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ORIGINAL ARTICLE

Lack of Circadian Pattern of Serum TNF-α and IL-6 in Patients with Fibromyalgia Syndrome

Ghizal Fatima · Abbas Ali Mahdi · Siddharth Kumar Das · Baby Anjum · Nar Singh Verma · Puneet Kumar · Ragini Shrivastava

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Abstract The present study was designed to test the hypothesis of a circadian variation in circulating levels of tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) in women with fibromyalgia syndrome (FMS). Serum levels of IL-6 and TNF-α were measured at 4 h intervals of the day in 50 women with FMS satisfying American College of Rheumatology criteria for FMS (age 36.68 ± 9.89) as well as 50 healthy control women (age 32.82 ± 10.53). Serum TNF-α levels were substantially increased in patients with FMS but showed no circadian variation. In contrast, no difference in the levels of IL-6 was found. Moreover, there was also no circadian variation in both the groups of patients and controls. We conclude that no circadian pattern exists in the circulating levels of serum IL-6 and TNF-α in patients with FMS, although TNF-α levels are found raised in patients with FMS.

Keywords Fibromyalgia syndrome · Circadian rhythm · Interleukin-6 · Tumor necrosis factor

G. Fatima · S. K. Das · P. Kumar · R. Shrivastava
Department of Rheumatology & Biochemistry, Chhatrapati Shahuji Maharaj Medical University, Lucknow, India

G. Fatima · A. A. Mahdi (✉) · B. Anjum
Department of Biochemistry, Chhatrapati Shahuji Maharaj Medical University, Lucknow, India
e-mail: mahdiaa@rediffmail.com

N. S. Verma
Department of Physiology, Chhatrapati Shahuji Maharaj Medical University, Lucknow, India

Introduction

Fibromyalgia syndrome (FMS) is associated with chronic widespread pain and at least eleven positive tender points out of 18 according to the 1990 criteria of the American College of Rheumatology (ACR) [1]. Additional symptoms like fatigue, pain associated sleep disturbance, depression, or gastrointestinal disorders are also frequently reported [2]. FMS is a common disorder which occurs more often in women than in men, with an estimated prevalence of 0.5–5.8 % in the general population of North America and Europe [3, 4]. In a study conducted in Lucknow (India), fibromyalgia was found in 4.45 % in rural (males 18.85 %; females 81.14 %) and 3.8 % in urban (males 9.79 %; females 90.20 %) areas, respectively [5]. Sleep disturbances has been recognized as one of the most probable cause of FMS [6] and this is strongly correlated to the severity of FMS symptoms [7]. An examination of circadian sleep–wake-related study shows that the symptoms of FMS may vary over the course of the day, like normal subjects have their lowest sensitivity of pain in the morning; patients with FMS have increased tenderness in the morning and no overnight improvement in pain due to disturbed sleep at night [8].

Diurnal rhythms in pain and fatigue symptoms have been reported in patients with fibromyalgia [9]. Interleukin-6 (IL-6) is a mediator of sleepiness and its circadian pattern reflects the homeostatic drive for sleep [10]. IL-6 and tumor necrosis factor-α (TNF-α) are fatigue-inducing cytokines. These cytokines are elevated during the daytime in disorders of excessive daytime sleepiness (EDS) [11, 12]. TNF-α levels exhibit distinct diurnal rhythms and are related inversely to the plasma cortisol rhythms [13]. The circadian rhythm of serum cortisol is known in patients with FMS [14, 15]. Therefore we hypothesized that the

level of TNF-α and IL-6 may also follow a circadian pattern. The goal of this study was to examine the 24-h pattern of IL-6 and TNF-α secretion in FMS patients and controls matched for age, sex and body mass index (BMI). We hypothesized that FMS would be associated with changed circadian rhythm of these two cytokines, which would explain the fatigue or disturbed sleep symptoms experienced by these patients. To test this hypothesis, serum IL-6 and TNF-α were measured at different intervals during the day and night in patients with FMS and control group.

