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Original Article

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Oxidative Stress Markers (8-Isoprostane and 8-Hydroxy-2-Deoxyguanosine) in Major Depression: A Case-control Study

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ABSTRACT

Introduction: Depression is associated with activation of innate immune response leading to oxidative damage. The 8-isoprostanes and 8-hydroxy-2-deoxyguanosine (8-OHdG) are biomarkers of oxidative damage to lipids and Deoxyribonucleic Acid (DNA), respectively. They have been independently linked to depression.

Aim: To study the oxidative stress markers (8-Isoprostanes and 8-OHdG) in subjects with major depression.

Materials and Methods: In this observational case-control study 42 cases of depression, 13-25 years of age were recruited from Psychiatry Out Patient Department (OPD) at a tertiary-care hospital in Delhi, India, along with 42 healthy controls. They were assessed clinically and using psychometric evaluation scores, Beck's Depression Inventory-II (BDI-II) and Hamilton Depression Rating Scale (HAM-D). All 42 subjects were on medication with antidepressants {33/42 with Selective Serotonin Reuptake Inhibitors (SSRI) 8/42 with Tricyclic Antidepressants (TCA) and 1/42 on a combination of both}. Routine laboratory investigations were done. Plasma 8-Isoprostane and serum 8-OHdG concentrations were measured in both cases and controls. The results obtained were analysed using relevant statistical tests on STATA version 11 (StataCorp, 2009).

Results: Clinically, all patients had moderate to severe depression. BDI-II and HAM-D scores were raised in all cases as compared to the controls (28.81±5.60 vs 1.62±1.59 for BDI and 20.88±4.67 vs 1.33±1.43 for HAM-D, respectively). The concentration (in depressed vs controls) of plasma 8-Isoprostane (107.70±54.48 pg/mL vs 77.78±60.15 pg/mL) and serum 8-OHdG (2103.03±154 pg/mL vs 2017±164.69 pg/mL) were significantly elevated (p-value <0.05). Though elevated in patients belonging to both genders, showed significant increase of 8-Isoprostane only in females and 8-OHdG only in males as compared to their healthy controls. No correlation of the levels of any of two markers was seen with clinical severity of depression of patients as assessed by BDI.

Conclusion: Evidence of oxidative stress to lipids and DNA are present in the peripheral blood. These can be explored further in establishing the biomarkers for diagnosis and prognosis of depression.

Keywords: Antidepressants, Beck's depression inventory, Biomarkers, Hamilton depression rating, Oxidative damage

INTRODUCTION

Oxidative stress is a state in which the endogenous antioxidant defenses of the host are overcome by toxic Reactive Oxygen Intermediates (ROI). Depression is associated with the activation of innate immune response. Activated phagocytes produce toxic ROI following injury and inflammation of tissues as a mechanism to kill invading microorganisms [1]. However, these ROI not only destroy the microbes but also destroy the lipid membranes, protein structures, thereby destroying the antigen bearing cells [2]. There is evidence for oxidative disturbances in major depression, as demonstrated by oxidative marker studies and those examining the anti inflammatory effects of antidepressants [3,4]. Isoprostanes and 8-OHdG are important biomarkers of oxidative damage to lipids and DNA respectively which have been independently linked to depression.

An 8-Isoprostane (8-iso Prostaglandin-F2α) is a Prostaglandin (PG) belonging to the F2-isoprostane class that is produced by free radical peroxidation of arachidonic acid in vivo. In humans, oxidative damage to lipids is best assessed via levels of F2-isoprostanes [5-7]. Levels of isoprostane are elevated in patients suffering from depression in plasma/serum [8,9] as well as urine [9,10]. The effect of antidepressant treatment on isoprostane levels has also been investigated [11]. Levels of 8-iso-prostaglandin F2α (8-isoPGF2α), are also found to be elevated in patients with metabolic syndrome [12], coronary heart disease as well as healthy adults with cardiovascular risk factors [13,14] (such as smoking, hypercholesterolemia, chronic infections, obesity, and diabetes). An 8-OHdG is a repair product of the oxidation of guanine in DNA, can be used to estimate the rate of oxidative DNA damage. The importance of this lesion stems from the fact that it is both abundant in DNA and it is mutagenic [15,16]. An 8-OHdG present in DNA during cellular replication causes somatic mutation that can cause carcinogenesis and also can contribute to the pathogenesis in formation of atherosclerotic plaques [17-19]. Some studies show that oxidative DNA damage over long period of time can be a common pathological mechanism for major depression and other medical co-morbidities [17,20,21]. An association between depression, its severity and levels of 8-OHdG has been examined in several cross-sectional studies. These studies have compared healthy group serum [20] as well as the urine sample 8-OHdG levels and found these were greater in people suffering from major depression [21]. A single study was identified examining changes in 8-OHdG following psychiatric treatment [22].

