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OXIDATIVE STRESS, α-TOCOPHEROL, ASCORBIC ACID AND REDUCED GLUTATHIONE STATUS IN SCHIZOPHRENICS

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ABSTRACT

A disturbance in the antioxidant defense system including α-tocopherol, ascorbic acid and reduced glutathione metabolism due to free radical induced oxidative injury has been implicated in various neuro-psychiatric disorders. The roles of these antioxidants, changes in their blood levels and correlation with oxidative stress were studied in a common psychiatric illness Schizophrenia. Fifty-eight subjects of either sex ranging in age from 18-60 years divided into two age groups (≤40 and >40 years) diagnosed for schizophrenia, and forty age and sex-matched normal subjects as controls were included in the study. Blood samples were analyzed for malondialdehyde (MDA), α-tocopherol, total ascorbic acid (TAA), dehydro ascorbic acid (DHAA), reduced ascorbic acid (RAA), leucocyte ascorbic acid (LAA) and reduced glutathione (GSH). A decrease in the levels of α-tocopherol, total ascorbic acid and reduced glutathione was found in schizophrenics compared to normal controls. Further a significant rise in oxidative stress and decreased antioxidant status was observed in the chronic stage of schizophrenia as compared to those in acute condition. A significant rise in dehydroascorbic acid with concomitant fall in reduced ascorbic acid suggests scavenging action of ascorbic acid and its utilization with increased oxidative stress as indicated by high blood malondialdehyde levels. Leucocyte ascorbic acid, a better index of ascorbic acid status was also found to be reduced in schizophrenics, suggesting depletion of body stores of ascorbic acid and the condition worsened with advancing age.

KEY WORDS

Oxidative stress, Malondialdehyde, α-tocopherol, Ascorbic acid, Dehydroascorbic acid, Glutathione, Schizophrenia

INTRODUCTION

A homeostasis between rate of formation of free radicals and the rate of their neutralization if not maintained oxidative damage accumulates known as oxidative stress (1). This radical induced damage may be important in disease like schizophrenia, as there is an increasing evidence that oxidative injury contributes to pathophysiology of schizophrenia (2, 3). The brain is most susceptible to attack by the free radicals as it is rich in polyunsaturated fatty acids and highly oxygenated (4) and the damage so caused to the neurons by these free radicals cannot be repaired (5).

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The potential toxicity of free radicals is counteracted by a large number of cytoprotective enzymes and antioxidants, that limit the damage. This protective mechanism functions co-operatively in form of a cascade in which the cellular antioxidants α-tocopherol, ascorbic acid and reduced glutathione act in combination (6). α-tocopherol is a chain breaking antioxidant that by neutralizing a free radical gets converted to α-tocopheroxyl radical. It can be reduced back to a-tocopherol by ascorbic acid (7). Dehydroascorbic acid formed in this reaction can be reconverted back to ascorbic acid by reduced glutathione (8). It is important that sufficient amounts of α-tocopherol, reduced ascorbic acid and reduced glutathione be present within the cell so as to provide protection against oxidative injury.

Thus, the present study is an attempt to examine oxidative stress and the status of protective antioxidants under condition of stress due to schizophrenia and the effect of age, sex, acute and chronic phase of disease on the level of these antioxidants.

MATERIAL AND METHODS

The study included fifty eight subjects without any organic illness of either sex (42 males and 16 non-pregnant, non-lactating females) ranging in age from 18-60 years and divided into age groups ≤40 years (n=30) and >40 years (n=28), newly diagnosed for schizophrenia, at OPD of Psychiatric Center, SMS Medical College, Jaipur by one of the authors, and forty age and sex matched healthy non-schizophrenics which served as controls. On the basis of duration and symptoms of illness and PANSS score (9,10) each schizophrenic was categorized as, in the acute (n=40) or chronic (n=18) stage of the illness. The complete clinical and personal history of the subjects was recorded. Subjects with history of receiving electro-convulsive therapy (ECT) in last one year, substance dependence for last one month and history or present symptoms of any other stress induced disorder were excluded.

