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Erythrocyte and Plasma Antioxidants in Bronchial Asthma Before and After Homeopathic Treatment

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Abstract

Objective: Oxidative stress is involved in the pathophysiology of bronchial asthma. The present study was done to assess the effectiveness of practicing homeopathy in modulating free radical toxicity in bronchial asthma by measuring some parameters of oxidant stress and antioxidant defenses in blood, before and after homeopathy treatment.

Methods: In the present study, erythrocyte lipid peroxidation (LP), erythrocyte antioxidants viz., glutathione (GSH), glutathione reductase (GR), superoxide dismutase (SOD), catalase (CT) and plasma antioxidants viz., ceruloplasmin, glutathione-S-transferase (GST), vitamin C, total antioxidant activity (AOA) have been determined in 41 patients with bronchial asthma and 53 control subjects. Twenty three patients who were treated with homeopathic remedies were considered for the follow-up studies.

Results: Erythrocyte LP (0 hour, $p<0.001$; 2 hours, $p<0.001$; and susceptibility to LP, $p<0.01$) and SOD ($p<0.05$) were significantly higher, whereas plasma vitamin C ($p<0.001$) and AOA ($p<0.001$) were significantly lower in bronchial asthma patients when compared to controls. In follow-up patients the erythrocyte LP (0 hour, $p<0.01$; 2 hours, $p<0.001$; and susceptibility to LP, $p<0.001$) and SOD ($p<0.01$) were significantly lower when compared to their pretreatment values. Plasma vitamin C attained a normal range. The AOA activity after treatment was not significantly different from that observed before treatment.

Conclusion: The present study showed an imbalance between antioxidants and oxidants in bronchial asthma. Oxidative stress had increased as indicated by increased LP, increased SOD, decreased vitamin C and decreased AOA. On homeopathic treatment the LP had decreased in the erythrocytes which shows that homeopathic treatment has some effect in reducing oxidative stress. This is further evidenced by returning of plasma vitamin C and erythrocyte SOD to the normal levels, but oxidant stress has not been completely overcome within the period of study as plasma AOA has still not returned to normal control levels. Thus, a prooxidant mileu exists in asthma patients which tends to normalize after homeopathic treatment.

Keywords: Free radicals; Homeopathy; Bronchial asthma

Introduction

Homeopathy, is a system of alternative medicine that strives to treat "like with like" [1,2]. It is used extensively throughout the world [3] being particularly popular in Europe and India [4,5]. The WHO definition of asthma is that it is a disease characterized by recurrent attacks of breathlessness and wheezing, which vary in severity and frequency from person to person. In an individual, they may occur from hour to hour and day to dayIn common with conventional medicine, homeopathy regards diseases as morbid derangements of the organism [1,2]. However, it differs in preferring to view each case of sickness as a strictly individual phenomenon. Homeopathy rests on the premise of treating sick persons with extremely diluted agents that in undiluted doses are deemed to produce similar symptoms in a healthy individual. Belief in the effectiveness of homeopathy in general is wide-spread and growing among the physicians and public [5-7]. Homeopathic treatment has also been found to be effective in treatment of respiratory tract disorders [4].

Asthma is a lower airways disease characterized by enhanced responsiveness to a variety of stimuli and manifested by airways obstruction that changes spontaneously or therapeutically [8]. Airways are unique in both their exposure to high levels of environmental oxidants and their unusually high concentration of extracellular antioxidants. Oxidative stress may play an important role in the pathophysiology of asthma [9-12] and may be a final common pathway leading to tissue damage. Variety of different substances such as allergens, gaseous pollutants, chemicals, drugs, bacteria and viruses [13] leads to the recruitment and activation of inflammatory cells which have an exceptional capacity for producing oxidants in asthmatic airways. Activated eosinophils, neutrophils, monocytes, macrophages and also resident cells such as bronchial epithelial cells, generate oxidants [10,14-19]. Allergen-specific reactions involving the acquired immune system are characterized by the production of interleukin (IL-5) and the subsequent recruitment and activation of eosinophils. In contrast, stimuli that act *via* the innate immune system lead to the production of IL-8 and the subsequent recruitment and activation of neutrophils. However, both of these pathways lead to the production of ROS, primarily due to the respiratory burst of activated inflammatory cells [20].