It cannot be ignored that the depressed young adults are more likely to engage in behaviours that bring about oxidative damage such as smoking, alcohol use and decreased physical activity [23]. Along with these factors, the increasing psychological stress and pressure in today's competitive world is associated with increased oxidative stress.

Thus, by promotion of inflammatory pathways, depression leads to increased levels of oxidised lipids and DNA. In this study, therefore, we aimed at comparing the status of stress biomarkers (8-Isoprostane and 8-OHdG) in peripheral blood of major depression patients along with healthy controls and correlate their levels with the disease severity.

MATERIALS AND METHODS

This was a case-control observational study in which the potential relationship of a suspected risk factor or an attribute to the disease (oxidative damage to Lipids and DNA) is examined by comparing the diseased and non-diseased subjects with regard to frequency of presence of the factor(s) or attribute(s) and its/their levels in each of the groups. The study design and the method plan were approved by the Ethics Committee (Ref No. IESC/T-26/04.01.2013) of All India Institute of Medical Sciences (AIIMS), Delhi, India.

In this case-control study, the levels of serum 8-OHdG and plasma 8-Isoprostane have been measured in cases diagnosed as major depression and compared to that of their matched healthy controls.

After clinical and psychometric evaluation of the cases and controls, blood samples (3 mL EDTA tube and 3 mL in Serum Separator Tubes (SST)) and urine sample was collected. Laboratory parameters studied in potential study subjects (patients and healthy controls) included plasma 8-Isoprostane and serum 8-OHdG. Other parameters included were haemogram, erythrocyte sedimentation rate, plasma glucose, renal function tests, liver function tests, serum calcium, serum phosphate, serum uric acid, serum total proteins, serum albumin, serum total cholesterol, urine routine chemistry and microscopy. These were carried out to rule out any co-morbid illness in the patients and for screening of the healthy controls for exclusion.

Inclusion and Exclusion criteria: The subjects were same as those included in our previous study where we have seen the effect of depression on blood brain barrier damage leading to the elevation of levels of marker S100B in serum [24].

Cases: Forty-two diagnosed cases of depression, 13-25 years of age were recruited from the Child-guidance and Walk-in-clinic in the Psychiatry Department of AIIMS, New Delhi during January 2013 to June 2014. Written informed consent was taken from subjects or guardians (or legally acceptable representative) of the patient in case of minor subjects before inclusion in the study. They were diagnosed with major depression by DSM-IV criteria on the basis of Structured Clinical Interview for DSM Disorders-nonpatient version (SCID-I/NP) [25,26]. Those suffering from any co-morbid medical illness, present or past history of psychotic disorders, post-traumatic stress disorder, behavioural disorder, substance abuse within the last 12 months were excluded from the study. Others with history of medication with antibiotics, immune modulators or steroids, any febrile illness (temperature >99 °F) within four weeks prior to blood collection or in females, a positive urine pregnancy test were also excluded. Detailed present and past clinical history along with routine hematology and biochemistry investigations were carried for the same.

Controls: A total of 42 healthy subjects of age 13-25 years including school going children and young adults were recruited as controls. They were subjects of comparable age, gender and socio-economic status (assessed by Modified Kuppuswamy's socio-economic scale) [27]. They had no past or present history of any DSM-IV Axis I disorder. Consent was taken from participant or guardian in case of minors. All of them were assessed using clinical interview along with General Health Questionnaire-12 [28]. Control subjects were also assessed using psychometric evaluation scores same as on depressed subjects. Subjects with any personal or family history of psychiatric illness, autoimmune disease, or with history of any substance abuse were excluded. All healthy controls were free of any acute/chronic illness within four weeks before the study. The controls thus, included in the study had normal laboratory findings including routine hematology and chemistry, renal and liver function tests. Females with positive urine pregnancy test were excluded from the study.

