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Adjuvant Use of Melatonin with Fluoxetine for Management of Fibromyalgia

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Abstract

Fibromyalgia (FMS) is a chronic musculoskeletal disorder characterized by generalized muscular pain and tenderness at specific anatomical sites. Although melatonin was effective in treating the pain associated with this syndrome, no defined clinical evidence supports this claim. The present study was designed to evaluate the clinical significance of using melatonin, alone or in combination with fluoxetine in FMS. A double-blind clinical study was conducted on 45 patients with FMS randomized into 4 groups; group A, treated with fluoxetine 20mg/day alone; group B, treated with melatonin 5mg alone; group C, treated with combination of fluoxetine 20 mg+3 mg melatonin; group D treated with combination of fluoxetine 20 mg+5 mg melatonin. Both fluoxetine and melatonin were given once daily in the morning and night time respectively for 8 weeks. Each patient clinically evaluated using Fibromyalgia Impact Questionnaire (FIQ). Serum levels of serotonin, malondialdehyde and nitric oxide were also evaluated. Using melatonin (3 and 5mg/day) in combination with 20mg/day fluoxetine significantly decreased total FIQ score values; the combination therapy significantly decreased serum serotonin level associated with reduction in the oxidative stress parameters (MDA and NO). In conclusion, adjuvant use of melatonin with fluoxetine improves the biochemical and clinical parameters of FMS patients.

Key words: Fibromyalgia; Oxidative Stress; Serotonin; MDA; Nitric oxide

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Introduction

Fibromyalgia (FMS) is a chronic musculoskeletal disorder characterized by generalized pain, fatigue, tenderness at specific anatomic sites called tender points. Patients with FMS have other clinical manifestations including altered sleep, headache and irritable bowel syndrome [1]. It is a common rheumatic disorder [2] and thought to be an inflammatory condition; however, evidence of inflammation has not been found [3]. Recently, FMS was considered as a disorder of pain regulation, due to heightened generalized pain sensitivity that arises from pathological processing of nociceptive stimuli; central and peripheral sensitization of nociceptive system and hypothalamic–Pituitary–Adrenal (HPA) axis dysfunction are also involved [4]. There is significant evidence for disturbances of the neurohormonal system in patients with FMS [5]. Serotonergic neurons have been implicated in pain perception through inhibitory effect on pain pathways in the spinal cord through inhibition of the release of sub P by afferent neurons responding to peripheral stimuli [6]. Low serum serotonin levels have an inverse correlation with clinical measures of perceived pain [7]. In addition to its role in pain perception, serotonin plays an important role in sleep regulation, and many studies show an abnormal low level of this hormone and its metabolite melatonin in people suffering of FMS; this may explain why people with this disorder have such trouble in getting a good night sleep [8]. Recent theory on the cause of FMS hypothesized that oxidative stress may play an important role in the disease. McCleaver *et al* (2006) found that women with FMS experienced a reduced flow of nutrient to the muscle after exercise, and this might be related to elevated levels of inducible nitric oxide synthase (iNOS) with consequent production of elevated level of nitric oxide [9, 10]. Central sensitization associated with FMS may be caused by stimulation of pain receptors in muscle, which may occur due to inflammatory cytokines that trigger iNOS in the muscle with inappropriate stimulation of pain receptors, and an increase in the production of reactive oxygen species (ROS) such as the peroxynitrite radical and others reactive oxygen species [11,12]. Melatonin is a ubiquitous natural compound produced by pineal gland; it is involved in numerous aspects of biological and physiological regulations, and chemically recognized as N-acetyl -5-methoxyserotonin. Although the antioxidant role of melatonin is partially based on receptor mechanisms, antioxidant capacity of melatonin also includes the indirect effect of up-regulating several antioxidative enzymes and down-regulating pro-oxidant enzymes, in particular 5- and 12-

lipo-oxygenases [13] and nitric oxide (NO) synthases [14]. It increases the level of several antioxidant enzymes including superoxide dismutase, glutathione peroxidase and glutathione reductase [15]. On the other hand, several studies have demonstrated that by inhibiting NO production or reducing the activation of NF- κ B, melatonin exerts important anti-inflammatory actions [16,17]. Melatonin exerts its anti-inflammatory effect at various molecular steps: reduces the expressions of nitric oxide synthase-2 (NOS-2) and prostaglandin (PG)-endoperoxide synthase-2 (COX-2) thereby inhibiting NO and PG production [18]. The present study was designed to evaluate the effect of different doses of melatonin, alone or in combination with fluoxetine, for the management of patients with fibromyalgia.