Blood samples were collected at the time of admission from the anticubital vein and serum was separated to estimate TAA and DHAA using 2, 4 – dinitrophenylhydrazine (11) and α-tocopherol by spectrophotometric method using bathophenanthroline (12) while, Leucocyte ascorbic acid an index of ascorbic acid deficiency was determined by the method of Denson and Bowers (13). Leucocyte count was performed with automated cell counter (PENTRA 120) and results were expressed as μg/10^8 leucocytes. Glutathione (GSH) was estimated by reducing DTNB (5,5', Dithiobis-2-nitrobenzoic acid) (14) and per oxidative stress was assessed by estimation of malondialdehyde (MDA) using thiobarbituric acid (TBA) assay method (15). Statistical analysis was done by Z- test and coefficient of correlation (r) was calculated between oxidative stress and α-tocopherol**,** ascorbic acid level & reduced glutathione.

RESULTS AND DISCUSSION

Examination of oxidative stress and antioxidant status revealed that the MDA level an indicator of oxidative stress was found to be significantly raised (P<0.001) in schizophrenics as compared to control subjects ranging in age from 18 to 60 years. Further, the elderly subjects in the age of >40 years had a greater degree of oxidative stress as compared to those below 40 years of age, and the effect was evident both in controls and schizophrenics, however elderly schizophrenics had greater degree of oxidative stress as compared to control (Table-1). This shows that age seems to affect the level of oxidative stress however there was no effect of gender on MDA level in controls as well as schizophrenics (Table-2). The raised MDA level reflects the oxidative injury due to schizophrenia, which is attributed to free radical formation that abstracts hydrogen atoms from lipoproteins causing lipid peroxidation, of which MDA is the main product (16, 17), and their delayed neutralization in the presence of low antioxidant concentration and condition is further aggravated by antipsychotic agents (18,19). Among all schizophrenics the value of PANSS was more in chronic patients as compared to acute ones (Table-3), and similarly the oxidative stress was found higher in chronic cases as compared to acute.

α-tocopherol ,ascorbic acid and reduced glutathione are important chain breaking antioxidants responsible for scavenging the free radicals and suppression of peroxidation in aqueous and lipid region of the cell (20,21). The level of α-tocopherol, the most potent lipid bound chain breaking antioxidant in serum was found to be significantly lower in schizophrenics (16) as compared to controls (P<0.001). Elderly subjects in both the groups of controls and schizophrenics as well as female subjects showed significantly decreased levels as compared to the male subjects (Table-1,2).This decrease is probably contributed to the increased consumption of α-tocopherol for free radical neutralization and its conversion to α-tocopheroxyl radical (1). Further on comparing the level of this nutrient vitamin in acute and chronic patients, its level depleted significantly in chronic subjects due to the increased oxidative stress levels found in chronic patients (Table-3).

Similarly ascorbic acid (TAA) the most effective water soluble vitamin was also found to decrease significantly (P<0.001) in elderly controls and schizophrenics as compared to young (Table-1). There was no significant change in both male as well as female subjects in both groups (Table-2).Ascorbic acid exists in blood as oxidised (DHAA) and reduced forms (RAA) and its transportation across cell membranes is in the form of DHAA, which is less ionized at physiological pH and has more membrane permeability (22). RAA reconverts tocopheroxyl radical to α-tocopherol, itself getting converted to ascorbate radical (DHAA). DHAA which normally constitutes five percent of serum TAA, has increased significantly (P<0.001) in Schizophrenics from 4.7 to 11.7 percent and consequently DHAA/TAA and DHAA/RAA ratio were significantly increased in Schizophrenics as compared to control subjects (Table-1). Significantly increased DHAA and DHAA/TAA & DHAA/RAA ratios were also found in elderly controls and schizophrenics.The increase in DHAA could perhaps be a mechanism to counteract the effect of increased oxidative stress or in other words increased conversion of ascorbic acid to dehydroascorbic acid reflects oxidative stress. Similarly the TAA and RAA levels in chronic schizophrenics showed a decrease as compared to acute, attributed to the antioxidant