Psychometric Evaluation

Psychometric evaluation was done for both the patients and control subjects. Based on the interview, the psychiatrist rated severity of depression on the BDI-II [29] and HAM-D [30]. For adolescents, Stressful Life Event Scale (SLES) was also used to assess any stressful events causing disturbance or anxiety to the patient [31]. A local language (Hindi) version of the psychometric evaluation scales was used wherever applicable. BDI is a 21-item, self-reported rating inventory that measures characteristic attitudes and symptoms of depression. HAM-D is a 21-item clinician-administered multiple-choice measure of depression symptom severity. Both these scales are easy to administer and their validity and applicability have been studied in Indian population [32,33]. Both these scales have been used in several other studies to assess the severity of depression as well as to assess the response to treatment. Also, SLES have been used to assess the presence of stressors/stressful events in an individual's life within the past one year. This scale has been used for the depressed adolescents too [31].

Laboratory Method

A 3 mL of blood in EDTA vial, 3 mL of blood in plain SST and an early morning urine specimen was collected after the clinical diagnosis was made. Serum was separated from the SST tube by centrifugation.

Routine medical assessment and laboratory investigations (complete blood count from EDTA sample, kidney and liver function tests from the serum and urine examination) were done for all participants to exclude those with any medical condition.

Rest of the EDTA sample was centrifuged to obtain Plasma. Following this, serum levels of 8-OHdG and plasma levels of 8-Isoprostane were estimated using the commercially available ELISA kits following the prescribed method by the manufacturer.

Plasma 8-Isoprostane and serum 8-OHdG were measured by competitive Immunoassay in duplicate for each serial aliquots using Cayman Chemical's ACE™ Enzyme Immunoassay (EIA) kit The absorbance was read in duplicate using a Bio-Rad xMark microplate reader (Bio-Rad Laboratories, Hercules, CA, USA) at 420 nm. The intra-assay and inter-assay coefficients of variation for all analyses were less than 4%. The mean of available duplicate sample values was used.

The specificity of measurement of serum 8-OHdG, indicative of oxidative damage of DNA/RNA, by EIA with monoclonal antibody, although was high, it had some cross reactivity with 8-hydroxyguanosine and 8-hydroxyguanine.

STATISTICAL ANALYSIS

STATA version 11 (StataCorp, 2009) was used for statistical analysis. Two-tailed unpaired Student's t-test was used if the distribution of response variable was normal (8-OHdG) and Mann-Whitney U test was used when the distribution of variable was non-normal (8-Isoprostane). The p-values of ≤0.05 were regarded as statistically significant. All the psychometric scales: BDI, HAM-D and SLES were considered ordinal variables, Mann Whitney U test was done for comparisons between the depressed and healthy subjects as well as gender-wise comparisons. For intergroup comparisons in more than equal to three groups, Kruskal-Wallis one-way analysis of variance was used. The Bonferroni correction method was used for post-hoc comparisons, the significance level for which was set at level of p ≤0.016. Pearson's correlation coefficient was used to assess the relation amongst the markers as well as the markers with psychometric evaluation score BDI.

RESULTS

The demographic and clinical characteristics of 42 cases and 42 controls were assessed [Table/Fig-1,2]. The study subjects were 13-25 years of age. There was no significant difference in

the age (19.81±4.26 years vs 20.62±4.0 years; p-value=0.186), male/female ratio (25 males/17 females vs 23 males/19 females; p-value=0.330) and BMI (23.29±1.34 kg/m^2 vs 23.68±1.68 kg/m^2; p-value=0.120) between the depressed and healthy controls, respectively. Thus, the two groups were satisfactorily comparable. Mean duration of illness of the depressed subjects was 15.95±11.53 months. The average duration of current episode was 4.71±1.63 months. All the 42 subjects were on antidepressants as mentioned in [Table/Fig-2].

