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# Effect of Methimazole on Eosinophil Granulocyte Changes and Uterus Layer Thicknesses

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**Abstract**

The purpose of this research was to evaluate the effects of methimazole used to create hypothyroidism in rats on the eosinophilic cells and uterus layer thicknesses. Uterus samples, 15 healthy Wistar Albino female rats aged 12-14 weeks were taken from control (C, n=6) and experimental (E, n=9) groups. Intraperitoneally, methimazole was injected as 10 mg/kg/day dose in the Group E for 2 weeks. After hypothyroidism was created in the Group E, feeding all the rats in this study was continued with normal pellet feed again for 2 weeks to see chronic effects on tissue. At the end of the 4th week, uterus samples were stained with Crossmon's trichrome staining. Uterus layer thicknesses were measuring with ImageJ Analysis using images of six different uterus regions including endometrium, myometrium and perimetrium layers. The mean number of eosinophil granulocyte in the endometrium was higher in Group E when compared to Group C ($P<0.05$). Its distribution was concentrated in the endometrium functionalis. However, there was no difference in uterine layer thicknesses between the groups ($P>0.05$). We concluded that methimazole might cause hypereosinophil granulocyte accumulation in the endometrium functionalis. This study is important for clinicians to evaluate methimazole-associated eosinophilic infiltration due to hypersensitive immune reactions to the drugs, especially used during pregnancy.

**Key Words:** Endometrium, hypereosinophilic infiltration, methimazole, rat

## Metimazolün Uterus Katman Kalınlıklarına ve Eozinofil Granülosit Değişimlerine Etkisi

**Öz**

Bu çalışmanın amacı ratlarda hipotiroidizm oluşturmada kullanılan metimazolün uterus katman kalınlıklarına ve eozinofil granülositler üzerindeki etkilerini değerlendirmektedir. Çalışmanın uterus örnekleri kontrol (K, n=6) ve deney (D, n=9) gruplarını oluşturan 12-14 haftalık 15 sağlıklı dişi Wistar Albino rattan alındı. Deney grubuna 2 hafta boyunca intraperitoneyal olarak 10 mg/kg/gün dozunda metimazol enjekte edildi. Deney grubunda hipotiroidizm oluşturulduktan sonra doku üzerindeki kronik etkileri görmek için çalışmadaki tüm ratların beslenmesine 2 hafta daha normal pelet yemle devam edildi. 4. haftanın sonunda alınan uterus örnekleri Crossmon's trikrom boyamasıyla boyandı. Endometriyum, miyometriyum ve perimetriyum katmanlarını içeren 6 farklı uterus görüntüsünün uterus katman kalınlıkları ImageJ analiz programı kullanılarak ölçüldü. Endometriyumdaki ortalama eozinofil granülosit sayısı kontrol grubuna kıyasla deney grubunda daha yüksekti ($P<0.05$). Bu dağılım endometriyum fonksiyonalis katında yoğunlaşmıştı. Fakat gruplar arasındaki uterus katman kalınlıklarında farklılık yoktu. Metimazolün endometriyum fonksiyonaliste hipereozinofilik granülosit birikimine sebep olabildiği sonucuna varıldı. Bu çalışma özellikle gebelik döneminde kullanılan ilaçların hipersensitif immün reaksiyonlara bağlı metimazol ilişkili eozinofilik hücre infiltrasyonunu değerlendirmede klinisyenler için önemlidir.

**Anahtar Kelimeler:** Endometriyum, hipereozinofilik infiltrasyon, metimazol, rat

## INTRODUCTION

Methimazole is one of the anti-thyroid drugs used to treat hyperthyroidism. Although how methimazole works is not known, some researchers have reported that it works similarly to carbimazole. The carbimazole is either directly effective on the muscle or causes a marked decrease in thyroid hormones by leading to relative local hypothyroidism in the muscle (1). Pharmacologically, hypothyroidism in rodents (2) is frequently induced using methimazole as an antithyroid drug (3). Hypothyroidism is the most common among thyroid disorders. This disorder is insufficient production of thyroid hormones (4).

In thyroid disorders, musculoskeletal pain is common (5). This pain is a result of an adverse reaction to the antithyroid medication. Methimazole preferred in the management of hyperthyroidism is known to have many adverse effects such as agranulocytosis, myalgia, arthralgia and arthritis (6).

The uterus is completely vital for pregnancy. The endometrium functionalis is a part shows cyclic changes of the lamina propria, and the endometrium basalis is the basal part of the lamina propria which has no cyclic changes (7). The

endometrium functionalis, which is discarded at the end of each cycle, is restored by the endometrium basalis (8).

