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Olfactory Bulb Lesion Induced Pineal Gland Calcification: a First Experimental Study

Olfaktör Bulbus Lezyonu ile İndüklenmiş Pineal Gland Kalsifikasyonu: İlk Deneysel Çalışma

Mehmet Kürşat KARADAĞ [1]
Atatürk University, Medical Faculty, Department of Neurosurgery Erzurum, Türkiye

Mehmet Dumlu AYDIN [1]
Atatürk University, Medical Faculty, Department of Neurosurgery, Erzurum, Türkiye

ABSTRACT

Objectives: There is no enough stereological study that seriously mentions maternal olfactory disorders among the causes of pineal gland insults. This study sought to examine the connection between lesions caused by olfactory bulbectomy and changes in the pineal gland in rats
Methods: A total of 24 male rats were used in the experiment, 5 control, 6 SHAM and 13 study. After a 1 mm burrhole was opened in the midfrontal-interpupillary line for the subjects in the SHAM and study groups, only the olfactory dura was opened in the SHAM group, while the olfactory bulbs were crushed in study group. They were sacrificed after a two-month follow-up. The number of calcified pineal cells (n/mm^3) determined. Results analyzed with the Mann-Witney U test.
Results: The mean calcified pineal cells numbers (n/mm^3) were measured as per cubic milimeters as: (7±2) x10^3/mm^3) in control (Group I); (15±3) x10^3 in SHAM (Group II); (34±9) x10^3 in study group (Group III). *P*<.005/(GI-GII); *P*<.0005/(GII-GIII); *P*<.00001/(GI-GIII).
Conclusions: Olfactory bulb lesion may be responsible for pineal gland calcification. Pathologies in the pineal gland resulting from olfactory bulbectomy may contribute to various endocrine, immune, and reproductive disorders with unclear etiologies.

Keywords: Olfactory bulb lesion, pineal gland, calcification

ÖZ

Amaç: Pineal bez hastalıklarının nedenleri arasında maternal koku bozukluklarından ciddi şekilde bahseden yeterli stereolojik çalışma bulunmamaktadır. Bu çalışma, olfaktör bulbektominin neden olduğu lezyonlar ile sıçanlarda pineal bezdeki değişiklikler arasındaki bağlantıyı incelemeyi amaçlamıştır.
Yöntem: Bu çalışmada 5 kontrol, 6 Sham ve 13 çalışma olmak üzere toplam 24 erkek sıçan kullanıldı. Sham ve çalışma grupları için midfrontal-interpupiller çizgide 1 mm'lik bir burrhole açıldıktan sonra, Sham grubunda sadece olfaktor dura açıldı, çalışma grubunda ise olfaktor bulbuslar hasarlandırılmıştır. İki aylık takipten sonra sıçanlar öldürüldüler. Kalsifiye edilmiş pineal hücre sayısı (n/mm3) belirlendi. Sonuçlar Mann-Witney U testi ile analiz edildi.
Bulgular: Ortalama kalsifiye pineal hücre sayısı (n/mm^3) milimetre küp başına şu şekilde ölçüldü: Kontrolde (Grup I) (7±2) x10^3/mm^3); SHAM'da (Grup II) (15±3) x10^3); (34±9) x10^3 çalışma grubunda (Grup III). *P*<.005/(GI-GII); *P*<.0005/(GII-GIII); *P*<.00001/(GI-GIII).
Sonuç: Olfaktör bulbus lezyonu pineal bez kalsifikasyonundan sorumlu olabilir. Olfaktor bulbektomi sonucu pineal bezde oluşan patolojiler etiyolojisi tam olarak bilinmeyen çeşitli endokrin, immün ve üreme bozukluklarına yol açabilir.

