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ORIGINAL RESEARCH—EJACULATORY DISORDERS

Serum Antioxidant Enzymes and Malondialdehyde Levels in Patients with Premature Ejaculation Before and After Pharmacotherapy

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ABSTRACT

Background. In our previous study, significantly higher antioxidant enzymes and malondialdehyde (MDA) levels in patients with premature ejaculation (PE) compared to healthy controls were found. In the present study, we planned to evaluate antioxidant enzymes and MDA levels of patients with PE before and after citalopram treatment.

Methods. The study comprised 30 patients with PE according to *Diagnostic and Statistical Manual of Mental Disorders Fourth Version* (DSM-IV) and 30 healthy controls. The patients were randomly assigned to two groups. Fifteen of them (group I) received 8 weeks of citalopram treatment but the remaining (group II) did not. The subjects were asked to determine the average intravaginal ejaculation latency time (IVELT). The fasting antioxidant enzymes and MDA levels were measured at baseline and after 8 weeks.

Results. No statistically significant difference in the mean IVELT between groups at baseline was found. IVELT considerably elevated after 8 weeks of citalopram treatment in group I with a mean of 209 ± 72.1 seconds but not in group II. Antioxidant enzymes and MDA levels did not differ between groups at baseline. At the evaluation of week 8, SOD, GSH-Px, and MDA levels significantly reduced during treatment in group I patients.

Conclusion. In conclusion, the results suggest that the ability for antioxidant enzymes and MDA to normalize through symptom alleviation reveals that they might be trait marker of PE. Further placebo-controlled studies are needed.

Key Words. SOD; CAT; GSH-Px; MDA; Premature Ejaculation; Citalopram

Introduction

Premature ejaculation (PE) is the recurrent or persistent orgasm with minimal sexual stimulation before, on, or after penetration and before the person desires it [1].

Reactive oxygen species (ROS) predominantly superoxide, hydroxyl ion, and nitric oxide (NO) are produced under physiological conditions during aerobic metabolism [2]. ROS can be indirectly evaluated by the measurement of some antioxidant

enzyme levels such as SOD, CAT, or GSH-Px, by products of lipid peroxidation such as malondialdehyde (MDA) or by some transition metals such as copper and zinc [3]. The oxidation of catecholamines such as dopamine and serotonin by monoamine oxidase (MAO) may result in increased radical burden. The inhibitory effect of serotonin on libido, ejaculation, and orgasm is well documented. This effect of serotonin has been attributed to serotonin-induced decrease in dopamine in central nervous system [4]. Selective

serotonin reuptake inhibitors (SSRIs) are effective in the treatment of PE. Probable mechanism of these drugs is the enhancement of net serotonergic transmission by blocking the presynaptic 5-hydroxytryptamine (5-HT) uptake site [5]. The results show that NO promotes erection in intact male rats, probably by mediating filling of the corpora cavernosa [6]. NO, an important FR, may mediate penile erection and allow vasodilatation of the corpora by inhibiting smooth muscle of the corpora cavernosa. In order to test the role of NO in the sexual function of intact male rats, one of the precursors of NO (L-arginine) or an inhibitor of its synthesis (NG-nitro-L-arginine methyl ester) was given before tests of copulation [7]. Initially recognized for a primary role in the relaxation of peripheral vasculature, NO has since been implicated in the control of several activities that are at least partially mediated by the preoptic area or hypothalamus [8]. NO may influence these behaviors through release of a variety of neurotransmitters and hormones, particularly dopamine whose role in sexual behavior is well established [9]. Systemic administration of the NOS inhibitor NG-nitro-L-arginine methyl ester impaired copulation in a dose-dependent manner [7]. This probably was due to an inhibition of erectile ability; drug-treated males were unable to achieve vaginal intromissions, and had fewer erections in an ex copula reflex test. These results indicate that NO promotes erection in intact male rats and suggest that NO inhibits seminal emission, probably by decreasing sympathetic nervous system activity, and consequently, it was suggested that this might help prevent PE [10]. On the other hand, it has been reported that the most important source of FRs is glial cells and FRs produced by these cells are associated with neuropsychiatric disorders such as schizophrenia, OCD, Sydenham Korea, and Parkinson disease [11,12]. These diseases are associated with hypothalamus and basal ganglia in respect to etiology which are particularly vulnerable to FR damage. These neuroanatomical localizations are also associated with sexual behaviors, particularly ejaculation [13]. The associations aforementioned previously prompted us to assess whether there is a relationship between FRs and PE and we found that there was a negative correlation between the duration of illness and SOD, CAT, or MDA values in the patient group and speculated that PE seemed to be associated with free radical damage [14]. It has not been studied whether antidepressant drugs in PE modify the alterations in antioxidant enzymes and

