Original Article

Preliminary study: Evaluation of melatonin secretion in children and adolescents with type 1 diabetes mellitus

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ABSTRACT

Objective: Melatonin is an indolamine hormone, synthesized from tryptophan in the pineal gland primarily. Melatonin exerts both antioxidative and immunoregulatory roles but little is known about melatonin secretion in patients with type 1 diabetes mellitus (T1DM). The aim of this study was to measure serum melatonin levels in patients with T1DM and investigates their relationship with type 1 diabetes mellitus. **Materials and Methods:** Forty children and adolescents with T1DM (18 boys and 22 girls) and 30 healthy control subjects (17 boys and 13 girls) participated in the study. All patients followed in Pediatric Endocrinology and Metabolism Unit of Gaziantep University Faculty of Medicine and also control subjects had no hypertension, obesity, hyperlipidemia, anemia, and infection. Blood samples were collected during routine analysis, after overnight fasting. Serum melatonin levels were analyzed with ELISA. **Results:** There were no statistically significant differences related with age, sex, BMI distribution between diabetic group and control group. Mean diabetic duration was 2.89 ± 2.69 years. The variables were in the equation. Mean melatonin level in diabetic group was 6.75 ± 3.52 pg/ml and mean melatonin level in control group was 11.51 ± 4.74 pg/ml. Melatonin levels were significantly lower in diabetic group compared to controls ($P < 0.01$). **Conclusions:** Melatonin was associated with type 1 diabetes mellitus significantly. Because of the varied roles of melatonin in human metabolic rhythms, these results suggest a role of melatonin in maintaining normal rhythmicity. Melatonin may play role in preventing process of inflammation and oxidative stress.

Key Words: Adolescent, children, melatonin, Type 1 diabetes mellitus

INTRODUCTION

Melatonin is a circulating neurohormone secreted predominantly at night. It is important in conveying the daily cycle of light and darkness to the body, thus regulating circadian rhythms. In addition to its' regulatory role, melatonin has antioxidative capacity, immunomodulatory potency, and also appears to be protective against certain types of cancers.[1-3] Type 1 diabetes mellitus (T1DM) is a T cell-mediated autoimmune disease characterized by excess inflammation, independent of adiposity and glycemic control. The incidence of T1DM diabetes is increasing at 3-5% per year worldwide,[4-6] and this increase cannot be accounted for by known genetic factors. It has been well known that both oxidative stress and inflammatory activity play crucial roles in the pathogenesis of T1DM. Little information is available on the different patterns of type 1 diabetes progression following diagnosis, particularly in the pediatric population. The relation between melatonin and T1DM in children and adolescents was not investigated before.

MATERIALS AND METHODS

Forty children and adolescents with T1DM (18 boys and 22 girls, mean age 10.43 ± 3.25 years) and 30 healthy control subjects (17 boys and 13 girls, mean age

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9.83 ± 4.36 years) participated in the study. All patients followed in Pediatric Endocrinology and Metabolism Unit of Gaziantep University Faculty of Medicine and also control subjects had no hypertension, obesity, hyperlipidemia, anemia, and infection. Blood samples were collected during routine analysis, after overnight fasting. Serum melatonin levels were analyzed with ELISA. They were receiving insulin for controlling hyperglycemia. Patients with other forms of diabetes (type 2, maturity-onset diabetes of the young, thiamine responsive megaloblastic anemia) were not included in the study. In the present study we enrolled only children who fulfilled the following eligibility criteria for control group: 1) Prepubertal and pubertal age; 2) absence of acute or chronic inflammatory and autoimmune diseases; 3) lack of diabetes mellitus, primary hyperlipidemia, hypertension, anemia and obesity; and 4) no current regular medications. The study received approval from the ethical committee of the hospital. Antropometric data included height, body weight, body mass index (BMI), and blood pressure. Height was measured in centimeters using a stadiometer. Weight was measured in kilograms using an electronic scale. BMI was calculated using measured weight (kilograms), hemoglobin, hematocrit, white blood cell count, free thyroxine, thyroid stimulating hormone and antithyroid peroxidase antibody levels were divided by measured height (meters) squared. Pubertal stage was classified according to Tanner. Blood samples were collected during routine analysis, after overnight fasting between 08:30 and 09:00 a.m. After separation, serum samples were immediately stored at -70°C until analyzed for measuring melatonin. Serum glucose, total cholesterol, high-density lipoprotein, low-density lipoprotein, triglyceride evaluated by chemical immunoassay method in patients and controls. Glycosylated hemoglobulin (HbA1c) was measured as a marker of glycemic control in diabetic patients. HbA1c levels were mathematically standardized to the Diabetes Control and Complications Trial reference range of 4.05-6.05% using multiple of the mean transformation. HbA1c was not measured in control group because there were no diabetic patients in control group. Thyroid peroxidase antibodies were defined as positive if higher than 40 U/mL (antibody titer). The diagnosis of Hashimoto thyroiditis was established by demonstrating high level of antithyroid peroxidase antibody levels. Tissue transglutaminase antibody level was analysed by enzyme-linked immunosorbent assay (ELISA). Celiac disease was suspected if tissue transglutaminase antibody level were higher than 15 U.