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Oxidative stress can have many detrimental effects on airway function including airway smooth muscle contraction [21], induction of airway hyper responsiveness [22,23], mucus hypersecretion [24,25], epithelial shedding [26] and vascular exudation [27,28]. Furthermore, ROS can induce cytokine and chemokine production through induction of the oxidative stress-sensitive transcription of nuclear factor-κB in bronchial epithelial cells [29].

In the present work, a study has been carried out on the levels of few oxidant and antioxidant parameters in plasma and RBC in order to find out whether they correlate with reported findings in respiratory airways and epithelium and also to find out whether homeopathic treatment has any influence on them.

Material and Methods

Study design

The study plan was approved by the Ethics Committee of the Medical Faculty, and all subjects volunteered for the trial.

Exclusion/inclusion criteria

Patients coming for the first time for homeopathic treatment were considered. They were advised not to take any other medications. Exclusion criteria included pregnancy, human immunodeficiency virus infection, and history of respiratory infection in the previous 6 weeks.

Blood samples were obtained from 41 bronchial asthma patients (males 17, females 24), aged 20-70 (mean age 36.71± 0.624) years. They were on homeopathic services at Fr. Muller Homeopathic Hospital during the period July 2004 to July 2006. These patients suffered from one or more of the following symptoms-wheezing, breathlessness, sneezing and cough. Classical homeopathy was followed where a comprehensive homeopathic history was taken, followed by prescription of a single individualized remedy in response to changing symptoms. For follow-up studies only 23 patients were available. From these patients, another blood sample was collected after 3 months of treatment. Different oxidant and antioxidant parameters were estimated in blood samples obtained before and after treatment. Following treatment with homeopathic drugs 75% of the patients who had come for followup studies felt a relief in their symptoms. The results were compared with those obtained in age and sex matched healthy non hospitalized individuals who were considered as normal controls. The control group consisted of 53 individuals (36 males, 17 females), aged 24 to 64 (mean age 45.42 ± 1.36) years. They had no history of bronchial asthma. They did not suffer from any one or more of the following symptoms-wheezing, breathlessness, sneezing and cough. A consent form was taken from them before blood samples were taken from them. Subjects who had come to the OPD for normal routine health checkups and had all parameters normal were taken for the study.

Methodology

Random blood samples were collected in heparinised bottles from normal subjects and bronchial asthma patients. Plasma and RBC's were separated. 50% erythrocyte suspensions were prepared according to the method of Kartha and Krishnamurthy [30]. These suspensions were used for some of the assays performed. The assays performed in the erythrocytes were lipid peroxidation (LP), glutathione (GSH), glutathione reductase (GR), catalase (CT), and in plasma were glutathione-S-transferase (GST), vitamin C, ceruloplasmin, antioxidant activity (AOA).

The hemoglobin content of the erythrocytes was determined by the cyanmethemoglobin method. Erythrocyte LP was determined by incubating RBC suspension in saline phosphate buffer containing 0.44M H_2O_2 at 0 hour and 2 hours. Aliquots were drawn from the above mixture at 0 hour and 2 hours. Lipid peroxidation in RBC was determined by estimating malondialdehyde (MDA) produced using thiobarbituric acid [31]. Erythrocyte GR activity was determined by recording the decrease in absorbance due to depletion of NADPH for a period of 5 minutes at 340nm [32]. SOD was determined according to the method of Beauchamp and Fridovich [33] based on inhibition of nitrozolium reduction. CT activity in the hemolysate was determined by adopting the method of Brannan *et al.* [34]. The assay is based on the disappearance of H_2O_2 in the presence of the enzyme source at 26˚C. The GSH content of erythrocytes was determined as described by Beutler *et al.* [35].

