Autologous Free Reverse Inlay Vein Graft in The Treatment of Cripple Hypospadias; An Experimental Rabbit Model

Kriple Hipospadias Tedavisinde Otolog Serbest Ters Inlay Ven Greftini; Deneysel Bir Tavşan Modeli

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ABSTRACT

Objective: The aim of this study is using the autologous reverse inlay jugular vein graft as a substitute for defective urethral plate and the incorporation of the graft to the urethral plate. Cripple hypospadias are the patients who have a history of previous multiple unsuccessful hypospadias surgery and still having anatomical and functional complications. Insufficient local or support tissue for the repair of defective urethral plate is always a problem.

Material and Methods: In the study fourteen male 8-10 months old which is weighting four kg, New Zealand type rabbit are divided into two equal groups. Juguler vein graft (1st group) and buccal mucosal graft (2nd group) harvested and transferred to defective area in the urethral plate as a free flap.

Results: In the histopatologic examination, epithelization, fibrosis and inflammation degrees were evaluated between the groups and in-groups. In the histopatological examination of the 1st group, uroepithelization is complete or nearly complete, fibrotic activity is (+) and the inflammation was found minimal. In the second group after 21st day, the uroepithelization on the graft is nearly thirty percent, fibrotic activity is (++) and the inflammation is also found minimal.

Conclusion: From the results of the study, in the treatment of defective urethra in cripple hypospadias cases, reverse free juguler vein graft can be used as an alternative for buccal mucosa graft as a substitution material which has been used for a long time.

Key Words: Graft enhancement, Hypospadias, Urethra

ÖZ

Amaç: Çalışmanın amacı hipospadias sakatlı olgularda defektif üretranın yerine juguler venin serbest reverse greft olarak getirilmesi ve bu dokunun üretral plak ile olan uyumunun incelenmesidir. Hipospadias sakatlı olgular, daha önce hipospadias nedeni ile tamir edilmeye çalışılmış, fonksiyonel ve anatomik komplikasyonları devam eden hipospadias grubu olarak tanımlanabilir. Bu hastalarda defektif üretral plak onarımında tübülarize edilecek lokal yada destek dokusunun bulunmayışı hemen daima sorun oluşturmaktadır.

Gereç ve Yöntemler: Çalışmada on dört adet genç erişkin, ortalama ağırlığı dört kilo olan, 8-10 aylık, erkek New Zealand cinsi tavşan; yedi tavşandan oluşan iki gruba bölündü. Birinci gruptan juguler ven grefti, ikinci gruptan bukkal mukoza grefti alındı ve alınan greftler üretral plakta oluşturulan defekte serbest flep olarak aktarıldı.

Bulgular: Histopatolojik incelemede grup içi ve gruplar arası epitelizasyon, fibrozis ve inflamasyon değerlendirildi. Juguler ven grefti kullanılan grubun (Grup 1) histopatolojik incelemesinde üroepitelizasyonunun tam veya tama yakın olduğu,

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fibrotik aktivitenin (+) ve inflamasyonun minimal olduğu izlendi. Bukkal mukoza grefti uygulanan grupta (Grup 2) ise üroepitelizasyonun yirmi birinci gün sonunda greft üzerinde yüzde otuz civarında olduğu, fibrotik aktivitenin (++) ve inflamasyonun minimal olduğu izlendi.

Sonuç: Çalışmamızın sonucunda hipospadias sakatlı olgularda defektif üretranın onarımında reverse serbest juguler ven greftinin uzun zamandır kullanılagelen bukkal mukozaya alternatif olarak kullanılabileceği sonucuna varılmıştır.

Anahtar Sözcükler: Greft alımı, Hipospadiyas, Uretra

INTRODUCTION

Hypospadias is the most common congenital anomaly of penis and is seen in approximately 19.4 per 1000 male live-births (1). It is an anomaly associated with abnormal localization of the external urethral meatus in the ventral of penis, abundant cape-like skin on the dorsum of the preputium, absence of the frenulum, and often accompanied by chordee in the penis (2). Many different surgical repair methods have been defined according to different clinical types of hypospadias (3). In the last two decades, hypospadias surgery has been successfully applied at an early age with the use of magnification loops, better suture materials, glandular hemostasis, artificial erection test and testosterone stimulation (4). Cripple hypospadias can be defined as the hypospadias group, which has been tried to be repaired before, and whose functional and anatomical complications continue (5). The absence of local or support tissue to be tubularized is almost always a problem in repair of defective urethral plate in these patients. Today, buccal mucosa is most commonly used for this purpose, but difficulties in obtaining it and reducing postoperative patient comfort are important shortcomings (6). For this purpose, it is aimed to use the easy-to-obtain vein graft in distal penile dorsal urethral plate grafting.