MDA. It has been reported that antidepressant treatment may suppress immune cells including natural killer cells [15]. Suppression of immune cells by means of the treatment with an antidepressant may cause decrease in FRs. This supported the results of Bilici et al. [16]. In the study by Bilici et al. in major depressed patients, subchronic SSRI treatment reduced the antioxidant enzyme and MDA levels. Furthermore, all SSRIs including citalopram inhibit P450 enzymes, which contribute overproduction of FRs. Hence, we decided to evaluate whether there would be a change in serum antioxidant enzymes and MDA levels of patients with PE before and after treatment with citalopram, an SSRI.

Method

The study comprised 30 married male patients (aged 23–42 years) who had applied to Firat University School of Medicine Departments of Psychiatry and Urology and had been diagnosed with PE according to *Diagnostic and Statistical Manual of Mental Disorders Fourth Version (DSM-IV)* [1]. A total of 15 patients (group I) received citalopram treatment of 8 weeks, while the rest (group II) did not receive any behavioral or pharmacological therapeutic approach.

The patients having any comorbid psychiatric disorder were not included. To be able to exclude organic sexual dysfunctions, complete blood count, fasting glucose level, urine analysis, sex hormones, and prolactin levels were obtained. All patients were free of all medications at least in the previous 2 weeks.

Exclusion criteria included the presence of erectile dysfunction and inhibited male orgasm, a severe physical illness, using of any antioxidant agent (i.e., E and C vitamins), presence of epilepsy and severe neurological disorder, presence of infectious disease, and excessive obesity.

The subjects were asked to determine the average intravaginal ejaculation latency time (IVELT) for three consecutive episodes of coitus verified by both patients and their wives via a chronometer. IVELT was defined as the duration between vaginal intromission and ejaculation. Group I patients received initially 20 mg citalopram per day for 1 week. Titration up to 60 mg per day was designed according to patient's tolerability and clinical response.

The blood samples were centrifuged at 4,000 rpm for 10 minutes at 4°C to remove plasma. The buffy coat on the erythrocytes sedi-

ment was separated carefully after plasma was removed and was used in the assay of MDA levels. The erythrocyte sediment was washed three times with 10-fold isotonic NaCl solution to remove plasma remnant.

Hemolysates of erythrocytes were used for measurement of total (Cu-Zn and Mn) SOD (EC 1.15.1.1) activity levels by the method of Sun et al. [17]. This method is based on reduction of superoxide, which is produced by the xanthine oxidase enzyme system, by nitroblue tetrazolium. GSH-Px (EC 1.6.4.2) activity levels in hemolysates of erythrocytes were measured using the method of Paglia and Valentine [18] in which GSH-Px activity was coupled to the oxidation of NADPH by glutathione reductase. CAT (EC 1.11.1.6) activity was determined by the method of Aebi [19]. The principle of the assay is based on the determination of the rate constant *k* (dimension: s⁻¹) of the hydrogen peroxide decomposition. Levels of plasma MDA were measured by the thiobarbutiric acid (TBA) method, which was modified from methods of Satoh [20] and Yagi [21]. Peroxidation was measured as the production of MDA, which in combination with TBA forms a pink chromogen compound whose absorbance at 532 nm was measured.

