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TO STUDY LEVEL OF SOD (SUPEROXIDE DISMUTASE), CATALASE, GSHPX (GLUTATHIONE PEROXIDASE) AND TBARS (THIOBARBITURIC ACID REACTIVE SUBSTANCES) IN PATIENTS WITH BRONCHIAL ASTHMA.

General Medicine

Mrs Meghna V. Pose MSc (Lab.Technician) Dept.of Medicine ,T.N.M.C.

Mr .Sudhir Dhokar Jr.Scientific Officer , Dept.of Medicine ,T.N.M.C.

Dr..Aher Sangeeta* MD,DHA ---Associate Professor ,Dept.of Medicine ,T.N.M.C & Nair hospital.
*Corresponding Author

ABSTRACT

Reactive oxygen species [2]might play an important role in the modulation of airway inflammation. There is evidence of an oxidant-antioxidant imbalance in asthma. Oxidant–antioxidant imbalance leads to pathophysiological effects associated with asthma such as vascular permeability, mucus hypersecretion, smooth muscle contraction, and epithelial shedding. Epidemiological data also support the scientific evidence of oxidant–antioxidant imbalance in asthmatics. Therefore, the supplementation of antioxidants to boost the endogenous antioxidants or scavenge excessive ROS production could be utilized to dampen/prevent the inflammatory response in asthma by restoring oxidant–antioxidant balance.

Aim : The aim of the study was to measure activity of antioxidant enzymes SOD,Catalase,GSHPx and extent of damage due to lipid peroxidation was measured in terms of TBARS in neutrophils in bronchial asthma patients.

Material and Methods:100 bronchial asthma patients and 50 normal subjects were included in study. Blood was collected and processed for PMNL separation and lysate preparation. SOD, Catalase, GSHPx and TBARs done be standard biochemical methods. Antioxidant status was evaluated by measuring red blood cell superoxide dismutase and catalase activity, total blood glutathione, and glutathione peroxidase activity in red blood cells and leukocytes and total antioxidant capacity in plasma.

Results: SOD presented marginal rise as compared to normals. Catalase showed considerable rise.Non significant changes observed in GSHPx.TBARS demonstrated sharp rise.

Conclusion: TBARS showed sharp rise indicating higher oxidative stress in asthma patients. Reactive oxygen species do play a role in Asthma.

KEYWORDS

Antioxidants, Asthma, Inflammation, Oxidative, Stress

1. Introduction:

Airway inflammation is thought to be prime cause for repeated episodes of airway obstruction in asthmatics. Several studies have shown that reactive oxygen species (ROS) play a key role in initiation as well as amplification of inflammation in asthmatic airways. Excessive ROS production in asthma leads to alteration in key enzymatic as well as nonenzymatic antioxidants such as glutathione, superoxide dismutases, catalase, and glutathione peroxidases ect. leading to oxidant–antioxidant imbalance in airways[1].

The production of reactive oxygen species (ROS) include superoxide anion(O2'-), hydrogen peroxide (H2O2), hydroxyl radical(OH-) is an important host defence function of phagocytic cells.However overproduction of these ROS has been implicated as a casual factor in a variety of diseases including asthma...1... Catalase,SOD and enzymes of the glutathione redox cycle are the primary intracellular antioxidant defence mechanisms to cope with increased oxidant stress[4]. They eliminate O2- and hydroperoxides that may oxidize cellular substrates. The cell damage due to lipid peroxidation was measured in terms of TBARS in patients with bronchial asthma[3].

2. MATERIAL AND METHOD:

In this study the activities of oxygen detoxifying enzymes of PMNL were measured in the patient's suffering from bronchial asthma (n=100) in the age group of 18 to 45 years. The study was carried out on the patients attending the asthma clinic at the B.Y.L.Nair charitable hospital, Mumbai.

Study design:

It was prospective observational cohort study conducted in B.Y.L.Nair hospital. Indoor and OPD patient total no 100 were included in the study after Ethics committee clearance .Subjects were divided into two study groups A included -100 bronchial asthma patients and group B - 50 normal subjects.

