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Research Article

Alterations in Antioxidant Profiles in Spirometry Proven Bronchial Asthma

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Abstract: Asthma is a complex, chronic inflammatory lung disease that may result in alterations in various anti oxidants. In this study superoxide dismutase (SOD), Vitamins C and E, Zinc, Copper and Magnesium were estimated using standard procedures in spirometry proven asthmatics selected at random. Ninety two (n=92) asthmatics, forty nine (n=49) females with mean age 33.98 ±11.52 standard deviation and forty three (n=43) males with mean age 35.91±12.88 between the ages of 16-70 years were enrolled and thirty healthy subjects (n=30), out of which nine (9) females with mean age 31.25±10.77 and twenty one (21) males with mean age 31.05±12.95 were taken as controls. Significant differences have been observed between the control and patient data for FEV1, FVC, FEV/FVC, PEF and Zn. Higher SOD value (19.92%) and lower Vit-C value (13.59%) have been observed in patients compared to control. The value of Vit-E has been increased to 2.06 times in patient data when compared to control. No significant difference for Cu (p=0.829), and Mg (p=0.277) has been observed in patient and control groups. Zn and Cu have been increased to 21.55% and 72.67% respectively in the samples of patients; while 10.81 % decrease in the amount of Mg in the samples of patients has been estimated compared to the samples of control. Discrimination between control and patient groups was evolved by principal component analysis. (PCA) The effects of asthma on antioxidant status showed significant alterations in exogenous antioxidants including Vit-E and Zn. Endogenous antioxidant SOD increase in asthmatics showed changes in antioxidant defenses.
Keywords: Asthma, SOD, Vitamins, Minerals, PCA.

INTRODUCTION

Asthma is a complex, chronic inflammatory lung disease that is characterized by a specific pattern of airway inflammation, airway smooth muscle (ASM) hypertrophy, hyperplasia and production of mucous [1]. Airway hyper responsiveness (AHR) is the exaggerated airway narrowing due to nonspecific irritants or pharmacological agonists, which is irreversible by bronchodilators that relax ASM [2]. Evidence suggests that specific inflammatory abnormalities exist in the airways of subjects suffering from mild to moderate persistent asthma, in which an inflammatory state is often associated with increased generation of reactive oxygen species [ROS] and the damaging effects of free radicals [3]. Antioxidants combat the damaging effects of ROS [4].

Superoxide Dismutase (SOD) catalyses the dismutation of superoxide to H_2O_2. The H_2O_2 must then be removed by catalase or glutathione peroxidase. Extracellular superoxide dismutase (Ec-SOD) is the major SOD detectable in the extracellular fluids [5].

The exogenous chain breaking antioxidants include vitamin E (Vit-E) vitamin A (Vit-A), vitamin C (Vit-C) and a large group of Flavenoids. Vitamin E, a fat soluble vitamin is one of the major antioxidants in cellular membrane where it acts by protecting polyunsaturated fatty acids against oxidation [6]. It was shown that vitamin E supplement could improve clinical manifestations and pulmonary function test in children with moderate asthma [7]. Vitamin C plays a role in quenching ROS to counteract ROS effect and render them harmless. It is the most abundant antioxidant substance present in the extracellular fluid in the lung and contribute to the regeneration of membrane bound oxidized Vit-E to function again [8].

Magnesium (Mg) has several biological effects of potential relevance to asthma, including broncho dilation when given intravenously in acute severe

asthma. There is also strong evidence of protection by dietary Mg against asthma. These effects of Mg are mediated by its properties of smooth muscle relaxation and mast cell stabilization. Copper (Cu) functions as a co-factor in various enzymes and in copper-based pigments. Zn has a structural stabilizing role while the Cu is directly involved in catalytic activity. There are evidences linking Cu, Zn and Mg with asthma. [9] Similarly an alteration in antioxidant defenses is observed in chronic obstructive pulmonary disease and cigarette smoking [10, 11].

The diagnosis of asthma is usually based on the characteristic symptoms. However, spirometric measurements of lung function, and particularly the demonstration of reversibility of lung function abnormalities, greatly enhance diagnostic confidence. Spirometry is the measurement of lung function which provides an assessment of the severity of airflow limitation, its reversibility and variability and confirmation of the diagnosis of asthma [12].

In recent years, substantial evidence has developed supporting a key role for free radicals in many fundamental cellular reactions and suggesting that oxidative stress might be important in the patho physiology of common diseases including atherosclerosis, chronic renal failure, and diabetes mellitus. Similarly the aim of our study is to evaluate antioxidant profiles in bronchial asthma and to find possible correlation with basic spirometric parameters in these patients.