Variables	Depressed		Healthy controls	
	n	%	n	%
Age (years)				
13-14	5	11.9	3	7.14
15-17	11	26.2	8	19.05
18-20	5	11.9	9	21.43
21-23	8	19.05	7	16.67
24-25	13	30.95	15	35.71
Gender				
Male	25	59.52	23	54.76
Female	17	40.48	19	45.24
Total	42	100	42	100

[Table/Fig-1]: Age and Gender distribution of depressed subjects and healthy controls.

Variables	Value
Mean duration of illness	15.95±11.53 months
Mean duration of current episode	4.71±1.63 months
Average number of episodes of major depression	1.79±0.72
Number of cases on treatment	42/42
Number of cases on treatment with Selective Serotonin Reuptake Inhibitors (SSRI) out of total	33/42
Number of cases on treatment with Tricyclic Antidepressants (TCA) out of total	8/42
Number of cases on treatment with combination SSRI+TCA out of total	1/42
Cases with family history of depression	12/42

[Table/Fig-2]: Clinical characteristics of depressed subjects.
*42 is the total number of depressed subjects included in the study

Psychometric evaluation scores: BDI scores, HAM-D scores and SLES, as expected were found to be significantly higher in patients as compared to the control groups (p-value <0.001 for all three respectively) [Table/Fig-3]. A total five (all males) out of 42 patients and four (all males) out of 42 controls were smokers and few consumed alcohol occasionally, taking an average of two drinks (60 mL) per week. None of our patients was a substance abuser.

Parameters measured (pg/mL)	Depressed		Healthy controls		p-value
	Mean	SD	Mean	SD	
BDI	28.81	5.60	1.62	1.59	<0.001
HAM-D	20.88	4.67	1.33	1.43	<0.001
SLES	6.17	2.98	1.53	1.30	<0.001
8-Isoprostane (pg/mL)	107.70	54.48	77.78	60.15	0.010
8-OHdG (pg/mL)*	2103.03	154.00	2017.09	164.69	0.008

[Table/Fig-3]: Psychometric evaluation scores, concentration of plasma 8-Isoprostane and serum 8-hydroxy-2-deoxyguanosine in depressed subjects as compared to healthy controls.
Mann Whitney U test used for comparison across the groups; *Unpaired t-test used

Levels of both markers namely 8-Isoprostane and 8-OHdG were significantly higher in the patient group when compared to the control group [Table/Fig-3].

The BDI and HAM-D scores were found equally significant in both female and male groups when compared to their respective healthy controls. Plasma 8-Isoprostane levels were 126.43±61.70 pg/mL in depressed females as compared to 87.22±35.12 pg/mL in healthy females (p=0.023). Serum 8-OHdG levels were 2153.72±136.07 pg/mL in depressed males as compared to 2042.25±145 pg/mL in male healthy controls (p=0.009) [Table/Fig-4].

Parameters		Depressed		Healthy controls		p-value
		Mean	SD	Mean	SD	
BDI	Male	29.20	5.74	1.30	1.40	<0.001
	Female	28.24	5.49	2.00	1.76	<0.001
HAM-D	Male	20.44	4.13	1.00	1.17	<0.001
	Female	21.53	5.42	1.74	1.63	<0.001
8-Isoprostane (pg/mL)	Male	94.96	45.99	69.99	74.78	0.166
	Female	126.43	61.70	87.22	35.12	0.023
8-OHdG (pg/mL)*	Male	2153.72	136.07	2042.25	145.00	0.009
	Female	2028.48	151.91	1986.64	185.18	0.467

[Table/Fig-4]: Gender wise comparison within depressed and healthy subjects.
Mann-Whitney U test used for comparison across the groups; *Unpaired t-test used

In patients group, Pearson's correlation coefficient was calculated to see the relation of severity of the patients as calculated by the BDI scores with the levels of both markers as shown in [Table/Fig-5].

BDI Vs. Markers		Depressed			Healthy controls		
		Females	Males	Overall	Females	Males	Overall
8-Isoprostane	Correlation	0.176	0.277	0.192	0.261	0.299	0.281
	p-value	0.499	0.180	0.222	0.280	0.165	0.071
8-OHdG	Correlation	0.299	-0.330	-0.027	-0.212	0.200	-0.070
	p-value	0.243	0.107	0.864	0.384	0.360	0.658

[Table/Fig-5]: Correlation of the markers with the clinical severity of the patient as assessed by BDI scores. Pearson's correlation coefficient was used to assess the relation.

