ORIGINAL ARTICLE

CLINICAL CORRELATION OF ALTERATION OF ENDOGENOUS ANTIOXIDANT-URIC ACID LEVEL IN MAJOR DEPRESSIVE DISORDER

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ABSTRACT

Derangement of antioxidant levels in major depressive disorder had been correlated with oxidative damage. The effect of Selective Serotonin Re-Uptake Inhibitors on endogenous antioxidant uric acid levels in major depressive disorder has never been examined. This was a prospective; open labeled, parallel, 12 weeks study, in which serum uric acid levels and Hamilton Rating Scale for Depression score were estimated in age and sex matched thirty-six healthy and forty major depressive disorder subjects before and after fluoxetine and citalopram treatment. Significant decrease in serum uric acid ($P < 0.0001$) was observed in newly diagnosed major depressive disorder subjects when compared to healthy subjects. The trend was reversed after 6 weeks more significantly after 12 weeks of treatment with improvement in Hamilton Rating Scale for Depression score. Also, Significant and negative correlation was found between Hamilton Rating Scale for Depression score and serum uric acid level ($r = -0.864$, $P < 0.001$) after 12 weeks of treatment. Treatment with fluoxetine or citalopram reverses endogenous antioxidants like uric acid and improves Hamilton Rating Scale for Depression score in major depressive disorder.

KEY WORDS

Uric acid, Citalopram, Fluoxetine, Hamilton Rating Scale for Depression score, Major depression, Selective Serotonin Re-Uptake Inhibitors.

INTRODUCTION

Mental health problems are among the most important contributors to the burden of disease and disability worldwide. A recent WHO report predicts that depression will be the leading cause of disability and premature death by 2020, second only to Ischaemic Heart Diseases (1). The illness has a poor outcome in spite of best available treatment. However the majority of patients respond to pharmacological treatment if diagnosed early. Hence development of novel strategies to

improve outcome, which include early diagnosis and treatment, will be of great benefit to patient of major depression.

Several investigators have implicated the role of oxidative stress in major depression. Abnormal levels of antioxidant enzymes and lipid peroxidation in major depression further substantiates the role of free radical in major depression (2, 3). Neurons are especially vulnerable to free radical attacks. Insufficient defenses with exposure to excess reactive oxygen species (ROS) can lead to neuronal dysfunction and death of neuron. Oxidative stress is one of the important mechanisms that causes the destruction of nerve cells and decrease the volume of hippocampus in patients of major depression (4). The major antioxidative defenses include both enzymatic and non enzymatic antioxidants. The levels of enzymatic antioxidants like superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSHPx) etc. are altered in major depressive patients (5).

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Selective Serotonin Re-Uptake Inhibitors (SSRIs) modify both enzymatic antioxidants and lipid peroxidation (3). Albumin, bilirubin, uric acid and ascorbic acid are some of the non enzymatic antioxidants in the body (6, 7). The non enzymatic antioxidant levels are also modified in major depression. Accumulating evidence suggest lowering of these antioxidants in patients of major depression (8, 9, 10). SSRIs also modify some of the non enzymatic antioxidant levels (11, 12).

Although incidence of major depressive disorder (MDD) is increasing the knowledge about its early diagnosis is inadequate. More often critical biochemical markers are overlooked due to lack of organized data available to clinicians. Evaluation of such biochemical markers would certainly be of useful and supportive for early diagnosis and monitoring of treatment response.

In recent years SSRIs have been used as first line drugs in the treatment of major depression. They lack cardiac toxicity and anticholinergic effect present in the older generation of antidepressants like Mono-amine Oxidase (MAO) inhibitors and Tricyclic Antidepressants (TCAs). They are more effective in long-term treatment as indicated by fewer cases of relapses in the patients taking these medicines (13).

So far the conducted studies did not bring unequivocal explanation of relation between non-enzymatic antioxidants and serotonin reuptake inhibitors. Non enzymatic antioxidants like albumin, globulin and total proteins were studied by some authors but in relation to acute phase response and MDD (9). Uric acid is a potent endogenous antioxidant in the body (6). As per our knowledge and recent internet search although alteration in the uric acid in MDD are reported (8, 10), no study has ever been reported the alteration of uric acid after SSRIs in particularly fluoxetine and citalopram treatment. Lack of conclusive information regarding the potential of such biochemical variable like uric acid as supportive prognostic indicator or predictor of treatment response in MDD has encouraged us to analyze changes induced by SSRIs on serum uric acid level in MDD.

MATERIALS AND METHODS

This prospective, open labeled, randomized, non-cross over 12 weeks trial was carried out in the Outpatient Department of Pharmacology and Department of Psychiatry, Government Medical College and Hospital (GMCH) Nagpur, India. The trial procedure was in accordance with the guidelines of 1964 declaration of Helsinki.