Anahtar Kelimeler: Olfaktör bulbus lezyonu, pineal bez, kalsifikasyon

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Sorumlu Yazar/Corresponding author:
Mehmet Kürşat KARADAĞ
E-mail: drkursatkaradag@gmail.com
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INTRODUCTION

The pineal gland, a small endocrine structure situated in the midline of the brain above the thalamus, plays a crucial role in regulating circadian rhythms through the secretion of melatonin. Postnatally, the number of pineal gland cells increases progressively. [1] While a direct anatomical or functional link between the pineal gland and olfactory nerves has not been fully established, studies suggest that peripubertal olfactory bulbectomy may lead to pineal gland degeneration, a reduction in pinealocyte numbers, hypothalamo-hypophyseal disruptions, loss of prolactin-secreting cells, and abnormalities in prolactin secretion. Histopathological changes, including calcification of the pineal gland, have also been observed following olfactory or light deprivation. The olfacto-pineal pathways are implicated in the modulation of feeding, reproductive, and sexual behaviours. [2–7] The olfato-pineal pathways are crucial in the modulation of feeding, reproductive and sexual behaviour.

METHODS

Ethics committee approval was obtained from Ethics Committee for Animal Experiments, Medical Faculty, Ataturk University of Turkey (Date: 09.011.2022, Number: 2200369071). The study involved 24 female rats, divided into three groups: 5 controls, 6 SHAMs, and 13 in the experimental group. The rats were housed individually in metal cages at room temperature, maintained under a 12-hour light cycle with 50% relative humidity, and monitored by a veterinarian. They were provided with standard laboratory feed and water ad libitum. The study design and permissions received approval from the Ethics Committee for Animal Experiments at the Medical Faculty of Atatürk University, Turkey. The care and experimental procedures adhered strictly to the guidelines established by this ethics committee.

Under general anaesthesia, after opening a 1mm burrhole on the midfrontal-interpupillary line for SHAM and the subjects in the study group, the dura covering only the olfactory bulbs was incised, and the olfactory bulbs in the study group were additionally injured with a clamp. After 1 month, the subjects were taken to their cages and lived with fertile male rats for 2 months. After the 2-month follow-up of the new-born pups, Sacrification was performed following the intracardiac formalin injection under inhalation anaesthesia and they were dehydrated in 10% formalin solution in groups.

Histopathological Procedures

The olfactory nerves were removed along with the entire brain and fixed in a horizontal position, and 20 consecutive sections were taken at 10 micron intervals for glomerulus and olfactory fila examination. The same procedures were performed for the pineal gland. Preparations were stained with hematoxylin-eosin and Calcium Stains (Von Kossa Calcium Set). Olfactory nerve volumes were estimated using ellipsoid volume formulas and calcified cell numbers were estimated stereologically.

Stereological analysis

Sampling of 30 consecutive sections of 5 micrometres taken from the pineal gland and calcified cell density (n/mm^3) were analysed using the physical dissector method.

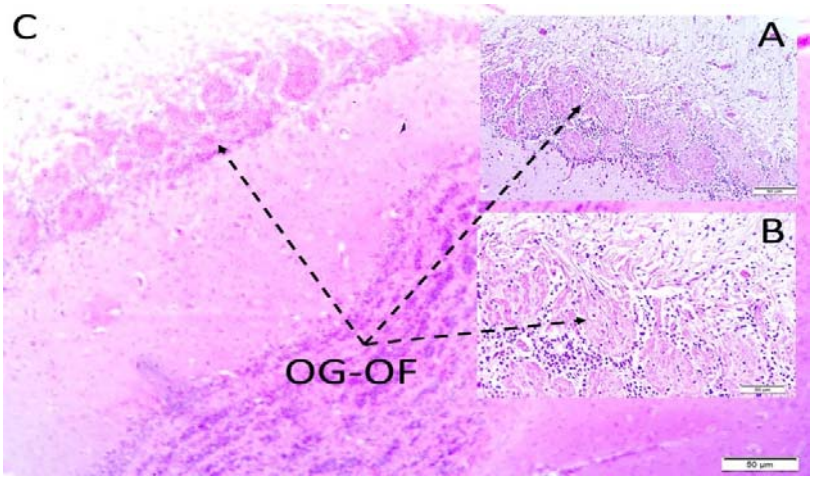

Figure-1: Normal olfactory glomeruli and normal ciliary processes (olfactory fila) were observed in the normal subject, while slightly small and atrophic olfactory glomeruli and reduced olfactory fila (OG-OF) were observed in the SHAM group, and severely atrophic olfactory glomeruli and greatly reduced olfactory fila were observed in the study group (LM, H&E, x20).

