Effect of Castration on the Thymus Gland and Spleen: Morphologic and Light Microscopic Level Study in Rats

SIÇANLARDA KASTRASYON SONRASI TİMUS VE DALAKTA GÖRÜLEN DEĞİŞİKLİKLERİN MAKROSKOPİK VE IŞIK MİKROSKOPİK DÜZEYDE DEĞERLENDİRİLMESİ

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Summary-

Objective: Effects of castration on histology and morphometry of two lymphoid organs (thymus and spleen) were studied, attempted to clarify agonistic relationship between thymus and spleen.

Material and Methods: 75 days old wistar albino male rats were used in this study. Animals in the castrate group were castrated 45 days before the sacrification. Thymic and splenic weights, splenic diameters and histologic examination of both organs were get in castrate and control groups.

Results: In castrate group, thymic weight (0.405±0.18 g) was significantly increased ($p<0.01$) compared to that of control group (0.185±0.007g). Splenic height and weight weren't significantly greater in the castrated group (0.691+0.034 g),(3.547±0.083 cm) than in the intact control group, (0.658±0.02g),(3.753±0.111 cm), ($p>0.05$) but the diameter of spleen was significantly greater (0.953±0.031 cm), than that of control group (0.827±0.041 cm) ($p<0.05$). By histologic examination, while there were no volume increase in the interlobuler connective tissue of thymus and there was no invasion to the parenchyma; thymic cortex (parenchyma) was hyperplasic and hypertrophic. In castrated group, stromal fatty cells were uniloc-ular, however, in control group stromal fatty cells were multilocular and also showed parenchymal invasions. There was no significant histologic differences in spleens of the castrated and control groups. Subcortical sinusoidal vessels of the spleen in the experimental group were found to be dilated.

Conclusion: Those findings suggest that castration of young, sexually mature male rats effect both lymphoid and non-lymphoid thymic components.

Key Words: Thymus gland, Spleen, Castration

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Ozet

Amaç: Bu çalışmada kastrasyonun iki lenfoit organ (dalak ve timus) histolojisi ve morfometrisi üzerine olan etkisi araştırılmıştır.

Materyal ve Metod: Bu çalışmada 75 günlük erkek Wistar-albino ratlar kullanılmıştır. Kastrasyon grubundaki hayvanlar sakrifiye edilmeden 45 gün önce kastre edilmişlerdir. Timus ve dalak ağırlıkları, dalak çapları, ve her iki organın histolojik incelemesi kastrasyon ve kontrol gruplarında gerçekleştirilmiştir.

Sonuçlar: Kastre edilen grupta timus ağırlığı (0.405±0.18 g), kontrol grubuna göre(0.185±0.007 g) anlamlı olarak artmıştır ($p<0.01$). Kastrasyon grubunda dalak vertikal çapı (3.547±0ç083 cm) ve ağırlığı (0.691±0.034 g), kontrol grubuna göre (3.75±0.111 cm), (0.658±0.02 g) istatistiksel olarak anlamlı artış göstermiştir ($p>0.05$). Deney grubunda dalak transvers çapı (0.953±0.031cm), kontrol grubuna (0.827±0.041) göre istatistiksel olarak anlamlı artış göstermiştir ($p<0.05$). Histolojik incelemede timus korteksi hiperplastik ve hipertrofık olarak görülmüş, interlobüler bağ dokusunda bir volüm artışı ve parankim invazyonu görülmemiştir. Kastrasyon grubunda stromal yağ hücreleri uniloküler olarak görülürken, kontrol grubunda multiloküler olarak görülmekte ve parankim invazyonu göstermektedir. Kastrasyon ve kontrol gruplarının dalaklarının histolojik incelenmesinde bir farklılık görülmemiş yalnızca deney grubunda subkortikal sinüzoidal damarlarda bir genişleme dikkati çekmiştir.

Sonuç: Bu bulgular göstermektedir ki, genç, seksüel olgunluğa erişmiş erkek ratların kastrasyonu tunusun lenfoit ve lenfoit olmayan bileşenlerini etkilemektedir.

