group-I). There was significant difference obtained in plasma Vitamin-C(P<0.01) of exposed individuals in Group-I. No significant differences obtained in serum levels of MDA and NO in Group-I.

Table-16, Figure-21 and Figure-22 depict the Mean ± SD of serum MDA, Serum NO and Plasma Vitamin-C of exposed and control individuals in Group-II. There were high serum levels of MDA (P<0.01), NO (P<0.001) and low level of Plasma Vitamin-C(P<0.001) of exposed individuals in Group-II.

Table-16, Figure-23 and Figure-24 depict the Mean ± SD of serum MDA, Serum NO and Plasma Vitamin-C of exposed and control individuals in Group-III. There were high serum levels of MDA (P<0.001), NO (P<0.001) and low level of Plasma Vitamin-C(P<0.001) of exposed individuals in Group-III.

Table 17 : Regression Analysis showing the Effect of Different Predictors on Dependant Variables in Exposed Group.

Dependant Variable: FEV_1/FVC Ratio				
Group	**R Value**	**R Square (r^2)**	**Constants**	**Significance**
Exposed	0.531 (53.1%)	0.282 (28.2%)	Exposure	0.028*
			Age	0.000***
			MDA	0.191
			Vitamin C	0.690
			Nitric Oxide	0.786
Controls	0.121 (12.1%)	0.015 (1.5%)	Exposure	Not Applicable
			Age	0.713
			MDA	0.232
			Vitamin C	0.515
			Nitric Oxide	0.744
Dependant Variable: MDA				
Group	**R Value**	**R Square (r^2)**	**Constants**	**Significance**
Exposed	0.840 (84.0%)	0.705 (70.5%)	Exposure	0.362
			Age	0.735
			Vitamin C	0.000***
			Nitric Oxide	0.370
Controls	0.121 (12.1%)	0.015 (1.5%)	Exposure	Not Applicable
			Age	0.362
			Vitamin C	0.419
			Nitric Oxide	0.584
Dependant Variable: NO				
Group	**R Value**	**R Square (r^2)**	**Constants**	**Significance**
Exposed	0.926 (92.6%)	0.858 (85.8%)	Exposure	0.350
			Age	0.004**
			Vitamin C	0.000***
			MDA	0.370
Controls	0.160 (16.0%)	0.025 (2.5%)	Exposure	Not Applicable
			Age	0.425
			Vitamin C	0.127
			MDA	0.584
Dependant Variable: Vitamin C				
Group	**R Value**	**R Square (r^2)**	**Constants**	**Significance**
Exposed	0.946 (94.6%)	0.895 (89.5%)	Exposure	0.895
			Age	0.820
			Nitric Oxide	0.000***
			MDA	0.000***
Controls	0.162 (16.2%)	0.026 (2.6%)	Exposure	Not Applicable
			Age	0.457
			Nitric Oxide	0.127
			MDA	0.419

Note: *-P<0.05, **-P<0.01, ***-P<0.001

Regression analysis was done to know the association of different predictors on dependant variables

On consideration of FEV_1/FVC Ratio as dependant variable and Exposure, Age, MDA, Vitamin-C and NO as constants, the R squared value(r^2) obtained was 28.2%. There was significant association of exposure($P<0.05$) and Age($P<0.001$) on FEV_1/FVC ratio of exposed individuals. There was no significant association of MDA, Vitamin-C and NO on FEV_1/FVC ratio of exposed individuals.

On consideration of MDA as dependant variable and Exposure, Age, Vitamin-C and NO as constants, the R squared value(r^2) obtained was 70.5%. There was significant association of Vitamin-C($P<0.001$) on MDA of exposed individuals. There was no significant association of Exposure, Age and NO on MDA of exposed individuals.

On consideration of NO as dependant variable and Exposure, Age, Vitamin-C and MDA as constants, the R squared value(r^2) obtained was 85.8%. There was significant association of Age($P<0.01$) Vitamin-C($P<0.001$) on NO of exposed individuals. There was no significant association of Exposure and MDA on NO of exposed individuals.

On consideration of Vitamin-C as dependant variable and Exposure, Age, MDA and NO as constants, the R squared value(r^2) obtained was 89.5%. There was significant association of MDA ($P<0.001$) and NO ($P<0.001$) on Vitamin-C in exposed individuals. There was no significant association of Exposure, Age Vitamin-C of exposed individuals.

There was no significant association of any of the dependant variables with the constants in control group individuals.

DISCUSSION

6. DISCUSSION:

Past 5 decades of “epidemiologic and clinical research has denoted a strong association between airborne particulates exposure to several environmental pollution episodes and cardiorespiratory diseases morbidity [RR (95% CI): 1.7 (1.0 to 3.0) and 1.12 (1.04 to 1.20) respectively] (*Hammar et al.* 1992) and (*Torén et al.* 2007)”[232] [54]. “During the past 10 years there was a developing interest about the cocept that atherosclerosis is an inflammatory disease and also about the finding that serum levels of inflammatory markers can be used to predict the risk of cardiovascular events and mortality. However, still there is no clarity whether occupational exposure increases this risk [(*Pope & Dockery*, 2006), (*Rückerl et al.* 2007) and (*Feng & Yang*, 2012)]. Previous epidemiological studies found that occupational exposure to wood dust was associated with an increased risk for ischemic heart disease”[48][233][234]. Present work was conducted to show the effect of occupational exposure on respiratory parameters, blood cell counts, ECG parameters, blood pressure and oxidative stress markers.

In the present study, we have obtained lower FVC(P<0.0001), FEV1 (P<0.0001)and FEV1/FVC ratio (P<0.0001)compared to control (table-3). But the obtained values are within the normal limits and are statistically significant. Our results are in agreement with the conclusions of *Gildea and McCarthy (2003)*. They obtained a low but not a significant decrease in FVC relative to the control groups. However, absence of alteration in pulmonary function can not be justified by FVC alone because in patients with obstructive lung diseases, FVC can be normal or slightly decreased” [235]. Similar results have been seen in the study carried out by *Anupriya Deshpande et al* [236] on saw mill workers.

Among the duration of dust exposure groups, we have obtained clinically significant lower values of FVC (P<0.0001), FEV1 (P<0.0001) and FEV1/FVC (P<0.0001) ratio in higher exposure duration groups(1-9years>10-18years>19-27years) compared to control group(table-7).Decrease in FVC and FEV1 may be due to obstructive impairment which further increases with increase in number of years of exposure. In other words, there is a dose exposure relationship. Similar research studies conducted by *Al-Neaimi et al.* 2001 and *Mwaiselage et al.* 2004. “The results also

showed a significant reduction in FEV1%, which is an indication of obstructive impairment it is in turn may be due to mechanical irritation caused by dust exposure and individual susceptibility. These changes in pulmonary volume suggest an urgent need of improvement in dust control measures and health awareness towards dust preventive measures" [237][238].

