Higher Levels of Melatonin in Early Stages of Adolescent Idiopathic Scoliosis: Toward a New Scenario

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Background: The melatonin deficiency hypothesis as a central mechanism in the pathogenesis of adolescent idiopathic scoliosis (AIS) is certainly intriguing. However, the actual role of melatonin remains unclear. The aim of this study was to assess the potential clinical value of melatonin serum level in the pathogenesis and the prognosis of AIS progression in patients who were treated nonoperatively.

Methods: Two groups of patients were enrolled. The study group consisted of patients with AIS aged below 14 years who were treated conservatively. In the second group, that is, the control group, age-matched, weight-matched, and height-matched healthy individuals were enrolled. Blood samples were collected from all patients on visit 1 and the serum levels of melatonin were evaluated with the enzyme-linked immunosorbent assay (ELISA) method. The blood sampling procedure was repeated exactly 1 year later (visit 2).

Results: Forty-two patients formed the study group (with AIS) and 29 served as the control group. The mean serum value of melatonin on visit 1 was 19.32 pg/mL for the AIS group and 12.23 pg/mL for the control group. This difference was statistically significant ($P = 0.014$). One year later, 34 patients from the AIS group and 23 from the control group were reevaluated and the mean serum levels of melatonin were 52.43 and 68.44 pg/mL, respectively. No statistically significant difference was found between the 2 groups ($P = 0.235$). Statistical analysis of the serum melatonin levels of patients with progressing AIS (>5 degrees of the Cobb angle in 1 y) when compared with patients with stable AIS ($P = 0.387$) or the control group ($P = 0.727$) failed to show that the deficiency of melatonin may be associated with the progression of AIS.

Conclusions: Higher melatonin levels were observed in conservatively treated patients with AIS, whereas melatonin deficiency was not associated with AIS progression in this study.

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Several theories have been introduced and many factors have been associated with adolescent idiopathic scoliosis (AIS). However, at present, its actual cause(s) remain(s) more or less unknown.[1]

The melatonin deficiency hypothesis in the pathogenesis of AIS is certainly intriguing. Several studies suggest that melatonin deficiency (especially in progressive AIS) may lead to the development of AIS.[2–4] In contrast, diseases which reduce melatonin levels do not necessarily lead to the development of AIS and certainly not all patients with AIS present sleeping disorders (associated with melatonin deficiency).[5] Overall, the actual role of melatonin in AIS remains unclear.[1–10]

The aims of this study were: (i) the assessment of the serum level of melatonin in AIS patients who were treated nonoperatively at a certain time frame and its reassessment 1 year later, and (ii) the evaluation of the potential effect(s) that the differences (if any) between the 2 consecutively assessed serum levels of melatonin might play in the pathogenesis and the prognosis of AIS.

METHODS

This prospective case-control study was approved by our Institution's Scientific Research Board and it was conducted between June 2008 and December 2010 in accordance with the World Medical Association Declaration of Helsinki of 1964, as revised in 1983 and 2000. The parents of all patients gave written informed consent.

Patients participating in this study were classified into 2 groups: the study group (AIS) consisted of patients under the age of 14 years with AIS who were treated conservatively (either with observation alone or with the application of a Boston brace); and the second group included age-matched, weight-matched, and height-matched healthy individuals who served as the control group. Children with congenital, neuromuscular, or scoliosis secondary to other disease that could interfere with the interpretation of the Adam bending test were excluded.

