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A new nonsurgical experimental model for Asherman syndrome created in rats

Sıçanlarda oluşturulan cerrahi müdahalesiz yeni bir deneysel Asherman sendromu modeli

Başak Büyük [1]

Abstract

Aim: Asherman Syndrome (AS) is a partial or complete obstruction of the uterine cavity with adhesions as a result of trauma. In pre-clinical studies, to be able to show the effectiveness of new treatment methods, first of all, the AS model needs to be created in the animals. The aim of this study is to develop a new and effective nonsurgical method for using in AS and intrauterine adhesions modeling, and through this way, to propose a more effective method for researchers in terms of safety and feasibility.

Methods: Twelve female Wistar Albino rats were divided into two groups. It was reached to the left uterine horn transvaginally by using pre-prepared pink color (20 gauge) cannula. While 0.2 ml normal saline was applied to the animals in Group I (control group), 0.2 ml (Trichloroacetic acid) TCA was applied to the animals in Group II (experiment group).The right uterine horns of the animals were left without treatment. After three menstrual cycles, the animals were sacrificed and Hematoxylin-Eosin and Masson's Trichrom stainings were performed and evaluated histopathologically. Inflammation was evaluated in Hematoxylin-Eosin staining and fibrosis was evaluated in Masson's Trichrom staining.

Results: Whereas the uterine sections of the Group I have normal histologic appearance, inflammation and fibrosis were found in the left uterine sections of the Group 2 by histopathological evaluation. Results were statistically significant (p=0.002).

Conclusion: The proposed nonsurgical AS modeling method created disease, and this was also revealed by histopathological examinations. Through this way, a new AS model is suggested without surgery, in which the disease is correctly created.

Key words: Asherman syndrome, rat, gynatresia, pathology, animal model.

Öz

Amaç: Asherman sendromu(AS) uterin kaviteye travma sonucu kavitenin adezyonlarla kısmi ya da tam tıkanması durumudur. Klinik öncesi çalışmalarla yeni tedavi yöntemlerinin etkinliğini gösterebilmek için öncelikle AS modelinin hayvanda oluşturulmasına ihtiyaç vardır. Bu çalışmanın amacı, AS ve intrauterin adezyon modellemesinde kullanılmak üzere cerrahi uygulanmaksızın yeni ve etkili bir yöntem geliştirmek, bu şekilde araştırmacılara güvenlik ve uygulanabilirlik açısından daha etkin bir metot önerebilmektir.

Yöntem: On iki adet dişi Wistar Albino sıçan rastgele 2 gruba ayrıldı. Daha önceden hazırlanmış olan pembe renk (20 gauge) kateterle transvajinal yoldan hayvanların sol uterin hornularına ulaşıldı. Grup I'deki hayvanlara 0,2 ml serum fizyolojik uygulandı, Grup II'dekilere ise 0,2 ml Triklorasetik asit (TCA) uygulandı. Üç menstruel siklus beklendikten sonra hayvanların uterusları çıkarılıp Hematoksilen-Eozin ve Masson Trikrom boyamalar yapılarak histopatolojik olarak değerlendirildi. Yapılan Hematoksilen-Eozin boyalı kesitlerde inflamasyon dereceleri değerlendirilirken, Masson Trikrom boyalı kesitlerde fibrozis dereceleri değerlendirildi.

Bulgular: Grup 1'e ait uterus kesitleri normal histolojik görünüme sahipken, Grup 2'ye ait sol uterus kesitlerinde inflamasyon ve fibrozis oluştuğu gözlendi. Bu sonuçlar istatistiksel olarak anlamlıydı (p=0,002).

Sonuç: Sonuç olarak, önerdiğimiz cerrahi müdahalesiz AS modelleme yöntemi, literatürde önerilen yöntemler ile benzer şekilde hastalık oluşturmuş ve bu etki, yapılan histopatolojik incelemelerle de ortaya konmuştur. Bu şekilde, AS için doğru bir hastalık modellemesi gerçekleştiren cerrahisiz yeni bir yöntem önerilmiştir.

Anahtar kelimeler: Asherman sendromu, sıçan, jinatrezi, patoloji, hayvan modeli

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Introduction

Asherman Syndrome (AS) is a partial or complete obstruction of the uterine cavity with adhesions as a result of trauma [1]. In clinical practice, AS may cause menstrual disorders, such as amenorrhea, dysmenorrhea, or hypomenorrhea, infertility, recurrent pregnancy losses, and placental disorders [2, 3]. In these patients, endometrial biopsies often give the appearance of postmenopausal endometrium. Looking at the pathology of intrauterine adhesions (IUA), it can be seen that it has a fibrous structure. The IUAs, which have a feature of fibrous tissue, may occlude the uterine cavity partially or totally. Clinical symptoms also occur according to the severity of these IUAs [4]. For women with normal menstruation and reproductive characteristics, the prevalence of AS was reported between 2.8% and 45.5% [3].