In comparison of FVC, FEV_1 and FEV_1/FVC ratio of study group with control group among different individuals with age groups(table-11), we have obtained significantly lower values of higher age groups (21-30years>31-40years>41-50years). This shows as the age advances there is decrease in flexibility hence loss of compliance and also increased airway resistance, which ultimately leads to decrease in FVC, FEV_1 and FEV_1/FVC ratio. Our results are in contrast to the results obtained by *Ghotkar et al* **[239]**. They have shown as there is no significant decrease in FEV_1/FVC ratio, though there is decrease in values of FEV_1 and FVC in groups of individuals with increase in age.

Entry of PM into the airways and lungs

↓

Increased oxidant levels and Inflammatory reactions

↓

Increased oxidative stress and Inflammation

↓

Increased constriction of airways and decrease in elasticity of lung tissues

↓

Increased airway resistance and loss of lung compliance

↓

Decrease in Forced Vital Capacity and Forced Expiratory Volume

Leucocytes are the important blood cells in immune response in case of inflammatory reactions. Neutrophils are found to have a significant role in acute inflammation and inflammatory reactions. Monocytes and Eosinophils are very important inflammatory cells in chronic inflammations and allergic reactions. Mast cells and Basophils also have their role in chronic inflammation,where they act by secreting

inflammatory mediators. We have found a significant increase in Absolute Lymphocyte Count ($P<0.05$), Absolute Monocyte Count ($P<0.05$),Absolute Basophil Count ($P<0.001$) and Absolute Eosinophil Count ($P<0.0001$) (Table-4). There was marked increase in Eosinophil count of exposed individuals. It is indicative of an allergic response. These abnormal variations in the counts of Eosinophils and Lymphocytes represent chronic inflammation.

Exposure to dust [PM]

↓

Entry of PM into Airway and Lungs

↓

Systemic Inflammation and Allergy

↓

Secretion of Inflammatory and Allergic mediators

↓

Increase in the counts of Neutrophils, Monocytes, Lymphocytes, Eosinophils and Basophils in blood.

Though, there was an abnormal variation in Differential Leucocyte Count, Total Leucocyte Count did not show any significant variation. It might be due to adaptation of body immune response for long term exposure to the irritant substances. This data shows that there is an immune response which is provoked due to dust exposure. Similar observations were made by various other researchers also. "*Ahinsa Tripathi et al.,* have shown there was a significant abnormal variation in haematological parameters of rice mill workers of Lucknow district" [16]. "HH Lim et al. have shown in their study, a significant increase in Eosinophil count of rice mill workers, which indicates allergic reactions and inflammation" [18]. "*Hiroyuki Kayaba et al* and *Weller PF et al.,* have found the exposure to rice husk dust causes increase in Eosinophil count as well as Eosinophil-Lymphocyte interaction in immune response" [17] [240]. Another study conducted by *S. Gripenback et al* on pine wood dust exposed individuals showed there was marked increase in Macrophages, Eosinophils and Lymphocytes due to airway inflammation[241].

E.Rubin et al reported entry of allergen or other toxic substances into the body causes activation of mast cells, which in turn produce chemical substances. In response to the stimulus, there is an increase in the eosinophil and basophil count which helps in providing immune response against allergic and chronic inflammatory conditions[242].

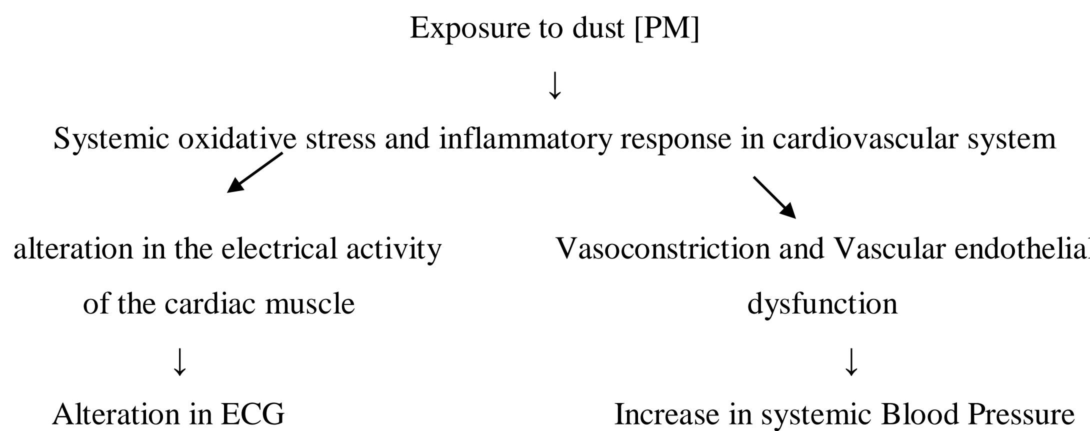
"There is a convincing evidence that monocytes possess preserved plasticity. Monocytes play an important role in immune defense, inflammation and tissue remodeling"[103]. "*Ross R* has shown increased monocyte count is associated with atherosclerosis, coronary artery disease and ischemic cardiomyopathy" [56].

In the present study, we have not found any significant decrease in RBC count, Haemoglobin Content and PCV which is in contrast with the findings of *Mohan Rao*[8] and *Ahinsa T*[16], where they have shown significant decrease in erythrocyte count, Haemoglobin Content and PCV.

We have also not obtained any abnormal variations in MCV, MCH and MCHC which is not in agreement with findings of *John Olusegun*[10], who observed significant variation in Blood indices of cement factory workers.

In our study, we also observed statistically significant difference in Systolic ($P<0.05$), Diastolic ($P<0.05$) and Mean Arterial Blood Pressures ($P<0.05$) in subjects of study group as compared to subjects of control group(Table-5). This indicates the change of vascular response to stress. These observations were in the agreement with observations made by other researchers. "*Ibald Mulli A et al., Chaung KJ et al.,* and *Liu L et al.*, suggested that there is an increased systolic blood pressure when density of particulate matter increases" [243-245]. "*Bellavi A* et al in their experimental study on controlled exposure of dust on human beings have shown that there was an increased blood pressure on short term exposure to fine particulate matter" [246]. "*Brook RD et al.*, have shown in their study there were an increased morbidity and mortality and also reduced life expectancy on long term exposure to increased density of particulate matter" [247]. "*Urch B et al.*, observed a significant increase in Diastolic Blood Pressure and Mean Arterial Blood Pressure of carpenters as compared to control" [248] . A very few studies have emphasized on effect of occupational exposure to dust on ECG parameters. In regard of ECG parameters, we noticed a significant difference in PR interval ($P<0.0001$),

QRS amplitude (P<0.0001), T wave amplitude (P<0.0001) and duration of T wave (P<0.05) in study group (Table-5). They are indicators of abnormal variation in the electrical activity of cardiac muscle of Rice mill workers. "A study carried out on traffic policemen exposed to traffic-related PM, increased resting diastolic blood pressure, alteration in the ST segment of the electrical activity of the heart and arrhythmias with exercise were observed in comparison with office workers" [249]. A study carried out by "*Henneberger et al*" reported changes in electrocardiogram (ECG) parameters for QT duration in response to exposure to organic carbon, changes in T-wave amplitude and complexity in patients with preexisting coronary heart disease in response to daily variations of PM levels" [250,251].