No significant relation was seen between the severity of depression in the patient group and the increase in levels of these markers.

Pearson's correlation to find out any relation between the raised values of two markers showed no significant difference (r-value: 0.056, p-value 0.616). Thus, the rise in their values in the depressed patients was independent of each other and are independently related to depression.

The time from diagnosis and initiation of treatment to inclusion of subject in the study was noted for these depressed subjects. Analysis of Variance (ANOVA)/Kruskal Wallis test were used to study the effect of treatment (t/t) with antidepressants for the duration of illness. Subjects were divided as those whose total duration of illness and medication was less than six months and those with more than six months.

On comparison it was found that in the initial six months, 8-Isoprostane was not significantly higher than healthy controls however as the duration increased 8-Isoprostane increased becoming significantly higher than healthy controls. However, 8-OHdG was higher than healthy controls throughout although significant only in first six months [Table/Fig-6].

Parameter (pg/mL)	≤6 months, A		>6 months, B		Healthy controls, C		p-value			
	Mean	SD	Mean	SD	Mean	SD	Overall	A Vs. B	A Vs. C	B Vs. C
8-Isoprostane	85.91	30.73	117.46	60.19	77.78	60.15	0.017	0.299	1.000	0.015
8-OHdG	2148.91	131.14	2082.46	161.06	2017.09	164.69	0.025	0.641	0.032	0.277

[Table/Fig-6]: Effect of duration of treatment on mean levels of the markers in study group as compared to the healthy controls.

DISCUSSION

The first appearance of depression occurs during childhood or adolescence. Depression in adolescents can be considered as an early-onset sub-form of the adult-equivalent form and has strong links with recurrence later in life [34]. This is the reason for choosing patients from young age group, including individuals in adolescence and early adulthood, which constitutes a considerably large portion of population of India.

Clinical depression is associated with increased oxidative stress that may represent a common pathophysiological mechanism making the patient vulnerable to comorbid medical illness. This may also be due to altered life-style behaviour in the young depressed patients such as smoking, reduced physical activity, inappropriate diet, alcohol/substance abuse etc., that may lead to increased oxidative stress and damage. In this study, we ruled out most of these life-style variables by taking SES-matched patients and controls along with comparison of their BMI, annual income, education, smoking habit and alcohol consumption. It was found that none of these were significantly different between depressed group and healthy groups of subjects except alcohol intake that was found to be slightly higher in the depressed subjects although the difference was not significant (p-value=0.030). However, we were not able to completely rule out the dietary differences between the two groups. The effect of illness modifying factors like BMI, smoking habit, and alcohol consumption which might have effect on the levels of these oxidative damage indicators was also studied by Yager S et al., 2010 [9].

Ruling out of comorbid acute infections and chronic disease using established exclusion criteria removed the possibility of confounding of result by any alternative disease process or treatment regime, thus further increasing the strength of the study.

As expected the psychometric evaluation scores BDI, HAM-D and SLES were significantly increased in the major depression patients as compared to the healthy controls. The degree of depression in these cases ranged from moderate to severe at the time of assessment.

On an average, the duration of current illness in months was 4.71±1.63 months and all the patients were on antidepressant medications, TCA/SSRI/both for a variable duration of time.

Oxidative damage to lipids is best assessed via levels of F2-Isoprostanes which are specific products of lipid peroxidation. Our results show a significant increase in plasma levels of 8-Isoprostane (107.70±54.48 pg/mL vs. 77.78±60.15 pg/mL; p-value=0.010) in patients with depression when compared to healthy controls. Concurring with the result of our study, serum and plasma levels of 8-Isoprostanes were found to be elevated in patients suffering from depression when compared to healthy comparison group in the study by Dimopoulos N et al., in 2008 and Yager S et al., in 2010, respectively [8,9]. High urinary concentrations of 8-iso-PGF2α and also its β-oxidation metabolite, 2,3-dinor-5,6- dihydro-15-F 2t-isoprostane (F2-isoPM) have also been reported in the depressed patients as compared to healthy controls [10,11]. Why lipid peroxidation would increase in the state of depression is not known. Whether it is specific for neurons, glia or peripheral cells is also not known. Further studies are essential to identify whether this lipid peroxidation is generalised or specific to parts of brain or happens elsewhere also in the body.