Anahtar Kelimeler: Timus, Dalak, Kastrasyon

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The thymus is the key organ for the development of T-cells that are responsible for the regulation of immune responses as well as cellular immunity. However, thymus as an important central lymphoid organ, begins to involute at about puberty in experimental animals and humans. The spleen is an other lymphoid organ for the production and development of s-cells that are responsible for the humoral immunity and also there is no involution with age in the spleen (1-3).

It was reported that the thymic involution can be delayed or reversed by castration before or after puberty. The effect of castration on the thymus has been explained by removal of sex hormones (4). Thymus has been also shown to have a humoral action on the lymphopoiesis of other lymphoid organs, like spleen (5).

Effect of castration on the pituitary gland, adrenals and prostate gland were studied by a variety of immunohistochemical and histologic methods. However, there are a few studies on the histology of thymus and, especially, of spleen after castration.

The present study was undertaken to asses the effects of castration on the histology and morphometry of thymus and spleen.

Material and Methods

Male, sexually mature, 30 days old wistar rats were purchased from Osmangazi University TICAM Farm, Eskişehir, Turkey. Rats were fed and housed on the same conditions, kept on a light-dark cycle (LD 12:12 hours), and weighing 190±10 gr. Food and water were available ad libitum.

After, skin cleansing with 0.1% Rivanol solution, rats were bilaterally orchidectomized thorough the scrotal route under ether (sirpa) anaesthesia. Incision was closed with 3/0 catgut (B.Braun Melsungen A.G.) Fourthyfive days after surgery, the rats were sacrificed by decapitation. After the decapitation, thymuses and spleens removed carefully, weighted and measured.

The thymus and spleen were fixed in neutralized tamponated formaldehyde solution (10%), dehydrated in alcohol, embedded in paraffin and serially sectioned at 5 urn. The slides were stained by haematoxylin-eosin. In splenic sections white pulp, red pulp, capsule and trabecular stromal structures were analysed individually. In thymic sections, the ratio of diffuse lymphoreticular connective tissue and interlobular connective tissue were compared in both castrate and control group. And also, interlobular connective tissue was examined for cellular and fibrillar structures. The histologic sections were photomicrographed by olympus PM-10A-5. For statistical evaluation Student-t test was used.

Results

Macroscopic findings of groups

In Table 1 the results of thymic and splenic weights, splenic diameters of both castrate and control groups were given. In castrate group, thymic weight was significantly increased compared to that of control group ($t=11.53, p<0.01$)

There is no statistically significant difference in regards of splenic weights ($t=0.431$, $p>0.05$).

There is no statistically significant difference in regards of splenic heights ($t=0.431$,$p>0.05$).

In castrate group, splenic width was significantly increased compared to that of control group ($t=2.483$, $p<0.05$).

In Table 2, the mean, standard deviation and standard errors of both groups were given.

Microscopic findings of groups

In thymic sections of control group, it is observed that there is a sliding to the interlobular connective tissue structures and to cellular elements in the diffuse lymphoreticular connective tissue (Figure 1). From the cellular elements macrophages, mast cells, and fibroblasts have started to proliferate and invading to the lymphoreticular connective tissue (Figure 2). In the connective tissue, fatty cells proliferated too much and changed it to the fatty tissue. It is evident that this fatty tissue and fatty cells are multilocular (Figure 3). In thymic sections of castrate group, there is hyperplasia of lymphoreticular connective tissue in thymic lobules (Figure 4), which is mostly in the cortical areas. Although, there is hyperplasia and hypertrophy in thymic parenchyma, there is normal structured interlobular connective tissue stroma (Figure 5). There is a normal amount of macrophages, fibroblasts and the other connective tissue elements fib-

Table 1. Results of thymic and splenic weight, splenic diameters of castrate and control groups