MATERIALS AND METHODS

Three hundred and seventy adult patients of both sexes between the ages of 16-70 years who presented with dyspnoea and history of bronchial asthma or subsequently diagnosed on spirometry were randomly evaluated. The patients were selected from different parts of a thickly populated city of Karachi by organizing various free asthma clinics/ medical camps over a period of about one year. Out of which ninety two patients were enrolled in the study who met the inclusion criteria. All other causes of dyspnoea including pneumothorax, pneumonia, pleural effusion, pulmonary fibrosis, anemia, cardiac failure and functional cases of dyspnoea were excluded from the study by carefully evaluating them on clinical grounds including detailed history and clinical examination. Thirty healthy adults of both sexes without any significant past medical/surgical history were selected as controls and subjected to spirometry. Their blood samples were drawn for enzymatic, vitamins and trace element analysis.

Portable handheld electronic Spirometer Micromedical plus model 1999 was used. The instrument was calibrated using 3.0 L calibration syringe by Vitalograph Limited prior to spirometry. Daily calibration was not required with the model used. Spirometry variables were measured for a series of at least 3 acceptable forced expiratory readings. The guidelines by American Thoracic Society (ATS) were followed for obtaining satisfactory spirometric values [13]. The best values were selected. Patients who presented with severe dyspnoea and unable to perform spirometry were excluded from the study.

Collection of blood was made under all aseptic measures and 11 milliliters of blood was drawn by veni puncture from a large vein using disposable syringe from Becton and Dikinson (BD). All chemicals were of analytical grade except where otherwise stated. Distilled water, double distilled water and de-ionized water were used for preparing solutions where required. Disposable plastic made test tubes, containers, pipette tips and centrifuge tubes were preferred. All plastic/ glassware was cleaned by soaking in 2% HNO_3 solution and then scouring with bristle brush, followed by extensive tap water rinsing and thereby at least three rinses with distilled water. Glassware was then oven dried at about 80^{o}C.Blood sample was then divided and prepared for estimation of vitamins, trace elements and SOD.

Detection of heavy metals and trace element analysis was carried out on atomic absorption spectrophotometer, Perkin Elmer 2380, equipped with Zeeman background correction and a data processor using air acetylene flame for Cu, Zn and Mg estimation. The instrument was optimized as per instrument manual.

Estimation of serum Tocopherols was done according to the method of Baker and Frank [14]. Ascorbate was estimated according to the method of Harris and Ray [15]. The activity of SOD is measured by the method of Marklund with some modifications [16], based on the ability of SOD to inhibit the auto-oxidation of pyrogallol by 50%.

Statistical Data Analysis

The mean values of different parameters for patient and control are shown in Table 1.Principal Component Analysis (PCA) was applied on the results to identify the similarity or difference between patient and control data. T test for parametric variables and Mann Whitney for non parametric variable (Cu) were applied for groups comparison analysis by using statistical software SPSS (ver. 20) and Minitab (ver.14).

RESULTS

Forty nine females (n=49) out of ninety two patients (n=92) were between the ages of 16 to 67 years with mean age 33.98 ±11.52. Forty three (43) out of ninety two male patients (n=43) age ranging between 16 to 67 with mean age 35.91±12.88 were included in the study. Thirty subjects (n=30) were taken as controls; out of which nine (9) were females with mean age

31.25±10.77 and twenty one (21) subjects were males with mean age 31.05±12.95.

Spirometry

The patients were subjected to spirometry and comparison of spirometric values including Forced Expiratory Volume in first second (FEV_1), Forced Vital Capacity (FVC), Peak Expiratory Flow (PEF) and Percentage Ratio (FEV_1/FVC) between patients and controls were applied to all the cases. FEV_1 was 1.91±0.83 in patients and 2.93±0.8 in controls with highly significant p<0.001showing 34.8% change. FVC was 2.2±0.9 in patients and 3.15±0.93 in controls with significant p<0.001 showing 30.2% change. PEF was 247.8±122.7 in patients and 428.2±150.02 in controls with significant p<0.001 showing 42.12% change. However, FEV_1/FVC was 86.21±16.6 in patients and 93.5±7.15 in controls, p- value being significant (0.028) with only 7.8% change in patients as expected. (Table1)

Table1: Comparison of FEV_1, FVC, PEF, FEV_1/FVC, SOD, Vit-C,Vit-E, Zn, Cu and Mg between Patients and Controls

