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Serum Melatonin in Juvenile Rheumatoid Arthritis: Correlation with Disease Activity

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Abstract: The study was conducted to investigate the abnormalities in early morning serum melatonin among patients with Juvenile Rheumatoid Arthritis (JRA) and to outline its relation to disease activity and severity. Twenty one patients with JRA and twenty healthy age and sex matched controls were enrolled in the study. Fifteen patients had polyarticular JRA, 3 had oligoarticular and 3 had systemic onset JRA. Evaluation was carried out clinically, functionally and radiologically by using disease activity score, Juvenile Arthritis Functional Assessment Report for Children (JAFAR-C score) and modified Larsen score, respectively. Laboratory investigations included Complete Blood Picture (CBC), The Erythrocyte Sedimentation Rate (ESR), C-Reactive Protein (CRP), classic IgM Rheumatoid Factor (RF), Anti-nuclear Antibodies (ANA) and melatonin estimation in serum. The serum levels of melatonin were significantly increased in JRA patients (mean±SD = 13.9±8 pg mL^{-1}) as compared to healthy controls (mean±SD = 8.1±2.7 pg mL^{-1}, $p<0.01$). A significant positive correlation could link serum melatonin levels to disease activity scores and ESR ($r = 0.91$, $p<0.001$ and $r = 0.55$, $p<0.01$, respectively). No significant correlation was found between melatonin and either Larsen or JAFAR scores ($r = 0.19$, $r = 0.15$, respectively). According to melatonin levels, there were 2 groups of patients: Group I with elevated melatonin level (more than 11 pg mL^{-1}) ($n = 15$) and group II with normal melatonin level (less than 11 pg mL^{-1}) ($n = 6$). Patients with elevated melatonin levels had higher ESR ($p<0.05$), higher disease activity scores ($p<0.01$) and Larsen scores ($p<0.05$), than the group of patients with normal serum melatonin. The results of GAFAR scores were comparable between the two groups ($p>0.05$). Hence the study conclude that the elevated melatonin levels among JRA patients with active synovitis and its close relation to disease activity rather than disease severity suggests that melatonin might play a promoting role in rheumatoid arthritis. Hence, inhibition of its synthesis and/or action by specific antagonists may be of therapeutic value.

Key words: Melatonin, juvenile rheumatoid arthritis, disease activity

INTRODUCTION

Juvenile Rheumatoid Arthritis (JRA) is the most common form of childhood arthritis. The cause is unknown. It is well known that some clinical symptoms and signs of JRA as swelling and stiffness vary within a day and between days. The circadian changes in the metabolism or nocturnal secretion of endogenous corticosteroids is certainly responsible, in part, for the time dependent changes that are observed in the inflammatory response and related clinical symptoms (Cutolo *et al*., 2005).

More recently, Melatonin (MLT), another circadian nocturnal hormone that is the secretory product of the pineal gland, has been implicated in time dependent inflammatory reactions, with effects that are opposite to those of corticosteroids. Therefore, altered functioning of the hypothalamic pituitary-adreno-cortical axis and of the pineal gland found in RA patients seem to be important factors in the perpetuation and clinical circadian symptoms of the disease (Cutolo and Masi, 2005).

In general, MLT displays an immuno enhancing effect. It is able to activate T lymphocytes, monocytes, natural killer cells and neutrophils. In addition, MLT activates antibody dependent cellular cytotoxicity and enhances antibody responses *in vivo* (Negrette *et al*., 2001). Furthermore, due to the antiapoptotic action of MLT at the level of B and T cell precursors, excessive levels of this hormone might promote survival of auto reactive cells clones (Maestroni *et al*., 2002).

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JRA is characterized by high serum levels of proinflammatory cytokines that reach the peak of concentration in the early morning, when MLT serum concentration is higher (Reghavendra *et al*., 2001). Due to its peculiar ability to enhance proinflammatory cytokine production, melatonin might play a pathogenic role in RA and possibly drive the circadian rhythm of RA clinical symptoms (Maestroni *et al*., 2005).

In the present research, we investigated the abnormalities in early morning serum melatonin among patients with juvenile rheumatoid arthritis. The relation of melatonin level to disease activity was evaluated.