Plasma ceruloplasmin was determined by p-phenylene diamine

Names of homeopathic Medications	Number of lines	Total homeopathic medications	
		%	Total (%)
Arsenicum alb	24	19.67	19.67
Pulsatilla	12	9.83	29.50
Antimonium tartaricum	10	8.20	37.7
Natrum sulphuricum	8	6.56	44.26
Kali carbonicum	8	6.56	50.82
Ferrum Phosphoricum	6	4.91	55.73
Ammonium carbonicum	6	4.91	60.64
Phosporus	5	4.1	64.74
Sepia officinalis	5	4.1	68.84
Lycopodium clavatum	4	3.28	72.12
Natrum muriaticum	4	3.28	75.40
Magnesia Phosphorica	4	3.28	78.68
Nihilina	4	3.28	81.96
Calcarea flourica	3	2.46	84.42
Rhus toxicodendron	3	2.46	86.88
Nux Vomica	3	2.46	89.34
Sulphur	3	2.46	91.80
Others	10	8.20	100.00
Total medications	122	100.00	.....

Table 1: The 17 most prescribed homeopathic medications in 41 patients.

Names of homeopathic Medications	Number of lines	Total homeopathic medications	
		%	Total (%)
Arsenicum alb	16	21.62	21.62
Pulsatilla	8	10.81	32.43
Antimonium tartaricum	6	8.11	40.54
Natrum sulphuricum	6	8.11	48.65
Ferrum Phosphoricum	7	9.46	58.11
Kali carbonicum	4	5.40	63.51
Sabedella	4	5.40	68.91
Magnesia Phosphorica	4	5.40	74.31
Ammonium carbonicum	3	4.06	78.37
Lycopodium clavatum	3	4.06	82.43
Nihilina	3	4.06	86.49
Others	10	13.51	100.00
Total medications	74	100.00	----------

Table 2: The 11 most prescribed homeopathic medications for the 23 patients whose follow up blood sample were taken.

oxidase activity [36]. Plasma vitamin C was determined chemically using dinitrophenyl hydrazine as a colour compound [37]. Plasma GST was determined by incubating CDNB (1 chloro 2, 4 dinitro benzene) with reduced GSH in the presence of serum containing glutathione-S-transferase. 2, 4-dinitrophenylglutathione (adduct) formed was read at 340nm [38]. AOA activity was measured as given by Koracevic *et al.* [39].

The package used for statistical analysis was SPSS/PC+ (version 11.0).

Homeopathetic Treatment of Bronchial asthma patients

A total of 122 prescription lines were prescribed for 41 patients i.e. 2.9 medications per patient, on an average. One prescription line corresponds to one medication prescribed to one patient at the inclusion visit. Medications were given simultaneously or sequentially depending on the condition of the patient. Homeopathic treatment was prescribed for all the patients.

Table 1 shows the 17 homeopathic medications most prescribed in the study group (i.e, 41 patients) during this study, the main ones are: *Arsenicum alb, Pulsatilla, Antimonium tartaricum, Natrum sulphuricum, Kali carbonicum, Ferrum Phosphoricum* and *Ammonium carbonicum*.

Arsenicum alb, Pulsatilla, Antimonium tartaricum, Natrum sulphuricum, Kali carbonicum and *Ammonium carbonicum* were most often prescribed at a dilution of 30^0c, whereas *Ferrum Phosphoricum* at 6x. 54% of the 41 patients received *Arsenicum alb* and 29% received *Pulsatilla*. *Antimonium tartaricum, Natrum sulphuricum, Kali carbonicum, Ferrum Phosphoricum* and *Ammonium carbonicum* were prescribed for 24%, 17%, 19%, 14% and 13% of the patients respectively.

Group	TBARS as nmol MDA/ g Hb (Mean ± SEM)		
	0 Hour	2 Hours	Susceptibility to LP
Normal Controls(NC) n=53	77.8 ± 4.46 (20.8 – 181.6)	384.5 ± 18.54 (102.8 – 898.7)	306.0± 16.65 (72.0-735.6)
Bronchial asthma n=41	101.9 ± 8.01 *** (20.3 – 299.1)	514.4 ± 31.32 *** (118.9 – 936.3)	412.5±30.00 ** (98.9-833.5)
% change	30.97%>NC	33.78% >NC	34.80%>NC

Statistical significance of results vs. NC: ** p< 0.01, *** p< 0.001.
Ranges of TBARS levels observed are given in parentheses
n= number of cases
(Mann-Whitney Test)

Table 3: Lipid peroxidation in bronchial asthma.

