

Effects of a Combined Oral Contraceptive: Histopathological Effects on the Thyroid Glands of Mice

*KOMBİNE BİR ORAL KONTRASEPTİF'İN ETKİLERİ:
FARE TİROİD BEZLERİ ÜZERİNDEKİ HİSTOPATOLOJİK ETKİLER*

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Summary

In this study, thyroid glands of female mice which were administered a combined oral contraceptive (COC) containing ethinyl estradiol+ethynadiol diacetate for one year were examined with a light microscope. The diameters of follicles were evaluated in the thyroid glands according to morphometric data in the treatment and the control groups. It was shown that the diameters of follicles were increased in the treatment group and it was correlated with histopathological findings and staining of colloid with H+E.

In conclusion, we observed some histopathological changes in thyroid glands of female mice which were administered COC for one year compared to the control group.

Key Words: Ethinyl estradiol, Ethynadiol diacetate, Thyroid glands, Oral contraceptives

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It is known that long-term use of oral contraceptives (OCs) can cause various side effects on endocrin functions (1,2). It has been reported that administration of estrogen leads to change in the binding capacity of TBG (thyroxin binding globulin) for T3 and T4 in the serum (3,4), to an increase in the histological activity of thyroid (5-7), to an increase in the weight of thyroid glands and in the height of thyroid epithelium (8); In addition, ad-

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Özet

Bu çalışmada, bir yıl süreyle ethinyl estradiol+ethynadiol diacetate içeren kombine bir oral kontraseptif(COC) verilen dişi farelerde tiroid bezleri ışık mikroskobu ile incelendi. Kontrol ve deney gruplarında tiroid bezlerinde folliküllerin çaplan morfometrik verilere göre değerlendirildi. Deney grubunda folliküllerin çaplarında artış olduğu ve bu artışın histopatolojik bulgular ve H+E ile kolloidin boyanması ile korelasyon gösterdiği gözlemlendi.

Sonuç olarak, bir yıl kombine oral kontraseptif verilen dişi farelerin tiroid bezlerinde kontrol grubuna göre bazı histolojik değişimler gözlemlendi.

Anahtar Kelimeler: Ethinyl estradiol, Ethynadiol diacetate, Tiroid bezleri, Oral kontraseptifler

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ministration of progesteron leads to a rise in the plasma FT3 (free T3) index (5). It has been suggested that low-dose OCs cause a significant elevation in TBG, TBI (thyroxin binding iodine), serum T4 (40%), FT4 (15-22%), serum T3 (17-28%) levels and a slight and effective stimulation of thyroid function (7-12). However in another study it has been suggested that there were no changes in the metabolic and endocrine functions in women taking OCs with a medium-dose for 6-12 month (13). In the present study, we have planned to evaluate histopathological changes in the thyroid glands of female mice administered ethinyl estradiol (EE)+ethynadioldiacetate for one year, in order to explain the functional changes with regard to morphological aspects of thyroid glands.

Materials and Methods

Forty albino female mice were used in this study. Mice (control group involving 10 and treatment group 30 mice) were kept in small groups in the laboratory under standard conditions and fed standard diet ad libitum. Mice in the treatment group were exposed to one year oral administration of low-dose combined oral contraceptive (COC) containing ethinyl estradiol (33 ug/kg) and ethynodioldiacetate (666 u.g/kg) (11,14). The mice were killed by decapitation and thyroid glands were quickly dissected out, fixed in 10% buffered formaldehyde solution and processed for histologic study. Five um thick paraffin sections were stained with Haematoxylin+Eosin (H+E) (15). Differences of histopathological findings were evaluated between control and treatment groups by "Fischer's X²-Test". Total thyroid lobe and central and peripheral follicles were measured in a microscope fitted with a scale in the eyepiece according to direct optical measurements as a classical method for small particles (16), and statistical significance of difference between control and treatment groups was tested by "Mann-Whitney U-Wilcoxon sum W Test". The differences were considered significant if the P values were lower than 0.05(17).

Results

a) Control group. The mouse thyroid gland was encapsulated by a moderately thick layer of connective tissue. Collagenous fibres were also found in the inter-follicular spaces. Generally two types of follicles were found. Peripheral follicles were larger than the central types. Furthermore, these follicles had small cuboidal cells and large lu-

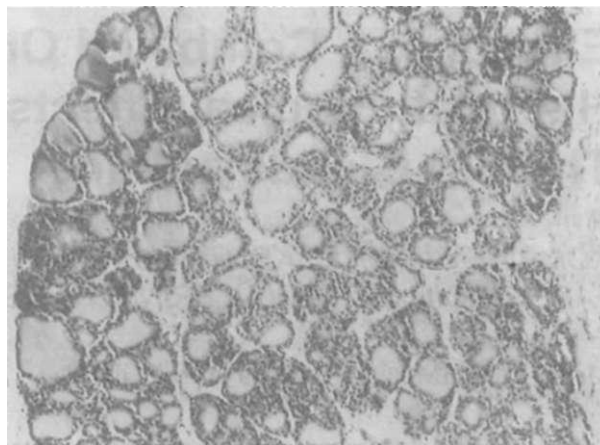


Figure 1. A cross-section of thyroid gland of a control mouse showing central and peripheral follicles. H+E.X100.

men (Figure 1) as compared to those of central types. Both types of follicles exhibited negligible amounts of colloid materials.

b) Treatment group. No significant differences were found according to size of total thyroid lobe between treatment and control groups (X 50). However, in the treatment group, mean diameter of total thyroid lobe was found greater than the control group. It was noticed that generally, peripheral and central follicles were in definite size in the control group, whereas the diameters of central and peripheral follicles were higher than the controls in the treatment group. It was also found that there was a significant increase in the diameters of peripheral follicles according to the control group (X100) (Table 1).