MATERIALS AND METHODS

This case control cross-sectional study took place in the Pediatric Allergy and Immunology Unit of the Children's Hospital of Ain Shams University, Cairo, during the period from January 2006 to June 2006.

Study population: The study comprised 21 subjects with JRA and 20 controls. Subject selection followed the systematic random method. An informed consent was obtained from their parents and from the National Research Center Ethical Committee before enrollment. Their demographic data were as follows:

Patients: The patients were fulfilling the American College of Rheumatology Criteria for diagnosis of JRA (Cassidy *et al*., 1986). The patients were 12 females and 9 males. Their ages ranged from 4 to 25 years with a mean of 15.2±5.5 years and their disease duration ranged from 1 to 20 years with a mean of 6.9±4.9 years. The JRA onset subtype was polyarticular in 15 patients, systemic in 3 patients and pauciarticular in 3 patients. All patients were receiving NSAIDs (Ibuprofen) 10-40 mg kg^{-1} day^{-1}. In addition, twelve of them were on low dose oral prednisone (0.25-1.5 mg kg^{-1} day^{-1}) and seven were receiving methotrexate (7-10 mg m^{-2} $week^{-1}$) either oral or IM.

Controls: Controls were 12 females and 8 males with an age range of 6 to 24 years (mean±SD = 15.3±5.6 years). Exclusion criteria for controls were presence of personal or family history of rheumatological diseases, chronic illness or weights or heights below the 5th or above the 95th percentiles for age.

Methods

Clinical evaluation: Evaluation was performed by detailed history through personal interview with patients and their parents besides the clinical examination and laboratory results obtained at the time of the study, aided by the medical records of the Pediatric Allergy and Immunology Outpatient Clinic.

Calculation of the activity score: The three clinical indices used to evaluate articular inflammation according to the American Rheumatism Medical Information System (ARAMIS, 1984) were:

- Joint swelling grades (0 = none, 1 = mild synovial swelling or effusion with visible bony landmarks, 2 = moderate swelling with definite obscuring of bony landmarks, 3 = severe swelling with no obvious landmarks).
- Pain on motion and/or joint tenderness grades (0 = none, 1 = mild pain, patient complains on joint movement or palpation, 2 = moderate pain, patient withdraws or changes facial expression on movement or palpation, 3 = severe pain, patient responds severely to movement or palpation).
- Limitation of motion grades (0 = full range of motion, 1 = 1 to 25% limitation, 2 = 26 to 50% limitation, 3 = 51 to 75% limitation, 4 = 76 to 100% limitation).

Joints examined were right and left metacarpophalyngeal, proximal and distal interphalyngeal, wrist, elbow, shoulder, temporo-mandibular, knee, ankle, small joints of the foot with exclusion of the sacroiliac and hip girdle joints. In addition to those indices, the total articular activity for each patient was then calculated according to Giannini *et al*. (1992) and Van Rossum *et al*., 1998):

Sum of the 3 clinical indices for each joint = x.

$$\text{Activity score} = \frac{\text{Sum of the x of all examined joints}}{\text{No. of affected joints}}$$

Assessment of physical activity of patients: Functional assessment of physical activity of patients with JRA was done using Juvenile Arthritis Functional Assessment Report for Children (JAFAR-C) (Howe *et al*., 1991), completed by the physician by asking the parents 23 items of activities if the child had been able to perform and responses were scored 0 for all the time, 1 for sometimes and 2 for almost never (0 best, 46 worst).

Radiological evaluation: Plain X-ray films were taken at the time of sampling. Evaluation was done after the method of Rau and Herborn (1995) for scoring soft tissue swelling, joint space narrowing, osteoporosis and erosions:

0 = Normal.
1 = Soft tissue swelling and/or joint space narrowing/ subchondral steoporosis.
2 = Erosions with destruction of the joint surface (DJS) < 25%.
3 = D J S (26-50%)
4 = D J S (51-75%)
5 = D J S (> 75%)

The Larsen's index score was applied to proximal IPs, MCPs and wrist joints of both hands (The Larsen indices of wrist joints were multiplied by five). Then the score were summed up giving a maximum score of 150 when all joints of both hands are fully destroyed (Larsen *et al*., 1977).

