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RESEARCH ARTICLE

ASSESSMENT OF SERUM VITAMIN C AND ERYTHROCYTE-REDUCED GLUTATHIONE LEVELS WITH INCREASED SERUM MALONDIALDEHYDE IN PATIENTS WITH COPD

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ABSTRACT

Chronic obstructive pulmonary disease (COPD) is one of the most common chronic diseases and represents an important cause of morbidity and mortality. The main aim of this study is no know the alterations in serum vitamin C, erythrocyte glutathione activity and MDA in controls and chronic obstructive pulmonary disease patients. A retrospective study includes 120 subjects, comprising of 60 healthy controls and 60 COPD cases. Among 60 COPD cases 30 were chronic bronchitis patients and 30 were emphysema patients. Vitamin C was estimated by 2, 4 – Dinitrophenyl Hydrazine Method, Erythrocyte reduced glutathione was estimated by Ernest Beutler *et al* method and malondialdehyde by Kei Satoh method. The Pearson's correlation coefficient for various antioxidants in COPD cases is evident that there is a positive correlation between vitamin C vs. erythrocyte reduced glutathione cases and is highly significant. It is also evident that there is a negative correlation between erythrocyte reduced glutathione vs. MDA and vitamin C vs. MDA cases and is highly significant. The present study revealed that there was an increased oxidative stress in patients with COPD, when compared with controls and also decreased levels of antioxidants like vitamin C and erythrocyte reduced glutathione activity in COPD patients, when compared with controls.

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INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is the fourth leading cause of death globally. The prevalence of COPD is higher in countries where smoking is highly prevalent. In India, there is and increasing tendency to abuse tobacco and COPD is emerging to be a major public health. Oxidative stress plays an important role in the pathogenesis of COPD. Oxidative stress is caused by an imbalance between the production of oxidants and the presence of antioxidants (WHO global burden of disease study, Rahman *et al*., 1996, Repine *et al*., 1997).

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There is an evidence that oxidative stress reaches the circulation by a fall in the plasma antioxidant capacity (vitamin C, vitamin E, carotene and sulphydryls) associated to smoking. In addition, a similar fall in plasma antioxidant occurs in exacerbations of COPD (MacNee W, 1999, MacNee 2005). Epidemiological studies have shown that high dietary intake of antioxidants vitamins C and E related to a lower prevalence of chronic bronchitis in smokers. (Rahman 2000) Experimental studies have provided evidence about an imbalance between oxidants/ antioxidants, in favor of reactive oxidizing species (oxidative stress), associated with COPD (Tuder *et al*., 2002, Rahman *et al*., 1996, MacNee *et al*. 1999, MacNee 2000, Hanta *et al*., 2006, Park *et al*., 2009).

Table 1. Comparison of Vitamin C, Erythrocyte reduced glutathione and MDA in controls and COPD cases

Vitamin C, GSH and MDA in controls and COPD cases							
		Vitamin C (mg/dl)		GSH (mg/dl)		MDA(nmol/ml)	
		Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD
Controls	60	0.82 -1.40	1.10 ±0.16	25.00 -38.02	33.55 ± 2.23	1.53 –3.58	2.62± 0.52
Cases	60	0.35- 0.81	0.56 ±0.12	24.17 -31.42	27.63 ± 1.96	3.58 -6.71	5.36 ±0.74
Mean Diff.		0.54		5.92		2.74	
t-value*		21.10		15.45		23.44	
p-value		<0.001, HS		<0.001, HS		<0.001, HS	

*Unpaired t-test

Table 2. Shows comparative analysis of Vitamin C, erythrocyte GSH and MDA in controls and in patients with chronic bronchitis cases