"Oxidative stress may be a mechanism whereby air pollutants induce inflammation in the airways and systemically leading to acute adverse cardio-respiratory responses (*Delfino et al.,* 2008)" [55].

"Under normal conditions, there is a steady state of balance between the production of oxygen free radicals and their destruction by the cellular antioxidant systems. The oxygen free radicals which accumulate via an imbalance between generation and scavenging are believed to induce many disease states" [252,253].

In our study, we observed significant increase in serum levels of MDA (P<0.001) and NO (P<0.001) of exposed group. There was a significant decrease in Ascorbic acid (P<0.001) level (Table-6). These observations indicated increase in levels of oxidants and

decrease in level of antioxidant vitamin-Ascorbic acid. "*Sayyed et al*., (2008) reported an increase in the level of oxidants with a simultaneous decrease in the level of antioxidant in association with smoking in cotton industry workers" [254].

Our study is in agreement with Syen Hernberg also shown that "there is a strong significant association between MDA level and oxidative stress. He also proved that ascorbic acid has significant role in reducing the oxidative stress"[255].In NIOSH Review, Khaleed S, carried out a research work on cement factory workers and found that "pollution due to cement factory causes increase serum levels of MDA and decrease in plasma ascorbic acid which leads to health problems" [11][8]. Similar study carried out on painters who exposed to Lead proved that there is a significant increase in MDA level as an oxidative stress marker[256].Several other studies supported the present study. *Babu Suresh et al*., who observed a tremendous increase in MDA levels in cardiovascular diseases due to oxidative stress[257].*Halliwell B et al*., in their study on acute coronary syndrome patients proved that malondialdehyde is one of the oxidative stress markers[29]. NO is found to have a role in mediating regulatory properties of the endothelium, its dysfunction and cardiovascular diseases [258] [30]. "Increased levels of Nitric Oxide cause loss of local vascular regulation, vasoconstriction and mechanical blockage of vessels all together cause a reduction in pulmonary vascular region" [259,260,261]. Studies on patients with Obsessive Compulsory Disorder (OCD)[262],Diabetes Mallitus[263], preeclampsia [264]Hypertensive[265],liver disease[266],Myocardial Infarction[267], atherosclerosis[28], Cancer, Asthma[268,269] provided the information that increased free radicals in these diseases cause increased oxidative stress, The important oxidants found in in these conditions are malondialdehyde and NO. In the other hand, these studies also showed the antioxidant role of ascorbic acid and association of diseases with decreased plasma levels of ascorbic acid. *Mojminiyi et al* found "significant elevation of serum malondialdehyde that was observed among carpenters compared with the control group. Significant reduction of blood antioxidant enzymes (glutathione and glutathione peroxidase) in the carpenters was also observed compared with the control group. Moreover, there was significant positive association between serum malondialdehyde and inflammatory markers among carpenters"[6]. "Vitamin C also known as ascorbic acid on the other hand

is the first line of antioxidant defenses (*Frei et al.,* 1988, 1989). This vitamin is susceptible to free radical oxidation. Ascorbic acid functions as an important component of cellular defence against oxygen toxicity and lipid peroxidation caused by free radical mechanism" [270] [271] [272].

6.1- Effect of duration of exposure:

Due to long term dust exposure, chronic health complications such as Atherosclerosis, Ischemic heart diseases, Coronary artery syndrome, Hypertension, dermatitis, asthma, allergic bronchitis, COPD etc may result. This effect may be due to toxicity of the dust components or inefficient defense system of the body which in turn cause an increased oxidative stress and oxidative damage.

In the present study, we have categorized the exposed individuals into three groups based on years of exposure. We have noticed that as the duration of exposure increased, there were marked variations in Absolute Counts of Eosinophils, Monocytes, Lymphocytes and Basophils. There was also marked increase in Oxidative stress markers like MDA and NO. Ascorbic acid level was significantly reduced in groups of individuals with longer duration of exposure. In the present study, we observed a strong positive correlation between duration of exposure and inflammatory markers, Oxidative markers and antioxidant-ascorbic acid. The present study is supported by the previous study conducted by *Debra L S et al*, They observed "as duration of exposure was increased, oxidative stress effect was also enhanced. They showed long term exposure to cotton dust results in inflammatory cell migration into the air spaces. This will generate ROS by opsonization, thereafter appear to precede increased lung permeability and reflect a loss of integrity of epithelial tight junction" [34].

Increased dust exposure

↓

Entry of dust into airways and lungs

↓

Increased migration of inflammatory cells

↓

Increased levels of ROS, Nitric Oxide and Lipid peroxide[MDA] by inflammatory and Oxidative reactions

↓

Increased oxidative stress

↓

Excessive consumption of plasma Vitamin-C for antioxidation

↓

Decrease in plasma levels of Vitamin-C

6.2- Effect of age:

As the age advances, the efficiency of the body for protection against infections decreases. With increasing age oxidative stress plays a major role in deterioration of the cellular functions. With the increasing age, levels of oxidants expected to be high and also decrease in antioxidant activities.

In the present study, we observed that there is an increase in Leucocyte count mainly Monocytes, Eosinophils, Lymphocytes in increasing age which indicate chronic inflammatory conditions.we have also observed the increase in serum levels of MDA and NO which are oxidants present in blood. There was decrease in levels of ascorbic acid which is a first line of defense as an antioxidant in the blood.

Our study is supported by *Mohan Rao et al* who has shown an increase in leucocyte counts with increase in age among cement factory workers[9]. A comparative study carried out by *Sayari Banarjee* on different age groups observed “an increase in oxidant levels and decrease in antioxidant levels in group of elderly workers” [273]. “*Kaspoglu and Ozben* protein oxidation is generally reported to increase during aging”

[274]. *Mezatti et al* showed that “plasma protein peroxidation products is higher in elderly than in younger subjects” [275].

In support of our study, Anderson et al observed an age related decrease in antioxidant activity[276]. “*Marjini A* in their study in regard of age related alterations in plasma lipid peroxidation and erythrocyte SOD in different age groups of Gorgan city of Iran observed that plasma lipid peroxidation (MDA) significantly increased with aging” [277].