Laboratory methods

Sample collection: Six milliliter of venous blood were collected under complete aseptic conditions and were used as follows:

- Two milliliter were added to EDTA anticoagulant and were used for ESR and CBC.
- Four milliliter were left to clot and the serum was obtained by flicking off after centrifugation for 15 min at 3000 rpm. This was used for assessment of ANA, CRP, RF and serum melatonin.

Laboratory investigations included:

- The erythrocyte sedimentation rate was measured by Westergren method after dilution of the EDTA blood sample with the standard 10^9 mmol L^{-1} (32 g L^{-1}) tri-sodium citrate in a ratio of 4 parts blood to 1 part citrate. Readings were obtained at room temperature (18-25°C) within 4 h of collection at 1 and 2 h interval.
- A complete blood count was performed using MaxM cell counter, Coulter electronics, Florida, USA.
- Serum ANA was assessed by indirect immunofluorescent microscopy (IMMCO Diagnostics, USA).
- CRP by latex agglutination.
- Classic IgM RF by latex agglutination.
- Assessment of melatonin level according to Wagner and Mathlens (2002).

Estimation of melatonin was done by Enzyme Linked ImmunoSorbent Assay (ELISA method) purchased from IBL, Immuno Biological Laboratories, Germany. An unknown amount of antigen present in the sample and a fixed amount of enzyme labeled antigen compete for the binding sites of antibodies coated onto wells. After incubation period, the wells are washed to stop the competition reaction. After the substrate reaction, the intensity of the developed color is inversely proportional to the amount of antigen present in the sample. The results were calculated on a standard curve. The cut-off value of melatonin in controls was (8.1±2.7) (11 pg mL^{-1})

Statistical analysis: The results were statistically analyzed using SPSS windows (version 9.05) package. The results were presented as mean and Standard Deviation (SD) values. For parametric data, independent t-student t-test was used for inter-group analysis and the correlation coefficient (r) for intra-group analysis. p-values <0.05 were considered significant and p<0.01 were highly significant.

RESULTS

This study included twenty one patients with JRA (12 females and 9 males). Their ages ranged from 4 to 25 years with a mean of 15.2±5.5 and their disease duration ranged from 1 to 20 years with a mean of 6.9±4.9 years. They were compared to twenty apparently healthy age and sex matched subjects as control group (12 females and 8 males aged between 6 and 24 years, mean 15.3±5.6 years).

The laboratory, radiological and functional assessment of the Juvenile Rheumatoid Arthritis Patients was shown at Table 1.

RF was positive in 5 patients only (23.8%) and ANA was positive in 2 patients (9.5%).The serum levels of melatonin were significantly increased in JRA patients (13.9±8 pg mL^{-1}) as compared to healthy controls (8.1±2.7 pg mL^{-1}) (p<0.01).

There was a statistically significant positive correlation between disease activity score of patients and both of ESR and CRP (p<0.05) in each comparison. (Table 2). Also, significant positive correlation could link activity scores and melatonin levels (p<0.001) (Table 2) (Fig. 1).

Table 1: Laboratory, radiological and functional assessment results of JRA patients

Variable tested	Minimum	Maximum	Mean	SD
ESR (mm h^{-1})	5.0	55.0	23.60	16.10
CRP (mg dL^{-1})	0.0	24.0	8.67	9.10
Hb (g dL^{-1})	7.4	13.6	10.21	1.50
WBC ($\times 10^3$ μL^{-1})	3.2	14.8	7.94	2.90
PLT ($\times 10^3$ μL^{-1})	223.0	584.0	375.80	108.80
Melatonin (pg mL^{-1})	4.0	30.0	13.90	8.00
Activity score	0.0	5.0	2.30	1.10
Larsen-score	20.0	150.0	60.95	46.00
JAFAR-Score	0.0	26.0	9.23	9.26

ESR = Erythrocyte Sedimentation Rate; CRP = C-Reactive Protein, Hb = Haemoglobin, WBC = White Blood Cells, PLT = Platelets

