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PULMONARY FUNCTION, ANTIOXIDANT ENZYMES, AND MELATONIN LEVELS IN PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE DURING STABLE AND EXACERBATION PERIODS

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ABSTRACT:
Background: In the development and progression of bronchial asthma and chronic obstructive pulmonary disease important role is played by an imbalance between oxidative stress and antioxidative capacity. The purpose of the study was to evaluate the BA and COPD systemic oxidant-antioxidant status during the exacerbation and the stable periods.
Methodology: This study was conducted at DHQ Hospital, Charsadda. 'The patients with BA and COPD' were 40, which were divided into 20 with BA and 20 with COPD. An investigation was carried out i-e "levels of malondialdehyde (MDA), activities of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), glutathione reductase (GRd), and catalase (CAT) in erythrocytes and serum melatonin concentrations" 'during the exacerbation' and the stable periods. Additionally, the respiratory functions, blood gases, and Blood counts were investigated.
Results: Bronchial asthma during an exacerbation period the % of eosinophils was higher significantly than in the stable period despite the declines in, GRd, GSH-Px, MDA, melatonin levels, white blood cell count, and CAT levels. We evaluate that increased during the stable period with, FVC/L, BA FEV^1/L pO^2, PEF/L/s, levels. GSH-Px, GRd, melatonin, pH, and pO^2 levels were lower in the exacerbation period than in the stable period while MDA and SOD values were higher in the exacerbation period than in the stable period. The change between the exacerbation and the stable period with COPD, blood counts, and respiratory function tests did not change significantly.
Conclusion: (BA or COPD) may be linked with elevated levels of oxidative stress, because of increased levels of oxidative stress in the exacerbation period with BA and COPD although the levels decreased antioxidant enzymes and melatonin.

Keywords: Pulmonary function, Antioxidant Enzymes, Chronic Obstructive Pulmonary Disease, Oxidant-antioxidant, Exacerbation, Stable periods

INTRODUCTION:

The commonest reason for 'bronchial asthma and chronic obstructive pulmonary disease' need for emergency care. The 4th worldwide mortality is COPD, ranked 3rd in those with smoking users in 2020. The male and female ratio is variable all over the world. Some causes are involved in the obstruction of the airway in BA which becomes harmful at night. Normal individuals exhibit both coincidental and physiological disorders, but the impact of these disorders is subclinical in all but a few cases of individuals who possess a BA (1, 2).

The etiopathogenesis of COPD is not known. Those people who smoke cigarette increases the risk of COPD including factors like 'dusty air in certain jobs, air pollution, gasses and smoke air, infections, familial and, a-1 antitrypsin deficiency, and genetical factors'. Antioxidant deficiency is accountable for the pathogenesis. Per year 'COPD patients have almost 2–4 exacerbations'. Morbidity and mortality ratios become higher and affect the life expectancy of the patient adversely with every exacerbation(3, 4).

In hospitals, dead rate is 3-4 percent while in care units it is 24.6 percent this means that exacerbations need hospitalization, the study reported that for COPD no medications 'prevent the development of the disease by affecting inflammation and destructive processes'. That's why it's important to diagnose it earlier and proper medication decreases the chances of exacerbations and treatment. The reactive oxygen species (ROS) gained an important role in 'tissue damage and the etiopathogenesis of most diseases i-e bronchopulmonary dysplasia emphysema, cancer, pneumoconiosis, diabetes, BA, and COPD'. Additionally, constriction on smooth muscles, tissue damage, mediator release, 'increase in vascular permeability, and Bronchoconstriction' are caused by ROS. In BA and COPD, they 'play a role in the pathogenesis of exacerbations' (5, 6).

Allergen signals and neutrophil infiltration occur during exacerbations of BA. A study observed that a BA patient's bronchial hyperactivity is linked with 'superoxide production in neutrophils'. "The mechanism may be related to the pathogenesis of the nocturnal exacerbations". The use of oxidative stress Malondialdehyde, a 'major product of lipid peroxidation'. 'Cell membrane homeostasis is destroyed by the increased LP production' (7).

'Reduction in intercellular gap junction (nexus) communication' can damage membranes and cause a 'loss in calcium and other transport systems'. "Enzymes including superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), glutathione reductase (GRd), and catalase (CAT) are major enzymes in the antioxidative defense system". Melatonin and antioxidant vitamins are examples of free radical scavengers that protect the lungs from oxidative stress. By acting as 'chain-breaking antioxidants or by scavenging and collecting' reactive oxygen species, antioxidants prevent oxidative damage (8).

