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Original article

Evaluation of human pineal gland acetylserotonin O-methyltransferase immunoreactivity in suicide: A preliminary study

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Abstract

Disorders of the serotonergic system are especially known to be present in the neurobiology of suicidal behavior. Studies investigating melatonin levels show that changes in pineal gland functions may also play a role in the pathogenesis of suicide. However, to our knowledge, there are no studies evaluating the activity of pinealocytes responsible for melatonin synthesis in suicide. This preliminary study aimed to investigate the relationship among pinealocyte, acetylserotonin O-methyltransferase (ASMT) immunoreactivity, and suicide. Samples of pineal gland, cerebrospinal fluid, blood, and urine were obtained from 21 suicide and 21 non-suicide cases on which medicolegal autopsies were performed. Expression of ASMT in human pineal gland was evaluated by immunohistochemical methods. A scoring system was used to define the anti-ASMT-positive staining in the sections. Enzyme-linked immunosorbent assays were employed to assess serum and cerebrospinal fluid melatonin levels and blood and urine noradrenaline levels. The ASMT-immunopositive pinealocyte count was observed to be lower in suicide cases compared to the non-suicide cases. With the exception of two cases (with moderate staining), all graded scores were 3 (strong staining) in non-suicide group, whereas scores were 1 (mild staining) or 2 (moderate staining) in the suicide group. Melatonin levels in the blood were lower among the suicide victims. These results support decreased pineal gland activity in suicide. However, further studies are needed to assess whether these changes are related to a psychiatric disorder.

Keywords

Suicide, autopsy, human pineal gland, pinealocyte, acetylserotonin O-methyltransferase, melatonin

Introduction

Suicide is a self-harming behavior, which constitutes a substantially important contemporary social issue. Because it is a complex behavior comprising social psychological and biological factors, there have been many studies conducted on suicide that aim for enlightenment on its etiopathogenesis and enable its prevention. In studies investigating neurobiological factors, the serotonergic, dopaminergic, and noradrenergic systems have been shown to be related to suicidal behavior. For example, serotonin (5-HT) and its metabolites in the brain have been observed to be decreased in suicide victims[1]; and melatonin levels have been evaluated in studies investigating its relation to the pineal gland.[2–4]

The pineal gland is an important neuroendocrine organ. It develops embryologically from the upper portion of the third ventricle (prosencephalon) and remains attached from a pedicle to the roof of the ventricle. Most of its cells are of a specific type, known as

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pinealocytes.[5] The main hormone they secrete is melatonin. Serotonin is the precursor, and noradrenalin is the positive regulator substance in melatonin synthesis. When impulses especially related to darkness reach the pineal gland, they bind to the adrenergic receptors in the noradrenalin pinealocyte membrane and initiate a series of reactions that lead to the activation of enzymes enabling melatonin synthesis. Melatonin results from the serotonin *n*-acetylation via the catalyzation of enzymes aralkylamine *n*-acetyltransferase and acetylserotonin O-methyltransferase (ASMT), also known as hydroxyindole-O-methyltransferase.[6] Once synthesized, melatonin is not stored in the pineal gland, but secreted into all bodily fluids. Therefore there is a strong correlation between pineal gland melatonin and plasma melatonin levels.[7] During the night, melatonin synthesis increases in relation to increased aralkylamine *n*-acetyltransferase activity. No significant changes are observed in ASMT activity during the 24-h period.[8] ASMT is a marker especially used to evaluate pinealocyte immunoreactivity in colocalization studies.[8]

Alongside studies that report increases in melatonin levels in suicide victims there are also studies that report decreases in melatonin levels.[2–4] Apart from these, to our knowledge, there are no studies that investigate pinealocyte immunoreactivity in victims of suicide.

The present preliminary study was carried out with the aim of evaluating the relationship between pinealocyte ASMT-immunoreactivity and suicide.