Materials and Methods

A double-blind clinical study was performed on 45 patients with fibromyalgia syndrome (FMS) who were attending the Rheumatology Clinic of Baghdad Teaching Hospital during the period from November 2008- September 2009. All patients fulfill the criteria of the American College of Rheumatology (ACR) of FMS [1]. Patients were included in the study if they met the following criteria: Agreed to participate in the study and signed the informed consent form, diagnosed with FMS, and have no other evident for overlapping diseases. Subjects were excluded from the study if they had a positive history of allergy to any one of the drugs used in the study or those who miss taking medication for more than 2 days and pregnant or lactating patients; all patients were informed to stop all previous forms of medications. The patients were randomized into 4 groups in a double-blind study for 8 weeks period as follow: group A: includes 11 patients treated with fluoxetine (Cipla, India) 20mg+placebo; group B: includes 12 patients treated with melatonin (Rupal Chemicals Ltd, Tarapur, India; specially formulated for this purpose) 5mg+placebo; group C: includes 12 patients treated with fluoxetine 20 mg+3mg melatonin; group D: includes 10 patients treated with fluoxetine 20 mg+5mg melatonin. Both fluoxetine and melatonin were given once daily in the morning and night time respectively. The four groups were matched for age and sex differences (Table 1). For all patients enrolled in the study, direct interview was performed to evaluate their disease manifestations and symptoms, their medical history, and previous laboratory findings. Assessment of the patient's outcomes was done using Fibromyalgia

Impact Questionnaire (FIQ) before starting the treatment (zero time) and 8 weeks after drug treatment. The FIQ has been extensively used as an outcome measure in fibromyalgia related studies. It appears to be a sensitive index of change in FMS related symptoms [19]. Ten ml blood samples were collected from all patients by vein puncture before and 8 weeks after starting treatment and kept in plain tubes for clot formation; serum was separated by centrifugation at 3000 rpm for 20 min and utilized for assessment of serum MDA [20], NO [21] and serotonin [22] levels.

Table 1. Summary of the patient's characteristics

Parameter	Group A n = 11	Group B n =12	Group C n =12	Group D n=10
Age (years)	37.45±3.37	43.42±3.35	34.38±3.13	39.60±3.11
Weight (kg)	78.27±4.97	75.50±3.39	75.67±4.07	72.0±3.59
Sex	♂ 9.10 % ♀ 90.9 %	♂ 8.30 % ♀ 91.7 %	♂ 8.30 % ♀ 91.7 %	♂ 10 % ♀ 90 %

Statistical Analysis

The results were expressed as mean± S.E. Student's *t*-test and analysis of variance (ANOVA) were utilized to examine the degree of significance, and *P* value less than 0.05 considered significant.

Results

Before enrollment in the study (zero time), all FMS patients demonstrated poor symptoms parameters which include (pain, fatigue, altered sleep, stiffness, anxiety, depression) and reduced health-related quality of life (HRQOL), these symptoms are manifested by high FIQ score in all groups which indicates severe or extreme symptoms of FMS (Table 2).

Table 2. Effects of treatment with melatonin, fluoxetine or their combination on total FIQ score after 8 weeks of therapy in FMS patients

Treatment groups	n	FIQ score	
		Pre-treatment	Post-treatment
Group A	11	61.18±3.15	48.0±3.88*
Group B	12	60.42±2.91	49.0±3.38*
Group C	12	59.42±1.20	42.33±1.87**
Group D	10	61.90±1.67	44.0±2.88**

Values are presented as mean±SEM; group A: 20 mg of fluoxetine+placebo; group B: 5 mg melatonin+placebo; group C: 20 mg fluoxetine+3mg melatonin; group D: 20 mg fluoxetine+5mg melatonin. * Significantly different ($P<0.05$) compared to pre-treatment within the same group; ** highly significant difference ($P<0.01$) compared to pre-treatment within the same group.