Today, development of a standard treatment model for AS is a condition that researchers are studying on. Although there are different treatment options for AS, standard treatment procedure has not been developed, so 100% success has not been achieved yet [1-3, 5-9].

In addition to this protocols, stem cell treatments have been also being tested in the literature for AS, but there isn't any protocol used in clinic applications yet [10-16]. In order to further development of these studies and to pass to the clinical application, more studies on animals are needed.

To be able to propose new treatment methods for AS, pre-clinical studies should be completed. In order to demonstrate the effectiveness of the treatment methods in pre-clinical studies, firstly, the AS model needs to be created in animal. After AS modeling is carried out in experimental animals, the effectiveness of the proposed method can be demonstrated by comparing with control groups.

There are various methods in the literature for accurate and effective modeling of AS in experimental animals. In all of these cases, under anesthesia, the uterine cavity is reached by opening the abdomen via abdominal incision, then, chemical, physical or infectious trauma is created and the uterus and the abdominal cavity are closed surgically again [10, 11, 17-29]. After enough time is waited for the development of fibrosis and IUAs, the experiment is terminated. However, when the abdomen is opened by surgical methods, the animals are exposed to the risk of infections, and if the wound care is not done correctly, especially rodent-group animals can damage themselves through their wounded areas. In addition, the anesthesia and surgical process are very difficult and time consuming operations for researchers, as well as they increase the costs.

The aim of this study was to develop a new and effective method for AS and IUA modeling without applying surgical techniques, and through this way, to be able to propose an effective method in terms of safety and feasibility.

Material and methods

Materials and experimental design

The study was approved by the Institutional Animal Use and Care Committee of Canakkale Onsekiz Mart University (COMU) with No: 2017/12-07 and performed in accordance with Turkish Law 6343/2, Veterinary Medicine Deontology Regulation 6.7.26 and with the Helsinki Declaration of World Medical Association recommendations on animal studies. Wistar albino rats were obtained from COMU Experimental Research Application and Research Center. Twelve female Wistar Albino rats were used in the study, with a mean age of four months and weight of 240–300 g. The animals were housed in stainless steel cages in an animal room maintained at a standard humidity (45%-50%) and temperature 22±2°C with 12 hours light periods (12 hours of daylight/12 hours of dark). All animals were fed standard food and water and twelve hours before the study procedure feeding was stopped and the rats were only allowed to drink water. The entire experiment was conducted under half-sterile conditions.

Experimental procedure

Before starting the study, 20 gauge cannula was taken, its metallic needle was removed and flexible tip of the cannula was bended to the left. Twelve female Wistar Albino rats were divided into two groups;

Group I: (Normal Saline (NS) group, n=6)

Group II: (Trichloroacetic acid (TCA) group, n=6).

After the anesthetization of the animals by giving 50 mg/kg ketamine hydrochloride (Ketalar®, Pfizer İlaçları Ltd, Şti, İstanbul, Türkiye) and 10 mg/kg xylazine (Alfazyne %2, Ege Vet San. Tic, İzmir, Türkiye) to them, it was reached to the left uterine horn through transvaginal route by using pre-prepared pink color (20 gauge) cannula. While 0.2 ml NS was applied to the animals in Group I, 0.2 ml TCA (IL-33, İstanbul İlaç San. Tic. AŞ, İstanbul, Türkiye) was applied to the animals in Group II (Figure 1). For both groups, the right uterine horn was left without treatment. After waiting three menstrual cycles, the uterus of the animals were removed and histopathologically evaluated.