Clinical status	TBARS as nmol MDA/ g Hb Mean ± SEM		
	0 Hours	2 Hours	Susceptibility to LP
Before treatment n=23	118.2±12.10 (20.3-299.1)	552.7±47.20 (118.9-936.3)	434.5±45.87 (98.6-833.5)
After treatment n=23	77.0 ± 7.52 ** (20.9 – 169.9)	354.7 ± 23.90 *** (140.8 – 605.2)	277.8±22.10*** (86.1-465.7)
% change	34.80%< before Treatment	35.82%< before treatment	36.07 %< before treatment

Statistical significance of values obtained after treatment vs. values before treatment: ** p< 0.01, *** p< 0.001.
Ranges of TBARS levels observed are given in parentheses
n= number of cases
(Paired T-Test)

Table 4: Lipid peroxidation in bronchial asthma before and after treatment.

Diagnosis	GSH (µmol/g Hb)	SOD (units/g Hb)	Catalase (units/g Hb)	GR (units/g Hb)
Normal Controls(NC)	4.71 ± 0.209 (2.36 – 10.25) N=53	9214 ± 492.5 (4046 – 21990) n=53	245996 ± 10410.2 (27920 – 413385) n=53	1.77 ± 0.153 (0.10 – 4.09) n=51
Bronchial asthma	5.39 ± 0.382 (1.46 – 10.38) n=41 NS	11787 ± 986.4 * (2396 – 36053) n=41	283870 ± 23404.0 (77978 – 881356) n=41 NS	1.88 ± 0.199 (0.22 – 5.79) n=39 NS
% change	12.52%>NC	27.92%>NC	15.39% > NC	6.21%>NC

Statistical significance of results vs.NC: p< 0.05, NS = Not significant.
The figures in the parentheses indicate the ranges of antioxidant levels observed
n= number of cases.
(Mann-Whitney Test).

Table 5: Erythrocyte antioxidant levels in bronchial asthma (Mean ± SEM).

Table 2 shows the 11 homeopathic medications most prescribed for 23 patients whose follow up blood sample was taken. *Arsenicum alb, Pulsatilla, Antimonium tartaricum, Natrum sulphuricum* and *Ferrum phosphoricum* were the main homeopathic treatments prescribed for the patients.

Results

Erythrocyte LP and susceptibility towards LP in bronchial asthma patients was significantly high compared to normal controls (Table 3). After treatment a significant decrease was observed in LP. Susceptibility was also decreased significantly (Table 4). SOD activity in the erythrocytes was found to be significantly increased in pre-treated asthmatic patients, compared with normal control subjects (Table 5). The enzyme activity decreased significantly in post-treated patients when compared to corresponding pretreated subjects (Table 6). A comparison of erythrocyte GSH, CT and GR in bronchial asthma patients with those in normal controls showed no significant change (Tables 5, 6).

Plasma vitamin C level and AOA were significantly decreased in asthmatic patients, when compared with that of normal control subjects (Table 7). A comparison of vitamin C levels before and after treatment showed a significant increase in the latter (Table 8). After treatment, AOA remained significantly low when compared to normal subjects. There was no significant difference in the ceruloplasmin levels and GST in asthmatic patients when compared to normal controls before and after homeopathic treatment (Table 7, Table 8).

Discussion

Asthma prevalence has increased dramatically in the recent years [40]. Epidemiological evidence suggests that changes in diet, in particular reduced antioxidant intake, have contributed to increases in asthma prevalence and severity and raises the

Clinical Status	GSH (µmol/g Hb)	SOD (units/g Hb)	Catalase (Units/g Hb)	GR (Units/g Hb)
Pre-treatment	6.02+0.470 (1.46-10.38)	13276+1527.8 (3274 – 36053)	283221+35677 (77978 -881356)	2.02+0.0300 (0.28- 5.79)
Post-treatment	4.73 ± 0.278 (2.82 – 8.70) n=23 NS	9991 ± 999.9 ** (2340 – 23545.2) n=23	260732 ± 17343 (117935– 390472) n=23 NS	1.34 ± 0.259 (0.00 – 5.10) n=22 NS
% change	21.42%< before treatment Treatment	32.87%<before Treatment	7.94%< before treatment	33.66%<before treatment

Statistical significance of values obtained after treatment vs. values before treatment: NS = Not significant, ** p< 0.01.
The figures in the parentheses indicate the ranges of antioxidants levels observed
n= number of cases.
(Wilcoxon Signed Ranks Test).