TABLE- 1

MDA and antioxidant levels of Control and Schizophrenic Young and Elderly Subjects
(Values are mean ± SD)

	Controls (n = 40)			Schizophrenics **(n = 58)**		
PARAMETERS	Age range 18-60 yrs. (n = 40)	Age range ≤ 40 yrs. (n = 22)	Age range > 40 yrs. (n = 18)	Age range 18-60 yrs. (n = 58)	Age range ≤ 40 yrs. (n =30)	Age range > 40 yrs. (n =28)
MDA (nmol/gmHb)	0.047±0.005	0.044±0.005	0.051±0.004*	0.068±0.017*	0.062±0.013	0.081±0.011*
α-tocopherol (mg /dl)	0.82 ±0.20	0.94±0.14	0.65±0.12*	0.54 ±0.21*	0.57 ± 0.21	0.49± 0.21*
TAA (mg /dl)	1.26 ± 0.43	1.60±0.21	0.87±0.28*	0.75± 0.21*	0.80+ 0.19	0.54±0.11*
DHAA (mg /dl)	0.06 ±0.02	0.04±0.009	0.08±0.01*	0.08 ± 0.02*	0.06 ±0.01	0.10± 0.01*
RAA (mg /dl)	1.19 ±0.45	1.55±0.22	0.84±0.36*	0.67±0.23*	0.73± 0.10	0.44± 0.14*
GSH (mg /dl)	41.39 ± 5.40	45.51±2.88	36.34±2.76*	35.8±3.91*	36.70±3.22	32.25 ± 3.09*
LAA ($\mu g/10^8$WBC)	28.32 ±4.20	31.20±2.57	24.53±2.39*	24.05±4.69*	25.04±3.91	19.71±3.77*
DHAA/TAA	0.06 ±0.05	0.02 ±0.01	0.10± 0.04*	0.13±0.08*	0.06 ±0.01	0.21± 0.07*
DHAA/RAA	0.07 ±0.06	0.02 ±0.01	0.12± 0.06*	0.16±0.13*	0.07 ±0.01	0.27 ±0.14*

Statistical comparison was done between: age-matched Controls and Schizophrenics (18-60 years);Control age ≤40 yrs. and >40 yrs.; schizophrenics age ≤40 yrs. and >40 yrs. *p<0.001

TABLE- 2

MDA and antioxidant levels of Control and Schizophrenic Male and Female subjects
(Values are mean ± SD)

	Controls (n = 40)			Schizophrenics (n = 58)		
PARAMETERS	Total (n = 40)	Male (n = 29)	Female (n = 11)	Total (n = 58)	Male (n =42)	Female (n =16)
MDA (nmol/gmHb)	0.047±0.005	0.047±0.005	0.047±0.002	0.056±0.016*	0.056±0.018	0.056±0.009
α-tocopherol (mg /dl)	0.82 ±0.20	0.82±0.22	0.78±0.06*	0.54 ±0.21*	0.57 ± 0.23	0.47± 0.11*
TAA (mg /dl)	1.26 ± 0.43	1.30±0.47	1.20±0.33	0.77± 0.21*	0.74± 0.21	0.83±0.19
DHAA (mg /dl)	0.06 ±0.02	0.06±0.02	0.06±0.01	0.09 ± 0.08*	0.07 ±0.02	0.07± 0.01
RAA (mg /dl)	1.19 ±0.45	1.23±0.49	1.13±0.34	0.69±0.23*	0.67±0.24	0.76±0.2
GSH (mg /dl)	41.39 ± 5.40	41.58±6.10	40.80±2.97	36.11±3.66*	35.68±3.96	37.22± 2.56
LAA ($\mu g/10^8$WBC)	28.32 ±4.20	28.72±4.77	27.75±2.01	24.30±4.47*	23.78±4.83	25.47±3.22