In this study, melatonin levels were detected with a sandwich enzyme-linked immonosorbent assay (ELISA). Melatonin was measured by DRG® Melatonin ELISA (EIA-1431) kit. The assay procedure follows the basic principle of competitive ELISA whereby there is competition between a biotinylated and a non-biotinylated antigen for a fixed number of antibody-binding sites. The amount of biotinylated antigen bound to the antibody is inversely proportional to the analyzed concentration of the sample.

Statistical analysis

Analysis was performed using SPSS version 13 software for Windows. Data are reported as means ± SD (range). The differences between groups were tested by the *t*-test for independent samples with normal data distribution or by the Mann-Whitney non-parametric test. $P < 0.05$ value was regarded as statistically significant.

RESULTS

The male/female ratio was 18/22 in patients. The mean diabetic duration was 2.89 ± 2.69 years and the mean age of diabetes was 10.49 ± 3.23 years. There was no obesity, hyperlipidemia, hypertension, thyroid dysfunction, anemia, and infection in diabetic patients.

Demographic and clinical characteristic of the diabetic patients are shown in Table 1. The male/female ratio was 17/13 in control subjects. The mean age of controls was 9.83 ± 4.36 years. There was no obesity, hypertension, hyperglycemia, hyperlipidemia, thyroid dysfunction, Hashimoto thyroiditis, celiac disease, anemia, and infection in controls. Demographic and clinical characteristic of the controls are also shown in Table 1.

There were no statistically significant differences in age, sex, BMI distribution between diabetic group and control group ($P > 0.05$). The variables were in the equation.

The serum level of melatonin was compared between diabetic group and control group. Mean melatonin level in diabetic group was 6.75 ± 3.52 pg/ml and mean melatonin level in control group was 11.51 ± 4.74 pg/ml. Melatonin levels were significantly lower in diabetic group compared to controls ($P < 0.01$) [Table 1].

Table 1: Characteristics of the patients and controls in the study population

	Diabetic group	Control group
N	40	30
Age	10.49±3.23	9.83±4.36
Sex (M/F)	18/22	17/13
BMI (kg/m²)	18.31±3.67	18.06±3.14
Total cholesterol (mg/dl)	144.87±33.23	135.40±27.51
Hemoglobin (g/dl)	13.75±1.08	13.11±1.05
HbA1c (%)	8.78±1.93	-
Diabetic duration (years)	2.89±2.69	-
Melatonin (pg/ml)	6.75±3.52*	11.51±4.74*

Data are expressed as mean±SD or median (interquartile range), and *Significant at *P*<0.05

Discussion

Type 1 diabetes mellitus is a chronic and serious metabolic disease. The present study looked for clues for preventing complications related with T1DM by investigating melatonin level in these patients. Melatonin is a neurotransmitter secreted predominantly by the pineal gland. There are extra-pineal sites of melatonin production, such as the retina, skin, bone marrow, lymphocytes and the gut, from where it may influence other physiological functions.[7] Melatonin is synthesized from its precursor, the essential amino acid tryptophan (TRP) in a series of four enzymatic steps.[8] It is quickly released into the blood and begins circulation. Melatonin concentration can be measured in other body fluids as saliva and urine. The rate of melatonin formation depends on the activity of enzymes-arylalkylamine-N-acetyl transferase and to a lesser extent, tryptophan hydroxylase. If intake of TRP is severely restricted, synthesis of melatonin is significantly reduced in humans.[9] Several vitamins and minerals like folate, B6 vitamin, and B12 vitamin act as co-factors in these processes.[10]