On regression analysis for effect of different predictors on dependant variables, we have obtained positive correlation between age and FEV_1/FVC percentage, negative correlation between exposure duration and FEV_1/FVC percentage. Other predictors showed no significant effect in exposed group. There was a negative correlation between Serum MDA, Serum NO and antioxidant Vitamin C. Age has shown positive correlation on serum level of NO among exposed group. There was no significant correlation between predictors and dependent variables in control group.

CONCLUSION

7. CONCLUSION:

- FVC,FEV_1 and FEV_1/FVC percentage were significantly decreased in exposed group compared to control group.This indicates the adverse effect of dust exposure on lung function parameters.
- Decrease in values of pulmonary function test parameters was observed in group with exposure duration of 19-27 years compared to groups with exposure duration of 10-18 years and 5-9 years. It is concluded that the duration of exposure is increased, the vital capacity of lungs decreased significantly. Expiratory capacity also decreased significantly which indicates that there is an increase in airway obstruction as the duration of exposure to dust is increased.
- Significant decrease in PFT parameters was observed in group III (age 41-50 years) compared to group II(age 31-40) and Group I (age 21-30). It signifies that as age advances, there is decrease in vital capacity along with increase in airway resistance. Therefore, abnormal decrease in efficiency of lungs may occur.
- There is significant increase in Absolute Counts of Eosinophil, Basophil, Monocyte and Lymphocyte compared to control group, which indicates chronic inflammation and significant immune response.
- There is increase in the amplitude of QRS complex and T wave as well as duration of T wave of standard bipolar limb lead II in Rice mill workers compared to Control group. It indicates mild variation in electrical activity of the Cardiac muscle in rice mill workers.
- There is significant prolongation of PR interval in standard bipolar limb lead II in Rice mill workers compared to Control group. It indicates mild alteration in conductive activity of the cardiac muscle in rice mill workers.
- We found significant difference in Systolic Blood Pressure, Diastolic Blood Pressure and Mean Arterial Blood Pressure in Rice mill workers compared to control group, which indicate the alteration in cardiovascular function may be due to increased stress.
- On comparison with control group, we found significant increase in serum levels of MDA and NO which are oxidants present in blood and are regarded as

oxidative stress markers. We found significant decrease in plasma level of Ascorbic acid which is a first line of defense among the antioxidants. These findings show there is an increased oxidative stress which could be the causative factor for cardiovascular dysfunction and heamatological abnormalities in rice mill workers on longstanding exposure.

- Changes in Hematological, ECG and Biochemical parameters are significantly varied in Group III rice mill workers(who are exposed to dust in rice mills for 19-27 years)compared to group II and group I rice mill workers, which proves long term exposure to dust hampers the homeostasis of the body system by increasing oxidative stress and causing cellular damage.
- Changes in Hematological, ECG and Biochemical parameters are significantly varied in higher age groups(31-40 years and 41-50 years) compared to younger age group(21-30 years) which indicates increase in age group is an associated factor for increase in oxidative stress and cellular dysfunction.

Though individuals are working in rice mills in dusty area, their blood counts, electrocardiographic recordings, blood pressure are not varied very significantly. Variations are within normal limits. Lung parameters, stressors and anti-stressors are also found in normal range. But, when individuals are grouped according to age and exposure duration, elderly age group and long term exposure groups have shown mild decrease in PFTs. These groups have also shown increased stressor level to upper normal values and decreased anti-stressor to lower normal values. As the age advances adverse effect of dust on serum NO level is enhanced. This is an alarming message for individuals working in rice mills that continued and longstanding exposure may lead to increased oxidative stress and oxidative damage.

7.1- Recommendations:

- It is recommended for rice mill workers that they must use all precautionary measures to prevent abnormalities in heamatological and cardiorespiratory parameters.
- They must use gloves and masks at working place to reduce the deleterious effect of dust on health.
- They are also advised to go for routine health check up at regular intervals and also for follow up.
- It may be advisable to estimate levels of oxidative stress markers like serum malondialdehyde, serum Nitric oxide and plasma Ascorbic Acid as routine investigations for industrial workers.
- Supplementation of Vitamin C may be recommended for Rice mill workers.
- Dietary supplementations of citrous fruits and vegetables may be advised for such workers.

7.2- Limitations:

- We have not estimated the Chemical and Physical properties of dust to which workers were exposed.
- We have not estimated the total antioxidant level.
- Female individuals were not included in the study.

7.3- Future Prospective:

- Study can be done to know the changes at molecular level by incorporating estimation of the Physicochemical properties of the dust.
- Study can also be conducted on exposure to dust along with dietary habits and supplementation of Vitamin C.

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ANNEXURES

PROFORMA

EFFECT OF OCCUPATIONAL EXPOSURE ON CARDIOVASCULAR AND HEMATOLOGICAL PARAMETERS OF INDIVIDUALS WORKING IN RICE MILLS AROUND RAICHUR URBAN AREA.

Name: **Age:**
Sex: **Occupation:**
H/O exposure to Rice husk:

Physical Parameters:

a. **Height-**
b. **Weight-**

Hematological Parameters:

a. **Hb (gm %)-** **MCV-**
b. **PCV (%)-** **MCH-**
c. **Platelets ($\times 10^9$/l)** **MCHC-**
d. **WBC-** **Neutrophil count-**
e. **RBC Count-** **Eosinophil count-**
f. **Monocyte count-** **Basophil count-**
g. **Lymphocyte count-**

(PCV- Packed Cell Volume, MCV-Mean Corpuscular Volume, MCH-Mean Corpuscular Hemoglobin, MCHC-Mean Corpuscular Hemoglobin Concentration, EMB-Eosinophil, Basophil, Monocyte)

Cardiovascular Function Parameters:

a. **ECG -**
   - **Heart rate-**
   - **P wave amplitude-**
   - **P wave duration-**
   - **P-R interval-**
   - **QRS amplitude-**
   - **QRS duration-**
   - **ST segment-**
   - **T wave amplitude-**
   - **T wave duration-**
b. **Systolic BP-**
c. **Diastolic BP-**
d. **Pulse pressure-**
   MABP(Mean Arterial Blood Pressure)-

Pulmonary Function Tests:

a. **Forced Vital Capacity(FVC)-**
b. **Forced Expiratory Volume(FEV_1)-**
c. **FVC/FEV_1-**

Biochemical Parameters:

a. **Serum MDA-**
b. **Serum NO-**
c. **Plasma Vitamin C-**

Signature of Ph.D Research Guide **Signature Ph.D Research Candidate**

B. L. D. E. U'S SHRI B.M. PATIL MEDICAL COLLEGE, HOSPITAL AND

RESEARCH CENTRE, BIJAPUR

Ph.D RESEARCH INFORMED CONSENT FORM

Title of the project: **"EFFECT OF OCCUPATIONAL EXPOSURE ON CARDIOVASCULAR AND HEMATOLOGICAL PARAMETERS OF INDIVIDUALS WORKING IN RICE MILLS AROUND RAICHUR URBAN AREA".**

Name of the Ph.D candidate: Mr. Praveen S Patil

Name of the Ph.D Guide : DR. MANJUNATHA AITHALA MD
PROF & HOD,
DEPARTMENT OF PHYSIOLOGY

1: PURPOSE OF RESEARCH:

I have been informed that this study will test EFFECT OF OCCUPATIONAL EXPOSURE ON CARDIOVASCULAR AND HEMATOLOGICAL PARAMETERS OF INDIVIDUALS WORKING IN RICE MILLS AROUND RAICHUR URBAN AREA.