Study groups consist of a control group of healthy subjects and that of major depression. Healthy adult subjects more than 18 years of age, of both sexes, more than 50 Kilogram body weight, taking good diet, preferably vegetarian, non-smoker, non-alcoholic, free of medication for at least one month prior to study were selected as controls. Controls were excluded for history of any psychiatric illnesses. Subjects of MDD were maintained on their usual dietary pattern. Subjects were kept on self-selected diets and were instructed not to make any major change in dietary pattern during the trial period. Compliance to the dietary restriction was determined by obtaining a twenty-four hour dietary recall from the subjects on two occasions during each one-month period.

The subjects included in the study were of both genders between the age group of 18-65 years and were able to comprehend and provided written consent prior to study. MDD was diagnosed by trained psychiatrist using DSM IV criteria and 21-item Hamilton Rating Scale for Depression (HRSD) score to measure the severity of disorder. The subjects with score less than 14 in 21 item HRSD scale and associated psychiatric disorders accompanying major depression were excluded from the study. Medical illnesses including endocrine, metabolic disorders known to affect free radical status and uric acid levels were also excluded. Further, subjects with abnormal liver and kidney function and on non-vegetarian diet like meat, fish etc., which are rich sources of uric acid and taking any other medication, were excluded from the study.

The trial was started after approval of institutional ethics committee GMCH Nagpur. Informed consent material about the drug trial was prepared in English and the local vernacular language. The information was read out to all the potential participants and doubts regarding the trial procedure were clarified. Thirty-six healthy subjects and forty major depressive subjects participated in the trial. The sample size was calculated on the basis of previous similar studies (3, 5) by using Graph Pad Prism Version 3.02 software and considering future drop outs during the trial.

After taking consent and screening with routine investigations, physical examination and obtaining HRSD scale, subjects suitable for drug treatment were allocated randomly to either fluoxetine (20 mg/day) or citalopram (20 mg/day) groups depending upon a computerized randomization programmer. Fasting blood samples were obtained at week 0 and at the end of 6th week and 12th weeks from the start of the treatment. For the follow up, similar parameters and routine laboratory analysis were performed to explore any adverse event after drug treatment.

Out of 40 MDD subjects who entered into the trial, four were withdrawn in citalopram group. The commonest cause was failure to attend the psychiatric OPD. One subject was dropped because of citalopram side effects like nausea, headache and sleep disturbances at the end of six weeks and was shifted to other drug with the consent of psychiatrist. Thus only 36 subjects completed the study (20 in fluoxetine group, 16 in citalopram group). No severe adverse event and abnormality in laboratory tests were reported during trial period.

5 ml of venous blood was collected individually from both control and study group after overnight fasting under aseptic precautions. The blood sample was obtained in a sterile bulb and allowed to clot at room temperature for 30 minutes and the serum was separated after centrifugation at 3000 rpm for 20 minutes. It was then used for assessment of various biochemical parameters. Estimation of uric acid was done by Uricase/PAP method.

Results were expressed as mean $\pm$ SEM. Statistical analysis of data was performed using a one-way analysis of variance (ANOVA) followed by Tukeys Post hoc test and unpaired t-test. Also, relationships between variables were ascertained by Pearson's correlation coefficient. Chi-square test was used for analysis of demographic data. Two tailed P value of < 0.05 was judged statistically significant. Graph Pad Prism, Version 3.02 software was used for statistical analysis.

RESULTS

There are no significant differences in age or sex ratio ($\chi^2 > 0.2$) between normal healthy subjects and major depression subjects. Further, no significant difference in age ($P > 0.3$) or sex ratio ($\chi^2 > 0.05$) is observed between fluoxetine and citalopram treated groups.

To test whether the major depression is associated with alteration serum uric acid levels, we compared the levels of serum uric acid in newly diagnosed major depression subjects

(3.75 ± 0.12) with that of healthy subjects (5.256 ± 0.17) with unpaired-'t' test. Uric acid levels in newly diagnosed MDS were significantly low ($P < 0.0001$) as compared to healthy subjects.

To study the effect of SSRIs like fluoxetine and citalopram on the endogenous antioxidant serum uric acid in subjects of major depression, we compared the pretreatment levels with that of post treatment levels at 6 weeks and 12 weeks. Fluoxetine treatment significantly ($P < 0.001$) increased serum uric acid at 6 weeks and 12 weeks when compared to before treatment concentrations. Further the levels were significantly ($P < 0.001$) elevated at 12 weeks as compared to that of 6 weeks after treatment with fluoxetine. Citalopram treatment caused significant ($P < 0.001$) rise in serum uric acid only at 12 weeks when compared to before treatment levels (Table 1).

To observe the comparative effectiveness of citalopram and fluoxetine we have calculated change from the baseline for both the drugs at 6 weeks and 12 weeks. It was observed that the effectiveness of citalopram did not alter this parameter significantly as compared to fluoxetine at both 6 weeks and 12 weeks of treatment.