The regulating system for the secretion of melatonin is complex. It is controlled by both an endogenous circadian clock and by environmental light. Light is the most influential environmental factor. Pineal melatonin levels begin increasing in the late evening, reaching the maximum in the early hours between 2:00 and 4:00 a.m., followed by a slow decline to lower daytime levels. Endogenous nocturnal melatonin production has been estimated to be about 10-80 μg per night, daytime production being significantly less. Circadian frequency implies that one repetition occurs every 24 hours. In humans, the following physiological variables attain peak levels during sleep: TSH, prolactin, melatonin, ACTH, FSH, LH, cortisol, and lymphocyte and eosinophil counts.[11] Circadian rhythms are generated by suprachiasmatic nucleus in the hypothalamus and are influenced by a variety of factors. These include sleep, cyclical hormone secretion and daily rhythms of core body temperature.[12] Disruption of the circadian rhythm and sleep-wake cycles are considered risk factors for a variety of health problems including obesity and cardiovascular disease. Several preclinical studies have identified dietary components, such as glucose, sodium, ethanol, or caffeine being capable of phase-shifting circadian rhythms by modifying the expression of genetic components of the biological clock, i.e. clock genes.[10,12,13] We estimate that alterations in metabolism like T1DM are able to entrain physiological clocks, resulting in changes to the rhythms.

This study showed that melatonin levels were significantly lower in diabetic group compared to controls. There was no any metabolic problem other than type 1 diabetes mellitus in patients. Blood samples were collected after overnight fasting. Even though melatonin levels were not at peak level and single blood sample in the morning may be insufficient to estimate total melatonin levels over a 24-hour period the conditions were the same for diabetic group and control group. Despite these limitations, we believe that the results of this study were significant. Lower melatonin level in diabetic group may be related with insulin treatment. Melatonin-insulin antagonism is well documented.[14,15] Melatonin influences insulin secretion mediated by G-protein-coupled melatonin receptor isoforms. Investigations showed that hyperinsulinemic Goto-Kakizaki rats, which are a rat model of type 2 diabetic rats, and humans have decreased melatonin plasma levels, whereas a streptozotocin-induced rat model of diabetes developed reduced insulin levels combined with increased melatonin levels. This finding is supported by the other study in Goto-Kakizaki rats that an increase of plasma insulin was combined with a decrease of plasma noradrenaline (norepinephrine), the most important activator of melatonin synthesis.[16]

Melatonin is anabolic hormone like insulin and it relates to the promotion of restorative or anabolic physiological processes. In humans, elevated melatonin levels have been associated with reduced core temperature, increased heat loss, decreased cardiovascular output, reduced alertness, and enhanced immune responsiveness; therefore, insulin treatment may affect melatonin secretion.

Exogenous melatonin has been used for the treatment of sleep disorders of circadian origin such as jet lag and delayed sleep phase syndrome and as a complement of other therapeutic drugs for the treatment of numerous diseases including glaucoma, irritable bowel disease, and certain types of cancers mainly to either enhance the therapeutic effect of conventional drug therapy or to reduce their toxicity thus ameliorating the side-effects.[17-19] In addition to melatonin's role as an endogenous synchronizer, growing evidence suggests its anti-oxidative activity as well as its having a role in modulating immune responses.

It is well known that oxidative stress is thought to be involved in both development of T1DM and its further complications. Poor glycemic control is frequently seen problem in T1DM.[20] Melatonin is a major scavenger of both oxygen and nitrogen-based radicals.[21] Melatonin has scavenging actions at both physiologic and pharmacologic concentrations. Melatonin defends cells against the hazards of hyperglycemia. Melatonin protects pancreatic β-cells and several diabetes-affected organs including kidney, brain, retina and vasculature system from the associated nitro-oxidative stress.[22] This preliminary study raises the

possibility that exogenous melatonin treatment may play role in preventing process of inflammation and oxidative stress patients with T1DM because melatonin levels were significantly lower in diabetic group. Our hypothesis must be supported by new studies.