This study will be useful academically as well as for clinically to find out association between Occupational exposure on functions of Cardiovascular system and Hematological Functions.

2: PROCEDURE:

I understand that , the procedure of the study will involve recording of various physiological & physical parameters. The procedure will not interfere with any of my physiological parameters and they are non invasive , excepting for determination of Complete Hemogram .

3: RISK AND DISCOMFORTS:

I understand this study which tests the effect of occupational exposure to Rice husk on functions of Cardiovascular system and hematological functions, will not cause any discomfort to me and do not involve any risk to my health.

4: BENEFITS:

I understand that my participation in the study may not have a direct benefit to me but this may have a potential beneficial effect in the field of Occupational Health in future.

5: CONFIDENTIALITY:

I understand that medical information produced by this study will become part of institutional records and will be subject to the confidentiality and privacy regulation of the said institute. Information of a sensitive personal nature will not be a part of medical record, but will be stored in investigators research file and identified only by a code number. The code key connecting name two numbers will be kept in a separate secured location.

If the data are used for publication in the medical literature and for teaching purposes no names will be used and other identities such as photographs, audio and video tapes will be used only with my special written permission. I understand I may see the photographs and the video tapes and have the audio tapes before giving this permission.

6: REQUEST FOR MORE INFORMATION:

I understand that I may ask more questions about the study at any time. Concerned researcher is available to answer my questions or concerns. I understand that I will be informed of any significant new findings discovered during the course of this study which might influence my continued participation. If during the study or later, I wish to discuss my participation in all concerns regarding this study with a person not directly involved, I am aware that the social

worker of the hospital is available to talk with me. A copy of this consent form will be given to me to keep for careful re-reading.

7: REFUSAL OR WITHDRAWAL OF PARTICIPATION:

I understand that my participation is voluntary and may refuse to participate or may withdraw my consent and discontinue participation in the study at any time without prejudice to my present or future care at this hospital. I also understand that researcher may terminate my participation in this study at any time after she/he has explained the reasons for doing so and had helped arrange for my continued care by my physician or physical therapist if this is appropriate.

8: INJURY STATEMENT:

I understand that in unlikely event of injury to me resulting directly from my participation in this study, if such injury were reported promptly, then medical treatment will be available to me, but no further compensation would be provided.

I understand that by my agreement to participate in this study I am not waiving any of my legal rights.

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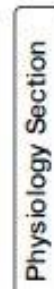
Original Article



Effect of Occupational Exposure on Blood Cell Counts, Electrocardiogram and Blood Pressure in Rice Mill Workers

PRAVEEN S. PATIL[1], MANJUNATHA AITHALA[2], KUSAL KANTI DAS[3]

ABSTRACT

Introduction: Under normal conditions, parasympathetic and sympathetic nervous systems interact to regulate the heart rate of about 70 beats per minute. Activation of sympathetic nervous system by emotional or physical stress increases heart rate and the force of heart beat. There are many factors which alter the heart rate. The chemical and mechanical stimulation of receptors can also cause change in blood pressure through autonomic nervous system. Exposure to dust also causes alteration in blood cell counts. This can be due to allergic reactions and inflammation which in turn evoked by dust entering the lungs.

Objectives: Aim of the study was to evaluate the effect of occupational exposure on haematological and cardiovascular parameters of rice mill workers by analysing Blood Cell Counts, ECG and Blood Pressure.

Materials and Methods: This cross-sectional study was carried on 134 rice mill workers and an equal number of age and sex matched healthy individual. The blood cell counts were determined by automated cell counter machine, ECG was recorded by using ECG machine and Blood Pressure was measured by using mercurial sphygmomanometer.

Results: Neurtrophil, Eosinophil and Lymphocyte count among haematological parameters were significantly increased in exposed individuals. Marked variation was seen in ECG and Blood pressure among cardiovascular parameters of exposed individuals compared with control group.

Conclusion: The findings of our study clearly indicate that the rice mill workers are under high level of dust exposure which has deleterious effects on their blood and tissues. It is due to high oxidative stress. There are abnormalities seen in cardiovascular system.

Keywords: Cardiovascular, ECG, Oxidative Stress

INTRODUCTION

Under normal conditions, parasympathetic and sympathetic nervous systems interact to regulate the heart rate of about 70 beats per minute. Activation of sympathetic nervous system by emotional or physical stress increases heart rate and the force of heart beat.

Abnormal rhythm of heart beat is called as arrhythmia. There are many factors which alter the heart rate. Clinically, the heart rate can be recorded by ECG machine. Brook RD [1] and Bhatnagar A [2] have proved that due to air pollution there is change in electrical activity and its regulation. Pope CA III et al., [3] and Brook RD [1] have also shown death can occur due to cardiopulmonary diseases which are consequences of increased levels of air pollution.

The chemical and mechanical stimulation of receptors can also cause change in blood pressure through autonomic nervous system. The studies were conducted to know the blood pressure changes in carpenters and non carpenters [4]. Similar studies were also carried out on sand stone mine workers [5]. These studies have shown statistically significant variation in blood pressure due to dust exposure.

There are evidences which show that exposure to dust causes alteration in blood cell counts. This can be due to allergic reactions and inflammation which in turn is evoked by dust entering the lungs. There are several studies which have been carried out to show the effect of exposure to cement dust on haematological parameters [6,7]. The exposure to dust and allergic reactions may also cause dermatitis and other hypersensitivity reactions.

MATERIALS AND METHODS

The study group comprised of rice mill workers of urban area in and around Raichur. Prior permission from owners of rice mills was sought. Consent was taken from rice mill workers.

A total of 134 rice dust exposed individuals were included in the study. The subjects who had been exposed to dust in rice mills more than 5 years were included in the study group where as another 134 unexposed individuals constituted control group. Both groups consisted of individuals who were matched with age and socio-economic status.

During collection of data, interviewer administered structured questionnaire was used. It helped to determine information in regard of duration of exposure, general health, disease history, details of habits like smoking and alcohol consumption.