Worldwide BA and COPD health issues and their prevalence are increasing region by region. There have been discrepancies about the oxidative stress caused by the 'degree of LP and antioxidant defense mechanisms' with these diseases. In the development and progression of bronchial asthma and chronic obstructive pulmonary disease, an important role is played by an imbalance between oxidative stress and antioxidative capacity (9). The purpose of the study was to evaluate the BA and COPD systemic oxidant-antioxidant status during the exacerbation and the stable periods. Additionally, the respiratory functions, blood gases, and Blood counts were investigated. It will explain the etiopathogenesis of diseases.

METHODOLOGY:

Study setting and Population: The study was conducted at DHQ Hospital, Charsadda. BA and COPD Inclusion criteria were those who quit smoking or were nonsmokers in the last 1 year. 'According to the Global Initiative for Asthma' diagnosis of BA diagnosis criteria "respiratory function tests, PEF, FEV1 the forced expiratory volume in 1-second levels and symptoms". According to the Global Initiative for Chronic Obstructive Lung Disease diagnosis COPD criteria "respiratory function tests; FEV1/FVC forced vital capacity, FEV1 levels and symptoms".

A total of 50 patients were included later it was 40 which was divided into 20 'BA and 20 COPD patients because patients with systemic and pulmonary diseases (except for BA and COPD) were eliminated from the research because diseases affect ROS production'.

The detail of the procedure for 'BA and COPD in our study was' that the initial day of hospitalization for study participants was designated as the exacerbation period, and the time following therapy was designated as the stable period. Within ten days, the patient showed a positive reaction to the medication, which was assessed by a physical examination, symptoms, lung function tests, blood gas, and blood count levels.

The BA and COPD blood was collected from the antecubital vein at about 10ml in the morning during exacerbations and stable periods. After that 3ml of blood for the 'measurement of erythrocyte MDA, SOD, GSH-Px, GRd, and CATactivities were transported EDTA tube'. In another EDTA 2ml blood was transported for analysis of blood count. for melatonin measurement, the remaining five ml was transported and 'centrifuged for 10 min at 2000 revolutions to obtain serum and was stored in deep frozen at 208^{C}'

Now for the erythrocyte suspension, Three rounds of washings in cold isotonic saline were performed on the erythrocyte samples. Following each washing and ten minutes of centrifugation at 3000 rpm, the upper part was taken out and thrown away. After adding two milliliters of cold, distilled water, 'the hemolyzed cells were gathered and kept at 808 degrees Celsius until the measurements were made'.

The method of Draper and Hadley was adopted for 'Lipid peroxidation levels were measured using thiobarbituric acid'. "The detection of erythrocyte MDA was based on its reaction with TBA and the measurements were made using a spectrophotometer"

The antioxidants 'method by using Randox commercial Kits measurement of erythrocyte SOD activity was detected by Woolliams et al'. When INT comes into contact with these radicals, red formazan dye is formed. The extent to which this process was inhibited served as a proxy for SOD activity. The activity of erythrocyte SOD was measured in units of 'U/ml and reported as U/g Hb'.

Using the 'method by using Randox commercial kits the' activity of erythrocyte GSH-Px. Hydrogen peroxide was added to the tube containing NADPH, reduced glutathione, sodium azide, and glutathione reductase (GRd) to start the enzyme reaction. A spectrophotometer was used to track the change in absorbance at 340 nm. The unit of measure for 'enzyme activity was U/g Hb'.

Using the method of 'Goldberg and Spooner Reagent' from Randox Laboratories was used to measure the erythrocyte GRd activity; the test was altered from the GRd powers the process conversion of GSSG to NADP by oxidation in the presence of NADPH. It was measured how much the absorbance decreased at 340 nm. U/g Hb was used to express the results.

Based on kinetic measurements, the Aebi approach was used to estimate the erythrocyte CAT activity. Hydrogen peroxide is broken down by 20 CAT into water and oxygen molecules. Using spectrophotometry, CAT activity was calculated by measuring the drop in Hydrogen peroxide at 240 nm during a stable phase. The results were given as kU/g Hb.