Study Groups	Animal Number	Thymic Weight* (gr)	Splenic Weight** (gr)	Splenic Length*** (cm)	Splenic Width**** (cm)
Castrate Group	1	0,45	0,80	3,2	0,8
	2	0,33	0,69	3,7	1,1
	3	0,39	0,72	3,4	1,0
	4	0,37	0,64	4,0	1,0 »
	5	0,47	0,79	4,1	1,1 *
	6	0,29	0,49	3,0	0,8
	7	0,50	0,42	3,3	0,7
	8	0,48	0,69	3,7	1,0
	9	0,33	0,78	3,6	0,9
	10	0,36	0,61	3,8	1,1
	11	0,41	0,76	3,2	1,0
	12	0,44	0,87	3,4	0,9
	13	0,50	0,51	3,6	1,0
	14	0,42	0,83	3,9	1,0
	15	0,33	0,77	3,3	0,9
Control Group	1	0,24	0,69	4,3	0,7
	2	0,21	0,78	3,5	1,0
	3	0,20	0,63	4,4	0,9
	4	0,16	0,81	3,2	0,8
	5	0,21	0,69	4,6	1,0
	6	0,13	0,55	3,3	0,8
	7	0,19	0,67	3,6	0,7
	8	0,21	0,64	3,8	0,9
	9	0,16	0,50	3,8	0,8
	10	0,20	0,55	3,3	0,9
	11	0,19	0,60	3,9	0,5
	12	0,17	0,61	4,0	1,1
	13	0,15	0,66	3,8	0,6
	14	0,17	0,80	3,3	0,8
	15	0,19	0,69	3,5	0,9

*	t=11,53	p<0.01
**	t=0,431	p>0.05
***	t= 1.487	p>0.05
****	t=2.483	p<0.05

Table 2. The mean, standard deviation and standard errors of castrate and control groups

Study Groups	Thymic Weight	Splenic Weight	Splenic Length	Splenic Width
Castrate Group				
X	0.405	0.691	3.547	0.953
SD	0.068	0.133	0.323	0.119
SE	0.018	0.034	0.083	0.031
Control Group				
X	0.185	0.658	3.753	0.827
SD	0.029	0.091	0.431	0.158
SE	0.007	0.024	0.111	0.041

X : Mean
SD : Standard Deviation
SE : Standard Error

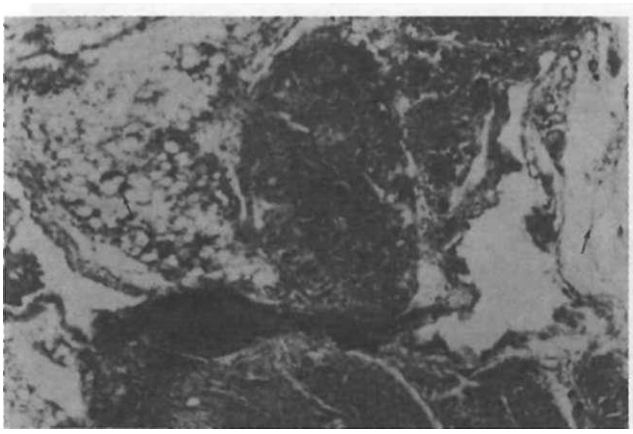

Figure 1. Thymic section of control group showing stroma and parenchyma. Haematoxylin-Eosin. Magnification:x33.

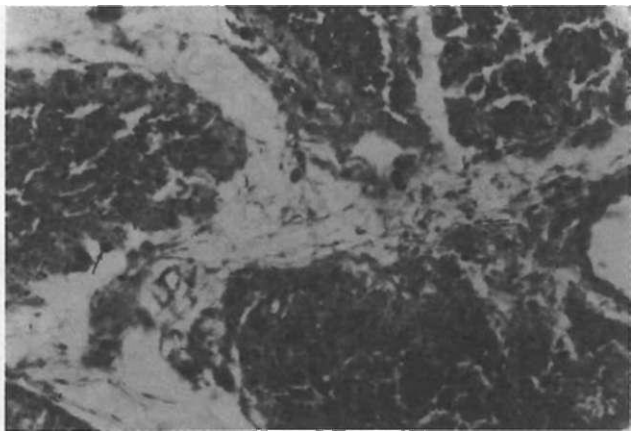

Figure 2. Invasion of proliferating elements to the diffuse lymphoreticular connective tissue in thymic sections of control group. Haematoxylin-Eosin. Magnification:x66.

