

[Abstract:0347] ADHD**Urinary 6-OH melatonin-sulfate levels in patients with attention deficit hyperactivity disorder**Ahmet Buber¹, Burcu Cakalo², Yetis Isildar³, Gulsen Unlu¹, Hayrani Eren Bostanci⁶, Hulya Aybek⁵, Huseyin Alacam⁴, Hasan Herken⁴¹Department of Child and Adolescent Psychiatry, Pamukkale University, Faculty of Medicine, Denizli-Turkey²Department of Child and Adolescent Psychiatry, Dr. Behcet Uz Children's Hospital, Izmir- Turkey³Department of Child and Adolescent Psychiatry, Dr. Sami Ulus Training and Research Hospital, Ankara- Turkey⁴Department of Psychiatry, Pamukkale University, Faculty of Medicine, Denizli- Turkey⁵Department of Biochemistry, Pamukkale University, Faculty of Medicine, Denizli- Turkey⁶Department of Pharmaceutical Basic Sciences, Cumhuriyet University, Faculty of Pharmacy, Sivas-Turkey

e-mail address: ahmetbuber@gmail.com

INTRODUCTION: Attention deficit hyperactivity disorder (ADHD) is one of the most common neurodevelopmental disorders of childhood that affects about 5% of children and about 2.5% of the adult population.

Melatonin is synthesized from tryptophan and secreted primarily by the pineal gland. Melatonin has a major role in the entrainment of the circadian rhythm and sleep onset and increases sleep efficiency.

Sleep disorders may be encountered in children with ADHD. The circadian rhythm of pineal melatonin secretion, which is controlled by the suprachiasmatic nucleus (SCN), is reflective of the mechanisms that are involved in the control of the sleep / wake cycle. The onset of melatonin production occurs with the decrease in SCN neuron firing rate late in the day. The SCN has an active role in promoting sleep, and melatonin is the principal neurochemical agent.

There is a complex relationship between dopamine and melatonin which play a major part in the etiology of ADHD. It has been reported that melatonin increases the activity of tyrosine hydroxylase, the rate-limiting enzyme in the synthesis of catecholamines. Furthermore, it was demonstrated that the MT1 melatonin receptor Messenger Ribonucleic Acid (mRNA) expression was also present both in the cells of the nucleus caudatus, putamen and nucleus accumbens, where the D2 dopamine receptor, which is involved in the etiology of ADHD, is positive, and in the cells of the ventral tegmental area where the tyrosine hydroxylase activity is positive. For this reason, melatonin may be involved in the etiology of ADHD. This study was aimed at detecting levels of 6-hydroxymelatonin sulfate (6-OH MS), the main urinary metabolite of melatonin, in patients diagnosed with ADHD.

METHOD: The case group consisted of 27 children ranging from 6 to 16 years of age with no axis I additional diagnosis apart from ADHD. The control group included 28 children according to DSM-4 as demonstrated by the results of a clinical interview and K-SADS-PL-T administered by a trained interviewer. Exclusion criteria for both of the groups were set as the presence of mental retardation in the child, administration of any psychotropic drugs during the last 6 months, presence of any chronic physical disease and history of infection during the past week.

The socio-demographic data questionnaire form was completed by the researcher employing a face-to-face interviewing technique. The Conners' Parent Rating Scale Short Version was completed for the purpose of supporting the diagnosis. WISC-R was administered to cases clinically suspected of mental retardation, and the cases diagnosed with mental retardation (two cases) were excluded from the study.

Urine Specimen Collection: Families were handed out written instructions regarding the procedure of urine specimen collection, and the urine specimens were collected from all the children at home under parental guidance within a period of 24 hours. It was ensured that the urine specimens were collected in two separate containers, one of which was to be designated for the specimen from daytime phase from 08:00 to 21:00 and the other for the nighttime phase from 21:00 to 08:00. It was requested that the bladder should be emptied between 20:45 and 21:00 before the nighttime phase started.

Biochemical Assessment: Urinary concentrations of 6-OH melatonin sulfate was measured using an ELISA kit. The urinary 6-OH MS concentration (ng/ml) was multiplied by the volume of the urine in milliliters.

RESULTS: The average age of the case group was 9.37 ± 2.69 (6-15) years, and that of the control group was 10.50 ± 2.71 (7-16) years. The difference in the average age between the case group and the control group was not statistically significant ($p=0.084$).