For haematological parameters, 2ml of intravenous blood was drawn and collected in EDTA tubes and processed in CBC Counter Machine. For the determination of heart rate and electrical activity, ECG machine was used. Blood pressure was recorded by mercurial sphygmomanometer.

The anthropometric parameters of subjects were expressed as Mean±SEM. The haematological parameters, blood pressure and ECG recordings were also expressed as Mean ±SEM.

STATISTICAL ANALYSIS

The statistical analysis of the 2 groups was carried out by using unpaired t-test and differences among means were calculated at a level of $p<0.05$.

RESULTS

In [Table/Fig-1], the anthropometric parameters of the exposed and unexposed individuals were presented. There was no significant difference between exposed and unexposed individuals. Mean age of control is slightly higher than those of exposed individuals. Mean duration of exposure of rice mill workers is 9.8±0.6 years.

[Table/Fig-2] shows comparison of mean SEM of Haematological parameters of study and Control groups.

Neutrophil count (p<0.05) of Rice mill workers was significantly lower than the control, where as Lymphocyte (P<0.05) and Eosinophil (p<0.0001) counts were significantly higher in Rice mill workers as compared to control. There was no significant difference in Haemoglobin concentration, RBC count, TLC, PCV, MCV and MCHC of exposed and non exposed individuals.

[Table/Fig-3] Mean SEM of Blood Pressure and ECG parameters of Exposed and non exposed individuals were compared.

QRS wave (p<0.0001) and T wave (p<0.0001) amplitudes of Rice mill workers were significantly higher than control, where as T wave duration (p<0.05), PR interval (p<0.0001) and ST segment duration (p<0.05) of Rice mill workers were significantly longer than control. There was no significant difference between P wave amplitude, P wave duration, QRS duration and QT interval of Rice mill workers and control. Systolic blood pressure (p<0.05), diastolic blood pressure (P<0.05) and mean arterial blood pressure (P<0.05) of rice mill workers were significantly higher than control. There was no significant difference in heart rate and pulse pressure of rice mill workers.

DISCUSSION

Present work was carried out to show the effect of occupational exposure on blood cell counts, blood pressure and ECG parameters. We found a significant increase in Absolute Lymphocyte count (P<0.05) and Absolute Eosinophil Count (P<0.0001). Eosinophil count of exposed individuals was markedly increased. It indicates allergic response. This abnormal variation of Eosinophils and Lymphocytes can lead to chronic inflammation. Though, there was an abnormal variation in Differential Leucocyte Count, Total Leucocyte Count did not show any significant variation. It might be due to adaptation of body immune response for long term exposure to the irritant substances. This data shows that there is an

Parameters	Mean SEM of Exposed	Standard Deviation	Mean SEM of Controls	Standard Deviation	p-value
Age (years)	29.24 ± 0.5096	5.8991	32.81 ± 0.7521	8.7058	-
Height (cm)	165.0 ± 0.4145	4.7985	164.6 ± 0.5329	6.1684	-
Weight (kg)	59.21 ± 0.7582	8.7771	60.87 ± 0.5900	6.8302	-
BMI (kg/m²)	21.75 ± 0.2608	3.0190	22.43 ± 0.1331	1.5407	-
Years of Exposure (years)	9.776 ± 0.5594	6.4756	-		-

[Table/Fig-1]: The anthropometric parameters of the exposed and unexposed individuals were presented

Parameters	Mean SEM of Exposed	Standard Deviation	Mean SEM of Control	Standard Deviation	p value
Haematological Parameters					
RBC count (millions/cumm)	4.826 ± 0.04408 N=134	0.5103	4.792 ± 0.04736 N=134	0.5482	0.6026
TLC (cells×10³/cumm)	7.765 ± 0.1853 N=134	2.1453	7.771 ± 0.1874 N=134	2.1692	0.9808
Hb(gms/dl) concentration	13.66 ± 0.1581 N=134	1.8303	13.85 ± 0.1304 N=134	1.5098	0.3447
PCV (%)	40.94 ± 0.4418 N=134	5.1142	40.99 ± 0.3492 N=134	4.0426	0.9399
MCV (fl)	85.09 ± 0.7670 N=134	8.8785	85.88 ± 0.5632 N=134	6.5197	0.4043
MCH (pg)	28.40 ± 0.3014 N=134	3.4885	29.05 ± 0.2297 N=134	2.6595	0.0878
MCHC (%)	33.36 ± 0.1983 N=134	2.2955	33.80 ± 0.1365 N=134	1.5806	0.0695
Neutrophils (cells/cumm)	4034 ± 149.2 N=134	1726.603	4560 ± 163.2 N=134	1889.319	0.0181*
Lymphocytes (cells/cumm)	2713 ± 76.59 N=134	886.6106	2464 ± 72.95 N=134	844.4318	0.0195*
Eosinophils (cells/cumm)	467.0 ± 36.24 N=134	419.4698	261.4 ± 20.82 N=134	240.9915	p<0.0001*

[Table/Fig-2]: Shows comparison of mean sem of haematological parameters of study and control groups
(*-Significant difference) Hb-Haemoglobin concentration, RBC-Red Blood Cell count, TLC-Total Leucocyte Count, PCV-Packed Cell Volume, MCV-Mean Corpuscular Volume, MCH-Mean Corpuscular Haemoglobin, MCHC-Mean Corpuscular Haemoglobin Concentration

Parameters	Mean SEM of Exposed	Standard Deviation	Mean SEM of Control	Standard Deviation	p value
Electrocardiograph Parameters					
P Wave amplitude(mV)	0.1552 ± 0.005562	0.0644	0.1449 ± 0.004889	0.0565	0.1655
P Wave duration (sec)	0.09134 ± 0.001778	0.0206	0.08896 ± 0.001507	0.0174	0.3065
QRS amplitude (mV)	1.230 ± 0.03585	0.4149	0.9619 ± 0.02856	0.3306	p<0.0001*
QRS duration (sec)	0.08567 ± 0.001350	0.0156	0.08478 ± 0.001274	0.0147	0.6300
T Wave amplitude (mV)	0.3187 ± 0.01122	0.1298	0.2530 ± 0.009863	0.1141	p<0.0001*
T Wave duration (sec)	0.1758 ± 0.003171	0.0367	0.1615 ± 0.002626	0.0303	0.0006*
PR Interval (sec)	0.1439 ± 0.002815	0.0325	0.1012 ± 0.004188	0.0484	p<0.0001*
QT Interval (sec)	0.3654 ± 0.002772	0.0320	0.3640 ± 0.002872	0.0332	0.7367
ST Segment (sec)	0.1000 ± 0.002638	0.0305	0.1143 ± 0.003118	0.0360	0.0005*
Heart Rate (bpm)	75.62 ± 0.8333	9.6462	76.42 ± 0.7901	9.1465	0.4874
Blood Pressure (mmHg)					
Systolic BP (mmHg)	123.9 ± 0.9893	11.4516	121.4 ± 0.7207	8.3424	0.0452*
Diastolic BP (mmHg)	80.19 ± 0.8284	9.5896	77.99 ± 0.5441	6.2983	0.0267*
Pulse Pressure (mmHg)	43.60 ± 0.9486	10.9812	43.42 ± 0.6412	7.4220	0.8758
Mean Arterial BP (mmHg)	94.83 ± 0.7631	8.8330	92.53 ± 0.5302	6.1371	0.0140*

[Table/Fig-3]: Mean SEM of blood pressure and ecg parameters of exposed and non exposed individuals were compared
(* -Significant difference), BP-Blood Pressure

immune response which is provoked due to dust exposure. Similar observations were made by various other researchers also.