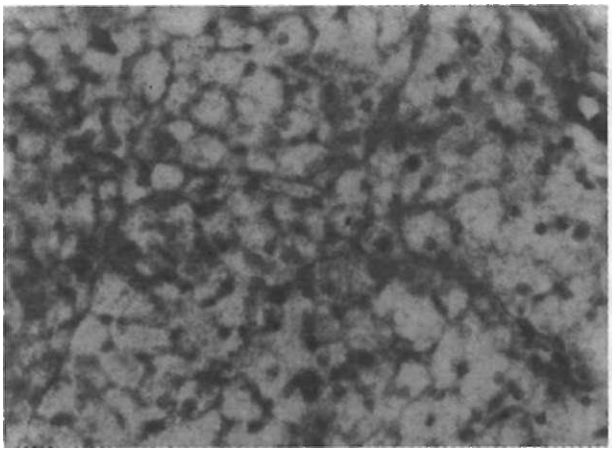

Figure 3. Multilocular fatty tissue in thymic section of control group. Haematoxylin-Eosin. Magnification:xl32.

Figure 4. Hyperplasie diffuse lymphoreticular connective tissue and normal stroma of thymic sections in castrated group. Haematoxylin-Eosin. Magnification:xl3.2.

rillar structures in thymic stroma; and no invasion to the thymic stroma (Figure 6,7,8). Interlobular fatty cells and fatty tissue formed of it is very much different from the control group and is unilocular and in normal amount (Figure 9). In the splenic sections of control group, there is normal appearing white and red pulp in splenic parenchyma. Elements of normal histologic structure is observed in splenic stroma (Figure 10). In splenic sections of castrate group, there is hypertrophied white pulp in paracortical areas in some of the animals. There is red pulp hypertrophy especially in subcapsular areas in much of animals. Splenic capsule is completely normal (Figure 11).

Discussion

Sex as well as age are known to effect the thymus and the weight of the thymus increases after castrations of rats (4,6). Studies reported that histology and weight changes of some organs changes after castration (1,5,7-18). Thymus also has been shown to have a humoral action on the lymphopoiesis of other lymphoid organs (Osoba, 1965; Shelton, 1966). The complex relationship between hypothalamo-pituitary-gonadal axis and the role of thymus in the immune and endocrine system tried to be explained by various authors. To our knowledge, there is no such study that gives the morphometry and light microscopic level structure of

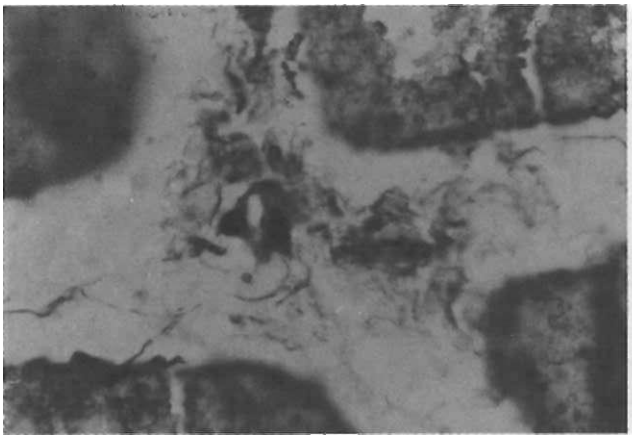

Figure 5. Normal appearing stroma in thymic sections of castrated group. Haematoxylin-Eosin. Magnification:xl32.