RESULTS

Clinical results: One animal in the study group (n=1) dead died within the seven days of surgery and they were changed new one. Neck stiffness, unconsciousness, convulsive attacks, fever, apnea, cardiac arrhythmia, and breathing disturbances were observed in animals. Two animals in the study group (n=2) and one animal in the SHAM group (n=1) were died within the seven days of surgery and they were switched with new ones. Neck stiffness, unconsciousness, convulsive attacks, fever, apnea, cardiac arrhythmia, and breathing disturbances were reported in dead animals. In control animals, the heart rate was 289±16/min, the respiratory rate was 32±6/min and the blood oxygen concentration was 93±7(%). OBX applied animals shown anosmia, memory loss.

Numerical results: The mean calcified pineal cells numbers (n/mm^3) were measured as per cubic millimetres as: (7±2)x10^3/mm^3) in control (Group I); (15±3)x10^3 in SHAM (Group II); (34±9)x10^3 in study group (Group III). *P*<.005/(GI-GII); *P*<.0005/(GII-GIII); *P*<.00001/(GI-GIII).

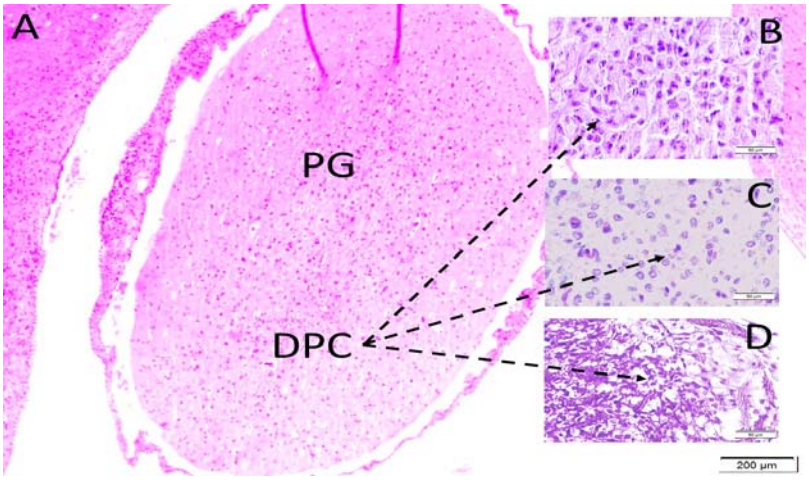

Figure-2: Normal pineal gland, normal and normal pineal cells are observed in the normal subject, slightly small, degenerated and atrophic pineal cells (DPC) in the SHAM group, and highly atrophic small and degenerated atrophic cells (DPC) in the study group (LM, H&E, x4/A; x20/B-D).

DISCUSSION

The number of pineal gland cell nuclei of rat continuously increased during post-natal life and differences between the lactation and after weaning periods were significant. It is possible that the supporting cells, fibres and new synapses are responsible for that PG late post-natal increase. [1] In the winter pineal gland sensitivity is increased. [8] Pineal denervation cause testicular regression. [9] Olfactory bulb lesions cause mammary gland and corpus luteum degeneration. [3,10] There are some indirect antitumor effects of olfactory bulbecrtomy on the suppression of growth of some prostatic tumour strains. [11]