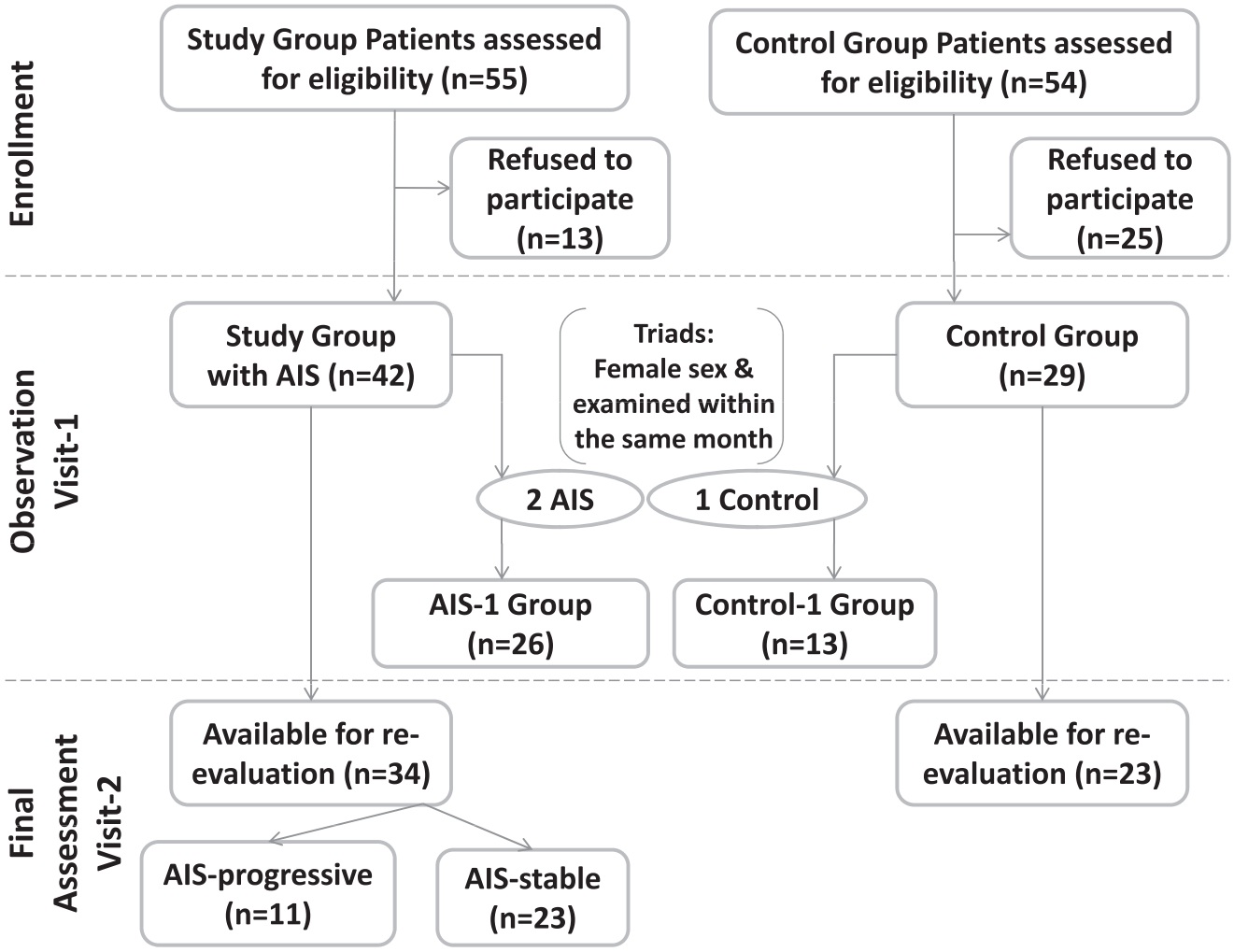

FIGURE 1. Flow diagram of our study.

Blood serum samples were collected from all patients (visit 1), using single serum tubes, at the same time interval (between 08:00 and 09:40 AM). The exact time and date of venipuncture was recorded to repeat the examination 1 year later. All samples were centrifuged (3500 rounds per minute for 6 min) in <30 minutes following the venipuncture and immediately frozen at $-21^{\circ}C$. Enzyme-linked immunosorbent assay (ELISA) was used to assess the concentration (pg/mL) of melatonin in the blood serum.

The height, weight, and body mass index (BMI) of all patients were recorded. Standard standing posteroanterior radiograph of the whole spine including the iliac crests were obtained to evaluate the scoliotic curve. A Cobb angle ≥ 10 degrees combined with rotational deformity of the involved vertebrae was defined as AIS.[11–13]

The blood sampling was repeated 1 year later (visit 2). Anthropometric measurements were collected and the Cobb angle was determined once again by obtaining new radiographs. An increase in ≥ 5 degrees of the Cobb angle 1 year after the initial evaluation was considered as a progression of the AIS.[4]

To specify the clinical power of our study, the daytime serum values of melatonin, as evaluated by Bagnall et al[6] were used. As this study was very similar to ours, we used it as a pilot study. By performing a post hoc analysis, the clinical power of our research was 83%. Standard statistical methods were used for descriptive statistics. The confidence intervals were set at 95%. Because of the samples' size, the control samples' distribution was performed using the Shapiro-Wilk Test. The Mann-Whitney test and the *t* test were used to compare the mean values, depending on the sample distribution. The Wilcoxon test was used to determine any changes in the serum levels of melatonin between the 2 evaluations.