Treatment with 20mg fluoxetine alone (group A) resulted in significant reduction in the total FIQ score by 21.5% after 8 weeks of treatment compared to pre-treatment value, while significant improvement in total FIQ score was reported in patients treated with 5mg melatonin alone (group B) manifested by 18.9% score reduction from the pre-treatment values; on the other hand, treatment with different doses of melatonin (3mg and 5mg/day) in combination with 20 mg/day fluoxetine (groups C and D) resulted in comparable and highly significant reduction in total FIQ score (28.8% and 28.9% respectively), compared to pre-treatment value. Table 3 showed that serum levels of serotonin are not significantly different in all patients groups at base line (zero time). Treatment with fluoxetine (20mg/day, group A) decreased serum serotonin (20.5%) after 8 weeks treatment, but this level was not significantly different compared to baseline value ($P=0.052$); meanwhile, treatment with 5mg/day melatonin results in 12.6 % elevation in serum levels of serotonin but it dose not significantly different compared to baseline value. Table 3 also indicates that using combination therapy that includes 20mg/day fluoxetine and 3mg/day melatonin produced highly significant reduction in serotonin levels after 8 weeks of treatment compared to baseline value (32.8%, $P<0.01$). However, increasing the dose of melatonin to 5mg/day in such combination produced 20% decrease in serum serotonin level, which was significantly different compared to pretreatment value.

Table 3. Effects of treatment with melatonin, fluoxetine, or their combination on serum serotonin levels after 8 weeks of therapy in FMS patients

Treatment groups	n	Serum serotonin (ng/ml)	
		Pre-treatment	Post-treatment
Group A	11	520.4±41.2	413.8±39.7
Group B	12	516.1±25.4	579.9±33.5
Group C	12	543.6±33.8	373.8±40.4**
Group D	10	541.8±21.6	432.9 ±39.6*

Values are presented as mean±SEM; group A: 20 mg of fluoxetine+placebo; group B: 5 mg melatonin+placebo; group C: 20 mg fluoxetine+3mg melatonin; group D: 20 mg fluoxetine+5mg melatonin. * Significantly different ($P<0.05$) compared to pre-treatment within the same group; ** highly significant difference ($P<0.01$) compared to pre-treatment within the same group.

Table 4 showed that there was no significant reduction ($P>0.05$) in the serum levels of MDA with fluoxetine (20 mg/day) therapy (group A), manifested by 9.2% reduction after 8 weeks of treatment compared to the baseline pre-treatment MDA level. Meanwhile, treatment with 5 mg/day melatonin (group B) results in a highly significant reduction (29.6%, $P<0.01$) in MDA levels after 8 weeks of treatment compared to pretreatment levels. However, the use of a combination therapy (fluoxetine 20 mg plus melatonin 3 mg; group C) significantly reduced MDA levels (26.6%, $P<0.01$) compared to pre-treatment levels, while increasing the dose of melatonin in such combination up to 5mg/day (group D) did not significantly alter MDA levels in this group (17.4%; $P>0.05$).

Table 4. Effects of treatment with melatonin, fluoxetine, or their combination on serum malondialdehyde (MDA) levels after 8 weeks of therapy in FMS patients

Treatment groups	n	Serum MDA ($\mu\text{mol/L}$)	
		Pre-treatment	Post -treatment
Group A	11	5.87±0.52	5.33±0.50
Group B	12	5.85±0.51	4.12±0.33**
Group C	12	5.70±0.59	4.18±0.35*
Group D	10	5.69±0.75	4.70±0.26

Values are presented as mean±SEM; group A: 20 mg of fluoxetine+placebo; group B: 5 mg melatonin+placebo; group C: 20 mg fluoxetine+3mg melatonin; group D: 20 mg fluoxetine+5mg melatonin. * Significantly different ($P<0.05$) compared to pre-treatment within the same group; ** highly significant difference ($P<0.01$) compared to pre-treatment within the same group.