Materials and Methods

Subjects

This study was initiated following the granting of approval number B.30.2.PAÜ.0.20.05.09/155 by the Clinical Trials Ethics Committee, Faculty of Medicine, Pamukkale University. The study included a total of 42 cases, comprising 21 non-suicide and 21 suicide cases, upon which medicolegal autopsies were performed at the Department of Forensic Medicine, Faculty of Medicine, Pamukkale University. Due to increased calcification of the pineal gland in older-aged individuals, the study was limited to cases within the range of 18–40 years.[9,10] Information was collected in regard to the age, gender, cause of death, time of death, and psychiatric history of the cases. Cases in which the cause of death resulted from medical intoxication or head trauma, and those who had a history of ongoing psychiatric treatment, were excluded.

Collection and conservation of samples

In all autopsy cases the skull was opened, and dura mater was lifted. After the corpus callosum was dissected, cerebrospinal fluid (CSF) was obtained from lateral ventricles via injection and placed in a Falcon tube (Corning, Corning, NY). The brain was removed and dissected horizontally, then the pineal pedicle on the roof of the third ventricle was dissected and the pineal gland was lifted. It was fixed with neutral formalin buffered with 10% phosphate. Blood obtained from the femoral artery was placed in ethylenediaminetetra-acetic acid tubes and centrifuged for 10 min at 3000 rpm. Urine was obtained by directly opening the bladder during autopsy, and the urine was collected in urine tubes. CSF, blood, and urine were kept at –80°C until analyses were carried out.

Human melatonin and noradrenaline enzyme-linked immunosorbent assay

A human melatonin kit was applied to serum and cerebrospinal fluid, and a human noradrenaline kit was applied to serum and urine samples taken from the suicide and the non-suicide groups. These tests were applied with the sandwich-based enzyme-linked immunosorbent assay method. Melatonin and noradrenaline protein levels were quantified using a Human Melatonin and Noradrenaline Kit (Cusabio Biotech, China; http://www.cusabio.com/). The procedure was carried out as described in the protocol provided by the manufacturer. Absorbance was measured at 450 nm using a Microplate Reader (BioTek Instruments, Winooski, VT). The melatonin and noradrenaline protein concentration in each well was calculated using the equation of the Melatonin and Noradrenaline protein standard.

Histomorphological evaluation

All histomorphological analyses described below were performed by two investigators blind to the cause of death. For histological examination, the tissue samples were fixed in 10% formalin in phosphate buffer for 3 d. Afterwards, the tissues were processed via routine histological methods and embedded in paraffin blocks. Paraffin blocks were placed in a rotary microtome (RM 2255; Leica Instruments, Nußloch, Germany), and sections of 5 μm thickness were obtained with disposable metal microtome blades (Type N35; Feather Company, Osaka, Japan).

Immunohistochemical assessment of ASMT expression

ASMT immunohistochemistry was applied to the paraffin sections. After deparaffinization and rehydration, sections were then treated with 10 mM citrate buffer (pH 6; Cat. No. AP-9003-125; Lab Vision, Thermo Fisher Scientific, Waltham, MA) in a microwave oven for 5 min. Then sections were incubated in a solution of 3% H_2O_2 for 5 min at room temperature to inhibit endogenous peroxidase activity. Sections were incubated with normal serum blocking solution at 37°C for 30 min. The sections were then incubated in a humid chamber overnight at +4°C with an anti-ASMT antibody (1:100; AP4790c; Abgent, San Diego, CA). The sections were incubated with biotinylated IgG and then with streptavidin for 30 min each, prepared according to the kit instructions (İnvitrogen-Plus Broad Spectrum 85–9043; Invitrogen, Carlsbad, CA). Sections were finally stained with DAB (Roche Diagnostics, Mannheim, Germany) and counterstained with Mayer hematoxylin (Sigma-Aldrich, St. Louis, MO) and analyzed with a light microscope.[11]

Semiquantification of immunostaining data

A scoring system was used to define the anti-ASMT-positive staining in the sections. The score was defined as: 0, no staining; 1, mild staining; 2, moderate staining; and 3, strong staining. Each section was graded by two investigators who were blinded to cause of death, and the average was taken.

Image analysis methods

Images were analyzed using a computer assisted image analyzer system consisting of a microscope (Model No. BX-51, Olympus, Tokyo Japan) equipped with a high-resolution video camera (Model No. DP-71; Olympus). All sections were digitally photographed.