.Variables	Range Patients	Patients n=92 Mean ± SD*	Range Control	Controls n=30 Mean ± SD*	**p-value
FEV_1 (l)	0.47-3.91	1.91±0.83	1.6-4.39	2.93±0.8	< 0.001
FVC (l)	0.42-5.33	2.2±0.9	1.63-5.32	3.15±0.93	< 0.001
FEV1/ FVC (%)	44-100	86.21±16.6	77-100	93.5±7.15	0.028
PEF (l)	54-595	247.8±122.7	148-798	428.2±150.02	< 0.001
SOD (U/ml)	13.6-92	65.01±16.59	15.33-77	54.21±16.20	0.0023
Vit-C (mmol/l)	5-13.6	8.90±1.83	6.2-13.2	10.30±1.92	0.004
Vit-E (mmol/l)	36.2-44.7	40.3±1.84	18.2-22.1	19.55±0.77	0.0012
Zn (ppm)	0.89-6.98	2.82±1.07	0.79-3.36	2.32±0.71	0.019
Mg (ppm)	0.29-2.04	0.66±0.32	0.32-2.27	0.74±0.41	0.277
Cu (ppb) Median (IQR)	5-9804	98.50 (5-235.2)	1-2217	77.5 (41-126.2)	0.829

**T-test and Mann Whitney U test were applied, $p \leq 0.05$ was considered as significant

Antioxidant enzymes

All the subjects were analyzed for SOD. The patients (n=92) showed SOD value ranging between 13.6 to 92 with mean value 65.01±16.59.The controls (n=30) showed SOD value ranging between 15.33 to 77 with mean value 54.21±16.20 had significant p-value (0.0023) showing 16.61% change.

Vitamins

All the subjects were analyzed for Vit-C and Vit-E. The patients (n=92) showed Vit-C and Vit-E value ranging between 5.00 to 13.6 with mean value 8.90±1.83 and 36.2 to 44.7 with mean value 40.3±1.84 respectively. The controls (n=30) showed Vit-C and Vit-E value ranging between 6.20 to 13.2 with mean value 10.30±1.92 and18.2 to 22.1 with mean value 19.55±0.77 respectively. The p-value was found significant for Vit-E (p=0.0012) showing 51.49 % change and significant (p=0.004) for Vit-C showing 13.59% change.

Trace elements

Zn, Cu and Mg were analyzed and results are shown in Table1.The patients (n=92) showed Zn, Cu and Mg value ranging between 0.89 to 6.98 with mean value 2.82±1.07, 5 to 9804 with Median (IQR) 98.50 (5-235.2) and 0.29 to 2.04 with mean value 0.66±0.32 respectively. The controls (n=29) showed Zn, Cu and Mg value ranging between 0.79 to 3.36 with mean value 2.32±0.71, 1 to 2217 with Median (IQR) 77.5 (41-126.5) and 0.32 to2.27 with mean value 0.74±0.41 respectively. The p-value was significant (0.019) showing 17.73% change for Zn and insignificant (0.829, and 0.277) for Cu and Mg respectively. (Table 2, Fig.1)

Table 2: Component matrix for contributing parameters

Variable	PCI	PCII	PCIII	PCIV
FEV1 (l)	-0.525	-0.001	0.103	0.033
FVC (l)	-0.488	-0.079	0.064	0.177
FEV1 / FVC (%)	-0.222	0.283	0.228	-0.518
PEF (l)	-0.482	0.166	0.059	-0.156
Cu (ppb)	-0.055	0.440	-0.294	0.523
Zn (ppm)	0.123	0.502	0.040	-0.323
Mg (ppm)	-0.049	0.572	-0.048	0.304
Vit-C (nmol/l)	0.144	0.167	-0.645	-0.468
Vit-E(nmol/µl)	0.373	-0.014	0.299	-0.050
SOD (U/ml)	0.178	0.316	0.578	0.029

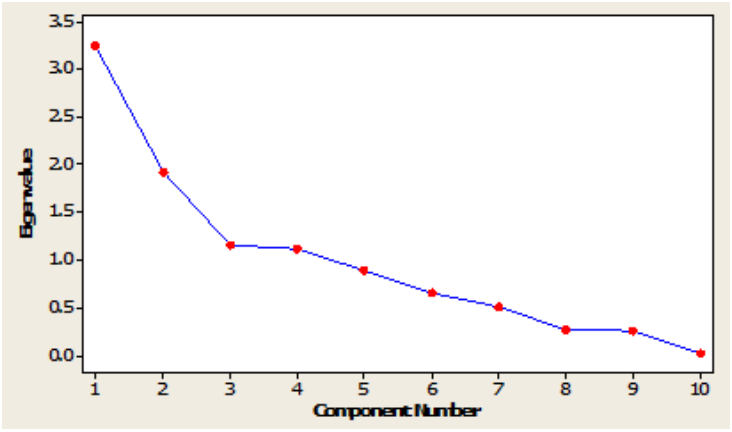

Fig. 1: Screen plot showing the significant principal component

Principal component analysis

Principal component analysis (PCA) was applied on the results to discriminate between control and patient groups. Three significant principal components were identified using eigen value equals or greater than one rule when the PCA is based on correlation matrix. A plot between the eigen values for each component against the component (screen plot) also confirms the number of significant components (Fig. 2).