Vitamin C, erythrocyte GSH and MDA in controls and chronic bronchitis cases				
Groups		Vitamin C (mg/dl)	GSH (mg/dl)	MDA(nmol/dl)
Controls	Mean ± SD	1.10 ± 0.16	33.54 ± 2.23	2.62 ± 0. 52
	Range	0.82 – 1.40	25.00 – 38.02	1.53 – 3.58
Chronic Bronchitis	Mean ± SD	0.65 ± 0.09	28.97 ± 1.11	4.83 ±0.51
	Range	0.47 – 0.81	26.76 – 31.42	3.58 – 5.89
Control vs. chronic	Mean Diff.	0.45	4.58	2.21
bronchitis	t-value*	17.67	13.01	19.20
	p-value	<0.001	<0.001	<0.001

*Unpaired test, p-value <0.001 highly significant.

Table 3. Shows comparative analysis of Vitamin C, erythrocyte GSH activity and MDA in controls and in patients with emphysema cases

Vitamin C, erythrocyte GSH activity and MDA in controls and in emphysema cases.				
Groups		Vitamin C (mg/dl)	GSH (mg/dl)	MDA(nmol/dl)
Controls	Mean ± SD	1.10 ± 0.16	33.54 ± 2.23	2.62 ± 0. 52
	Range	0.82 – 1.40	25.00 – 38.02	1.53 – 3.58
Emphysema	Mean ± SD	0.47 ± 0.08	26.29 ± 1.70	5.89 ± 0.52
	Range	0.35 – 0.75	24.17 – 30.98	4.42 – 6.71
Control vs.	Mean Diff.	0.63	7.26	3.27
Emphysema	t-value*	25.34	17.17	28.11
	p-value	<0.001	<0.001	<0.001

*Unpaired test, p-value <0.001 highly significant.

Table 4. Comparative analysis of Vitamin C, erythrocyte GSH activity and MDA levels between chronic bronchitis and emphysema cases

Vitamin C, erythrocyte GSH activity and MDA levels between chronic bronchitis and emphysema cases.				
COPD groups	No	Vitamin C (mg/dl)	GSH (mg/dl)	MDA(nmol/dl)
Chronic Bronchitis	30	0.65 ± 0.09	28.97 ± 1.11	4.83 ±0.51
Emphysema	30	0.47 ± 0.08	26.29 ± 1.70	5.89 ± 0.52
Mean Diff.		0.18↓	2.68↓	1.06↑
t-value*		8.44	7.23	7.98
p-value		<0.001	<0.001	<0.001

*Unpaired test, p-value <0.001 highly significant.

Table 5. Relationships between various antioxidants in COPD cases

Correlation between antioxidants	Pearson's correlation coefficient 'r' value	Significance p- value
Vitamin C vs. GSH	+0.58	<0.001
GSH vs. MDA	-0.68	<0.001
Vitamin C vs. MDA	-0.64	<0.001

The main aim of this study is to know the alterations in serum vitamin C, erythrocyte glutathione activity and MDA in COPD patients.

MATERIALS AND METHODS

The present study was conducted in MNR Medical Collage & Hospital situated in Sangareddy, part of Medak District, Telangana state, India (600 beds teaching hospital catering to rural population). A total of 120 samples include 60 COPD cases and 60 controls.

Study approved by the Institutional ethical committee and Informed consent was taken from the all participating patients.

Estimation of Serum Vitamin C

1. Method: 2, 4 – Dinitrophenyl Hydrazine Method. (Kaplan In clinical chemistry. 3rd ed.
Chapter 39, p. 786-787.)

Principle: Ascorbic acid is oxidised by copper to form dehydroascorbic acid and diketogulonic acid. These products are treated with 2, 4- dinitrophenyl hydrazine (DNPH) to form the derivative bis2, 4-dinitrophenyl hydrazone. This compound, in strong sulphuric acid, undergoes rearrangement to form a coloured product which is measured at 520nm.this reaction is run in the presence of thiourea to provide a mildly reducing medium, which helps to prevent interference from non-ascorbic acid chromogen.