Statistical analysis

SPSS Version 16.0 for Windows (IBM Analytics; https://www.ibm.com/analytics/) was used for statistical analyses. Continuous variables were presented as mean ± SD. The independent samples test was used when parametric test assumptions were used to compare independent group differences, whereas the Mann–Whitney U-test was used when parametric test assumptions were not provided. Pearson correlation analysis was used when the relations between numerical variables were examined; $p < 0.05$ was considered as statistically significant.

Results

The suicide cases were 15 males and 6 females with an average age of 34.2 ± 7.8 years (minimum: 18; maximum: 40). The non-suicide cases were 16 males and 5 females with an average age of 30.9 ± 5.7 (min: 20; max: 40). The suicide method in 17 of the cases was by hanging, and in the remaining four cases by gunshot wound. In the non-suicide group, cause of death was natural death in 12 of the cases, injury by sharp object in four of the cases, gunshot wound in three, and electric shock in two of the cases. Seven of the suicide victims had a diagnosis of depression but had dropped out of treatment. Four of these had a history of prior suicide attempts. The medical history of non-suicide cases did not include any psychiatric diagnosis. The postmortem interval ranged from 3 to 24 h.

Anti-ASMT immune positive pinealocytes were presented in Figure 1. In the suicide group, expression of ASMT was significantly decreased compared to the non-suicide group ($p = 0.000$). With the exception of two cases (with moderate staining), all graded scores were 3 (strong staining) in the non-suicide group, but in the suicide group, graded scores were either 1 (mild staining) or 2 (moderate staining). The means of

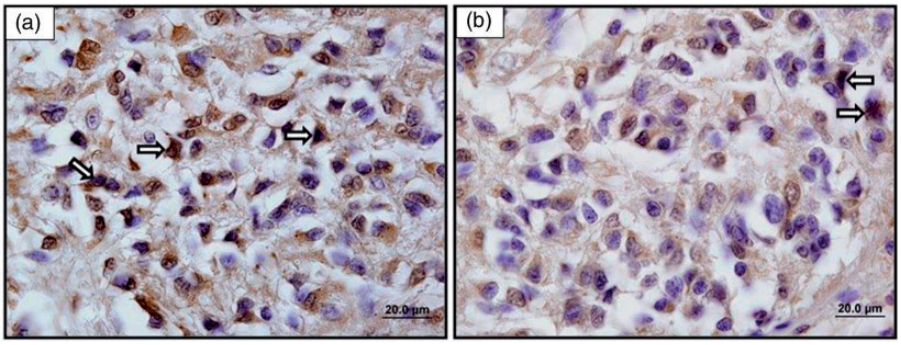


Figure 1. Representative light-microscopy images of anti-ASMT immunoreactivity in pineal tissue.
(a) Non-suicide group; (b) suicide group. Anti-ASMT immunoreactivity was decreased in non-suicide group when compared to control group. Arrows (→) indicate anti-ASMT immunoreactivity.

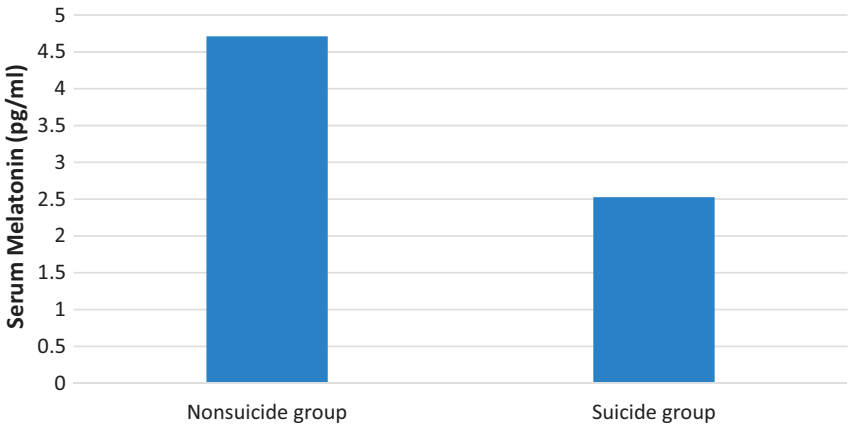


Figure 2. Serum melatonin concentrations in suicide and non-suicide groups. $p < 0.05$.

scoring in the suicide group was 1.7 ± 0.57, and in the non-suicide group it was 2.78 ± 0.42.