Tripathi A et al., have shown there was a significant abnormal variation in haematological parameters of rice mill workers of Lucknow district [8]. HH Lim et al., have shown in their study, a significant increase in Eosinophil count of rice mill workers, which indicates allergic reactions and inflammation [9]. Kayaba et al., Weller PF et al., have found the exposure to rice husk dust causes increase in Eosinophil count as well as Eosinophil-Lymphocyte interaction in immune response [10,11].

In our study, we also observed statistically significant difference in Systolic ($p<0.05$), Diastolic ($p<0.05$) and Mean Arterial Blood Pressures ($p<0.05$) in subjects of study group as compared to subjects of control group. This indicates the change of vascular response to stress. These observations were in the agreement of observations of studies conducted by other researchers. Ibald Mulli A et al., Chaung KJ et al., and Liu L et al., suggested that there is an increased systolic blood pressure when density of particulate matter increases [12-14]. Bellavi A et al., in their experimental study on controlled exposure of dust on human beings have shown that there was an increased blood pressure on short term exposure to fine particulate matter [15]. Brook RD et al., have shown in their study there were an increased morbidity and mortality and also reduced life expectancy on long term exposure to increased density of particulate matter [16]. Urch B et al., observed a significant increase in Diastolic Blood Pressure and Mean Arterial Blood Pressure of carpenters as compared to control [17].

A very few studies have emphasized on effect of occupational exposure to dust on ECG parameters. In this regard of ECG parameters it was noticed a significant difference in PR interval ($p<0.0001$), QRS amplitude ($p<0.0001$), T wave amplitude ($p<0.0001$) and duration of T wave ($p<0.05$), in study group. They are indicators of abnormal variation in the electrical activity of cardiac muscle of Rice mill workers.

CONCLUSION

The rice mill workers were exposed to high concentration of particulate matter. It can be inorganic or organic chemical substance or can be toxins produced by bacteria or can be gases. The deleterious effect also depends upon the sizes of the particles that are inhaled by the individual. There was a significant increase in Neutrophil, Eosinophil and Lymphocyte counts. There was no significant difference in Haemoglobin concentration, PCV, RBC Count, TLC and MCHC.

There was a significant abnormal variation in PR interval, QRS amplitude, ST segment and T wave. There were no significant differences in other components of ECG. There were significant differences in the Mean values of systolic blood pressure, diastolic blood pressure and mean arterial blood pressure of Rice mill workers as compared to control. The findings of our study clearly indicate that the Rice mill workers are under high level of dust exposure which has dangerous effects on their blood and tissues. It is due to high oxidative stress. There are abnormalities seen in cardiovascular system also. The rice mill workers should be educated regarding health hazards of dust exposure. They should be provided with masks and gloves which can reduce the consequences of dust exposure.

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ORIGINAL ARTICLE

Effect of Occupational Exposure on WBC Count and Oxidative Stress in Rice Mill Workers

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Abstract:

Background: There are several occupations where individuals are exposed to high concentration of dust particles. The exposed dust can be organic, inorganic, bacterial toxins, viruses or lipopolysaccharides. Exposure to rice husk causes activation of eosinophils and inflammation reactions. Cardiovascular diseases are associated with increased lipid peroxidation and decrease in levels of antioxidants. *Aim & Objectives:* Aim of the study was to evaluate the effect of occupational exposure on haematological and biochemical parameters of rice mill workers by analyzing blood cell Counts, Malondialdehyde (MDA), Nitric Oxide (NO) and Ascorbic acid. *Material and Methods:* A total of 134 eligible individuals who had been exposed to dust in rice mills for more than 5 years were included in the study group where as another 134 unexposed individuals constituted control group. Estimation of serum MDA, serum NO and plasma Ascorbic acid were done by using semiautoanalyzer. *Results:* There was no significant difference between Total Leucocyte Count of exposed and unexposed individuals. Lymphocyte, monocyte, eosinophil and basophil counts are significantly increased in exposed individuals. Serum levels of MDA, NO and Ascorbic acid are also significantly increased in exposed group compared with control group. *Conclusion:* The findings of our study clearly indicate that the rice mill workers are under high level of dust exposure which has deleterious effects on their blood and tissues. Increased MDA, NO and decreased Vitamin-C are probably due to high oxidative stress.

Keywords: Rice mill workers, White Blood Cell Counts, MDA, Nitric Oxide, Vitamin-C, Oxidative Stress

Introduction:

There are several occupations where individuals are exposed to high concentration of dust particles. The exposed dust can be organic, inorganic, bacterial toxins, viruses or lipopoly-saccharides. Exposure to rice husk causes activation of eosinophils and inflammation reactions as well as aggravation of asthma [1, 2]. Individuals who are exposed to cement dust were shown to have decrease in hemoglobin concentration with rise in lymphocyte count [3]. Martin-Ventura *et al* observed role of blood cells in chronic vascular disease. Pathological vascular modeling of arterial wall is found to occur in such diseases [4]. Cardiovascular diseases are associated with increased lipid peroxidation and decrease in levels of antioxidants [5]. On longstanding oxidative stress, high levels of lipid peroxides cause to the oxidative DNA damage and individuals will be at a greater risk of developing

cardiovascular diseases. Serum levels of Malondialdehyde (MDA), Nitric Oxide (NO) and Vitamin C are considered as markers of oxidative stress. Due to oxidative stress, MDA levels will be increased tremendously in cardiovascular diseases. NO related endothelial dysfunction is seen in cardiovascular deseases. Ascorbic acid, amost affective water soluble antioxidant is considered as first line of defense against oxidative damage [6-11]. Vitamin C inhibits initiation of lipid peroxidation and also reduces lipid peroxidation activated by leucocytes during inflammation [12, 13]. Hence, the present study was undertaken to determine the effect of dust induced oxidative stress by measuring leucocyte count and oxidative stress markers such as MDA, NO and Ascorbic Acid.