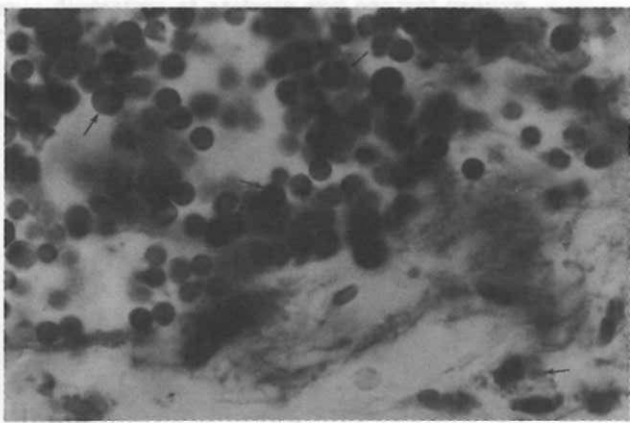

Figure 6. Diffuse lymphoreticular connective tissue and stroma cells in thymic sections of castrated group. Haematoxylin-Eosin. Magnification:x330.

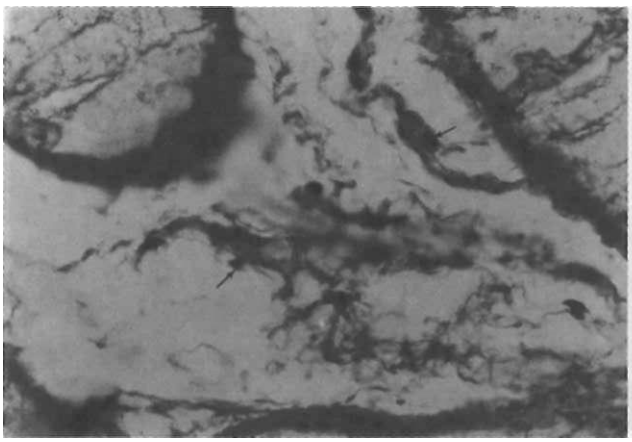

Figure 7. Connective tissue structures in thymic sections of castrated group. Haematoxylin-Eosin. Magnification:x66.

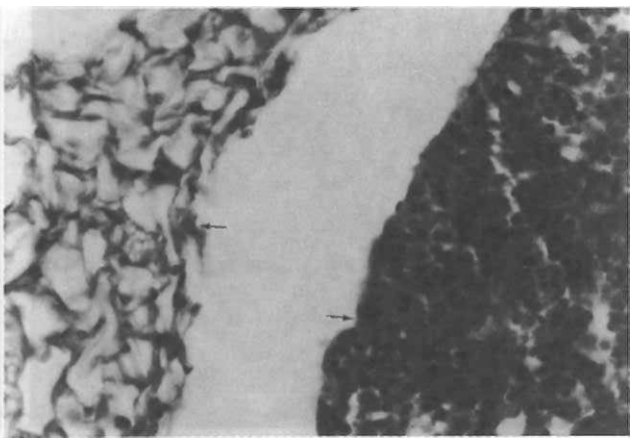

Figure 8. Diffuse lymphoreticular connective tissue (parenchyma) and stroma in thymic sections of castrated group. Haematoxylin-Eosin. Magnification: x!32.

both spleen and thymus after castrations of adult male rats, that may help understanding this complex relationship.

Mean thymic weight increase in our 4-week-old rats is 74.9% after 45 days of castration. In the study of Dean et al, both thymus (organ weight) and spleen (nucleated cell count) were larger in castrated as compared to control mice. Seven weeks after castration 10 and 19 month old mice had mean thymic weight increases of 34% and 128%, respectively, compared to sham-operated controls (9). In another study of Dean et al., significant splenic hyperplasia (organ weight) was inconstantly observed in castrated mice (8). Although, there was an increase in splenic weight as nucleated cell count and organ weight in these two studies, we cannot confirm splenic hyperplasia in our study as organ weight and histologicaly. Masse et al. reported that correlations between body weight and lymphoid organ weights were highly significant (spleen: $r=0.938$ $p<0.05$). when it is thought with the finding that the diameter of spleen was significantly increased in castrated group in our study, the differences for the current literature could be attributed that there could be also decrease-increase in weight