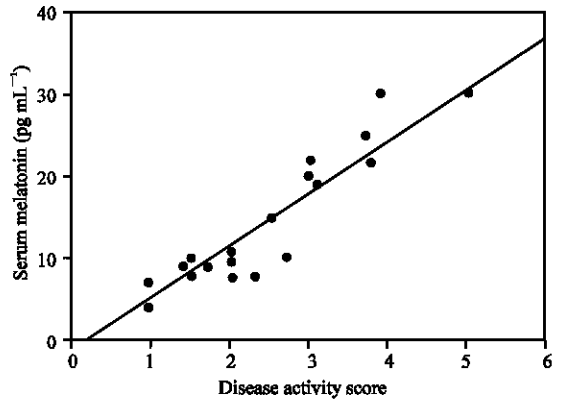

Fig. 1: A significant positive correlation between melatonin level and disease activity score among JRA patients

Table 2: Correlation between disease activity score, ESR, CRP and melatonin levels among JRA patients

Variable tested	Disease activity score
ESR	r = 0.47 p<0.05
CRP	r = 0.47 p<0.05
Melatonin	r = 0.91 p<0.001

ESR = Erythrocyte Sedimentation Rate; CRP = C-Reactive Protein

Table 3: Comparison between two groups of patients according to melatonin levels

Variable tested	Group (1) elevated melatonin >11 pg mL^{-1} (n = 15) Mean±SD	Group (2) normal melatonin <11 pg mL^{-1} (n = 6) Mean±SD	t	p
Age	16.2±5.4	12.5±4.8	1.64	> 0.05
Duration	7.1±5.3	6.5±4.3	0.24	> 0.05
ESR (mm h^{-1})	28.4±16.5	11.6±6.1	2.38	< 0.05
CRP (mg dL^{-1})	10.06±9.3	5.2±8.3	1.10	>0.05
Hb (g dL^{-1})	10.1±1.7	10.4±0.9	0.49	>0.05
WBC (×103 µL^{-1})	7.7±3.2	8.4±2.2	0.46	>0.05
PLT (×103 µL^{-1})	396.4±110.6	324.1±92.9	1.40	>0.05
Activity score	2.7±1.03	1.3±0.8	3.06	<0.01
Larsen score	74±48.8	28.3±4.08	2.25	<0.05
JAFAR score	11.1±9.57	4.5±6.9	1.53	>0.05

p>0.05 = Not significant; p<0.05 = Significant; p<0.01 = Highly significant

Also, there was a significant positive correlation between melatonin level and ESR (r = 0.55, p<0.01). No significant correlation was found between melatonin level and either Larsen or JAFAR scores (r = 0.19, r = 0.15, in each correlation).

While, there was a highly statistically significant positive correlation between Larsen and JAFAR scores (r = 0.87, p<0.001).

Patients with elevated melatonin levels had higher ESR (p<0.05), higher disease activity scores (p<0.01) and Larsen scores (p<0.05) than the group of patients with normal serum melatonin. The results of age, duration of disease, CBC, CRP and GAFAR scores were comparable between the two groups (p>0.05) (Table 3).

DISCUSSION

The results of the present study revealed that the mean melatonin levels among JRA patient were significantly increased as compared to healthy controls in early morning (p<0.01). This was in accordance with the study done by Sulli *et al.* (2002) who found that MLT serum levels at 8 PM and 8 AM were higher in RA patients than in controls (p<0.05).

Also, Cutolo and Maestroni (2005) found that MLT serum levels at 8 pm and 8 am were significantly higher in patients who had RA than in healthy controls (p<0.05). Cutolo *et al.* (2005) hospitalized for active RA, 10 patients compared to 6 controls. They found that the concentration of MLT was significantly higher in RA sera at 8 pm and 8 am (p<0.02). These results indicate that melatonin production in RA patients seems to be higher than in healthy controls at the beginning of the night and in early morning. All these patients with active RA showed the typical circadian variation of symptoms with joint stiffness and pain being more prominent in the early morning. Interestingly, the plasma concentration of melatonin was higher in patients in the early morning too, when maximal inflammatory reactivity was present after awakening in the morning than in the afternoon or evening.