The patients were asked to determine the average IVELT for three consecutive episodes of coitus verified by both patients and their wives via a chronometer throughout 2 weeks. IVELT was defined as the duration between vaginal intromission and ejaculation. The subjects and their wives were encouraged to perform at least twice coitus in a week and record IVELT. Written consent to participate in the study was obtained from the subjects after they were thoroughly informed about the research details. Local Ethics Committee of the Firat University School of Medicine approved the study protocol.

Statistical analysis was performed using the statistical package for social sciences (SPSS/PC 9.05 version, 1998). In the statistical analysis, Student's *t*-test and Pearson's method of correlation were

used. Differences were considered significant at *P* < 0.05 for all these tests.

Results

Groups I and II did not differ in regard to the mean age (31.5 ± 7.9 vs. 27.8 ± 5.3 years), age of wives (30.3 ± 6.8 vs. 28.6 ± 4.9 years), and duration of illness which was 82.3 ± 15.6 and 79.8 ± 13.8 months in group I and group II, respectively (*P* > 0.05). At baseline, the mean IVELT for group I and group II patients was 38.5 ± 14.7 and 36.2 ± 16.1 seconds, respectively (*P* > 0.05). The mean time after 8 weeks of treatment with citalopram in group I was 247.9 ± 117.8 seconds whereas it was 41.1 ± 13.3 seconds in group II. Paired *t*-test revealed that the mean IVELT considerably increased after citalopram treatment (*P* < 0.001). At 8 weeks, the mean dose of citalopram received in group I was 30.7 ± 9.3 mg/day.

Table 1 demonstrates the antioxidant enzymes and MDA measurements in groups I and II. As can be seen in Table 1, at baseline, there was no significant difference between groups I and II in each of parameter measured. In patients, for whole group, the SOD and GSH-Px values were significantly and negatively correlated with the IVELT (*r* = 0.55, *P* < 0.05 for SOD; *r* = 0.58, *P* < 0.05 for GSH-Px).

Table 1 also demonstrates the results of MDA, SOD, GSH-Px, and CAT levels in groups I and II at the beginning and end of the 8-week period. SOD, GSH-Px, and MDA levels significantly reduced during treatment in group I, as shown in Table 1. Significant and negative correlations were observed between increase in IVELT, and decreases in SOD or GSH-Px levels (*r* = 0.52, *P* < 0.05; *r* = 0.62, *P* < 0.05, respectively).

No patient withdrew from the study because of adverse events. Totally eight patients experienced 14 various adverse events. Most frequently reported events were gastrointestinal complaints (N = 4) and insomnia (N = 3).

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Table 1 Antioxidant enzyme and MDA levels in the groups at baseline and week 8

	SOD (U/g Hb)	GSH-Px (U/g Hb)	CAT(k/g Hb)	MDA (nmol/mL)
Group I (N = 15)				
Before treatment	1,239.1 ± 198.4	28.3 ± 3.7	267.6 ± 40.1	4.1 ± 0.9
After treatment	1,022.4 ± 179.4 ^a	24.9 ± 3.1 ^a	232.6 ± 41.3 ^a	3.1 ± 0.7
Group II* (N = 15)	1,263.1 ± 203.6	26.9 ± 2.8	259.5 ± 36.7	4.6 ± 1.1
Statistical values	^a <i>t</i> = 9.42, <i>P</i> < 0.01	^a <i>t</i> = 7.94, <i>P</i> < 0.01	^a <i>t</i> = 3.28, <i>P</i> < 0.05	^a <i>t</i> = 8.78, <i>P</i> < 0.01

* There was no difference between groups regarding antioxidant enzyme and MDA levels (*P* > 0.05).

Discussion

The present study evaluating antioxidant enzymes and MDA changes in patients with PE who were treated by citalopram showed the following important preliminary findings: (i) patients treated with citalopram had noticeable decrease in SOD, GSH-Px, and MDA levels compared with patients who did not receive any treatment; and (ii) significant and negative correlations were observed between increase in IVELT, and decreases in SOD or GSH-Px levels.