INCLUSION CRITERIA:

Adult patients suffering from bronchial asthma (n=100) in the age group of 18 to 45 years were selected for the study:

EXCLUSION CRITERIA:

Individuals who had history of any other diseases ie. Hypertension, Diabetes, and liver disease were excluded from the study ,The result of study were compared against age and sex matched healthy individuals (n=50). Table 1

Table 1 Clinical characteristics of the COPD patients.

Character	Mean ± SD
Age	54.9 ± 9
Sex (M/F)	27/7
Cigarette (pack-years)	46.4 ± 1.7
FEV1 (%)	59 ± 3.7

The blood was collected in the tube containing anticoagulant Acid Citrate Dextrose.(ACD).And samples were immediately processed for PMNL separation and lysate preparation. Estimation of SOD, Catalase, GSHPx and proteins done from neutrophil lysate and TBARS done fromW.B.C.suspension.

A.Method: Epinephrine inhibition method. Mishra and Fridovich(1972)...3...

Principle:

Epinephrine can be auto oxidised to adrenochrome by superoxide radicals.Maximum autoxidation of epinephrine takes place at PH 10.2. The ability of superoxid dismutase to inhibit the auto oxidation of epinephrine to adrenochrome at PH 10.2. has been used as the basis for the assay of this enzyme.In this method epinephrine acts both as the source of superoxide radical (O2-) and as the detecting system giving adrenochrome which can be monitored at 480 nm.

B.Catalase : Method : Beutler E.(1986)...4..

Principle : Catalase catalyses the breakdown of hydrogen peroxide according to the following reaction.

catalase

2H2O2. ____----^ 2H2O+O2

The rate of decomposition of H2O2 by catalase is measured spectrophotometrically at 230 nm,since hydrogen peroxide absorbs light at this wavelength.

C.GSHPx. :. Beutler E.(1986)...5..

Principle :Glutathione peroxidase catalyses the oxidation of GSH to GSSG

GSHpx

2 GSH+ROOH_____,,,,,^GSSG+ H2O+ R-OH

Where ROOH is the peroxide t-Butylhydroperoxide is the most

suitable substance for assay of the enzyme. The rate of reduction of GSSG by glutathione reductase (GR) is measured.

GR
GSSG+NADPH____^2GSH+NADP
The oxidation of NADPH is followed at 340 nm.

D.TBARS. :. Stock J.et al (1972)...6...

Principle : An accelerated form of non-enzymatic oxidative breakdown of polyunsaturated fatty acids (PUFA) can be induced in PMNL by exposure to hydrogen peroxide.The formation of TBARS is measured ,being a secondary fragmentation product of PUFA peroxides.It is a more specific and sensitive measure of lipid auto oxidation.

The reaction depends on the formation of a coloured complex between TBARS and thiobarbituric acid (TBA), having an absorption maximum at 532 na,B-formylpyruvic acid also reacts with TBA to produce a coloured complex having a normal maximal absorption at 550 nm, sufficiently close to the TBA-MDA complex ,acting as a potential source of error in measuring lipid peroxidation.In order to avoid this interference prior to the treatment with TBA, The proteins are precipitated out using trichloro acetic acid arsenite.Azide present in the buffer inhibits the activity of catalase which reduces hydrogen peroxide to water.

E.Proteins : Modified Colin Lowry Method (1952)...7...

Principle : Proteins react with two reagents,the alkaline copper reagent and the Folin Phenol reagent. The final colour obtained is a result of:

1). The biuret reaction of protein with the copper ions of the alkaline copper reagent and
2). A reduction of the phosphotungstic acid to tungsten blue and molybdenum blue both by the Cu-protein complex and by the tyrosine and tryptophan of the protein.

The blue colour obtained as a result of the above reactions is read at 650 nm.