The endometrium is exposed to many physiological and pathological cell changes (9). One of the most common endometrial changes is eosinophilic cell changes. Eosinophils play an important role in cellular immunity such as atopic/allergic reactions, helminthic parasitic infections, drug side effects, malignancies and endocrinopathies (1). Eosinophils are non-specific destructive and cytotoxic cells (10). These cells are capable of specifically regulating both innate and acquired immune responses. According to the studies, these granulocytes have always been seen as very complex cells (11).

The information on the effect of methimazole on eosinophilic cells and uterus layer thickness was very limited in the literature review. The aim of this research was to detect the effects of methimazole on the eosinophilic cells and the layer thicknesses of the uterus.

## MATERIAL AND METHOD

### Animals

15 Wistar Albino female rats showing proestrus stage of the sexual cycle (12-14 weeks old, initially weighing 198-250 g) were in this study. The rats were randomly divided into two groups: control group (C, n=6) and the hypothyroid-induced experimental group (E, n=9). The rats were housed in thea same laboratory conditions. The temperature was 20–25°C with a 12-hour light/dark cycle per day and ad libitum water and food. The approval for investigation was obtained by Karamanoglu Mehmetbey University Faculty of Health Sciences Ethics Committee (2018/35).

### Creating of Hypothyroidism

Control group was untreated. Hypothyroidic rats were created with 10 mg/kg/day intraperitoneal methimazole injection for two weeks (12). At the end of the 2nd week, total serum tetraiodothyronine (T4) and triiodothyronine (T3) concentrations were measured with the ADVIA Centaur CP Immunoassay System (detection kits provided by Siemens). While average serum T3 and T4 levels were measured as 3.22 pg/ml-2.35 ng/dl in Group C, these values were found as 2.83 pg/ml-1.61 ng/dl respectively. According to the measurements obtained, the rats of the experimental group were accepted as hypothyroid. After, feeding of all rats in the study was continued with normal pellet feed again for 2 weeks to see chronic effects on tissue. At the end of the 4th week, all rats were sacrificed by cervical dislocation under general anesthesia.

### Histologic Procedure

Taken uterus samples were fixed in 10% neutral buffered formaldehyde for 24 hours at room temperature. The fixed samples washed overnight in running water to remove the fixation solution. Then, the samples were dehydrated, cleared, and embedded in paraffin. Serial sections were taken transversely at regular intervals (5 µm thickness) from uterus samples and were stained with Crossmon's trichrome staining (13).

### Histomorphometric Evaluation

All specimens were examined under the light microscope and were photographed. As shown in Figure 1 and 2, eosinophils are polymorphonuclear leukocytes, and granules in the cytoplasm are stained pink-red. In the images taken at 40 x lens magnification, eosinophil granulocyte count was performed in a total area of 1mm2 from 10 different endometrium regions. Uterus layer thicknesses were obtained by measuring from images of six different uterus regions, including endometrium, myometrium and perimetrium layers of each preparation with ImageJ Analysis Program in the images taken at 10 x lens magnification (Figure 3 and 4) (14).

### Statistical Analysis

The variables were investigated using visual (histograms, probability plots) and analytical methods (Kolmogorov-Simirnov/Shapiro-Wilk's test) to determine whether or not they are normally distributed. Kruskal-Wallis test applied to compare the data obtained from the uterus and Mann-Whitney U test applied to compare the data between the groups ($P < 0.05$) (SPSS 2018). Results were considered to be significant at $P<0.05$. Data are expressed as means ± standard error (SE).

## RESULTS

As a result of the statistical analysis (Table 1), the mean eosinophil granulocyte counts of the Groups C and Group E were 67±17.60/1mm2 and 318.67±180.62/1mm2 respectively. Statistically, the number of eosinophil granulocytes in Group E was higher than Group C ($P<0.05$). In addition, it was observed that the distribution of these cells was mostly concentrated in the endometrium functionalis (Figure 1). As shown in Table 1, the uterus layer thicknesses of the groups, C and E were 279.55±23.05 and 252.47±13.73 respectively. Whereas there was no statistical difference in these thicknesses between the groups, uterus layer thickness of the Group E was thinner than Group C. (Figure 2).

**Table 1.** The mean eosinophil granulocyte counts in the endometrium ±SE (1 mm2), and uterus layer thicknesses (µm), (Mean±SE)

| Groups | Eosinophil granulocyte | Endometrium | Myometrium | Perimetrium |
|---|---|---|---|---|
| **Control (n=6)** | 67±17.60[a] | 279.55±23.046 | 113.86±4.87 | 6.18±0.64 |
| **Experimental (n=9)** | 318.67±180.62[b] | 252.47±13.73 | 142.38±13.97 | 7.68±0.84 |

a,b: Values within a column with no common superscripts are significantly ($P<0.05$) different.