The effect of antidepressant treatment on isoprostane levels was investigated in one of the studies which showed that the urinary excretion of F2-Isoprostanes increased significantly following eight weeks of treatment with bupropion or sertraline in patients with major depression associated with improvement in depression severity [11]. This was in contrast to the hypothesis that antidepressants should play a role to decrease the levels of oxidative stress and their biomarkers. However, the number of subjects included in this study was 18 and those followed-up were only 9, is a very small number to develop any generalised conclusion [11]. Moreover, one of the limitations of this kind of study as by Chung CP et al., in 2013 was that the markers of inflammation and metabolic variables beyond body mass index were not collected to rule out the association of oxidative stress and depression independent of any other inflammatory process in the body [11].

An 8-OHdG is a repair product of the oxidation of guanine in DNA, and its level can be used to estimate the rate of oxidative DNA damage. We found a significant increase in serum levels of 8-OHdG (2103.03±154.0 pg/mL vs. 2017.09±164.69 pg/mL; p-value=0.008) in patients with depression when compared to healthy controls. Other studies have compared 8-OHdG levels in serum [20] as well as the urine sample [21] of depressed patients and found the levels greater in people suffering from major depression. Another study on unipolar and bipolar patients with severe depression found that there are no differences in urinary 8-OHdG between depressed and healthy samples, however, its Ribonucleoside (RNA) analogue, 8-oxo- 7,8-dihydroguanosine (8-oxoGuo), was higher in depressed patients [22]. In community based populations suffering from depressive symptoms, no differences were found in 8-OHdG levels when compared with those without depressive symptoms [35,36]. These findings may indicate the decreased or complex interactions between mild forms of depression and systemic oxidative DNA damage and repair in normal population. As mentioned, it is also not known whether the DNA damage is neuron- specific or originates from the peripheral non-neuronal tissues/cells. A study that examined the changes in 8-OHdG following treatment found that there was no change in the urinary excretion of 8-OHdG levels on treatment with Electroconvulsive Therapy (ECT). But excretion of its RNA analogue, 8-oxoGuo, was found increased significantly in urine of participants with major depression [22].

In contrast to all these older findings and our study results, a recent study on a large cohort by Black CN et al., however, concluded that elevated oxidative stress marker of 8-OHdG and F2 Isoprostanes was not seen in major depression and anxiety disorders. But antidepressant use was associated with lower oxidative DNA damage, suggesting the antioxidant effect of antidepressants [37].

The present study has also shown that the elevation of two markers, namely 8-Isoprostane and 8-OHdG, are not dependent on each other and thereby are independently related to depression. The study of Forlenza MJ and Miller GE on 8-OHdG in 2006 and the study of Yager S et al., in 2010 in same group of depressed population on 8-Isoprostane show similar results indicating the rise were independent of each other [9,20]. The explanation for this could be brought from facts that DNA is inside the nucleus of cells while oxidative damage to the lipids takes place in plasma or cell membranes. Within these two different compartments the source and type of reactive oxygen species as well as the antioxidants differ. Another factor is worth mentioning here that the antioxidant scavenging mechanisms and DNA repair mechanism can possibly be different within individuals.

Gender bias for serum 8-OHdG level, which was significantly higher in males as compared to the females in the present study. When similar gender subjects, depressed and healthy controls, were compared it was found that significantly higher levels of 8-Isoprostane were present only in females as compared to their healthy control counterparts and not in the males. Regarding oxidative damage, not many studies have noted the gender bias/differences for the levels of 8-Isoprostane or 8-OHdG. However, in one study, depressed elderly men were found to have higher urinary concentrations of 8-iso-PGF2α as compared to non-depressed men even after adjustment for multiple socio-demographic, lifestyle and health factors [10] whereas this association was not present in women. Exact reason for these differences is not known. The reasons could be the differences in coping mechanisms for stress in male and female, their different endocrine milieu, role of family, and

differences in cognitive ability of male and female. But we wonder why oxidative damage to lipids occur mostly in females and to DNA mostly in males.