We used commercial kits to assess serum melatonin levels. This test's fundamental workings rely on the melatonin radioimmunoassay (RIA) technique. The serum sample in one-milliliter and two-milliliter solution was vortexed for a minute after being incubated for 15 minutes at 378C. 'The mixture was centrifuged for ten minutes after that 500 ml of the upper fluid phase was extracted using 4 ml of diethyl ether following centrifugation'. In a nutshell, the 'upper organic phase evaporated while under vacuum'. The mixture was then vortexed for one minute while being resuspended in a buffer. This mixture was utilized in 200 microliters for the RIA method of measuring melatonin. Pg/ml was used to express the results**.**

The analysis of blood count was obtained from the Department of Pathology (Hematology)

Furthermore, 'on the day of blood collection, during the exacerbation and stable periods with BA and COPD, pulmonary function tests were performed'. These examinations were carried out in the morning using a spirometer. Three sphyrometric readings were performed, and the best outcome was considered. Every individual had measurements of the following ventilatory parameters: "PEF/L/S,

FVC/L, FEV1/L, and FEV1/FVC". Also, the percentages of expected values for "FVC%, FEV1%, FEV1%/FVC%, and PEF%" were measured for these parameters.

Arterial blood samples were taken in the morning for blood gas analysis from patients with COPD and BA during both their stable and exacerbation phases. Before the process, syringes were cleaned with heparin, and samples were delivered straight to the lab to avoid any air interaction.

Data analysis

Software called SPSS 15.0 was used to statistically evaluate all of the data. The Wilcoxon Signed Ranks test was employed to compare these illness durations. The median and range are used to express data. The statistically significant thresholds were determined to be $p < 0.05$.

Results:

Table 1 displays certain clinical and demographic data for patients with COPD and BA. When the hematological parameters were compared between the patients' exacerbation and stable periods, it was discovered that patients with BA had significant variations in their WBC and eosinophil percentage ($p < 0.04$), while patients with COPD did not show significant variations in these values. Patients with COPD did not significantly differ in their pulmonary function tests between the stable and exacerbation phases. In patients with BA, significant variations were seen for "FEV1/L ($p < 0.05$), FVC/L ($p < 0.05$), and PEF/L/s ($p < 0.05$)". Compared to the exacerbation, these values were greater during the stable phase. 'Patients with COPD and BA had pO2 levels that were considerably lower ($p < 0.02$) during the exacerbation period compared to the stable period'. Conversely, pH readings dropped when COPD patients were experiencing an exacerbation. LP levels of erythrocytes in individuals with COPD and BA during periods of exacerbation and stability. Compared to the stable phase, BA p 0.02 and COPD p 0.002 had significantly higher erythrocyte LP levels during their exacerbation episodes.

Table 1: The features of individuals suffering from 'chronic obstructive pulmonary disease, also known as (COPD) and asthma of the bronchi'

Parameters	**Exacerbation period BA (n=20)** Median	**Stable period BA (n=20)** Median	**Exacerbation period COPD (n=20)** Median	**Stable period COPD (n=20)** Median
Gender **Male/Female**	11/9	11/9	4/17	4/17
Age (years)	56.50 (34.00–79.00)	56.50 (34.00–79.00)	67.00 (52.00–73.00)	67.00 (52.00–73.00)
Smoking history **(last 1 year)**	Na	NA	NA	NA
WBC	15.12 (8.30–21.70)þ	10.40 (7.10–19.27)	10.60 (5.40–27.80)	13.01 (6.30–17.10)
NE%	70.25 (28.50–84.70)	71.60 (53.40–93.80)	67.40 (38.70–89.80)	72.40 (52.30–85.90)
RBC	6.93 (4.30–8.30)	6.98 (4.24–9.75)	7.11 (6.60–8.05)	6.85 (6.02–8.09)
EOZ%	5.90 (1.70–17.90)þ	2.95 (1.10–5.80)	4.10 (1.40–6.60)	3.70 (1.30–5.80)
HCT%	44.90 (34.10–47.30)	44.65 (38.40–51.90)	46.20 (37.60–59.40)	44.70 (36.90–56.60)
Hb (g/dl)	16.50 (12.90–17.90)	16.25 (14.20–19.60)	17.20 (13.60–21.00)	15.90 (13.70–20.60)
FVC (L)	4.05 (2.13–6.57)	4.51 (2.32–5.85)-	3.58 (1.84–5.22)	3.64 (2.02–5.85)
MCV	89.50 (70.30–101.00)	89.85 (68.30–117.00)	88.60 (71.90–101.10)	88.70 (71.90–98.60)
FEV1 (L)	3.37 (1.69–4.81)	3.70 (1.87–4.75)-	3.04 (1.47–5.01)	3.12 (1.47–5.07)
FVC%	73.00 (28.00–101.00)	81.5 (32.00–101.00)	46.00 (24.00–79.00)	48.00 (31.00–89.00)