RESULTS

Fifty-five AIS patients were initially screened but 13 refused to participate (Fig. 1). As a result, 42 patients formed the study group (AIS). Their Cobb angle ranged from 11 to 43 degrees. Twenty of these patients were only observed and underwent no treatment whatsoever (Cobb angle range, 11 to 29 degrees); in the remaining 22,

TABLE 1. Demographics and Melatonin Serum Values of AIS and Control Groups Plus Corresponding *P*-values

Groups	n	Girls [n (%)]	Boys [n (%)]	Age [Mean (SD)] (y)	Menarche [Mean (SD)] (y)	Weight [Mean (SD)] (kg)	Height [Mean (SD)] (cm)	BMI [Mean (SD)] (kg/m^2)	Venipuncture Time	Visit 1 Melatonin Serum Levels [Mean (SD)] (pg/mL)	Visit 2 Melatonin Serum Levels [Mean (SD)] (pg/mL)
AIS	42	39 (92.9)	3 (7.1)	12.3 (1.5)	11.8 (1.1) n = 31*	50.0 (12.1)	159 (11)	19.5 (3.3)	08:34 AM	19.32 (25.95)	52.43 (58.33)
Control	29	21 (72.4)	8 (27.6)	11.7 (1.8)	12.3 (1.3) n = 9*	46.2 (14.3)	154 (12)	19.0 (3.7)	08:42 AM	12.23 (20.91)	68.44 (72.27)
P		**0.041**		0.188	0.384	0.248	0.066	0.580		**0.014**	0.235

*Mean time of menarche onset is based only on the female individuals who have had menarche during our study.
Bold values indicate for the statistically significant results.

because of the severity of the deformity, a Boston Brace was applied (Cobb angle range, 20 to 43 degrees). It is certainly worth noting here that all patients who were enrolled in the study group with more severe forms of AIS were offered either the application of a Boston brace or operative treatment depending on the magnitude of scoliosis. Nevertheless, none of the patients (or their parents) decided to proceed to operative treatment. In 9 cases there was also a family history of AIS. Another 54 individuals were initially screened clinically and, when in doubt, radiologically for participating in the control group. After their briefing on the protocol of the study, 25 refused to participate. As a result, 29 age-matched, weight-matched, and height-matched healthy volunteers served as the control group (Table 1). The initial blood samples were collected between June 2008 and November 2009, whereas the reevaluation samples were collected between June 2009 and December 2010.

There was no statistically significant difference between the 2 groups as far as the age, the weight, the height, the BMI, and the age of menarche in female patients were concerned. However, there was a statistically significant difference with regard to the sex of the patients belonging to the 2 groups. The mean times of venipuncture of the 2 groups were quite similar (AIS group, 08:05 to 09:00 AM; control group, 08:20 to 09:35 AM). All patients enrolled in the study were born and living in the same territory of our country, thus reducing the possible influence of the different duration of daylight (because of the different latitude) on the serum value of melatonin. The mean serum values of melatonin (visit 1) of the 2 groups are presented in Table 1. The study group had higher values and the difference was statistically significant. No correlation between the Cobb angle and the serum level of melatonin at visit 1 was found ($P = 0.501$, the Spearman correlation coefficient = -0.107).

One year later, 34 of the initial 42 patients who formed the study group and 23 of the 29 controls were available for reevaluation. Fourteen individuals were lost to follow-up (5 moved away, 7 could not be reached by telephone or mail, and 2 refused to further participate in the study). In total, 51 individuals (enrolled to both the study and control group) were reevaluated 1 year $\pm$ 1 week after the initial examination, 56 (51 + 5 additional patients) after 1 year $\pm$ 2 weeks, and only 1 patient was examined 1 year + 18 days after the initial examination. The mean serum levels of melatonin for AIS patients and for the controls who were available for reevaluation (visit 2) had no statistically significant difference (Table 1).

To further minimize the factors that may affect the levels of melatonin, stricter matching between the 2 groups was attempted. This was achieved by creating triads, each one formed by 2 patients from AIS group and 1 control patient, so that: (i) each triad comprised patients of the same sex (females); and (ii) all 3 patients who formed each triad were examined within a period of a certain month of the year. As a result, the influence of the male sex and of the season of the year during which the evaluation took place on the hormonal status of the patients were neutralized. The 2 newly formed groups (AIS-1, control-1) consisted of 26 and 13 female individuals included from the initial AIS and control groups (Table 2).