3. STATISTICALANALYSIS

All statistical analysis was carried out using the SPSS (statistical package for the social science software) statistical package version 20.0 for Windows, and the software Microsoft Excel V.5 (2003). USA Quantitative data were expressed as mean and standard deviation (X + SD) and analyzed by applying Student's *t*-test for comparison of two groups of normally distributed variables. The results of the "*t*"-value are then checked on Student's "*t*"- table to find out the significance level (*p*-value) according to the degree of freedom. All these tests were used as tests of significance at $p < 0.05$

4. RESULTS AND ANALYSIS:

Percent Rise / Fall in enzymatic and non enzymatic antioxidants in PMNL of asthma patients.

Assay.	Control.	Asthma.	P value
SOD.	84.17+-12.35	8.35%^.	P^0.01
Catalase. (U/mg of protein)	15.66+-3.99.	22.79%^.	P^0.001
GSHPx. NS (U/ mg of protein)	0.052+-0.015.	1.53%^.	NS
Percent Rise in lipid peroxidation **(TBARS)** levels.In PMNL of Asthmatic patients (nmoles/mg of proteins) with Azide	3.44+-1.05.	63.37%^.	P^0.001
TBARS (nmoles/mg of proteins) Without azide	2.37+-0.96	55.27%^.	P^0.001

DISCUSSION:

The lungs have several natural antioxidant mechanisms to neutralize the effect of oxidants, which include enzymatic as well as nonenzymatic antioxidants. Enzymatic antioxidants include catalane, glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) and nonenzymatic antioxidants are vitamin E, vitamin C, albumin, uric acid, ceruloplasmin, and glutathione (GSH ROS production and oxidative stress in asthma Over the past decade, a number of studies have clearly demonstrated that (a) oxidative stress is an important consequence of the inflammatory response in asthma [Nadeem *et al*. 2005, 2003; Wood *et al*. 2003], (b) oxidative stress is associated with an alteration in antioxidant activity in the lung and blood SOD presented marginal rise while catalyse showed considerable rise.Non significant changes were observed in GSHPx activity. However the product of lipid peroxidation TBARS demonstrated sharp rise indicating thereby higher oxidative stress in asthma patients[7]. Since many studies have shown associations between airway obstruction/severity of the disease and systemic oxidant–antioxidant imbalance Red cells are considered to be important reservoirs of antioxidants such as SOD, catalase, GSH-Px and Red cells exposed to oxidants also adhere more to endothelial surface[4] .These data clearly indicate that airway oxidant–antioxidant imbalance, probably as a consequence of the inflammatory response, could play an important role in the development of asthmatic symptomatology and possibly in the etiology of asthma NADPH maintains catalase in the active form and is used as a cofactor by thioredoxin as well as GSH reductase, which converts oxidized glutathione (GSSG) to GSH, a co substrate for the GSH-Pxs[6]. Intracellular NADPH, in turn, is generated by the reduction of NADP[b] by glucose-6-phosphate dehydrogenase (G6PD), the first and rate-limiting enzyme of the pentose phosphate pathway.By generating NADPH, G6PD is a critical determinant of cytosolic GSH buffering capacity (GSH/GSSG), and therefore, can be considered an essential, regulatory antioxidant enzyme [5]

6.CONCLUSION.

Asthmatic inflammation is characterized by ongoing inflammation and accompanied by increased oxidative stress and subsequent lung injury.Reactive oxygen species do play a role in Asthma. G6PD is at the nexus of many essential metabolic pathways. We are only at the beginning of understanding G6PD, NADPH and their inter relationships with cellular systems.

Rise in TBARS indicating thereby higher oxidative stress in asthma patients Using the results of the present study as base to evaluate further the role of antioxidants in asthma it us required to study the activities of antioxidant enzymes and lipid peroxidation In conclusion, Using the results of the present study as base, the role of antioxidants in asthma is required to study the activities of antioxidant enzymes and lipid peroxidation after the antioxidant supplementation such as Vit C, Vit E alone and in combination as well as minerals namely Zinc, Copper, Manganese and Selenium in sufficiently larger homogeneous study population.

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