Materials and Methods

Twenty-four hour serum cytokine patterns were determined in 50 females with FMS (mean age 36.68 ± 9.89, BMI 25.5 ± 3.93) and control group comprised of 50 age matched healthy females without FMS (mean age 32.82 ± 10.53, BMI 23.9 ± 3.33), who were nonalcoholic, non-smokers, non-diabetic without any kind of cardiac, respiratory and endocrinal disease. Subjects were excluded if they met criteria for rheumatoid arthritis and psychiatric disorders. Patients of FMS were recruited from the Department of Rheumatology, at the Chhatrapati Shahuji Maharaj Medical University, Lucknow, India. Diagnosis of FMS was made using 1990 ACR criteria [1]. Control subjects were recruited from relatives of the patients and other normal persons of Lucknow. All the patients and controls were screened to exclude any recent chronobiological disruptions, such as shift work or travel across various time zones. Before enrolling in the study, written informed consent was obtained from both the subject groups using documents approved by the Institutional Ethics Committee and all applicable institutional and governmental regulations concerning the ethical use of human volunteers were followed during this study. All data was coded to remove any identifiable information. Both the group of patients and controls were admitted to the Department of Rheumatology, where the subjects completed a structured questionnaire, which assessed the biographical information, medical, personal and family history.

Quality of life was assessed using Fibromyalgia Impact Questionnaire Revised (FIQR) (a patient self-reported instrument that assesses the impact of fibromyalgia symptoms and functional impairment, where patients were required to indicate a score between range of 0–10 (with 10 indicating very severe) [16]. 5 ml of intravenous blood was collected at 06:00, 12:00, 18:00 and 24:00 h. Blood samples were centrifuged at 3,000 rpms for 10 min; all the samples collected in the day were spun within half an hour of collection, serum was separated and was aliquoted into labeled storage tubes and frozen at −40 °C until assayed. Erythrocyte sedimentation rate (ESR) westergren and serum alanine aminotransferase (ALT) were also done together with the day time sample. Evening and night time samples were kept at 4 °C and processed next morning. IL-6 and TNF-α were assayed by standard capture enzyme-linked immunosorbent assay using Krishgen biosystems kits (Shah & Nahar Ind Premises Ltd., Worli, Mumbai, India). The assays were performed according to the manufacturer's instructions and absorbance was measured at $\lambda = 450$ nm on a Microplate ELISA reader (BIO-RAD, i-MARK). Body weight and height were measured in kilograms and meters. BMI was calculated using a standard formula of weight in kilograms divided by height in meters square.

Statistical Analysis

Statistical analysis was carried out using the INSTAT 3.0 (Graph Pad Software, San Diego, CA) [17]. Quantitative variables are presented as the mean ± standard deviation. Welch's corrected unpaired t test was performed to assess the difference in cytokines among the two groups and the association between clinical characteristics among patients and control group was expressed as odds ratio (OR) with 95 % confidence interval (95 % CI). All Statistical tests were two-tailed, and $p < 0.05$ was chosen as the level of significance.

Results

Clinical and Laboratory Parameters

Clinical assessments of FMS patients and control groups like muscle twitching, lack of energy, morning tiredness, night tiredness, disturbed sleep, morning stiffness, morning fatigue, headache, disequilibrium in climbing stairs and anxiety were more commonly seen in patients with FMS than in controls. However, weight loss, jaw pain, abdomen pain and fever fell within normal reference value ranges. No statistical differences were observed between the groups (data not shown).

The FIQR score, BMI, and tender points count was significantly higher in women with FMS than in controls. All the patients had normal laboratory tests regarding ESR, and serum ALT (Table 1).

Interleukin-6

The quantification of serum levels of IL-6 in the FMS and control groups by ELISA is shown in Table 2. Serum IL-6 levels were measured at 6:00, 12:00, 18:00 and 24:00 in FMS patients and control group. The serum levels of IL-6 were not found significant among both the groups of patients and controls throughout the day. Therefore, no

Table 1 Clinical and biochemical characteristics among study and control groups

Parameters (SI)	Study = 50 (Mean ± SD)	Controls = 50 (Mean ± SD)	*p* Value
Age (years)	36.68 ± 9.89	32.82 ± 10.53	0.069
BMI (kg/m^2)	25.5 ± 3.93	23.9 ± 3.33	0.038
ESR	27.24 ± 9.77	24.94 ± 8.15	0.196
ALT	39.87 ± 14.19	37.68 ± 14.41	0.383
FIQR	91.97 ± 8.07	5.03 ± 8.33	0.0001
Tender points	16.76 ± 1.97	1.88 ± 2.40	0.0001

Study group = females with FMS, control group = females without FMS

Table 2 Serum IL-6 levels at different time intervals

Time of day	Study (n = 50) (Mean ± SD)	Controls (n = 50) (Mean ± SD)	*p* Value, by *t* test
Morning (6:00)	3.03 ± 2.40	2.35 ± 1.74	0.108
Afternoon (12:00)	2.91 ± 2.28	2.19 ± 1.68	0.077
Evening (18:00)	2.68 ± 2.17	2.24 ± 1.45	0.233
Night (24:00)	2.94 ± 2.48	2.14 ± 1.46	0.052

circadian rhythm was observed in the circulating levels of IL-6 among patients with FMS and control group.