Table 5 showed that in all groups of patients serum nitric oxide levels did not significantly different at base-line (pretreatment); after 8 weeks of treatment, fluoxetine 20 mg/day produced slight elevation in serum NO levels (5.9%; $P>0.05$) compared to pre-treatment values, while treatment with melatonin 5mg/day alone produced highly significant reduction in serum NO levels (44.8%) compared to pre-treatment value. However, combination therapy in both groups C and D results in non-significant decrease in serum NO levels compared to pre-treatment levels.

Table 5. Effects of treatment with melatonin, fluoxetine, or their combination on serum nitric oxide (NO) levels after 8 weeks of therapy.

Treatment groups	n	Serum NO ($\mu\text{mol/L}$)	
		Pre-treatment	Post-treatment
Group A	11	281.7 \pm 32.5	298.2 \pm 36.2
Group B	12	435.0 \pm 60.3	240.4 \pm 22.8**
Group C	12	286.61 \pm 28.41	238.6 \pm 28.1
Group D	10	368.27 \pm 43.05	288.9 \pm 47.7

Values are presented as mean \pm SEM; group A: 20 mg of fluoxetine+placebo; group B: 5 mg melatonin+placebo; group C: 20 mg fluoxetine+3mg melatonin; group D: 20 mg fluoxetine+5mg melatonin. * Significantly different ($P<0.05$) compared to pre-treatment within the same group; ** highly significant difference ($P<0.01$) compared to pre-treatment within the same group.

Discussion

Although various pharmacological approaches are evaluated and tried for treating FMS, no single drug or group of drugs has proved to be useful in this respect, this may be attributed to the complexity of this syndrome or to the difficulty in the measurements of treatment outcome [23]. FIQ has been used extensively as an outcome measure in fibromyalgia related studies. It appears to be a sensitive index of change in FMS related symptoms, and has good correlation with similar questionnaires as SF-36 [19]. The present study showed that the use of fluoxetine alone produced significant improvement in the FIQ score, which is compatible with data previously reported by others [24,25]. On the other hand, the use of melatonin as single treatment for FMS patients significantly improves the total FIQ scores. This result was found comparable to that reported by Citera *et al* (2000) who demonstrate that treatment with

3 mg melatonin at bed time produced significant improvement in the pain score, measured by Visual Analog Scale (VAS), and sleep parameters were the mostly affected parameters compared to other variable, but without reaching significant level [26]. Moreover, the results of another clinical trial indicated that using 6 mg/day melatonin at night for 30 days, all the included FMS patients developed a relatively normal sleep/wake cycle, which accompanied by significant reduction in pain and improvement of behavioral symptoms [27]. Such type of findings related to the components of FIQ, which are comparable to that reported in the present study, may highlight the importance of long-term use of effective doses of melatonin for management of FMS patients. Since there are no specific medication available that successfully treating FMS, treatment remains inadequate to resolve the syndrome in most patients; such poor outcome may be attributed to the complex interplay between pain, sleep disturbances and depression, the most characteristic symptoms of FMS [28]. Fibromyalgia is a musculoskeletal disorder associated with lowered pain threshold, non restorative sleep and depression which supports the concept of decreased flux through the serotonin pathway in these patients [29]. The majority of studies on serotonin levels in the peripheral blood suggest a role for serotonin in FMS [30,31]; subjects with FMS have exceptionally high intensity pain messages sent to the brain along with deficiency in pain inhibition, this idea was supported by data indicating abnormal levels of serotonin [32]. In many multicenter studies, serum serotonin levels founded to be consistently low in patients with FMS. Recently, the existence of antibodies against serotonin and its receptors in FMS patients were reported, and gene polymorphism related to serotonin metabolism and transmission also has been reported, which provide an indirect evidence for abnormalities in serotonin metabolism [33]. Antidepressants belongs to SSRIs class enhance serotonin activities through increasing the neurotransmitter levels in the synapse area. Although SSRIs are widely used in the clinical practice for ameliorating FMS symptoms, only few studies conducted to estimate the changes in serum serotonin concentration following therapy; Alvarez, *et al* (1999) reported that treatment of depressed patients with fluoxetine (20mg/day) resulted in significant reduction of serum serotonin to 17.5% and 34.1% of its pre-treatment value after 14 days and 28 days of treatment respectively [34]. The possible explanation for this finding is that fluoxetine and its active metabolite norfluoxetine induce blockade of platelet serotonin uptake mechanism after 28 days leading to dramatic decrease in serum serotonin level, providing at the same time significant amount of serotonin to the brain resulting in improving FMS symptoms [35]. From the other point of view, melatonin is a product of a two-step conversion of serotonin,