To test the clinical benefit with SSRIs, we compared the HRSD score after citalopram and fluoxetine treatment at 6 weeks and 12 weeks with baseline levels. Significant ($P < 0.001$) decrease in HRSD score was observed after 6 weeks and 12 weeks of drug treatment compared to the baseline (Table 2). Significant and negative correlation was found between HRSD score and serum uric acid ($r = -0.864$, $P < 0.001$) after 12 weeks of treatment.

To test whether these drugs are better at reversing the alteration in uric acid level, we compared the levels of uric acid (both group combined) after 12 weeks of treatment (4.82 ± 0.06) with that of healthy subjects (5.256 ± 0.17) with unpaired-'t' test. It was observed that the significance of difference of the values of uric acid between newly diagnosed MDD subjects and that of healthy subjects was more

Table 1: Serum uric acid levels in major depressive subjects at different time intervals of fluoxetine and citalopram treatment

Treatment Group	Before Treatment (A)	After Treatment		*P	F
		6 weeks (B)	12 weeks (C)		
Fluoxetine Group	3.64 $\pm$ 0.12	4.20 $\pm$ 0.11 ^a	4.82 $\pm$ 0.08 ^{a,b}	0.0001	33
Citalopram Group	3.89 $\pm$ 0.22	4.28 $\pm$ 0.17	4.83 $\pm$ 0.09 ^a	0.001	7.85

Values expressed as mean $\pm$ SEM in mg/dl, in Fluoxetine group : n=20, df 2,57 and in Citalopram group : n=16, df 2,45, ^aP < 0.001 Vs A, ^bP < 0.001 vs B, *P as determined by One Way ANOVA with Tukeys post-hoc test.

Table 2: Hamilton Rating Scale for Depression (HRSD) scores before and after SSRIs treatment

HRSD	n	Before Treatment (A)	After Treatment		*P (ANOVA)	F
			6 weeks (B)	12 weeks (C)		
Citalopram	16	25.81±0.65	22.81±0.56 ^a	16.31±0.22 ^{a,b}	<0.001	91.14
Fluoxetine	20	24.95±0.43	22.15±0.42 ^a	17.55±0.27 ^{a,b}	<0.001	96.77

Values expressed as mean ± SEM, ^aP < 0.001 vs A, ^bP<0.001 Vs B; using one way ANOVA with Tukey's post-hoc test.

(P<0.0001) when compared to the significance of difference of values between subjects after 12 weeks of SSRIs treatment with that of healthy subjects (P<0.05). Thus the result indicates reversal of decreased uric acid in SSRIs treated subjects as compared to newly diagnosed subjects.

DISCUSSION

Uric acid can be oxidized following a non-enzymatic degradation and has been proved to be a selective antioxidant capable of reaction with hydroxy radicals and hypochlorous acid. In plasma, uric acid, albumin and ascorbic acid accounts for more than 85% of total antioxidant capacity (14). Uric acid is a major contributor to total radical trapping capacity (TRAP) accounting between 38-47% of the entire total in contrast to vitamin C and vitamin E which contributes 13-17% and 2-8% respectively (15). Uric acid is present in plasma, in relatively large concentration. Urate radicals (UH₂⁻) unlike ascorbate do not react with oxygen to give another peroxy radical which make it a good antioxidant (16). The present study demonstrates lower levels of serum uric acid in major depression. It is well known that the major depression is associated with increased free radical production and decreased levels of antioxidant defenses (5).

The lower levels of serum uric acid in the present study could be due to the increased utilization of uric acid for scavenging free radicals in the brain. Our findings are in consistent with most of the previous reports on serum uric acid in major depression (8, 10). The different mechanisms have been proposed for action of uric acid as an antioxidant

Uric acid spares ascorbic acid by changing its electron content (6). Inactivation of Nitric oxide and peroxynitrite radicals which are increased in brain cells and are responsible for degeneration and cytotoxic effects (17, 18). Uric acid and dopamine undergo protective reaction leading to fast chemical repair of oxidative free radical induced damage of DNA in certain brain cells (19). Uric acid was shown to reduce oxidative damage to DNA by reacting with guanyl radical.

The present study also demonstrated increased serum uric acid levels after drug treatment with SSRIs like citalopram and fluoxetine. Although, no literature is available regarding the direct effect of these SSRIs on serum uric acid levels, the rise could be due to decreased consumption of uric acid required for scavenging free radicals. SSRIs are known to decrease lipid peroxidation in major depression (2). Similarly, they also increase plasma ascorbic acid levels and up regulate SOD1 gene in depressive subjects and produce neuroprotective effects against free radical induced damage in major depression (20). Uric acid spares ascorbic acid from utilization. Rise in ascorbic acid after the treatment with SSRIs in depressive subjects may decrease utilization of uric acid, thus increasing the uric acid levels.

Serum uric acid level may be affected by diet. But in the present study all the subjects were advised to avoid meat, seafood and also not to alter dairy product intake significantly during the period of the study. Hence it is unlikely that the alterations in serum uric acid levels were due to diet.

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