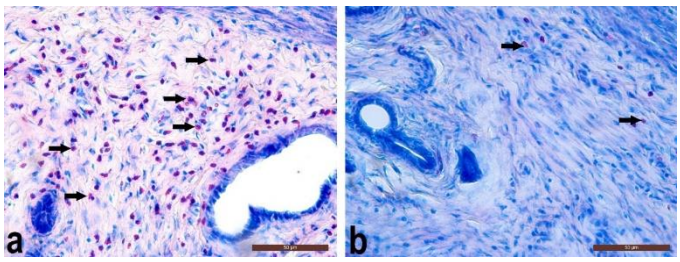

**Figure 1.** Eosinophil granulocyte infiltration in the endometrium of Group E (a) and Group C (b). Crossmon's trichrome staining. Arrows: Eosinophil granulocytes. Bar: 50 µm.

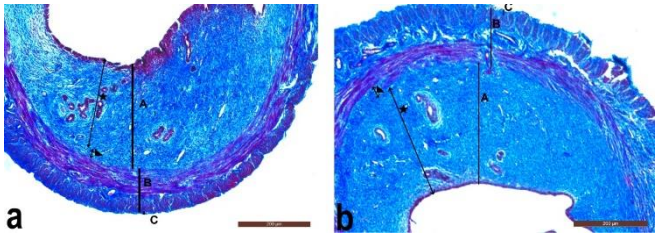

**Figure 2.** The layers of the uterine wall in Group C (a) and Group E (b). Crossmon's trichrome staining. Endometrium (A), myometrium (B), perimetrium (C), endometrium functionalis (★), endometrium basalis (◣). Bar: 200 µm.

## DISCUSSION AND CONCLUSION

Gynecological problems such as infertility and recurrent spontaneous abortions are possible with a good understanding of the interactions between the drug and the immunity. This study was conducted with the idea that it could aid in the investigation and management of many immunological conditions related to this drug. Antithyroid drugs are important in the treatment of hyperthyroidism. These drugs have varied patient responses and potentially serious side effects (15). Thyroid hormones are known to stimulate some of the functions of polymorph nuclear leukocytes such as chemotaxis, phagocytosis and cytokine synthesis (16).

Methimazole is one of the anti-thyroid drugs used to treat hyperthyroidism (1). Gaspar-da-Costa et al (17) reported that eosinophilic pleuritis was formed as a result of hypersensitivity immune drug reaction due to methimazole. Bou Khalil et al (6) declared that methimazole leads to diffuse perimysial and endomysial eosinophilic infiltration, described as focal eosinophilic myositis in the gluteus muscle. These symptoms disappear when the patient stops taking methimazole (6,17). Endometriosis correlates with autoimmune diseases involving hypothyroidism. Infertility is a distressing symptom associated with endometriosis. One of the most common endometrial changes is eosinophilic cell changes. Blumenthal et al (18) were reported that degranulating eosinophils in endometriosis involved in general tissue remodeling and wound healing. In this study, we observed that methimazole might cause hypereosinophil granulocyte infiltration in the endometrium. It is known that most drugs can cause allergic reactions as a side effect. It is thought that the use of methimazole may cause hypersensitive reactions as a result of eosinophil cell increase by affecting the immune system.

Uterus is an important organ for maintaining a healthy pregnancy. Uterus layer thickness correlates with successful implantation of embryos (19). Although Al-Qudsi and Linjawi (20) said endometrial thickness of 3-month-old rats at proestrus stage was 0.1 mm, Ye et al (21) reported endometrial thickness of rats with normal estrous cycle was intervals of 0.6-0.8 mm of eight-week-old rats. A study of Zhao et al (22) shown endometrial thickness of rats with estrous cycle was 359.13 ± 49.70 µm. In our histological examination, it was observed that the uterine glands in the endometrium were small in size and the secretory contents were low. These data showed that rats were at proestrus stage as described by Ekizceli et al (23). Moreover, when thickness measurements were taken into consideration, in addition to the fact that all animals were in the same sexual cycle, methimazole was also found to have no effect between the groups.

In conclusion, the root of gynecological problems such as infertility and recurrent spontaneous abortions can be explained with a good understanding of the interactions between the drug and the immunity. Immunological changes that occur between maternal blood and endometrial tissue are important for maintaining a healthy pregnancy, and this can be impeded by the use of methimazole, a hyperthyroidism medication. Because methimazole has especially teratogenic effect in the first trimester of pregnancy, this drug should be carefully implemented in terms of mother, fetus and neonate. Finally, the methimazole-induced hypereosinophilic infiltration is important for clinicians to evaluate due to the possibility of a hypersensitivity immune reaction to the drug, especially with regards to pregnancy. Most drugs could cause allergic reactions as a side effect. In this study, we observed that the use of methimazole might cause hypersensitive reactions as a result of eosinophil granulocyte increase by affecting the immune system. Also, we thought that methimazole might cause hypereosinophil granulocyte accumulation in the endometrium functionalis. In addition to this method used, the application of other methods may help to define hypereosinophilia.

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