In the present study, there was a statistically significant positive correlation between disease activity score of patients and ESR, CRP (p<0.05) with a highly significant positive correlation existed between disease activity score and melatonin level (p<0.001).

Also, patient with elevated melatonin level had higher disease activity score and ESR level than patients with normal melatonin level (p<0.01, p<0.05).

These findings suggest that MLT might play a promoting role in RA by its ability to stimulate. The cells responses, counteract the immunosuppressive effect of steroids. Also, it enhances the production of inflammatory cytokines (as $IL_{1,2,4,6}$), TNF and IFN). These cytokines are strongly involved in the synovial immune and inflammatory responses in RA (Maestroni *et al.*, 2005).

Drazen and Nelson (2001) stated that there may be a defect in the multisynaptic pathway connecting the retina and the retino hypothalamic tract to the pineal gland. This explains the significant difference in MLT level observed especially at the beginning and end of the dark period in RA patients.

In addition, Straub and Cutolo (2001) found that MLT inhibit testosterone secretion by acting at the hypothalamo-pituitary gonadal axis and by decreasing the potential immunosuppressive and anti inflammatory activities exerted by androgens.

On the other hand, West and Oosthuizen (1992) suggest that MLT is methoxy-indole, known as N-acetyl-5-methoxy tryptamine which is structurally related to indomethacin, a derivative of methylated indole which possesses anti inflammatory effect.

This hypothesis was tested by determining MLT levels by radioimmunoassay in RA patients. They found that the daytime melatonin levels were significantly lower in patients (mean 5.76 pg mL^{-1}) than healthy controls (33 pg mL^{-1}).

These results were in discrepancy with the present study, this may be due to the fact that they did not exactly determine the day time at which samples were collected. Also, their normal mean values of MLT were higher than the mean value of controls in the present study which may be due to different radioimmunoassay technique used.

In the present study, there was no significant correlation between melatonin level and Larsen or JAFAR scores while there was a highly significant positive correlation between Larsen and JAFAR scores denoting disease severity ($p<0.001$). This was in accordance with Oen (2002) who examined the relationship between radiographic damage and disability in Juvenile chronic arthritis. He reported significant positive correlation between joint changes and greater levels of disability assessed by children Health Assessment Questionaire (CHAQ). These results indicated that there is a close relationship between structural damage and functional impairement over the course of juvenile arthritis.

Ravelli and Martini (2003) found a high colinearity among the functional status measures specifically used for children with juvenile arthritis including the CHAQ and JAFAR scores. This finding showed that the assessment of functional disability give similar results either when the questionaires are completed by the physician by asking the parent (JAFAR score) or by the parent himself (CHAQ). However, JAFAR scores potentially have great value because of its strong measurement characteristics, the ease with which they can be administered and their relevance to actual patient concerns. They showed that a large number of the children with JRA had very low levels of disability.

The present study showed that patients with elevated melatonin levels had higher ESR ($p<0.05$), higher disease activity scores ($p<0.01$) and Larsen scores ($p<0.05$) than the group of patients with normal serum melatonin. The results of GAFAR scores were comparable between the two groups ($p>0.05$). This was in accordance with Bloom *et al.* (2002) who found that there was no correlations between CHAQ scores and JRA disease activity.

Also, Magni-Manzoni *et al.* (2003) concluded that the measures of disease activity including the subjective assessments made by the physician, the ESR and CRP values were not related to the radiographic progression. Since, patients had different disease durations at study entery, it was likely that the amount of radiographic damage at baseline would be different. Because, there may often be a significant lag time between clinical manifestations and the occurrence of radiographic changes. However, the radiographic progression was strongly related to the number of joints with active disease which suggests that greater articular damage was associated with continued joint inflammation (Felici *et al.*, 2002).

In conclusion, the melatonin-RA relationship might explain why clinical symptoms of active synovitis are more evident in the early morning rather than its relation to disease severity suggesting that melatonin might play a promoting role in RA. Hence, inhibition of its synthesis and/or action by specific antagonists can provide a conceptual basis for the development of novel therapeutic strategies in rheumatoid arthritis.

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