TABLE 2. Demographics and Melatonin Serum Values of AIS-1 and Control-1 Groups on Visit 1 Plus Corresponding *P*-values

Groups	n	Girls	Age [Mean (SD)] (y)	Menarche [Mean (SD)] (y)	Weight [Mean (SD)] (kg)	Height [Mean (SD)] (cm)	BMI [Mean (SD)] (kg/m^2)	Venipuncture Time	Melatonin Serum Levels [Mean (SD)] (pg/mL)
AIS-1	26	26	12.4 (1.4)	11.8 (1.3) n* = 21	51.1 (11.2)	160 (10)	19.8 (3.3)	08:32 AM	23.04 (31.16)
Control-1	13	13	11.2 (1.6)	12.9 (1.2) n* = 4	42.6 (13.3)	151 (12)	18.4 (3.3)	08:38 AM	4.68 (4.25)
P			**0.023**	0.111	0.061	**0.031**	0.200		**0.005**

*Mean time of menarche onset is based only on the female individuals who have had menarche during our study.
Bold values indicate for the statistically significant results.

TABLE 3. Mean Serum Concentrations of Melatonin at Visit 1 (First Evaluation) and Visit 2 (Reevaluation) for Subgroups AIS Progressive and AIS Stable and Corresponding *P*-values

		Mean (SD)	
Group	**N**	**Visit 1**	**Visit 2**
AIS progressive	11	16.16 (24.24)	53.67 (42.38)
AIS stable	23	21.03 (24.35)	51.84 (65.46)
P		0.232	0.387

Melatonin values are expressed in pg/mL.

There was no statistically significant difference in the age of menarche, the weight, or the BMI of the patients belonging to AIS-1 and control-1 groups. Nevertheless, there was a statistically significant difference with regard to the mean age and the mean height of the patients belonging to these groups. The mean times of venipuncture for AIS-1 group and for the control-1 group were also similar. The mean serum values of melatonin (on visit 1), which corresponded to the 2 matched groups, are presented in Table 2. The difference between them was also found to be statistically significant, with $P < 0.01$.

On the basis of the threshold of ≥ 5-degree increase of the Cobb angle on the second evaluation, AIS patients who were available for reevaluation were further divided into 2 groups: AIS progressive (n = 11) and AIS stable (n = 23). On their initial evaluation (visit 1), there was no statistically significant difference as far as the melatonin levels were concerned between the AIS progressive and the AIS stable patients (Table 3), or between the AIS progressive patients and the controls (Table 4). No statistically significant difference was found either on the second evaluation (visit 2) of the serum melatonin levels. However, in all patients (both patients and controls) who were available for the second evaluation (N = 57), there was a statistically significant increase in the serum levels of melatonin, when compared with the values obtained on the initial evaluation ($P = 0.000$).

DISCUSSION

To investigate the possible fluctuations in the serum level of melatonin among individuals with mild AIS, only patients who were treated nonoperatively (observation alone or application of a Boston brace) were included in the study group. The young age of the patients enrolled in this study (just before or after menarche) and the fact that all AIS patients had milder forms of AIS, differentiates this study from other similar ones that examined the influence of melatonin on the development and progression of AIS in patients with severe scoliosis.[6]

The role of melatonin in the pathogenesis and prognosis of AIS has been under experimental investigation over the last 20 years.[14] A more recently performed study in pinealectomized Rhesus monkeys[15] failed to validate previously conducted promising studies.

Early case-control studies in humans did not provide clear evidence regarding the role of melatonin, either by measuring its serum levels,[2–4,6,7] or its metabolic product in urine (6-sulphatoximelatonin).[8–10] However, several studies provided indications that the serum level of melatonin may be deficient in patients with progressive AIS.[2–4] Melatonin receptors located in paraspinal muscles were found to be asymmetric on the 2 sides of the scoliotic curve. However, it was believed that this asymmetry was secondary because of the different loads exerted on the 2 sides of the spine.[16–18] Several genetic studies failed to identify mutations in the genes responsible for the production of melatonin.[19–24] There is only one study suggesting that the MTNR1B gene responsible for a melatonin receptor is a predisposing factor for the development of AIS.[25]

Recent studies focus on the role of Gi proteins on the cellular membrane, which are in contact with the melatonin receptors and play an important role in the phosphorylation of other cell proteins, such as protein kinase A and C. The role of Gi proteins seems to be crucial, leading to a transmembrane dysfunction, which in turn affects the transfer of the melatonin hormonal message inside the cell by altering c-AMP levels.[26–30] c-AMP levels,[26–29] especially in osteoblasts, and the cellular dielectric spectroscopy on peripheral blood mononuclear cells[31–33] are 2 different methods, based on the theory of Gi protein stimulation, which are both under evaluation for the screening of asymptomatic individuals and for the prognosis of AIS progression.