Calculation: Serum vitamin C= O.D of Test / O.D of Standard x 2 mg/dl.

Estimation of Erythrocyte Reduced Glutathione

Method: Ernest Beutler *et al*. method. (Beutler et. al, 1963)

Principle

All of the non-protein sulfhydryl groups of red blood cells are in the form of GSH. 5, 5'-Dithiobis-2-nitrobenzoic acid (DTNB) is a disulfide compound, which is readily reduced by sulfhydryl compounds, forming a deep yellow-coloured com-pound. Optical density (OD) that is measured at 412 nm is directly proportional to the GSH concentration.

Calculation: Concentration of erythrocyte GSH = OD of test/ O.D of Standard × 100 mg/dl.

Estimation of Serum Malondialdehyde

Method: Kei Satoh method. (Santosh *et al*., 1978)

Principle: Auto-oxidation of unsaturated fatty acids involves the formation of semi stable peroxides, which then undergo a series of reactions to form MDA. MDA reacts with thiobarbituric acid (TBA) to form pink-coloured chromogen. The resulting chromogen is extracted with 4.0 mL of n-butyl alcohol and the absorbance of which is measured at 530 nm.

Calculation: Concentration of serum MDA (nmol/ml) = Absorbance of test (A) × 51.28 nmol/ml.

Statistical Analysis: Results are expressed as Mean ± SD and range values. Unpaired' test is used for comparing different biochemical parameters between cases and controls. Pearson's correlation co-efficient used to assess the relationship between different variables. P value of < 0.05 was considered as statistical significance.

RESULTS

A total number of 120 subjects were included in this study. Among them 60 were controls who were normal healthy individuals and 60 were chronic obstructive pulmonary disease (COPD) cases. Of the 60 controls, 35 were male and 25 were female and their mean age is 57.7 ± 7.4 years. Among 60 chronic obstructive pulmonary diseases cases, 46 were male and 14 were female and their mean age is 62.3± 7.8 years. Based on clinical and radiological findings chronic obstructive pulmonary disease cases were divided in to two groups as chronic bronchitis and emphysema. Of the 60 cases, 30 cases had chronic bronchitis and 30 cases had emphysema.

It is observed from the above table that the mean levels of vitamin C, Erythrocyte reduced glutathione and MDA in controls were in the range of 1.10 ± 0.16 mg/dl, 33.55 ± 2.23 mg/dl and 2.62 ± 0.52 nmol/ml, respectively. (Table 1) In COPD cases the mean levels of Serum Vitamin C, Erythrocyte reduced glutathione and MDA in the range of 0.56 ± 0.12 mg/dl, 27.63 ± 1.96 mg/dl and 5.36 ± 0.74 nmol/ml, respectively. Mean levels of vitamin C, erythrocyte reduced glutathione activity were significantly decreased ($p<0.001$) and mean levels of serum MDA was significantly increased in COPD cases. When compared to healthy controls and are statistically highly significant ($p<0.001$). (Table 1)