References

1.   Zawilska JB, Skene DJ, Arendt J. Physiology and pharmacology of melatonin in relation to biological rhythms. Pharmacol Rep 2009;61:383-410.
2.   Mediavilla MD, Sanches-Barcelo EJ, Tan DX, Manchester L, Reiter RJ. Basic mechanisms involved in anti-cancer effects of melatonin. Curr Med Chem 2010;17:4462-81.
3.   Grant SG, Melan MA, Latimer JJ, Witt-Enderby PA. Melatonin and breast cancer: Cellular mechanisms, clinical studies and future perspectives. Expert Rev Mol Med 2009;11:e5.
4.   Vehik K, Hamman RF, Lezotte D, Norris JM, Klingensmith G, Bloch C, *et al*. Increasing incidence of type 1 diabetes in 0-to 17-year-old Colorado youth. Diabetes Care 2007;30:503-9.
5.   Fourlanos S, Harrison LC, Colman PG. The accelerator hypothesis and increasing incidence of type 1 diabetes. Curr Opin Endocrinol Diabetes Obes 2008;15:321-5.
6.   Harjutsalo V, Sjöberg L, Tuomilehto J. Time trends in the incidence of type 1 diabetes in Finnish children: A cohort study. Lancet 2008;371:1777-82.
7.   Pandi-Perumal SR, Srinivasan V, Maestroni GJ, Cardinali DP, Poeggeler B, Hardeland R. Melatonin: Nature's most versatile biological signal? FEBS J 2006;273:2813-38.
8.   Malhotra S, Sawhney G, Pandhi P. The therapeutic potential of melatonin: A review of the science. MedGenMed 2004;6:46.
9.   Zimmermann RC, McDougle CJ, Schumacher M, Olcese J, Mason JW, Heninger GR, *et al*. Effects of acute tryptophan depletion on nocturnal melatonin secretion in humans. J Clin Endocrinol Metab 1993;76:1160-4.
10.   Peuhkuri K, Sihvola N, Korpela R. Dietary factors and fluctuating levels of melatonin. Food Nutr Res 2012;56.
11.   Burns RE, Sateia MJ, Lee-Chiong TL. Basic principles of chronobiology and disorders of circadian sleep-wake rhythm. In: Lee-Chiong TL, Sateia MJ, Carskadon MA, editors. Sleep Medicine. 1st ed. Philadephia: Hanley and Belfus, Inc; 2002. p. 245-52.
12.   Boivin DB, James FO. Insomnia due to circadian rhythm disturbances. In: Szuba MP, Kloss JD, Dinges DF, editors. Insomnia: Principles and Management. 1st ed. Cambridge; 2003. p. 155-91.
13.   Sherman H, Gutman R, Chapnik N, Meylan J, le Coutre J, Froy O. Caffeine alters circadian rhythms and expression of disease and metabolic markers. Int J Biochem Cell Biol 2011;43:829-38.
14.   Hirota T, Okano T, Kokame K, Shirotani-Ikejima H, Miyata T, Fukada Y. Glucose down-regulates Per1 and Per2 mRNA levels and induces circadian gene expression in cultured Rat-1 fibroblasts. J Biol Chem 2002;277:44244-51.
15.   Peschke E, Schucht H, Mühlbauer E. Long-term enteral administration of melatonin reduces plasma insulin and increases expression of pineal insulin receptors in both Wistar and type 2-diabetic Goto-Kakizaki rats. J Pineal Res 2010;49:373-81.
16.   Wang JP, Liu IM, Tzeng TF, Cheng JT. Decrease in catechol-O-methyltransferase activity in the liver of streptozotocin-induced diabetic rats. Clin Exp Pharmol Physiol 2002;29:419-22.
17.   van Geijlswijk IM, Korzilius HP, Smits MG. The use of exogenous melatonin in delayed sleep phase disorder: A meta-analysis. Sleep 2010;33:1605-14.
18.   Mills E, Wu P, Seely D, Guyatt G. Melatonin in the treatment of cancer: A systematic review of randomized controlled trials and meta-analysis. J Pineal Res 2005;39:360-6.
19.   Sánchez-Barceló EJ, Mediavilla MD, Tan DX, Reiter RJ. Clinical uses of melatonin: Evaluation of human trials. Curr Med Chem 2010;17:2070-95.
20.   Kim H, Elmi A, Henderson CL, Cogen FR, Kaplowitz PB. Characteristics of children with type 1 diabetes and persistent suboptimal glycemic control. J Clin Res Pediatr Endocrinol 2012;4:82-8.
21.   Tan DX, Manchester LC, Terron MP, Flores LJ, Reiter RJ. One molecule, many derivatives: A never-ending interaction of melatonin with reactive oxygen and nitrogen species? J Pineal Res 2007;42:28-42.
22.   Korkmaz A, Ma S, Topal T, Rosales-Corral S, Tan DX, Reiter RJ. Glucose: A vital toxin and potential utility of melatonin in protecting against the diabetic state. Mol Cell Endocrinol 2012;349:128-37.