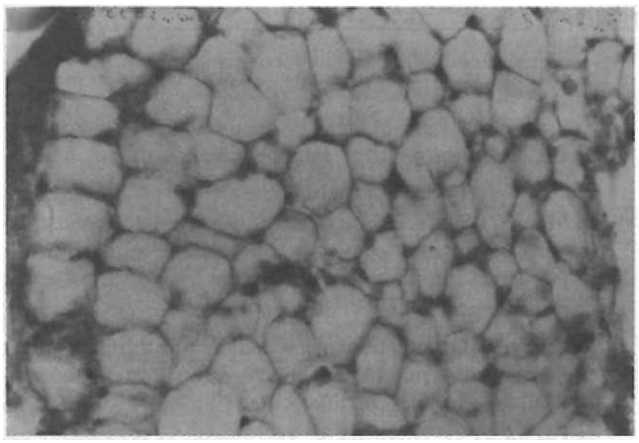

Figure 9. Unilocular fatty tissue formed of unilocular fatty cells that belongs to the interlobular connectie tissue forming stroma in thymic sections of castrated group. Haematoxylin-Eosin. Magnification:X132.

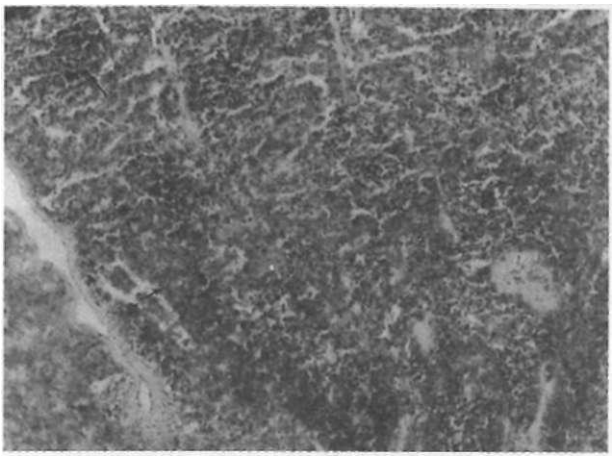

Figure 10. Normal appearing red and white pulp in splenic sections of control group. Haematoxylin-Eosin. Magnification: x33.

period like the thymus as shown by Karapetrovic et al (13). Effects of castration on the lymhocytes of the thymus, spleen and lymph nodes were studied by Windmill and Lee and they reported that castration of 5-week-old male rats have produced a significant increase in thymic weight compared to age-matched intact animals (19).

Physiological and biochemical observations suggest that androgenic hormones modulate the function of the thymus gland. In different studies it was given that androgen levels, especially of testosterone decreases after castration (1,12,14,20,21). Female and castrated male mice produce more antibodies to a variety of antigens than do male mice. Administration of superphysiological dose of sex steroid hormones in a short period can bring about thymus atrophy, lymphopenia, and decreased humoral as well as cellular immunity. Although, their cellular location has been disputed, thymus contains specific androgen receptors. According to some investigators, androgen receptors are considered to be located either in subpopulations of thymocytes or in the epithelial cells of the thymus (5). When the effect of sex steroids has gone, the thymus gland which is formed mostly of addipose tissue and not very much distinct cortex and medulla in non-castrated group reorganizes into very much distinct cortex and medulla and still much fatty tissue between the lymphoid areas and the capsule. And the most striking difference was the changing

Figure 11. Dilated subcapsular sinuzoids in splenic sections of castraed group. Haematoxylin-Eosin. Magnification:x66.

of multilocular fatty tissue, which forms most of the thymic gland, into unilocular fatty tissue. The unilocular fatty tissue also known as embriologic fatty tissue. This finding gives the impression that postpubertal involusion is the reversal of intrauterine thymic developmental steps. The expected finding was the reappearance of lymphoid structures. This unexpected finding of changing character of fatty structure brings the question that, what could be the role of fatty tissue in this interstisial millieu. The precise target of androgen action within the thymus are still under investigation and we offer to look for the androgen receptors and nature of them within this fatty tissue.