It was determined that in treatment group, some follicles of epithelium was hyperplased in five cases and there were cystic follicular nodules

Table 1. The mean \pm SD values of total thyroid lobe diameters and central and peripheral follicle diameters in the control and the treatment groups

Variables (u)	Control group (n=10)	Treatment group (n=30)	P values
Total thyroid lobe	210.25 \pm 29.61	217.50 \pm 36.41	0.47
Diameters of central follicles	60.00 \pm 10.35	98.12 \pm 50.75	0.08
Diameters of peripheral follicles	151.87 \pm 43.00	230.26 \pm 110.73	0.02*

*There was a significant difference statistically when it was compared with the control group (PO.05).



Figure 2. Note cellular hyperplasia of follicular epithelium in the peripheral follicles of the thyroid gland in the treatment group. H+E. X400.

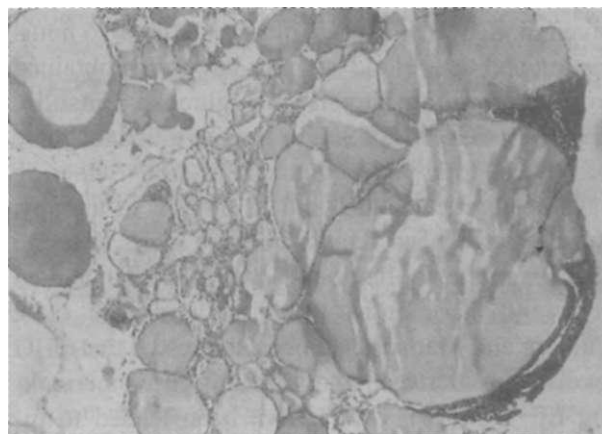


Figure 3. Note cystic follicular nodules in the peripheral follicles of the thyroid gland in the treatment group. H+E. X100.

in one case (Figure 2, 3). Hyperplasia rate was 16% and cystic follicular nodule rate was 3%. In hyperplasia the follicles were lined by tall cuboidal cells. Hyperplastic epithelium of the follicles projected into the lumen in the form of papillary in foldings. It was observed that hyperplased follicles had a few colloid materials whereas cystic follicles (nodules) had a rich colloid material and stained darker and intensive. Some of the follicles were found to be varied in size and shape and contained varying amounts of colloid. The larger follicles seemed to compress the small follicles. In terms of statistical values no significant differences were found in terms of histopathological findings in the treatment group.

Discussion

Most of the studies on the thyroid gland are associated with physiological activity (1-13). Leatherland and Renfree have considered in adult tammar wallabies (*Macropus eugenii*) after the corpus luteum (CL) had been removed, progesteron injections given for 14 days significantly elevated the plasma FT3 index, but had no effect on other thyroid parameters measured; in other groups, estrogen and androstenedione given for 14 days after corpus luteotomy had no significant effect on any of the thyroid parameters measured, despite the fact that in both groups injected with these steroids, the histological appearance of the thyroid was suggestive of an increased activity (5). Gopinath and Kitts indicated that increased total and FT4 plasma con-

centrations in steers due to the implantation of Synovex-S, a non-significant increase due to DES implantation suggest that it could be one of the mechanism by which they improve growth and metabolism of the organism. The investigator's results demonstrated a stimulation of thyroid gland activity, as reflected by the elevated T4 concentration in steers treated with estrogenic compounds, especially Synovex-S and DES 86). Kuhl et al, investigated that the effect of the two low-dose combinations of EE with levonorgestrel (EE/NG) and desogestrel (EE/DG) on thyroid function and other biochemical parameters. Both the EE/NG and EE/DG preparation elevated serum concentrations of T4 (40%), FT4 (15-22%), T3 (17-28%) and TBG (20%) significantly, whereby the effect was more pronounced during the second treatment period after washing-out. The increase in T4 and T3 is probably due to a rise in estrogen induced TBG production, the data seem to indicate that there was a slight, but effective stimulation of thyroid function during treatment with low-dose (7). Pansini et al. have observed increased blood levels of T4, T3 and TBG and no change in the levels of the free forms of the hormones. Their data demonstrated that the rFT3 blood level is significantly increased during the estrogen-progestin treatment. They have reported that even reverse T3, the main peripheral catabolite of T4 is significantly increased during therapy with OCs (9). Smith et al. have observed that the influence of OCs on hormonal and metabolic homeostasis in young adolescents. Norinyl 1/50 has been