FEV1%	62.00 (23.00–98.00)	70.00 (28.00–98.00)	47.00 (15.00–92.00)	40.00 (15.00–94.00)
FEV1/FVC (L)	73.35 (46.70–88.80)	79.75 (45.20–88.50)	64.80 (36.80–95.50)	66.20 (28.10–99.20)
FEV1%/FVC%	88.50 (58.00–109.00)	97.00 (54.00–108.00)	81.00 (45.00–119.00)	83.00 (34.00–125.00)
PEF (L/s)	4.48 (2.00–7.67)	5.10 (2.85–10.00)-	4.28 (2.50–8.33)	4.16 (2.57–7.07)
pO2	66.05 (39.20–90.90)	81.90 (53.30–6.70)--	56.30 (34.90–80.00)	67.70 (47.40–8.50)--
pH	7.48 (7.43–7.54)	7.47 (7.39–7.53)	7.44 (7.39–7.58)	7.48 (7.40–7.56)-
pCO2	33.35 (26.90–56.10)	33.75 (26.80–11.10)	41.80 (25.80–67.00)	37.70 (29.00–61.80)

The statistical analysis was carried out using the 'Wilcoxon Signed Ranks test; values are expressed as median and range'. 'The variables include pO2, carbon dioxide pressure; pO2, oxygen pressure; hematocrit; MCV, mean corpuscular volume; $p < 0.04$ and $p < 0.02$ (higher stable period); $p < 0.04$ higher exacerbation period'.

Table 2. 'Erythrocyte superoxide dismutase (SOD), glutathione peroxidase (GSH-PX), glutathione reductase (GRd), and catalase (CAT) activities in the exacerbation and the stable period of patients with bronchial asthma (BA) and chronic obstructive pulmonary disease (COPD)'

Groups	**Parameters**	**Exacerbation period Median (range)**	**Stable period Median (range)**
BA (n=20)	GSH-PX	540.30 (246.60–634.30)	563.55 (253.60–671.80)
	SOD (IU/g Hb)	1390.20 (1260.90–1998.40)	1670.15 (1091.90–1670.90)
	CAT (kU/g Hb)	3621.85 (2036.50–4676.20)þþþ	2532.35 (1734.90–4282.60)
	GRd	335.85 (310.40–566.70)	855.35 (791.30–1027.50)
COPD (n=17)			
	SOD	1541.30 (850.65–2202)þþ	1478.10 (836.40–1700.20)
	GRd	341.34 (278.64–821.46)	517.86 (352.46–902.20)
	GSH-PX (U/g Hb)	271.70 (175.70–520.10)	282.30 (270.00–556.70)
	CAT	2211.10 (149.25–2571.90)	2001.80 (1427.90–1136.20)

'Values are expressed as median and range the statistical analysis was performed using the Wilcoxon Signed Ranks test'. '-$p < 0.04$, $p < 0.02$, $p < 0.002$ (higher stable period). - þþp < 0.02, þþþp < 0.002 higher exacerbation period'.

Table 2 displays the erythrocyte activities of antioxidant enzymes "SOD, GSH-Px, GRd, and CAT" and the blood melatonin concentrations.

While GSH-Px p 0.02 and GRd p 0.002 activities in patients with BA were considerably lower in the exacerbation periods than in the stable period, CAT activity was found to be significantly p 0.002 greater in the exacerbation period than in the stable period. Compared to the steady period, SOD activities in COPD patients were considerably p 0.01 higher during the exacerbation period. In patients with BA and COPD, GSH-Px ($p < 0.02$) and GRd p 0.04 activities were significantly lower during the exacerbation period compared to the stable period. In a similar vein, patients with COPD and BA had significantly higher serum melatonin concentrations p 0.002.

DISCUSSION

Globally 'BA and COPD are global problems with' an impact on the economy and society. 'Mortality and morbidity rates'. 'Acute exacerbations influence both BA and COPD. role for chemokines, a class of cytokines' Evidence points to a key that is important in, inflammatory cell activation, leukocyte recruitment, 'increase in oxidative stress, T-cell differentiation, and oxidant-antioxidant imbalance' (10, 11).

'During inflammation and respiratory diseases BA, COPD, idiopathic pulmonary fibrosis, and acute respiratory distress syndrome oxidative stress imbalance due to oxidant-antioxidant and also due to environmental oxidants, cigarette smoke, toxic gases, and ozone are exogenous sources of oxidants while are inflammatory cells endogenous oxidants' (12).