thus when serotonin level decreased; melatonin synthesis may also decreased. The present study, showed that melatonin therapy alone resulted in non significant increase in serum serotonin levels after 8 weeks therapy; meanwhile, combination therapy using (fluoxetine 20 mg with 3mg and 5mg melatonin) decreases serum serotonin level; a possible explanation is that exogenous melatonin (in pharmacological doses) will save endogenous melatonin, and there is no need to consume more endogenous serotonin for the biosynthesis of endogenous melatonin; the gained clinical benefit may be attributed to the elevation of both serotonin and melatonin in the CNS in those patients after 8 weeks of therapy, as activity was increased in any part of CNS that uses this neurotransmitter [36]. Oxidative stress and nitric oxide theory may play an important role in FMS Pathophysiology, but it is still not clear whether oxidative stress abnormalities in FMS are the cause or the consequence. However, one of the suggested mechanisms for the pathogenesis of FMS is the influence of local hypoxia due to disturbed microcirculation leading to vasoconstriction in the skin of tender points [37]. It's well known that hypoxia may result in both ROS production and decreased antioxidant levels and efficacy, which may contribute to the signs and symptoms of FMS related to oxidative stress [38]. There are numerous studies indicating that ROS-induced muscle and neuron damages have an important role in the pathophysiology of muscular disorders. Many previously reported data indicated that lipid peroxidation level in patients with active FMS were higher than controls [39,40]. The present study clearly showed that treatment of FMS patients with melatonin or its combination with fluoxetine resulted in highly significant reduction in MDA levels compared with pretreatment value; combination therapies using fluoxetine 20mg plus melatonin 3 mg and 5 mg also reduced MDA level compared to its pretreatment level. This can be explained on the bases of the strong antioxidant activity of melatonin. Besides this direct radical scavenging effect, melatonin has a number of indirect actions, it may reduce oxidative stress including stimulation of antioxidant enzymes, in addition to the down regulating prooxidant enzymes such as 5 and 12-lipoxygenase and nitric oxide synthase, and stimulation of glutathione synthesis [15]. Excessive NO production can be toxic and cause nitrosative stress leading to damages of proteins, DNA and to cell injury and death. Consequently, it has shown to play a role in many important human diseases [41]. Many studies investigated the role of NO in the etiology of FMS [42,43], where FMS has been described as a disease characterized by abnormal sensitization of the spinal cord and CNS, this sensitization may occur because of inflammatory cytokines that trigger inducible nitric oxide synthase (iNOS) in muscle tissues; this isoform causes inappropriate stimulation of

pain receptors and an increase in oxidants such as the peroxynitrite radical and other ROS [44]. Previous studies have reported such oxidative damage in FMS, which are consistent with the proposed idea [45,46]. The most characteristic feature of FMS is multiorgan pain, and it is known that excessive NO can generate pain through stimulation of some but not all of the nociceptors providing an explanation for the pain generation. In the present study, administration of melatonin with fluoxetine resulted in reduction of serum NO levels but failed to reach statistically significant difference compared to pretreatment levels which may be due to limitation of small patient's sample. The reduction in serum NO level could be attributed to the up-regulation of several antioxidative enzymes and down-regulation of pro-oxidant enzymes, in particular nitric oxide (NO) synthases. Melatonin also may act at intracellular sites through binding to a cytosolic calmodulin, where experimental evidence has clearly demonstrated the interaction of melatonin with Ca^{+2} -calmodulin resulting in inhibition intracellular Ca^{+2} -calmodulin-dependent enzymes, such as nitric oxide synthase and hence inhibits NO production [47]. Finally, melatonin may exert its anti-inflammatory effect by reducing the expression of NOS-2 and COX-2, thereby inhibiting NO and PG production [18]. In conclusion, the use of melatonin as adjuvant therapy with fluoxetine improves the clinical signs and symptoms and biochemical markers of FMS.

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