Selective serotonin reuptake inhibitors have been found to be effective in the treatment of PE [5,6]. In the present study, our results suggest that citalopram, also known as the most selective SSRI [22], revealed a considerable improvement in patients with PE, even though the study had methodological limitations such as having small sample size. NO, one of the predominant FRs, may mediate penile erection by inhibiting smooth muscle of the corpora cavernosa, thereby allowing vasodilatation of the corpora. In order to test the role of NO in the sexual function of intact male rats, one of the precursors of NO (L-arginine) or an inhibitor of its synthesis (NG-nitro-L-arginine methyl ester) was given before tests of copulation [7]. In this study, the results indicate that NO promotes erection in intact male rats and suggest that NO inhibits seminal emission, probably by decreasing sympathetic nervous system activity, and consequently, it was suggested that this might help prevent PE. In another study [6], the relationship between PE and the levels of seminal magnesium (Mg) was evaluated. Significantly lower seminal plasma Mg levels in men with PE were determined. Decreased level of magnesium gives rise to increased NO. This may lead to premature emission and ejaculation processes. On the other hand, the oxidation of catecholamines such as dopamine and serotonin by MAO may result in increased radical burden. The inhibitory effect of serotonin on libido, ejaculation, and orgasm has been attributed to serotonin-induced change in dopamine level in central nervous system [23]. Consequently, these relationships mentioned above support that FRs might have an important role in the etiopathogenesis of PE. The question whether citalopram exerts a direct effect on FRs and antioxidant enzymes, and MDA levels has arisen. However, for this to be determined exactly, the control group should have been treated. Some speculations may be done regarding this issue, as done in our previous study [24]. It has been reported that antide-

pressant treatment may suppress immune cells including natural killer cells [15]. Suppression of immune cells by means of the treatment with an SSRI, citalopram, may cause decrease in FRs. This supported the results of Bilici et al. [16]. In the study by Bilici et al. in major depressed patients, subchronic SSRI treatment reduced the antioxidant enzyme and MDA levels. Furthermore, all SSRIs including citalopram inhibit P450 enzymes, which contribute overproduction of FRs. It may be concluded that citalopram may decrease antioxidant enzyme levels via reducing immune cells and inhibiting P450 enzyme system. Citalopram is metabolized by CYP2C19 to *N*-desmethylcitalopram, which is further interacted with CYP2D6 [25]. It has been reported citalopram to be a weaker inhibitor of CYP2D6 when compared with the other SSRIs [26] and drug–drug interactions with citalopram may not be as much of a problem as with other SSRIs. However, it should be noted that *N*-desmethylcitalopram, a metabolite of citalopram, is 5–10 times stronger than the parent drug at inhibiting CYP2D6 in vitro in human liver microsomes and is almost as potent as fluoxetine at inhibiting 2-hydroxylation of imipramine in vitro in such a model [27,28]. There is a paucity of information available at present about the significance of this degree of inhibition of CYP2D6 in the clinical situation. On the other hand, decreases in MDA values may be explained by means of SSRI's inhibiting effect on iron-dependent lipid peroxidation. Consequently, it may be speculated that antioxidant enzymes and MDA are state markers of SP because of returning

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normal values through treatment.

There are some methodological limitations to the present study that should be acknowledged. First, the lack of placebo-controlled design and short duration of treatment are the main limitations of the present study. Second, the relatively small sample size might not be representative of the patients with PE. Moreover, we could not test some confounding factors related to outpatient habits, that is, exercise, lifestyle, and so on, which might be related to antioxidant enzymes and MDA values and dietary changes which may affect the production of FRs although the patients and controls came from similar socioeconomic level. We found that antioxidant enzyme and MDA levels in patients with PE following treatment with citalopram decreased, as hypothesized, and that this decrease seems to be linked to the therapeutic effect. This effect should be tried in patients on other SSRI treatments as well. However, our find-

ing is only a suggestion and more comprehensive and detailed studies are needed to decipher the exact roles of antioxidant enzyme and MDA levels in PE.

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