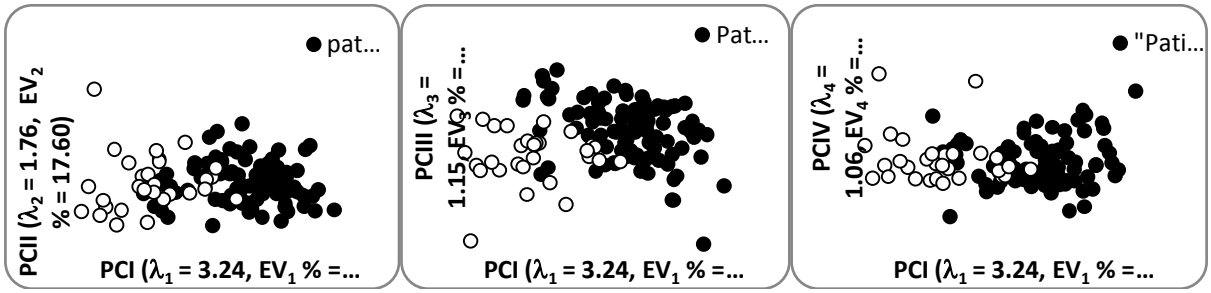

Fig. 2: Principal Component Analysis (PCA) applied on patient and control sample. λ_1 and λ_2 are eigen values and $EV_1$and EV_2 percentage explain variance

Analysis of means

Analysis of means (ANOM) was applied on the data to identify the factors responsible for significant difference (alpha 0.05) between the values of patient and control (Fig. 3).

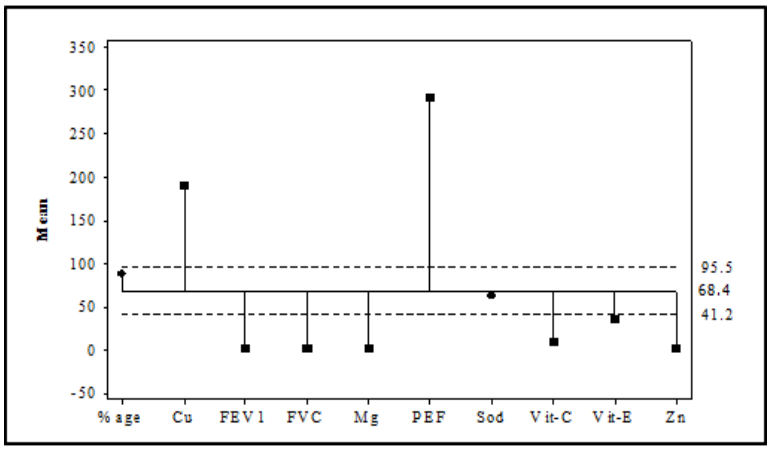

Fig. 3: One-way normal ANOM for patient and control data at α = 0.05

DISCUSSION

Oxidative stress is commonly used to explain the imbalance between the production of oxidants and endogenous antioxidant defenses. In general, mammalian cells undergo apoptotic death when there is an imbalance between oxidants and antioxidants. Recent studies using experimental allergic asthmatic mouse models and peripheral cells and tissues from asthmatic humans have revealed antioxidants as promising treatments for people with asthma [17]. It was hypothesized to detect alterations in antioxidant profiles of spirometry proven asthmatics. The profiles

included endogenous antioxidant enzymes like extracellular Cu/Zn SOD, exogenous antioxidant vitamins C and E and antioxidant trace elements Zn, Cu and Mg. In this study, there was overall 17% increase in patients Ec-SOD as compared to the controls. Yvonne and coworkers demonstrated in bronchial brush material obtained from mild asthmatics that SOD inactivation or knockdown is sufficient to cause apoptosis [18]. However they did not show any evidence of Ec-SOD changes in the serum. Whether mitochondrial or cytosol SOD changes has any influence on the serum SOD has to be found. In our study, we measured Ec- SOD and showed that it increased in patients as compared to controls. This quantification increase of serum SOD may be partly reactionary due to decrease in cellular SOD or due to its inactivation. The other study point which is related to decrease cellular activity and cell apoptosis needs further work because asthma mortality is comparatively low despite disability caused by the disease. Decreases in SOD activity in asthmatics were found to correlate with decreased lung function [19]. Similarly in our study spirometric parameters were significantly reduced as compared to normal healthy controls indicating decreased lung function. Therefore it was logical to conclude that the SOD activity was decreased and hence the increase in Ec-SOD found in asthmatics compared to normal controls.