The use of tubularized vein grafts has been tried in recent years due to complications in skin grafting such as hair growth, diverticulum, fistula and stone formation, which are generally used in urethroplasty (7). Vein grafts are preferred because they can be grafted with a simpler technique, are easily accessible and have a diameter of approximately as much as the urethra. External jugular, internal jugular or saphenous veins can be used for grafting, but internal jugular vein is preferred both as having no valves and sufficient diameter (8).

MATERIAL and METHODS

This experimental study was approved as a research project of Gülhane Military Medical Faculty Research Scientific Committee, numbered AR 2005-63, and was approved by the Ethics Committee of the Experimental Research Center ARGE-9029-9-06/ Ethics Committee

Fourteen male New Zealand rabbits, 8-10 months old, average weight four kilograms; divided into two equal groups of seven rabbits. Jugular vein graft was taken from the first group and buccal mucosa graft from the second group. All rabbits were sedated with preanesthetic intramuscular Alfazyne 3-5 mg/kg (Xylazine HCL, 20 mg/ml, 30 ml vial, Ege Vet Livestock) and Alfamine 20-40 mg/kg (Ketamine HCL, 100 mg/kg) ml, 10 ml vial, Ege Vet Animal husbandry) were placed in the supine position.

After the skin preparation, the internal jugular vein was freed by crossing the layers with a vertical neck incision, and a 1-centimeter graft was first incised in the middle and then excised from both ends by ligating from the lower and upper ends (Figure 1). The graft was left in 0.9% saline solution, while excess surrounding tissue was removed. Under sterile conditions, the urethra was catheterized with an 8 Fr feeding catheter and a suspension suture with 5/0 prolene was placed on the glans of the penis. The hypospadiac urethra was incised ventrally from the tip to the base of the penile body, sagittally, and the dorsal urethral plate was exposed. Then, with using a 2.5X magnification loop, a region of about 1 cm of the urethral plate was excised through the midline. The reverse jugular vein graft was anastomosed to the defective area one by one with eight 7/0 polyglactone sutures (Figure 2). After the anastomosis was completed, the ventral urethra was tubularized over the catheter with a 6/0 polyglactone by continuous locking technique. The catheter was fixed with 5/0 prolene suture and the operation was completed.

In the buccal mucosal graft group, sufficient oral cavity space was provided with the help of the injector placed between the teeth and a suspension suture with 6/0 polyglactone was placed on the four corners of the buccal mucosal graft. After the incision with a scalpel, the buccinator muscle was dissected with the help of sharp scissors (Figure 3). The prepared graft was left in 0.9% saline solution and excess subcutaneous tissue and glands were removed. A suspension suture with 5/0 prolene was placed on the glans of the penis and catheterized with 8 Fr feeding tube. The urethra was prepared ventrally in the same way and the urethral plate was exposed. Then, an area of approximately 1 cm from the midline of the urethral plate was excised, and the buccal tissue graft was anastomosed one by one with eight 7/0 polyglactone sutures to the defective area created with the help of magnification loop (Figure 4). After the anastomosis was completed, the ventral urethra was tubularized over the catheter with a 6/0 polyglactone suture by continuous locking technique. The catheter was fixed with 5/0 prolene suture and the operation was terminated.