Olfactory bulbectomy resulted in increased morning gonadotropin levels and ovarian weight in animals in reproduction phases in animals on a short photoperiod. [12] Olfactory bulbectomy leads to hypothalamo-hypohiseal insults in lactating animals. [4] The pineal gland causes mammotroph hypotrophy and hypoplasia in blind-anosmic female rats. [13] Olfactory bulbectomy prevents the testicular regression with the antigonadotropic effect of melatonin. [14] Olfactory bulbectomy have antigonadal actions. [15] Pineal inhibits prolactin synthesis, storage and release in both female and male blind-anosmic rats. [5] There is inhibitory effect of the pineal gland on the prolactin synthesis cells of blind-anosmic female rats following olfactory bulbectomy. [6] Photoperiodic control can be loss oin reproduction time in olfactory-bulbectomized rats. [2] Anosmic rats shows prolactin secretion abnormalities. [13] Anosmic male rats have an increased sensitivity to antigonadotrophic and prolactin -inhibitory effects of melatonin. [16] Olfactory deprivations have antigonadal actions. [15] Pubertal prolactin cell development inhibited in anosmic female rats. [17] Olfactory bulbectomy seems to be responsible for Onuf's nucleus degeneration. [18] Onuf's nucleus degeneration secondary to olfactory bulbectomy seems to be responsible for reduced sperm numbers. [19]

Olfactory bulbectomy may lead to mammary gland degeneration, intestinal immunodeficiency causing by olfaction loss induced denervation injury of Peyer's patches, diminished thyroid hormone secretion and serious behavioral, neurochemical, neuroendocrine, and neuroimmune alterations. [20–23]

Histopathological alterations occur in blind-anosmic female rats. [5] Peripubertal olfactory bulbectomy may lead to pineal gland degeneration. [17] Olfactory bulb lesions cause decreased pinealocyte numbers. [24] Pineal gland may have calcified following olfactory or light deprivation. [7] The pineal gland calcified in the aging rats. [25] Olfaction deficits creating COVID-19 may cause pineal gland dysfunction. [26] So, damage to the olfactory pathways may cause functional cytoarchitectural disorders in the pineal gland.

Limitation: This study does not include biochemical data.

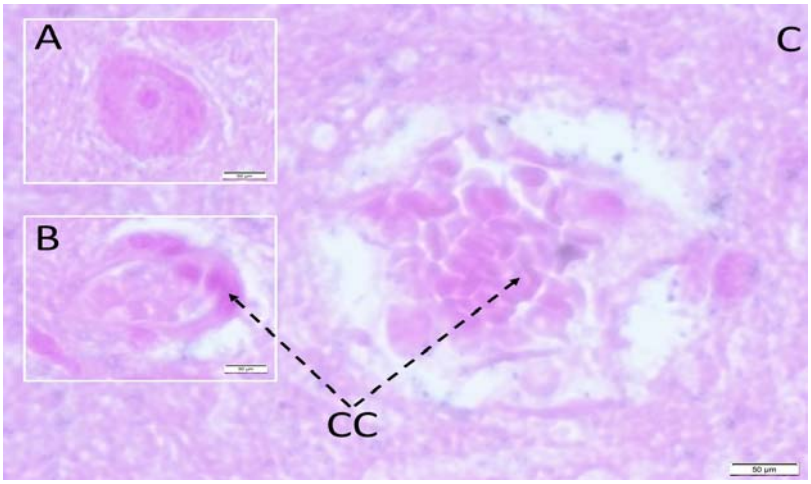

Figure-3: Normal pineal cells are observed in the normal subject, slightly calcified pineal cells in the SHAM group, and highly calcified pineal cells in the study group (LM, H&E, x20).

CONCLUSION

Therefore, various pathologies that cause damage to the olfactory pathways can cause functional histopathological changes in the pineal gland. Chemicals that damage the olfactory nerves should also be avoided.

Future Insights: Olfactory bulbectomy leading pineal gland pathologies may be responsible for many endocrine, immune and reproductive diseases with obscure etiology.

Ethics Committee Approval: Ethics committee approval was obtained from Ethics Committee for Animal Experiments, Medical Faculty, Ataturk University of Turkey (Date: 09.011.2022, Number: 2200369071)
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