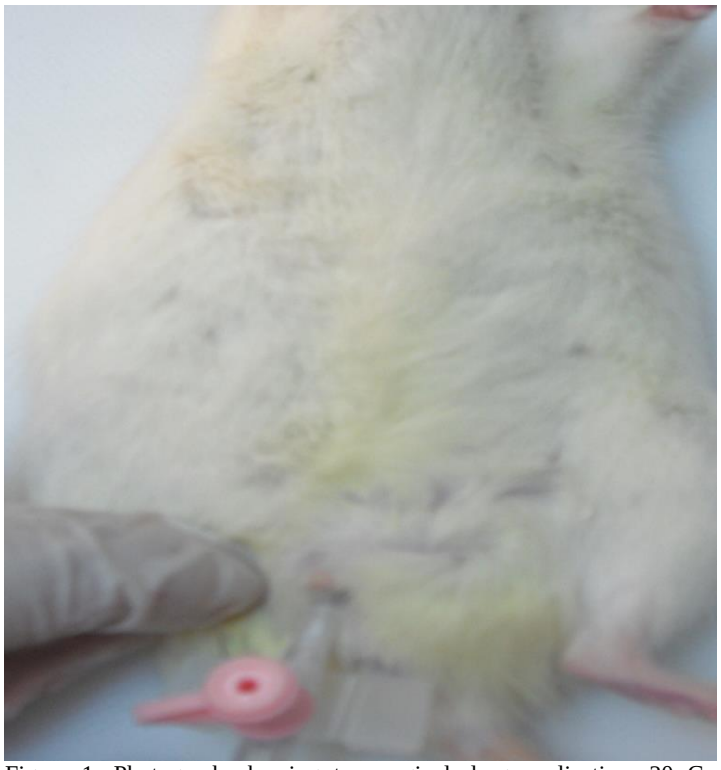

Figure 1. Photograph showing transvaginal drug application. 20 G cannula is used for reaching the uterine cavity via transvaginally.

Histopathological examinations

To investigate histopathologic changes, uterine tissue samples were consecutively numbered and placed in 10% neutral buffered formalin. After fixation with formaline, uterine tissues were embedded in paraffin. The paraffin blocks were cut in 5 mm thickness on Rotary Microtome (Leica RM2125 RTS) and the sections were stained with hematoxylin and eosin (H&E) and Masson's Trichrome methods. The histopathological sections were examined under a light microscope (Zeiss AxioScope A1) for the presence of fibrosis, inflammation, then rated on a modified semi-quantative scale of 0-3[14]. Simple columnar

epithelium-covered endometrium is considered normal if it consists of a basal layer adjacent to myometrium and a functional layer with a spongiform lamina propria, including spiral arteries and uterine glands (30). Evaluation of the specimens was done by a histologist who was blind to the study groups.

Statistical analysis

Statistical analysis was performed using SPSS for Windows 19.0 (Chicago Inc., Chicago, IL). All continuous variables were expressed as mean ±standard deviation (SD) and median (minimum–maximum). Because of the small sample size and non-normal distribution of the data non-parametric tests were used to evaluate the results. The mean values were compared by Kruskall–Wallis and Mann–Whitney U tests. A p value less than 0.05 were considered as statistically significant.

Results

As a result of the evaluation of H&E stained sections of the tissues of animals in each group, it was observed that the right uterine horns had a normal histological appearance. While the endometrial glands and the epithelial structures were evaluated as normal, there was no finding encountered in the myometrium. The left uterine horns belonging to the animals in Group 1 also had a normal microscopic appearance. In the sections of the left uterine horns taken from the animals in Group 2, it was also microscopically observed that IUAs had been developed in all animals' sections (Figure 2). In the Masson's Trichrome staining, the levels of fibrosis were evaluated (Figure 3). In animals of group 2, while IUAs were observed in the tissue sections of the left uterine horn, the fibrosis and inflammation scores were determined as grade 3 (Figure 4). Inflammation and fibrosis scores of group 2 are significantly higher than group 1 (p=0.002). No fibrosis was observed in the uterine horns of group 1 animals and the right horns of group 2 animals.

Table 1. Comparison of group 1 and group 2 in terms of inflammation.

	Group 1 (n=6)	Group 2 (n=6)	
Grade	n(%)	n(%)	p
0	5 (83.33)	0 (0)	0.002
1	1 (16.66)	0 (0)	
2	0 (0)	2 (33.33)	
3	0 (0)	4 (66.66)	

Table 2. Comparison of group 1 and group 2 in terms of fibrosis

	Group 1 (n=6)	Group 2 (n=6)	
Grade	n(%)	n(%)	p
0	6 (100.00)	0 (0)	0.002
1	0 (0)	0 (0)	
2	0 (0)	2 (33.33)	
3	0 (0)	4 (66.66)	

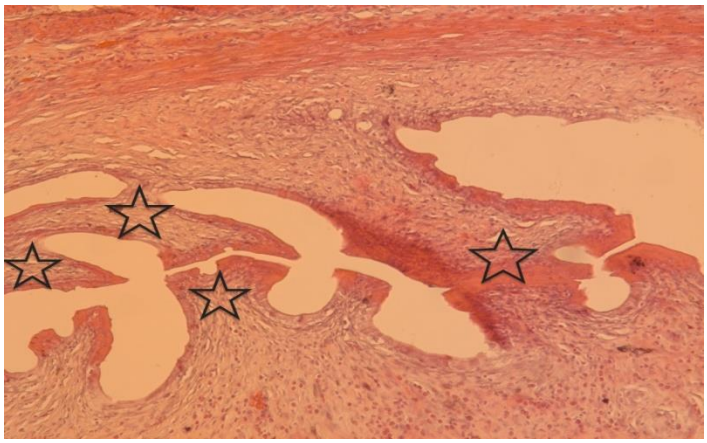

Figure 2. Intrauterine adhesions are seen in sections belong to the left uterine horn in Group 2 Stars show fibrotic bands which generate IUAs (H-E staining x100 magnification).

