ORIGINAL RESEARCH PAPER
Volume - 10 | Issue - 01 | January - 2021 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

TO STUDY LEVEL OF G6PD,NADPH OXIDASE AND TBARS (PERCENT RISE IN LIPID PEROXIDATION) IN PATIENTS WITH BRONCHIAL ASTHMA

General Medicine

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ABSTRACT

G6PD deficiency is the most common gene mutation in the world and the numerous mutations have been classified by the World Health Organization Glucose-6-phosphate (G6P) may be utilized in glycolysis to produce energy in the form of ATP and NADH, used to store energy in the form of glycogen, or used by the pentose phosphate pathway (PPP). **Aim:** The aim was to study levels of G6PD, NADPH Oxidase , and the extent of damage due to lipid peroxidation was measured in terms of TBARS (Percent Rise in lipid peroxidation) in neutrophils in Bronchial asthma patients.**Material and method:**100 bronchial asthma patients and 50 normal subjects were included in study. Blood was collected and processed for PMNL separation and lysate preparation G6PD , NADPH Oxidase and TBARS done by standard biochemical methods.**Result:**G6PD showed considerable rise as compared to healthy individuals.NADPH Oxidase both stimulated and unstimulated presented marginal rise.TBARS demonstrated sharp rise compared to normal's.**Conclusion:**Reactive oxygen species do play a role in Asthma. Rise in TBARS indicating thereby higher oxidative stress in asthma patients.

KEYWORDS

Antioxidants, Asthma, Inflammation, Oxidative, Stress

1. INTRODUCTION:

Lipid peroxidation is a very important process in free radicals pathology as it is very damaging to cell .The extent of damage due to lipid peroxidation was measured in terms of TBARS in patients with bronchial asthma.

G6PD and 6-phosphogluconate dehydrogenase in the hexose monophosphate shunt are important antioxidant enzymes by virtue of their catalyzed reactions that supply NADPH for completion of the GSH redox cycle by generation of GSH...4...5...

O2'- is produced by neutrophils through the action of NADPH oxidase.This oxidase which is dormant in resting neutrophils but is activated when the cells are exposed to bacterial targets.

2. MATERIALAND METHOD

2.1 Aim. The aim of the study was to measure activity of antioxidant enzymes GSHPx, GR,GSH and G6PD in neutrophils of bronchial asthma patients.

2.2 Material and Method:

The study was carried out on the patients attending the asthma clinic at the B.Y.L.Nair charitable hospital, Mumbai.

Sample size:100 bronchial asthma patients and 50 normal subjects were included in study.Blood was collected and processed for PMNL separation and lysate preparation.GSHPx, GR,GSH and G6PD done by standard biochemical methods. In this study the activities of oxygen detoxifying enzymes of PMNL were measured in the patients suffering from bronchial asthma (n=100) in the age group of 18 to 45 years.

Study design: It was prospective observational cohort study conducted in tertiary care 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

2.3 Experimental methods:

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. The blood was collected in the tube containing anticoagulant Acid Citrate Dextrose. (ACD).And samples were immediately processed for PMNL separation and lysate preparation.

A. Estimation of G6PD, NADPH Oxidase and proteins done from neutrophil lysate and TBARS done fromW.B.C.suspension. Glucose -6- Phosphate Dehydrogenase : Block and McLean Method(1953)...7

Principle: Glucose 6 Phosphate dehydrogenase catalyses the oxidation of glucose 6 phosphate to 6 phosphogluconate (6-PGA). 6 phosphogluconate dehydrogenase catalyses the oxidative decarboxylation of 6 PGA to ribulose 5 phosphate and carbon dioxide , using NADP as the acceptor of hydrogen.

B.NADPH Oxidase : NBT Reduction Method. Hedley D.W.and Currie G.A.(1978)...8..

Principle : The redox dye NBT is reduced by the action of NADPH oxidase by phagocytic cells.The product is the insoluble violet crystalline compound formazan. The violet colour extracted with Dioxan is read at 520 nm.This assay is also used for the indirect measurement of the Hexose Monophosphate Shunt.

C .TBARS. :. Stock J.et al (1972)..9...

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.

D. Proteins : Modified Colin Lowry Method (1952)...10...
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. Statistical analysis
All statistical analysis were 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 Asthmatic patients.

Assay.	Control.	Asthma.	P value
NADPH oxidase . Stimulateded (nm NBT reduced/ mg of protein/min)	4.088+-0.749	10.32%^.	P^0.01
NADPH oxidase Unstimulated (nm of NBT reduced/ mg of protein/min)	3.452+-0.778	10.83%^	P^0.01
G6PD.(U/gm protein)	6.63+- 2.2.	17.64%^.	p^0.001
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

5.DISCUSSION.
Glucose-6-phosphate dehydrogenase (G6PD) deficiency, an X-chromosome-linked genetic disorder, is the most prevalent mutation in humans, affecting more than 400 million people worldwide [1]. This disorder is characterized by decreased activity of the G6PD enzyme, which is the central factor of the antioxidant defence system in red blood cells (RBCs). The enzyme is responsible for maintaining high levels of reduced glutathione (GSH) and nicotine adenine dinucleotide phosphate (NADPH), which protect the cell from the oxidative damage caused by reactive oxygen species. Because G6PD-deficient RBCs are unable to generate NADPH through other pathways, these cells lack the ability to tolerate excessive amounts of oxidative stress [2, 3].

It has been traditionally taught that G6PD is regulated by the NADPH/NADP ratio so that as the ratio decreases, activity increases to provide more NADPH. The most common clinical manifestation associated with G6PD deficiency is hemolytic anemia, which is generally triggered by the intake of oxidative drugs or foods. Occasionally, the defect can result in such complications as kidney failure, severe neonatal jaundice, or gallstones, and may require blood transfusion

NADPH is mainly produced by four enzymes in mammalian cells, G6PD, PGD, malic enzyme, and isocitrate dehydrogenase. All have been studied extensively and play critical cellular roles. G6PD appears to be of unique importance to many cellular processes that utilize NADPH, as inhibition of G6PD impairs many cellular processes that are dependent on NADPH

NADPH Oxidase presented rise of 10-11% in both stimulated and unstimulated activities.While G6PD activity showed significant rise of 18% in bronchial asthma patients.

TBARS demonstrated sharp rise indicating thereby higher oxidative stress in asthma patients.

CONCLUSION:
In conclusion, G6PD is at the nexus of many essential metabolic pathways. We are only at the beginning of understanding G6PD, NADPH and their interrelationships with cellular systems. A full understanding of G6PD, its role as a metabolic nexus for many cellular systems, and how it is regulated should provide critical insights into many intracellular processes and disease mechanisms.

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