Another interesting facet of such study was to look into the relation of the levels of these markers with the severity of depression. Our study had shown no correlation of 8-Isoprostane (p-value=0.222) and 8-OHdG (p-value=0.864) markers to BDI scores in the depressed individuals. HAM-D severity ratings within the depressed cohort in the study by Yager S et al., in 2010 have also showed that there was no significant association between the severity of symptoms and levels of 8-iso-PGF2α suggesting probably that the increase in stress marker is threshold-dependent rather than bearing a dose response relationship [9]. In some studies, levels of 8-OHdG have shown a positive correlation with the severity of depression [20,22]. Patients having major depression had higher levels than those with minor depression. Patients with recurrent episodes of depression had higher levels than those with single episodes [20]. The reasons for such difference among these studies and our study may be due to our small sample size and heterogeneity in the clinical characters of the depressed patients. It may also be because the previously mentioned studies had compared non-medicated individuals. In our study, all the patients were on treatment for an average duration of their illness. Moreover, most of the patients had moderate to severe degree of depression at the time of assessment by psychometric evaluation scores.

To study the effect of treatment, the levels of plasma 8-Isoprostane and serum 8-OHdG were compared against that of healthy control between those who were on treatment for less than and more than six months. It was seen that the levels of 8-Isoprostane were significantly higher in the depressed patients with more than six months of illness and medication than the healthy controls (p-value=0.015) whereas the serum levels of 8-OHdG in depressed patients with less than six months of depression and treatment were significantly higher than the controls (p-value=0.032). Patients who were on treatment for more than six months did not have elevated 8-OHdG levels as compared to the controls (p-value=0.277). Whether this difference is an effect of treatment or it is because of the duration of illness could not be ascertained in the present study. The previous studies have shown decrease in markers of oxidative stress following antidepressant treatment [38,39]. One study on the urinary excretion of F2-Isoprostane showed an increase in its excretion after effective treatment of depression [11]. But their sample size was too small to state definitively any possible reasons but they postulated that effective treatment of depression could result in another change like obesity or improved appetite and resulted in weight gain which could be the reason for increased oxidative stress. This might cause increased excretion of F2-Isoprostane post-medication. Single study examining the changes in 8-OHdG following psychiatric treatment found no change in 8-OHdG levels following ECT although its RNA analogue, 8-oxoGuo, increased significantly [22].

The effect of therapy on biomarkers has primarily focused on antidepressant medication and ECT. Research in other alternate treatments such as psychological therapy and lifestyle interventions (e.g., sleep, diet and exercise) has been lacking. The influence of more targeted anti-inflammatory treatments and antioxidant therapies on depressive symptoms and biomarker levels are also required. Not enough literature regarding the effect of antidepressants on oxidative damage could be found. There is a need for large longitudinal studies to see the antioxidant effect of antidepressants.

Nevertheless, the strength of our study had been that it consisted of a homogeneous population of depression patients in adolescence and early adulthood of Indian origin from a uniform socio-economic status and comparable controls were taken up for the study. Two oxidative stress markers, 8-Isoprostane and 8-OHdG, were studied together in a moderately large number of depressed patients and controls. We could exclude in our subjects most of the factors which could lead to comorbidity. It removed the possibility of confounding by alternative disease process or treatment regimens further increasing the strength of the study.

Limitation(s)

Since the study was carried out in a tertiary care hospital where almost all the patients are referred from other places, we could not get any medication-naive patients. Thus, a baseline level of these markers prior to medication could not be established. The heterogeneity in the duration of illness and medication status in these patients was a limitation of the study. Patients included in the study were all on antidepressants for a variable duration of time. The effect of other antioxidants in diet could not be studied; they might play a role in reducing the effect of oxidative stress in depression. Also, we were not able to completely rule out the dietary differences between the two groups. Another limitation was the small sample size of the study.

CONCLUSION(S)

Major depression subjects have higher levels of oxidative stress markers 8-Isoprostane and 8-hydroxy-2-deoxyguanosine in peripheral blood in comparison to healthy controls of comparable age and gender. The gender difference was specific in females for plasma 8-Isoprostane, and in males for serum 8-OHdG when compared to their respective healthy control group. No significant relation was seen between the severity of depression in the patient group and the increase in levels of these oxidative markers. More multicentric and population-based studies are required to conclude 8-Isoprostane and 8-hydroxy-2-deoxyguanosine as the biomarkers of stress in depression.

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