Table 6: Follow up studies of various erythrocyte antioxidants in bronchial asthma patients.

Diagnosis	Vitamin C (µmol/L)	Ceruloplasmin (g/L)	GST (IU/L)	AOA (mmol/L)
Normal Controls(NC)	22.5 ± 1.23 (3.5 – 49.5) n=53	0.479 ± 0.0268 (0.225 – 1.400) n=53	4.31 ± 0.450 (0.41 – 15.41) n=53	1.03 ± 0.060 (0.32 – 2.20) n=53
Bronchial asthma	12.4 ± 1.40*** $ (0.7 – 42.3) n=41	0.580 ± 0.055 (0.046 – 2.140) n=41 NS	4.27 ± 0.583 (0.00 – 20.80) n=41 NS	0.54 ± 0.035 *** # (0.22 – 1.05) n=36 NS
% change	44.88%<NC	21.08>NC	0.92%<NC	47.57%<NC

Statistical significance of results vs. NC: ** p< 0.01, *** p< 0.001, NS = Not Significant.
The figures in the parentheses indicate the ranges of antioxidant levels observed
n= number of cases.
($=Anova, # =Mann-Whitney Test)

Table 7: Plasma antioxidant levels in bronchial asthma patients (Mean± SEM).

Clinical Status	Vitamin C (µmol/L)	Ceruloplasmin (g/L)	GST (IU/L)	AOA (mmol/L)
Pre-treatment	13.1 ± 2.12 (3.1- 42.3)	0.597 ± 0.8300 (0.150 – 2.140)	3.84 ± 0.485 (0.50 -10.41)	0.47 ± 0.043 (0.22-1.02)
Post-treatment	26.0 ± 2.55 ** (4.2 – 46.6) n=23	0.519 ± 0.0875 (0.75 – 2.300) n=23 NS	4.62 ± 0.612 (0.31 – 11.45) n=23 NS	0.53 ± 0.045 (0.22 – 1.04) n=21 NS
% change	98.47%>before treatment	13.06%<before treatment	20.31%>before treatment	12.76%>before treatment

Statistical significance of values obtained after treatment vs. values before treatment: NS = Not significant.
The figures in the parentheses indicate the ranges of antioxidant levels observed
n= number of cases.
(Wilcoxon Signed Rank test)

Table 8: Follow up studies of plasma antioxidants in bronchial asthma patients (Mean± SEM).

possibility that dietary interventions may improve asthma [41]. Lipid peroxidation is of particular significance inasthma. Recent studies have demonstrated [42] elevated plasma lipid peroxidation in asthma, as measured by 8-iso-$PGF_{2\alpha}$. Elevated MDA levels have been observed in both plasma [43-46] and breathe condensate in asthmatics [47]. Studies done by Nadeem *et al.* [48] showed increased plasma levels of lipid peroxidation products, measured as TBARS in asthmatic patients. The results of present work are also in agreement with these findings as judged by increased RBC lipid peroxidation. *In vitro* lipid peroxidation of RBC has significantly increased at 0 hour in asthmatic patients when compared to normal controls. The TBARS concentration at 2 hours is also significantly increased in asthmatic patients when compared to control subjects. Even the susceptibility towards lipid peroxidation has increased significantly.

Important antioxidants in the respiratory tract lining fluid include reduced GSH, mucin, uric acid, vitamin C and albumin [49]. GSH is a key antioxidant in the lining fluid of the respiratory tract. It is 100-fold more concentrated in the airway epithelial lining fluid compared with plasma. The glutathione system is a central mechanism for reducing H_2O_2. It complements catalase as a reducing system for H_2O_2 but exceeds catalase in its capacity to eliminate additional varieties of toxic peroxides [50]. Disturbed GSH status is reported in asthma, with total [51] and oxidized [52] GSH being elevated in bronchoalveolar larvage (BAL) fluid and reduced GSH being elevated in erythrocytes [53]. Studies done by Nadeem *et al.* [48] showed similar results suggesting that GSH synthesis and/or transport has increased in response to the presence of excess oxidants and has subsequently been oxidized as it performs its antioxidant role. However, in the present study no increase was observed in the GSH levels in asthmatic patients.