It is observed from the table that the mean levels of, Vitamin C, erythrocyte GSH and MDA in controls were in the range of 1.10 ± 0.16 mg/dl, 33.54 ± 2.23, and 2.62 ± 0.52 nmol/dl, respectively. The estimated mean levels of serum vitamin C, erythrocyte reduced glutathione activity and MDA in chronic bronchitis were in the range of 0.65 ± 0.09 mg/dl, 28.97 ± 1.11 mg/dl and 4.83 ±0.51, respectively. Chronic bronchitis patients had significantly lower levels of vitamin C, erythrocyte reduced glutathione activity than those of controls ($p<0.001$) and significantly higher levels of serum MDA than in controls ($p<0.001$). (Table 2) It is observed from the table that the mean levels of Vitamin C, erythrocyte reduced glutathione activity and MDA in controls were in the range of 1.10 ± 0.16 mg/dl, 33.54 ± 2.23 and 2.62 ± 0. 52 nmol/ml, respectively. Emphysema patients had significant lower levels of Vitamin C, erythrocyte reduced glutathione activity than those of controls ($p<0.001$) and significantly higher level of serum MDA than in controls ($p<0.001$). (Table 3) The estimated mean levels of Vitamin C, erythrocyte GSH activity and MDA in chronic bronchitis were in the range of 0.65 ± 0.09 mg/dl, 28.97 ± 1.11 mg/dl and 4.83 ±0.51 nmol/ml, respectively. Emphysema cases had significantly lower levels of vitamin C, erythrocyte reduced glutathione activity ($p<0.001$) and significantly higher levels of serum MDA than chronic bronchitis ($p<0.001$). (Table 4) The Pearson's correlation coefficient for various antioxidants in COPD cases is evident that there is a positive correlation between vitamin C vs. erythrocyte reduced glutathione cases and is highly significant. It is also evident that there is a negative correlation between erythrocyte reduced glutathione vs. MDA and vitamin C vs. MDA cases and is highly significant. (Table 5)

DISCUSSION

Vitamin C is a water soluble free radical scavenger, can directly scavenge O^{-}_{2} and OH^{-} radicals and helps to neutralize physiological oxidant burden created by both exogenous and endogenous sources. The mean value of serum vitamin C is 1.10 ± 0.16 mg/dl, in controls and 0.56 ± 0.12 mg/dl, in COPD

cases. When compared to controls COPD patients have significantly (p value < 0.001) decreased level of vitamin C. This is accordance with studies Raghunath R. Rai *et al*., 2006, Sargeant *et al*., 2000; Calikoglu *et al*., 2002. The mean value of vitamin C is 0.65 ± 0.09 mg/dl in chronic bronchitis patients and 0.47 ± 0.08 mg/dl in emphysema patients. Vitamin C is significantly decreased (p value < 0.001) in emphysema patients when compared to chronic bronchitis patients. This is in accordance with the study of Papaioannou *et al*., 2010.

Vitamin C functions as an important free radical scavenger. The mechanism involved in the reduction of vitamin C level in COPD is due to rapid oxidation of ascorbic acid by free radicals. The negative relationship between vitamin C and MDA may be due to the depletion of vitamin C when the oxidant burden is increased (Padayatty *et al*., 2003). Vitamin C functions as an antioxidant by donating its electrons it prevents other compounds from being oxidized, however by the very nature of this reaction. Vitamin C itself is oxidised in the process. The species formed after the loss of one electron is a free radical i.e., ascorbyl radical. As compared to other free radical is relatively stable with half life of 10^{-5} seconds and is fairly. Dietary antioxidant supplementation is one of the simplest approaches to boost antioxidant defense systems. Supplementation of vitamin C, vitamin E, and β-carotene has been attempted in cigarette smokers and patients with COPD (Cross *et al*., 1993, Calikoglu *et al*., 2002). Depletion of total antioxidant capacity in smokers is associated with decreased levels of major plasma antioxidants. Studies show depletion of vitamin C, vitamin E, β-carotene, and selenium in the serum of chronic smokers and in patients with COPD (Antwerpen *et al*., 1993, Mezzetti *et al*., 1995, Rahman *et al*., 1996).

Conclusion

The present study demonstrates that there is increased oxidative stress in patients with COPD when compared to controls and it is higher in emphysema patients when compared to chronic bronchitis patients. This study also emphasizes the decreased antioxidants namely erythrocyte reduced glutathione, serum vitamin C activity in COPD patients when compared to controls. Antioxidants are particularly decreased in emphysema patients when compared to chronic bronchitis patients. This study demonstrates that tobacco smoke induces oxidative stress in smokers which results in chronic obstructive pulmonary disease. Hence by advising diet rich in antioxidants or supplementation of antioxidants may prevent the further oxidative damage in COPD patients.

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