'An estimate of free radical activity provides the amount of MDA as a measure of LP and its measure. Our research reported that the LP levels of erythrocytes of BA and COPD were significantly higher in the exacerbation period than in the stable period' (13).

In accordance, the study observed BA blood level increased during the acute exacerbations period. Another study reported that COPD oxidative stress acute exacerbation was linked to higher levels of LP. The study reported stable and exacerbated COPD, and healthy smokers' plasma MDA level was increased.

The results of the mentioned studies prove that the 'MDA level elevation of the erythrocyte in acute exacerbation and inflammatory cells in patients with BA and COPD is the result of the degeneration of polyunsaturated fatty acids on cell membranes by increased oxidative stress' (14, 15).

Endogenous agents of the lungs are known as antioxidants, 'the first lines of defense against oxidants, and are the major non-enzymatic antioxidants of the lungs' are "glutathione, melatonin, vitamins C and E, b-carotene, uric acid, and the enzymatic antioxidants are SOD, GSH-Px, GRd, and CAT". for 'protection against the increase in a ROS production GSH-Px' is the enzymatic antioxidant defense system. A study found that GSH-Px in rats has "protected alveolar epithelial cells against hyperoxia-induced injury and the activity is much lower in asthmatic children compared to that in normal children".

'Protecting cells and tissues against oxidative stress' SOD is important. Only a few research reported that COPD and in healthy smokers SOD erythrocyte enzyme activity was increased than in healthy nonsmokers. 'In contrast, because of an increase in consumption of antioxidants, COPD SOD erythrocyte enzyme activity was reduced' (16, 17).

Our study reported that, exacerbate COPD was elevated SOD erythrocyte activity was increased in than in stable COPD. Research conducted reported that in asthmatic airways 'down-regulation of bothCuZnSOD and MnSOD'. Our study reported, 'between the exacerbation and the stable periods for BA' no significant SOD erythrocyte activity while 'during the exacerbation period, the levels elevated'. "GRd plays an important role in protecting hemoglobin, red cell enzymes, and biological cell membranes against oxidative damage by increasing the level of reduced glutathione in the process of aerobic glycolysis". 'A study reported that the GRd erythrocyte activities were not changed in the epithelial lining fluid of severe asthma' (18).

Our study reported, that significant erythrocyte CAT enzyme activity increases were observed during the exacerbation periods of BA. furthermore, "erythrocyte GSH-Px and GRd activities decreased during the exacerbation period of a patient with both BA and COPD". 'BA and COPD are linked with instabilities in antioxidants and indicate that oxidant stress occurred'. In addition to developmental changes, differentiation and aging influences, inflammation, and hormonal regulation of antioxidative enzymes have been reported. Additionally, several antioxidants and cell protectors are believed to regulate gene expression and antioxidant enzyme activity.

'A powerful and effective endogenous hydroxyl radical scavenger is melatonin which is a homeostasis regulator of circadian rhythm in humans'. One study reported that melatonin does not affect 'the activity of stimulated macrophages, while rats pinealectomy significantly reduces airway inflammation' after the "ovalbumin inhalational challenge, and melatonin administration to the pinealectomized rats seems to restore airway inflammation, which further supports the pro-inflammatory effect of melatonin" (19).

The current investigation found that during the periods of exacerbation, blood melatonin levels 'in patients with COPD and BA were significantly lower'. 'Melatonin stimulates GSH-Px activity, which is an important antioxidative enzyme'.

Pinealectomy caused melatonin deprivation, resulting in lower 'GSH-Px activity levels in several tissues in rats. Melatonin stimulates GSH-Px activity, hence decreased levels of melatonin during BA exacerbation times could be one of the causes of low GSH-Px activity' (20).
This study compared BA and COPD patients during stable and exacerbation periods, yielding novel findings. 'Blood counts, respiratory function tests, and arterial blood gases' are essential for diagnosing and treating patients with BA and COPD in clinics, emergency rooms, and intensive care units. Proper usage and assessment of these tests are essential for treating follow-up patients.

CONCLUSION:

Patients with BA and COPD exhibit increased LP and decreased antioxidant enzymes in erythrocytes and serum melatonin, indicating oxidative stress. The findings support the idea of an imbalance between ROS generation and antioxidant defense in inflammatory and obstructive lung illnesses. The findings recommend using antioxidants like melatonin and selenium to treat these disorders.

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