Reports on SOD enzymatic antioxidant status in asthma are inconsistent. Studies done by Kurosawa *et al.* [54] in platelets of bronchial asthmatic patients showed significantly higher levels of SOD activity than those of normal healthy subjects. Nadeem *et al* [55] have shown an increase in SOD activity in the erythrocytes. In the present work also an increase in SOD activity in the erythrocytes was observed and this is in agreement with the above findings. This increase in SOD in the RBC cells might be a compensatory mechanism for increased oxidative stress. However, Powell *et al* [55] did not find increased activity of erythrocyte SOD in their study. Smith [56] reported unchanged levels of SOD in BAL. Tekin et *al.* [57] and Fenech [58] have reported decreased SOD activity in erythrocytes of asthmatics compared with controls. De Raeve et *al* [59] have reported decreased SOD activity in bronchial epithelial cells. Zn, a cofactor of SOD, has also been reported to be decreased [60] or unchanged [61].

Catalase is most effective in the presence of high H_2O_2 concentrations. In the present study, the red blood cell catalase activity was not found to be changed. Similar studies done by Nadeem *et al.* [48] and Tekin *et al.* [57] in the erythrocytes of asthmatics showed no change in catalase activity when compared to control subjects. This might be because the hydrogen peroxide formed after dismutation of superoxide anion by SOD can be actively scavenged by normal levels of catalase.

A study by Yang *et al.* [62] have emphasized on the critical role of the copper containing enzyme, ceruloplasmin in defence against oxidative damage and infection in the lungs. However in the present study, no significant alteration in plasma ceruloplasmin levels was observed when compared to control subjects.

Olusi *et al.* [63] have reported a decrease in plasma vitamin C level in asthmatic patients when compared to control subjects. Similar results were obtained in the present work. There was a marked decrease in plasma vitamin C level in asthmatic patients when compared to control subjects. Olusi *et al* [63] have also reported decrease vitamin C levels in leucocytes of asthmatics. Recent studies found that supplementation with vitamin C reduced ozone related decrement in lung function in asthmatic subjects, particularly in those with genetically determined increased susceptibility to oxidative stress [64].

In the present study, the plasma total antioxidant capacity in asthmatic patients was significantly lower than that in control subjects. Rahman *et al.* [65] also reported a decreased total antioxidant capacity of plasma in asthmatic patients. The decrease in the total plasma antioxidant level may partly be because of decrease in plasma vitamin C which is an important antioxidant. Following treatment with homeopathic drugs 75% of the patients who had come for followup studies felt a relief in their symptoms. The concentration of lipid peroxides in these patients decreased significantly both at 0 hour and 2 hours. This indicates that the homeopathic drugs had the effect of reducing lipid peroxidation in asthmatics. However, they had no significant antioxidant activity *invitro.*

The erythrocyte antioxidant enzyme SOD significantly decreased after treatment compared to pre-treatment values almost reaching normal levels. The antioxidant vitamin of plasma i.e. vitamin C increased to normal range. But total antioxidant activity remained significantly low. This seems to indicate that other antioxidants which contribute to total antioxidant activity are not affected by the treatment. Glutathione reductase activity was increased after treatment. Probably this is a reflection of increase in antioxidant capacity of erythrocytes to counter the oxidant change.

While it is difficult to compare the data due to differences in the disease severity it is clear that, overall status of the antioxidant enzymes and their cofactors is often altered in asthma, indicating a disturbed oxidant/antioxidant balance. Following homeopathic treatment the oxidative stress is decreased at least partially. This is evidenced by significant decrease in lipid peroxidation, increase in vitamin C and decrease in SOD.

Limitation of this study

Although this study is the first of its kind in homeopathy it has certain limitations. Biomarkers like eosiophil count (Eosinophilia), NF-KB, neutrophils, monocytes, macrophages, macrophage degranulation assay, B-cell, IgE estimation, allergen-specific reactions (interleukin) IL-5, IL-8 IL-4, CD^4, CD^8 cell or secondary messenger assays etc. could have been performed to make the study more complete. However, these assays could add on to the future scope of this study.

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