given in 46 of 12-17 years old girls after a 16 hour-fasting and then blood samples have been obtained for determining levels of glucose, insulin, glucagon, GH, LH, TSH, prolactin, gluconeogenic substrates, total lipids and cholesterol. Sampling have been repeated at 6 and 12 months of therapy. Results have been suggested that there were no changes in metabolic or endocrin functions studied at 6 and 12 months on a medium-dose (13). Culberg and Mattson had been suggested that TBG levels were increasing significantly in women taking EE+DG (10). COCs have been led to increase in the TBG and TBI levels due to estrogens, however it has been reported that these effects have no great clinical significance (11,12). Chadhuri et al. have been asseyed the incidence and determined the molecular characteristics of steroid hormone receptors in human thyroid tissue specimens. Estrogen has been found in 23 of 45 specimens including 8 of 8 papillary cancers. The mean estrogen receptor has been found higher in neoplastic than non-neoplastic thyroid tissue. The findings have been suggested that steroid hormones might influence the development and growth of thyroid tumors (18). It has been reported that administration of estrogen leads to an increase in the weight of thyroid glands and increased in a height of thyroid epithelium (8). Maiti has been reported that the treatment of norethisterone with a daily dosage of 0.5 mg/rat, consecutively for 75 days increased gland weight and caused hyperthyroidism in albino rat. These findings have been suggested that even only progestogen contraceptives have untoward effects upon thyroid gland function in albino rats (19). Morphological appearance of thyroid follicles show differences due to localization and functional activities of thyroid gland. There may be height cuboidal follicular epithelium and small lumen with negligible colloid material in the small follicles besides low cuboidal follicular epithelium and large lumen with amount of colloid material in the large follicles. It is considered that flattened epithelium of many follicles are hypoactive (20-22). Thyroid are known to show cyclic variations with reproductive activities in normal birds. Histologically, during the breeding season thyroid showed an active condition with enlarged thyroid follicles lined by cells of increased height. Many of thyroid follicles were empty of their colloidal con-

tent while other follicles were with varying quantity of colloidal material. During the non-breeding season, in the thyroid, the follicles were reduced in size with reduced cell height and accumulated colloid material (23). Gupta et al. revealed that there is a great deal of change in thyroid gland in adult from that of juveniles in the turtle species studied. The absolute weight of the gland in adult from that of juveniles in the turtle species studied. The absolute weight of the gland was greatly increased in adults from that of juvenile animals. Simultaneously, follicular size and lumen size also increased in adults (non-breeding and breeding) from that of juveniles, while the follicular epithelial height was decreased in adults (non-breeding and breeding) in comparison to that of juvenile animals. These observations suggest that there may be an inverse relationship between the follicular size and the follicular epithelial height observed in juvenile and adult animals (24). Girod et al. have observed histological appearances showing hyperactivity in the monkey thyroid glands which were administered estrogen for 21-24 days (25). It is known that follicular epithelial height increase while the amount of colloid material decrease and diameter of follicle is similarly decreased after drug administration, thereby stimulate synthesis of thyroid hormone (12,20-22). Yardimoglu and Misirlioglu have observed that histopathological effects of EE+DG containing COC on the rat thyroid glands. EE+DG has been given in different doses and times. A remarkable increase has been found at the diameters of follicle in the high dose treatment groups in comparison to the control groups. Differences in staining characteristics have been observed in the increased colloid substance in the treatment groups (26). We report that hyperplasia was seen on thyroid follicle epithelium and amount of colloid material decreased in thyroid gland from some mice which were administered COC containing EE+ethynadioldiacetate with low dose for one year. Some of cystic follicles had a rich colloid content and stained darker and intensive. Although the histopathological findings were not statistically significant, even long-term COC with low-dose effects may have pathologic effects.

Relative data obtained by morphometric techniques must be related to a reference system so that it can be reported as an absolute quantity. Methods

of histometry contain manual, semi automatic and automatic systems. Histometry should therefore be used selectively on structures which are difficult to assess accurately and on tissue samples which would benefit from such investigations. The dimensions of particles are often difficult to obtain. If the particles are spherical and all about the same size, the diameter of the largest profiles observed will be equal to the equatorial diameter (16). In the present study according to micrometric measurements total thyroid lobe and central and peripheral follicles have increased in the treatment group as compared to the control group. But there was a significant increase statistically only the diameters of peripheral follicles in the treatment group according to the control group. Because we couldn't examine total thyroid lobes under the magnification of X 100. When we examined the staining properties of the colloid, it has been observed that in the treatment group the amount of colloid material had decreased and stained pale acidophilic. These findings suggested that, there is an accelerating uptake of colloid material in to the blood stream.

In conclusion, we observed some histopathological changes on thyroid glands of female mice which were administered COC for one year compared to the control group. In the treatment group hyperplasia were seen in 6 cases and cystic nodules in 1 case of 30 mice and there was a significant increase of peripheral follicles in the treatment group. As a result the present findings show functional change of the thyroid gland and is affected by hormonal homeostasis.

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