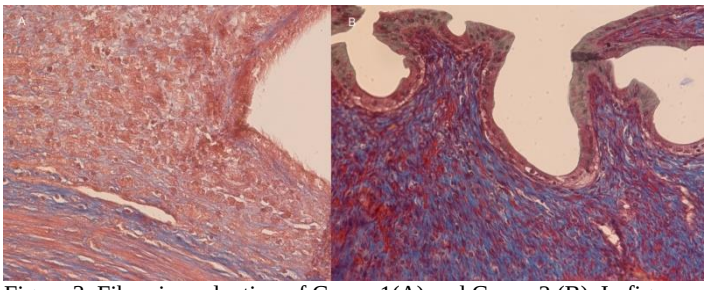

Figure 3. Fibrosis evaluation of Group 1(A) and Group 2 (B). In figure A, no fibrosis is seen. In figure B, fibrosis is seen in dark blue coloured areas (Masson's Trichrome staining x200 magnification).

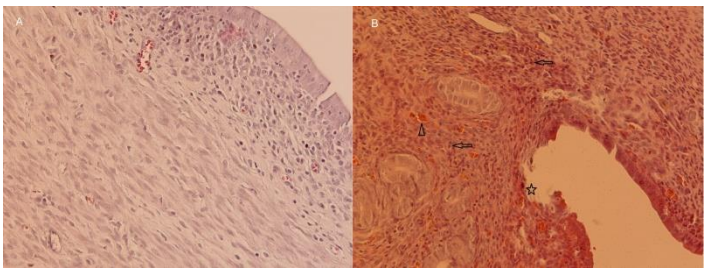

Figure 4. Histopathological evaluation of Group 1(A) and Group 2 (B). Inflammation is seen in figure B. Arrows show inflammatory cells, arrowhead shows vascular congesion and star shows epithelial erosion (H-E staining x200 magnification).

Discussion

AS is a condition that develops as a result of endometrial trauma, followed by IUAs, and causes clinical results such as menstrual disorders and infertility [31-33]. Pathological endometrial thinning is the result of damage to the basal layer, which results in the failure of the functional layer proliferation [2].

Today, development of a standard treatment model for AS is a condition that researchers are studying on. There are a number of methods proposed for the AS modeling in the literature which AS modeling was carried out by endometrial scratching [10, 11, 21, 23, 25, 26]. In some of these studies, it is seen that additional procedures are needed to create endometrial damage that is adequate to create IUA [25]. Especially in the AS modeling method recommended for the rabbits, scratching was performed with a 4 mm endometrial curette and it was attempted to create an infection by using infectious agents. It has been reported that a better result was achieved by the binary method like this way [25]. In all these studies, operations were performed by laparatomy under anesthesia. At the end of the waiting period determined for modeling process, the abdomen was opened again and tissue samples were taken. All of these surgical procedures can make the researchers to loss both time and money. Moreover, because of the importance of the care of animals after the surgery, additional workload emerges. In addition, if appropriate care is not given after surgery, animal deaths can be too great and the experiment can be unsuccessful.

In other studies suggested in the literature for the formation of IUA and AS models, endometrial trauma was attempted to create by using 24 G [18] or 27 G [16, 19, 20] needles. In those studies, the abdomen was opened surgically, the uterine horns were reached and trauma was created by entering the lumen with a needle. Since those studies also require surgery, they can bring additional load in terms of time and cost. After the operation, animal care and especially the wound follow-up may be trouble again and require being more careful. Moreover, if surgery is required for the treatment method to be tried after the modeling, laparotomy may be needed several times and may cause negative results for wound healing. Therefore, animal

follow-up and care are easier in our model that can be done non-surgically.

In addition to physical trauma, in the literature, there are also some studies in which AS is created chemically [17, 22, 24, 29]. In those studies, the experimental procedure is almost the same as in the previous ones; under anesthesia, the abdomen is opened and the related uterine horn is reached. With ethanol or TCA injection into the uterine horn, endometrial damage is attempted to create. All the side effects of the surgery are also valid for those studies, and animal care processes still needs special interest.

In our proposed modeling, the uterine cavity is reached through transvaginal route in the rats, with the TCA injection [14] used earlier in the literature, endometrial trauma is created, and after waiting enough time to prevent the fluid from escaping back, the catheter is withdrawn. In this model, it was microscopically shown that all pathological features of the AS were obtained. In addition, after the operation, no problems were observed in the follow-up process of animals and no special care was required.

In conclusion, similar to the methods suggested in the literature, the proposed nonsurgical AS modeling method created disease, and this effect was also revealed by histopathological examinations. Through this way, a new AS model is suggested without surgery, in which the disease is correctly created.

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