Tumor Necrosis Factor-α

In contrast to the IL-6 levels, the serum levels of TNF-α morning, ($p = 0.0001$) afternoon, ($p = 0.0001$) evening, ($p = 0.0001$) and night ($p = 0.0001$) were found to be significantly higher among FMS patients compared with the control groups (Table 3). However, no circadian pattern was found in the circulating levels of TNF-α in both the groups of patients and controls.

Table 3 Serum TNF-alpha levels at different time intervals

Time of day	Study (n = 50) (Mean ± SD)	Controls (n = 50) (Mean ± SD)	*p* Value, by *t* test
Morning (6:00)	5.84 ± 3.63	2.99 ± 2.05	0.0001
Afternoon (12:00)	5.86 ± 3.48	3.11 ± 2.02	0.0001
Evening (18:00)	5.90 ± 3.50	2.95 ± 2.10	0.0001
Night (24:00)	5.87 ± 3.43	2.78 ± 1.99	0.0001

Discussion

Neuro-endocrine hormone secretion is characterized by circadian rhythmicity, for example, cortisol is secreted in periodic bursts [18], and these bursts cannot be seen unless sampling is done rapidly enough to capture the basic structure of the burst. In contrast to the knowledge on circadian rhythm of various hormones there is little information available on cytokine circadian rhythm in FMS patients and this was a major reason for our doing this study. As a result, we now report that statistical evaluation of cytokine levels over time does not reveal any circadian patterns. Therefore, our findings demonstrate that the circulating levels of TNF-α are increased in the patients with FMS but showed no circadian pattern in its circulating levels. On the contrary, the circulating levels of IL-6 were not increased and remained stable during the day. In other words the IL-6 levels were low while the TNF-α levels were increased but showed no diurnal variation in FMS patients and control groups. Collecting repeated samples over time and using an assay method that allows determination of levels of IL-6 and TNF-α allows us to determine whether these cytokines are secreted in a circadian pattern. We found the above mentioned results. This result argues against the idea that Cytokine levels were uniformly low but characterized by bursts of secretion meaning it follows a circadian pattern in its secretion of IL-6 level but having no circadian pattern in the secretion of TNF-α level [19]. The results of this finding were partially similar with our result of having no circadian pattern in the circulating levels of TNF-α. In addition, the levels of TNF-α in patients with FMS differed from those of healthy controls, primarily having high levels in patients than in controls throughout the day.

Moreover, these correlations did not match with the study findings of Hernandez et al. [20] who have reported the decreased level of TNF-α and significant increase in IL-6 in patients with FMS. One of the latest study by Topal et al. [21] reported the significant increased level of serum TNF-α in patients with FMS compared to controls. Furthermore, they found no significant difference in the levels of IL-6 between the two groups, therefore, our findings of decreased serum levels of IL-6 and increased serum levels of TNF-α are in concordance with this study. TNF-α levels increased in FMS patients significantly in our study, in contrast to reports by other groups, wherein TNF-α levels rose in untreated patients in one of the studies and were unchanged in the other reports [22–24]. This discrepancy might be due to variations in inclusion criteria for the patients, age or detection methods.

Furthermore, we found no significant change in the level of IL-6 in women with FMS throughout a 24 h period, which may reflect the fact that IL-6 has no soporific properties [25]. However, our findings of serum IL-6 agrees with the report of Kim et al. [26] who found no significant difference in serum IL-6 in FMS patients and

control groups, on the other hand it differs from other studies revealing the high level of IL-6 in patients with FMS than in control group [27, 28]. One of the study reported the higher level of IL-6 and TNF-α in the serum of FMS patients compared to control group [29]. We cannot explain this discrepancy except to note our use of a substantially larger sample size due to repeated measures over time within subjects. In conclusion, our results suggest that there were no perturbations in the circadian pattern of serum levels of IL-6 and TNF-α in patients with FMS. However, the findings of the present study may be a very small step put forward. Further research will be needed to explore what may be a fundamental problem in cytokine organization in FMS.

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