Material and Methods:

The study group comprised of rice mill workers of urban area in and around Raichur district. Prior permission from owners of rice mills was sought. Consent was taken from volunteered rice mill workers. A total of 134 eligible individuals who had been exposed to dust in rice mills for more than 5 years were included in the study group where as another 134 unexposed individuals constituted control group. Information in regard of duration of exposure, general health, disease history, details of habits like smoking and alcohol consumption were collected. For haematological parameters, 2ml of intravenous blood was drawn and collected in Ethylene Diamine Tetraacetic Acid (EDTA) tubes and processed in Complete Blood Cell (CBC) counter machine. For biochemical parameters, 6ml of intravenous blood was collected in plain tubes for estimation of serum MDA and serum NO. Two ml of blood was collected in EDTA tube for estimation of plasma ascorbic acid.

Estimation of serum MDA [14], serum NO [15] and plasma ascorbic acid [16] were done by using semiautoanalyzer. Sample size was calculated by taking proportion as prevalence with the help of the formula- $n= 4Pq/E^2$, where, 'n' is sample size, 'P' is prevalence(75%), 'q' is non prevalence(25%) and 'E^{2}' is probable error(7.5^2). A statistical analysis was done by using unpaired t-test with the help of Graph Pad Prism software. The anthropometric parameters of subjects were expressed as mean ± Standard Deviation (SD). The haematological and biochemical parameters were also expressed as mean ± SD with standard deviations.

Results:

Table 1: Anthropometric Parameters of Exposed and Unexposed Individuals

Parameters	Mean Values of Controls	Mean Values of Exposed
Age (years)	32.81	29.24 ***
Height (cms)	164.6	165.0
Weight (kgs)	60.87	59.21
BMI (kg/m^2)	22.43	21.75 *
Years of Exposure (years)	-	09.78

Note: *-$P<0.05$, **-$P<0.01$, ***-$P<0.001$

There was no significant difference between mean values of exposed and unexposed individuals (Table 1) except that the mean age of control individuals was higher than those of exposed group. Mean duration of exposure of rice mill workers was 9.78±0.56 years.

Table 2: Hematological Parameters of Study and Control Groups

Parameters	Control Mean ± SD (N=134)	Exposed Mean ± SD (N=134)
TLC (cells×10^3/cumm)	7.77 ± 0002.16	7.77 ± 002.14
Neutrophils (cells/cumm)	4560 ± 1889.31	4034 ± 1726.6*
Lymphocytes (cells/cumm)	2464 ± 844.43	2713 ± 886.61*
Eosinophils (cells/cumm)	261.4 ± 240.99	467.0 ±419.46***
Basophils (cells/cumm)	34.55 ± 20.69	43.06 ± 018.91***
Monocytes (cells/cumm)	450.0 ± 170.60	508.7 ± 208.71***

*Note: *-P<0.05, **-P<0.01, ***-P<0.001*

Neutrophil count (P<0.05) of Rice mill workers was significantly lower than the control, where as lymphocyte (P<0.05), eosinophil (P<0.001), monocyte (P<0.001), basophil (P<0.001) counts were significantly higher in Rice mill workers as compared to control (Table 2).

Table 3: Biochemical Parameters of Study and Control groups

Parameters	Control Mean ± SD (N=134)	Exposed Mean ± SD (N=134)
Serum MDA (µmol/l)	5.64 ± 2.090	7.01 ± 1.89***
Serum NO (µmol/l)	36.73 ± 14.15	51.03 ± 16.81***
Plasma Vitamin C (mg/l)	1.17 ± 0.260	0.86 ± 0.32***

*Note: *-P<0.05, **-P<0.01, ***-P<0.001*

Serum levels of MDA (P<0.001) and NO (P<0.001) of exposed individuals were significantly higher than those of control. Ascorbic acid (P<0.001) levels of exposed were significantly lower than those of control.

Discussion:

Present work was carried out to show the effect of occupational exposure of dust from rice mills on leucocyte count and oxidative stress. We found significant increase in absolute counts of lymphocyte (P<0.05), eosinophil (P<0.001), monocyte (P<0.001) and basophil count (P<0.001). Eosinophil and basophil counts of exposed individuals were significantly increased. It indicates allergic response. Lymphocyte and monocyte counts were also significantly increased. It indicates increased inflammatory reaction. These abnormal variations of eosinophil, basophil, monocyte and lymphocyte counts can lead to chronic inflammation and oxidative stress.

In the present study there was an increase in differential leucocyte count; total leucocyte count did not show any significant variation. This data shows that there is dust exposure related immune response too.

Tripathi *et al* [17] have shown that there was a significant abnormal variation in haematological paramaters of Rice mill workers of Lucknow district. Lim *et al* [1] have shown in their study, a significant increase in Eosinophil count of Rice mill workers, which indicated allergic reactions and inflammation. Kayab *et al* [2], Weller *et al* [18] have found the exposure to rice husk dust caused increase in eosinophil count as well as eosinophil-lymphocyte interaction in immune response. Study carried out by Olusegum *et al* [19] on cement factory workers also showed significant variations in Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin Concentration (MCHC), Mean Corpuscular Haemoglobin (MCH), lymphocyte and eosinophil counts.

In our study, we observed significant increase in Serum levels of MDA ($P<0.001$) and NO ($P<0.001$) of exposed group. There was a significant decrease in Ascorbic acid ($P<0.001$) level. These observations indicated increase in level of oxidants and decrease in levels of antioxidant vitamin-Ascorbic acid.

Several other researchers carried out studies on oxidative stress in cement factory workers, on painters, thermal plant workers, cotton mill workers and also on exercise. They observed increase in serum levels of MDA [20-23, 26], increase in serum level of NO [23-25] and also decrease in plasma Ascorbic acid level [20, 24, 26] in the study groups.

Conclusion:

The rice mill workers are exposed to high concentration of particulate matter. There is a significant increase in counts of eosinophils, monocytes, basophils and lymphocytes. There is no significant difference in neutrophils and total leucocyte counts. This data indicates the Rice mill workers have increased oxidative stress.

There is a significant increase in serum levels of MDA, NO and significant decrease in plasma concentration of ascorbic acid. MDA and NO are oxidants and Ascorbic acid is an antioxidant. To combat the increased oxidation, antioxidant ascorbic acid is utilized. When oxidant activity overwhelms the activity of antioxidants, antioxidant level will automatically be reduced.

Recommendations:

The rice mill workers should be educated regarding health hazards of dust exposure. They should be provided with masks and gloves which can reduce the consequences of dust exposure. The rice mill workers must undergo routine health check up.

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Recording of Pulmonary Function Tests

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Reagents used for Estimation of MDA, NO and Vitamin-C

Color of the solution in the estimation of serum NO

Color of the solution in the estimation of Plasma Vitamin-C