The case group was divided between 14.8% girls ($n=4$) and 85.2% boys ($n=23$), while 25% of the control group were girls ($n=7$) and 75% boys ($n=21$). The difference in sex between the case group and the control group was not statistically significant ($p=0.345$).

An examination of the diagnosis-distribution in the case group found ADHD – Subtype Predominantly Inattentive at 22.2% ($n=6$), ADHD – Subtype Predominantly Hyperactive-Impulsive at 7.4% ($n=2$) and ADHD – Subtype Combined at 70.4% ($n=19$).

A comparison of the data obtained via the Conners' Parent Rating Scale – Short Version (CPRS-48) with respect to the case and control groups revealed differences among the subscales of impulsivity / hyperactivity, learning problems, oppositional defiant disorder and conduct problem, but no difference among the subscales of psychosomatic problems and anxiety.

There was no difference in 24-hour creatinine excretions between the groups, either (15.15 ± 3.08 mg/kg and 15.07 ± 3.58 mg/kg, respectively [$p=0.690$]).

The analyses performed employing MWU test yielded significantly higher nighttime levels of 6-OH MS in the case group as compared to the control group ($p=0.045$). The daytime 6-OH MS levels were found to be higher than in that of the control group ($p=0.018$). 24-hour (daytime + nighttime) 6-OH MS levels were also higher in the case group as compared to the control group ($p=0.018$).

DISCUSSION: The literature includes only few studies focusing on the relationship between ADHD and melatonin. The results of the study conducted by Molina-Carballo et al. on the effect of methylphenidate treatment in children with ADHD on serotonin and melatonin levels demonstrated, in a similar way to our study, that the nighttime urinary 6-OH melatonin sulfate levels were higher in the ADHD group than in the control group. Another study on the subject detected high nighttime urinary melatonin levels in four adults recently diagnosed with ADHD who had not received a drug treatment.

Ours is the first comprehensive study known to have researched the daytime, nighttime, and 24-hour 6-OH MS levels in ADHD.

The data obtained in our study may suggest a higher melatonin production in children with ADHD. To modulate pineal melatonin production, axons originating from SCN neurons project to the PVN of the hypothalamus where they release gamma-aminobutyric acid (GABA), and GABA has an inhibitory effect here. A study indicated that the administration of the bicuculline (BIC), an antagonist of GABA, caused the disinhibition of PVN neurons in the daytime, resulting in a stimulating effect on the melatonin synthesis from the pineal gland. In one study, the GABAergic component of ADHD was researched by using magnetic resonance spectroscopy. It was found that GABA levels in children with ADHD were significantly lower. One of the reasons for the high levels of 6-OH MS excretion in children with ADHD may be these low GABA levels in such children.

The SCN is responsible for regulating the circadian rhythm by means of a series of transcription and translation processes with positive and negative feedback effects. Clock genes such as Clock, Bmal1, Period (Per1-3) and Cryptochrome (Cry1-2) are involved in the regulation of this rhythm. The expression of Striatal Per1 requires a functional pineal gland and melatonin, its product. The rhythm of diurnal striatal Per1 mRNA and Per1 protein is observed in normal rats but not in pinealectomised rats. Another study conducted on rats, on the other hand, indicated that Per1 was involved in the regulation of rhythmic melatonin synthesis from the pineal gland and melatonin concentration increase in the shortage of Per1. Considering the data obtained from those studies which focused especially on Per1 among the clock genes together with the results of our own study, it may be presumed that a deficiency in the function of clock genes, and particularly of Per1 gene, might be a matter of relevance in children with ADHD.

It has been indicated that sleep problems are frequently encountered in children with ADHD and that chronic sleep onset insomnia is one of the basic characteristics of circadian rhythm problems. There is a close relationship between the circadian rhythm and sleep-wake cycle and the melatonin concentration. A study suggested, although hypothetically, that shorter melatonin signals might be a matter of significance in children with ADHD. There are also studies indicating that children with ADHD benefit from exogenous melatonin administration with respect to sleep disorders. When the data we obtained from our study and such information are considered cumulatively, this situation might mean that melatonin is catabolized in such children to a greater extent, although there is no data supporting this argument.

In conclusion, the higher level of 6-OH MS excretion in children with ADHD as compared to the control group suggests that melatonin synthesis or other possible conditions that affect the melatonin synthesis may be different in children with ADHD as compared to healthy children.

Keywords: attention deficit hyperactivity disorder, melatonin, etiology

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Bulletin of Clinical Psychopharmacology 2015;25(Suppl. 1):S31-S2