Average serum melatonin levels were measured to be 2.52 ± 2.77 pg/mL in suicide cases and 4.71 ± 1.75 pg/mL in non-suicide cases. The decrease in serum melatonin levels among suicide cases was found to be statistically significant ($p < 0.05$) (Figure 2). No statistically significant difference was found between CSF melatonin levels in the suicide group (mean $\pm$ SD: 3.48 ± 2.21 pg/mL) and in the non-suicide group (mean $\pm$ SD: 3.80 ± 2.22; $p > 0.05$). No statistically significant differences were observed between the groups in respect to serum and urine noradrenaline levels. There was no correlation between serum melatonin levels and postmortem interval.

Discussion

In this preliminary study, in which we investigated ASMT immunoreactivity in the postmortem pineal gland of suicide victims, the immunopositive pinealocyte count was observed to be lower in the suicide group compared to the non-suicide group. Furthermore, melatonin levels in blood were lower among suicide victims. There are other studies that investigated melatonin levels in relation to the pineal gland in suicide victims. In one study, an increase was detected in melatonin levels, and lobular disposition in pinealocytes, arborization in connective tissues and glia, reduction in cell nucleus volumes, and non-specific variations such as irregular distribution of astrocytes and "acervulina" formations have been defined. These morphological variations have been reported to be possible markers of pineal gland functional activation in the presuicidal period .[3] On the contrary, in another study a decrease was found in pineal gland melatonin levels among suicide victims.[2] Sandkyr and Awerbuch[4] have detected low melatonin in suicide attempts in multiple sclerosis patients and have reported implicating pineal gland in the pathogenesis of suicidality in multiple sclerosis. There are also studies in the literature reporting that blood melatonin levels are reduced in psychiatric disorders such as depression and mood disorders in which the suicide risk is high.[12,13]

Decreases in melatonin levels in suicidal cases may be related to changes in melatonin synthesis. Melatonin is created via the conversion of tryptophan to serotonin through hydroxylation and decarboxylation, and the *n*-acetylation of serotonin. The last step of this process is catalyzed by ASMT. ASMT is a key enzyme in melatonin biosynthesis and its activity is not affected by diurnal rhythm.[8,14] It has been shown that this enzyme, which is located at the last step of the melatonin biosynthesis, may be associated with diseases such as depression and mood disorders that themselves have high risk of suicide.[15,16] Galecki et al.[16] showed that the ASMT-gene polymorphism affects the expression of the enzyme and a decrease in ASMT mRNA expression in depressed patients. They reported that this gene may be the sensitivity gene for recurrent depressive disorders. Melke et al.[17] showed a positive correlation between ASMT polymorphism (rs4446909) and ASMT enzyme activity and melatonin concentration. However, pineal gland ASMT activity has not been previously studied in completed suicides. The difference of our study from the others is to show that human pineal gland ASMT immunoreactivity is decreased in completed suicides, and that it may affect melatonin levels. This study findings support a change in pineal gland activity in suicide.

Limitations

The relatively low number of cases in our study is a limiting factor. Only seven of the suicide cases had

psychiatric disease diagnosed before the death. Another limiting factor is that the suicide group is not divided according to different psychiatric diagnosis groups.

Conclusion

Medicolegal evaluation of suicide cases comprises a compound of many parameters, including investigation of the suicide scene, medicolegal autopsy, witness testaments, suicide notes, and additional criminalistic investigations. No matter how difficult it may seem for the evaluation of data obtained in our study to become a routine postmortem examination, we consider it to be contributory in the medicolegal domain as a supportive factor along with the above-mentioned parameters.