Statistical comparison was done between gender matched: Controls and Schizophrenics; Controls and Schizophrenics (male) and Controls and Schizophrenics (female). *p<0.001

TABLE- 3
MDA and antioxidant levels of acute and chronic Schizophrenics (Values are mean ± SD)

PARAMETERS	Acute Schizophrenics (n = 40)	Chronic Schizophrenics (n = 18)
PANSS score	151.10 ± 16.78	175.10 ± 8.70*
MDA (nmol/gmHb)	0.058 ± 0.017	0.076 ± 0.011*
α-tocopherol (mg /dl)	0.55 ± 0.21	0.33 ± 0.05*
TAA (mg /dl)	0.78 ±0.19	0.52 ±0.19*
DHAA (mg /dl)	0.09 ± 0.02	0.11 ± 0.01*
RAA (mg /dl)	0.69 ± 0.20	0.40 ± 0.20*
GSH (mg /dl)	36.02 ±3.78	32.03 ± 2.79*
LAA ($\mu g/10^8$WBC)	24.34 ± 4.57	19.33 ± 3.38*

Statistical comparison was done between acute and chronic Schizophrenics
*p<0.001

Table-4
Correlation between oxidative stress and antioxidants

Correlation between	Correlation Coefficient (r)	Significance
MDA- a-tocopherol	-0.89	P<0.01
MDA-TAA	-0.83	P<0.01
MDA-DHAA	+0.88	P<0.01
MDA- Reduced AA	-0.85	P<0.01
MDA -GSH	-0.93	P<0.01
MDA - LAA	-0.94	P<0.01

deficit due to chronic phase of schizophrenia. A consequent increase in DHAA levels in chronic schizophrenics was also observed (Table-3). Ascorbate is again regenerated by reduction with GSH in the process of free radical scavenging (6). Glutathione level was found to be significantly decreased (P<0.001) in all schizophrenics, and in elderly controls and schizophrenics as compared to young (Table-1) and no change was found due to effect of gender (Table-2) in controls as well as schizophrenics indicating its increased utilization to counteract oxidative stress by way of converting DHAA to RAA. This low level is insufficient to regenerate AA from DHAA, therefore it can be assumed as a reason for increased level of DHAA in schizophrenics (8). Heavy cumulative burden of free radicals also depleted GSH in chronic condition of schizophrenia (Table-3).

Ascorbic acid is a water-soluble vitamin and normally its excess is excreted in urine and its concentration in serum is affected by recent intake. While ascorbic acid concentration in leucocytes is stable, and not affected by recent dietary intake, hence considered a better index to assess vitamin C status of an individual (22,23). Schizophrenics were found to have significantly reduced (p<0.001) LAA (24.05±4.69µg/10^8 WBC) as compared to controls (28.32±4.20µg/10^8 WBC) indicating increased utilization and the decrease was similar in elderly controls and schizophrenics as compared to young (Table-1), but not significant in male and female subjects of both groups (Table-2). The level also decreased in chronic schizophrenics (Table-3).

The above results show a positive correlation between MDA and DHAA indicating the increased consumption of TAA to combat stress and a negative correlation between MDA and α-tocopherol, TAA, RAA, LAA & GSH indicating the loss of vital antioxidants of the body due to stress (Table-4), and changes in the concentration of different fractions of ascorbic acid sensitively reflects oxidative stress in tissues (24).

Thus, it is evident from the study that increased oxidative stress in schizophrenics leads to decrease in the level of antioxidants; α-tocopherol, AA and GSH, and disturb their metabolism, that weaken their ability to fight the growing stress. Further advancing age worsens the condition. Hence their supplementation in treatment protocol could be beneficial.

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