Our results (using ELISA) showed that the blood serum melatonin levels proved to be significantly higher in patients with AIS when compared with age-matched, weight-matched, and height-matched controls. However, we failed to find any indication that the deficiency of melatonin may be associated with the progression of AIS. Higher serum melatonin levels in people with less severe AIS may indicate either an early stage of this clinical entity or a milder clinical form of AIS. Given this hypothesis, it could be that at the beginning of AIS and while deformity is still limited, thus requiring only conservative treatment, melatonin levels rise, perhaps in response to the signaling dysfunction on the cell membrane. Later on, when severe AIS is observed, the increased production of melatonin probably is no longer feasible and can, therefore, no longer counterbalance this dysfunction. The majority of patients enrolled in similar studies were those who were either about to undergo

TABLE 4. The Mean Serum Concentrations of Melatonin at Visit 1 (First Evaluation) and Visit 2 (Reevaluation) for Subgroup AIS Progressive and Controls (Comprising Only the Controls Who Were Reevaluated on Visit 2) and Corresponding *P*-values

		Mean (SD)	
Group	**N**	**Visit 1**	**Visit 2**
AIS progressive	11	16.16 (24.24)	53.67 (42.38)
Controls	23	14.37 (22.98)	68.44 (72.27)
P		0.699	0.727

Melatonin values are expressed in pg/mL.

operative treatment and/or those who had been previously operated on. This may have contributed to the different results presented in these studies, as far as the role of melatonin on the pathogenesis of AIS is concerned. The neurohormonal concept may play a part in the early stages of AIS but in a different direction. Maybe at a more progressed stage, the cellular dysfunction can no longer be compensated, leading to increased Cobb angles and further rotational distortion. This theory may further enhance results presented in recent studies which "re-locate" the pathogenesis of AIS inside the human cell.[26–30] This is only a theoretical hypothesis, which needs to be verified by future studies.

One important limitation of this study is the samples' size, especially when subgrouping. Although the determination of the samples' sizes was based on similar studies, it is true that failure to find statistically significant differences in progression's prognosis can be due to underpowering the groups into smaller subgroups (progressive and stable). However, it is worth mentioning that the clinical power (G power) of this study has been calculated $\geq 80\%$. Another issue was that the patients were lost to follow-up. Fourteen patients in total, belonging to both groups, failed to present at visit 2. This may influence the statistical analysis. However, we retrospectively decided to exclude from further evaluation any patient who did not come for visit 2 within a time frame of $\pm$ 3 weeks from the exact calendar date of visit 1. This was carried out to deal with the influence of the yearly/infradian rhythm of melatonin. Retrospective triad formation also led to significant differences in height and age. One morning sample instead of the mean integrated hormonal concentration was used to achieve better compliance. Nevertheless, 19.7% of the initially evaluated patients were lost to follow-up. Another limitation was the systemic bias between the first and the second serum melatonin values. On the basis of the relative literature, melatonin can routinely be measured by either RIA or ELISA, although RIA is considered as more precise.[34,35] The ELISA kit used in this study had an analytical sensitivity of 1.6 pg/mL, which is well below the expected daytime melatonin values. However, to overcome these issues, same single kits provided by the same manufacturer were used in both measurements. Furthermore collection, storage, and evaluation of the samples were performed in the same way in both visits (1 and 2), and all evaluations took place approximately at the same time and almost on the same time of the year. An extra measurement by using bank samples from both visits in the same kit suggested that the bias resulted from the different effect of each kit. Further, the reevaluation was closer to later stages of AIS in which melatonin deficiency compared with controls is reported.[2–4]

In conclusion, this longitudinal case control study showed that blood serum melatonin levels proved to be significantly higher in patients with milder AIS/or earlier stages of AIS when compared with matched controls. This result was more obvious when we further excluded the sex as a factor and the effect of the infradian rhythm of melatonin. Finally, we failed to correlate AIS progression to melatonin deficiency.

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