In different types of clinical studies, melatonergic system functions are investigated to illuminate the etiology of depression. However, there is not a sufficient amount of research on this subject in completed suicide cases. In this preliminary study, the evaluation of pineal gland function in completed suicide cases revealed a decline in ASMT enzyme activity in melatonin synthesis, and it was shown that pinealocyte function disorder (and, therefore, melatonergic system dysfunction) may be related to suicide inclination, and can be considered to be one of the neurobiological risk factors in completed suicide. Nonetheless, we believe further research is needed on this topic, and our findings may contribute to future studies.

Author contributions

AKD, GND, YD, and KA contributed to the conception and design of the study. AKD, KA, and BB collected biological materials. YD analyzed the melatonin and noradrenaline with an enzyme-linked immunosorbent assay. SO, UC, GND, and EA performed histological examination of the tissue. AKD, GND, YD, KA, SO, and UC wrote the first draft of the manuscript. AKD, GND, and KA drafted the article, which was revised critically and approved for publication by all authors.

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References

1. Mann JJ. Neurobiology of suicidal behavior. *Nat Rev Neurosci* 2003; 4: 819–828.
2. Stanley M and Brown GM. Melatonin levels are reduced in the pineal glands of suicide victims. *Psychopharmacol Bull* 1988; 24: 484–488.
3. Pacchierotti C, Iapichino S, Bossini L, et al. Melatonin in psychiatric disorders: a review on the melatonin involvement in psychiatry. *Front Neuroendocrinol* 2001; 22: 18–32.
4. Sandyk R and Awerbuch GI. Nocturnal melatonin secretion in suicidal patients with multiple sclerosis. *Int J Neurosci* 1993; 71: 173–182.
5. Kuehnel W. *Color atlas of cytology, histology, and microscopic anatomy*. New York & Stuttgart: *Thieme Medical Publishers*, 2003, p.256.
6. Zawilska JB, Skene DJ and Arendt J. Physiology and pharmacology of melatonin in relation to biological rhythms. *Pharmacol Rep* 2009; 61: 383–410.
7. Macchi MM and Bruce JN. Human pineal physiology and functional significance of melatonin. *Front Neuroendocrinol* 2004; 25: 177–195.
8. Rath MF, Coon SL, Amaral FG, et al. Melatonin synthesis: acetylserotonin O-methyltransferase (ASMT) is strongly expressed in a subpopulation of pinealocytes in the male rat pineal gland. *Endocrinology* 2016; 157: 2028–2040.
9. Kunz D, Schmitz S, Mahlberg R, et al. A new concept for melatonin deficit: on pineal calcification and melatonin excretion. *Neuropsychopharmacology* 1999; 21: 765–772.
10. Humbert W and Pevet P. The pineal gland of the aging rat: calcium localization and variation in the number of pinealocytes. *J Pineal Res* 1995; 18: 32–40.
11. Kumral A, Tuzun F, Ozbal S, et al. Lipopolysaccharide-preconditioning protects against endotoxin-induced white matter injury in the neonatal rat brain. *Brain Res* 2012; 1489: 81–89.
12. Brown R, Kocsis JH, Caroff S, et al. Differences in nocturnal melatonin secretion between melancholic depressed patients and control subjects. *Am J Psychiatry* 2006; 142: 811–816.
13. Venkoba RA, Parvathi DS, Kota RT, et al. Depression, cognitive disorder and melatonin profile. *Ann Nat Acad Med Sci (India)* 1988; 25: 41–49.

14. Ackermann K, Bux R, Rüb U, et al. Characterization of human melatonin synthesis using autoptic pineal tissue. *Endocrinology* 2006; 147: 3235–3242.
15. Etain B, Dumaine A, Bellivier F, et al. Genetic and functional abnormalities of the melatonin biosynthesis pathway in patients with bipolar disorder. *Hum Mol Genet* 2012; 21: 4030–4037.
16. Gałecki P, Szemraj J, Bartosz G, et al. Single-nucleotide polymorphisms and mRNA expression for melatonin synthesis rate-limiting enzyme in recurrent depressive disorder. *J Pineal Res* 2010; 48: 311–317.
17. Melke J, Goubran Botros H, Chaste P, et al. Abnormal melatonin synthesis in autism spectrum disorders. *Mol Psychiatry* 2008; 13: 90–98.