The role of nutrition in bronchial asthma is related to antioxidant vitamins A, C, and E by counteracting oxidants and reducing external attacks for example bacteria, virus, toxins and xenobiotics in the lung, antioxidant vitamins modulate the development of asthma and the impairment of pulmonary function [20]. In this study Vit-E levels were significantly increased in patients when compared to controls although they remained within the normal range. This may indicate impairment in utilization of the vitamins. However, vitamin E intake was generally unrelated to asthma status but was significantly lower in severe asthma than in mild asthma [21]. This is not in agreement to our results possibly because of the inclusion of mild to moderate asthmatics in our study.

In our findings Vit-C levels were significantly decreased in patients when compared to controls. It indicates that ascorbate plays an important role in progression of asthma. Similar to our results, relatively low dietary intakes of vitamins A and C are associated with statistically significant increased odds of asthma and wheeze [21]. It has been reported that the administration of Vit-C can sometimes lead to an increase in oxidative damage, particularly if iron is also administered. This might be crucial in ensuring that tocopherol concentrations are maintained in lipoproteins and membranes [22]. Therefore increased serum concentration of Vit-E and simultaneous decrease in concentration of Vit-C may be pertinent in this study on asthmatics.

Antioxidants are important in restoring the disturbed oxidant/antioxidant equilibrium in patients with asthma. Antioxidants selenium, magnesium, vitamin A and C as well as glutathione are lower in asthmatics than in people without asthma [23]. Loss of Zn from biological membranes increases their susceptibility to oxidative damage and impairs their function. Zn deficiency may also lead to an enhanced Th2 immune response. Similarly in our findings Zn had direct relationship with asthma. Zn has a structural stabilizing role while the Cu is directly involved in catalytic activity. Cu and Zn have role in antioxidant defense as cofactor in SOD. Mg has several biological effects of potential relevance to asthma, including bronchodilation when given intravenously in acute severe asthma [9]. There is also strong evidence of protection by dietary magnesium against asthma.

Principal component analysis

Fig. 1 shows that only the first four components retained with 72.20 % cumulative variance because the subsequent eigen values were less than one. Fig. 2, plot (a), (b) and (c) show distinct separation between patient (filled circle) and control (empty circle) groups. PCA explained 32.40 % of the total variability for first component which is a measure of a function of FEV_1. Negative value of FEV1 indicates that PCI increased with decrease in FEV1. This component tells that the lungs of patients can exhale lesser amount of air in the first second of a forced exhalation.

Second principal component is positively correlated with Zn, and Mg; and showing 17.60 % of the total variance. This component suggests that high concentration of zinc was found in the patients who have high concentration of Mg.

The third component is comprised of Vit-C and SOD showing 11.60 % of variation. A negative correlation between Vit-C and SOD shows that a high amount of Superoxide Dismutase enzymes break Vit-C molecule; hence, lesser amount of Vit-C has been observed in the patients who show higher SOD activity. Fourth component is made up of Cu and FEV1/FVC with 10.60 % variance. A negative correlation between Cu and FEV1 and FVC show that an increase in Cu level in patient decreases FEV1/FVC ratio showing obstructive and restrictive lung disease (Table 2).

Fig.3 shows one way ANOM with significance level 0.05. It is evident from figure that percentage and SOD are within the decision limit. Therefore, it is concluded that there is no significant difference between the values of percent and SOD of patient and control groups. Other parameters which cross the decision limits are responsible for the significant difference between the values of patients and controls. PEF was identified as the parameter with the highest variability between the groups. Other parameters like FEV_1, FVC, Vit-C, Cu, Mg, and Zn moderately contributed in the distinction

between patient and control groups. Vit-E was found as the least contributor in the discrimination between patient and control groups. PEF and Cu were identified as the major contributor in the discrimination between patient and control groups. It is also evident from the figure that there is a significant difference between the patients and control groups in terms of FEV1, FVC, Mg, Vit-C, and Zn. No difference has been observed for %age and SOD values in control and patients.

CONCLUSIONS

It has been concluded that various exogenous antioxidants including Vit-E and Zn showed significant changes on the antioxidant status in asthma. Endogenous antioxidant SOD increase in asthmatics showed alteration in antioxidant defenses.

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