The rabbits were controlled daily for urinating, the catheters placement and infection. Postoperatively for five days,

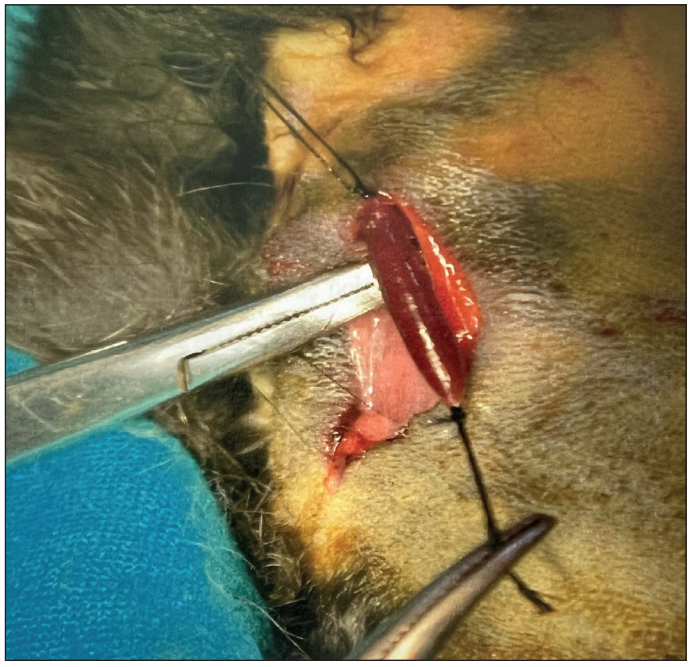

Figure 1: After the skin preparation, the internal jugular vein.

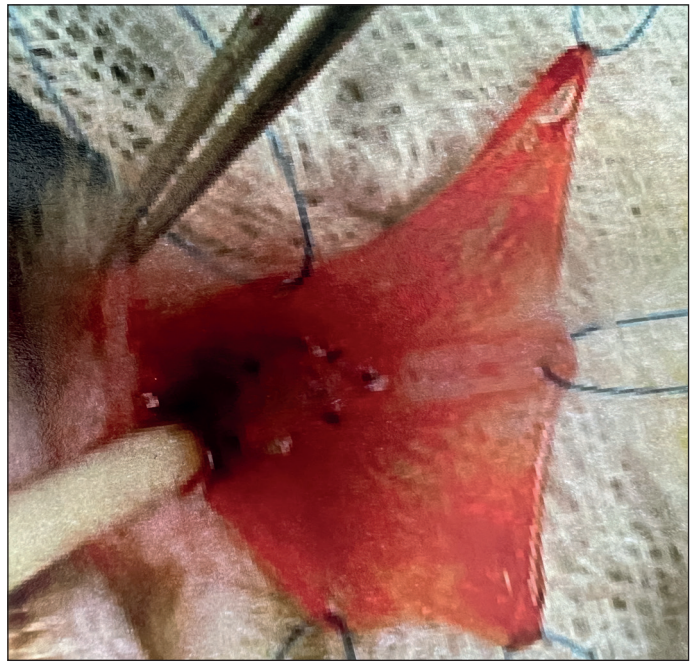

Figure 2: The reverse jugular vein graft was anastomosed to the defective area.

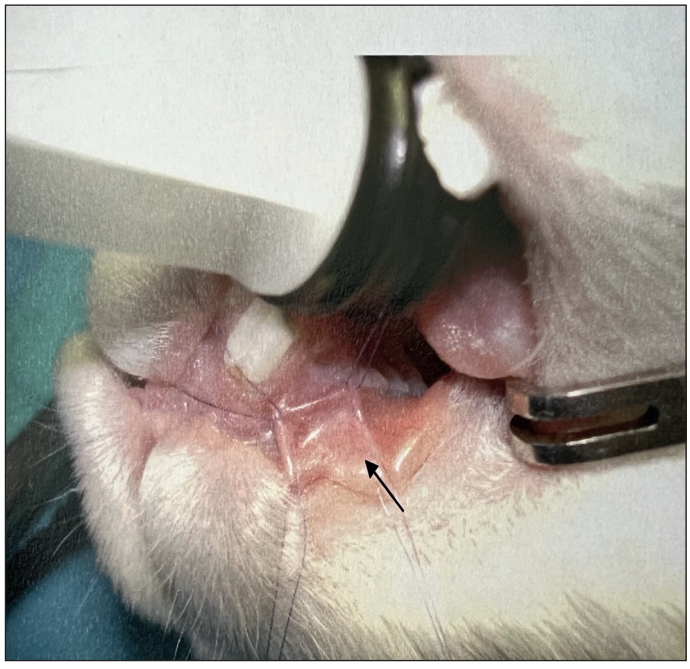

Figure 3: After the incision with a scalpel, the buccinator muscle was dissected with the help of sharp scissors.

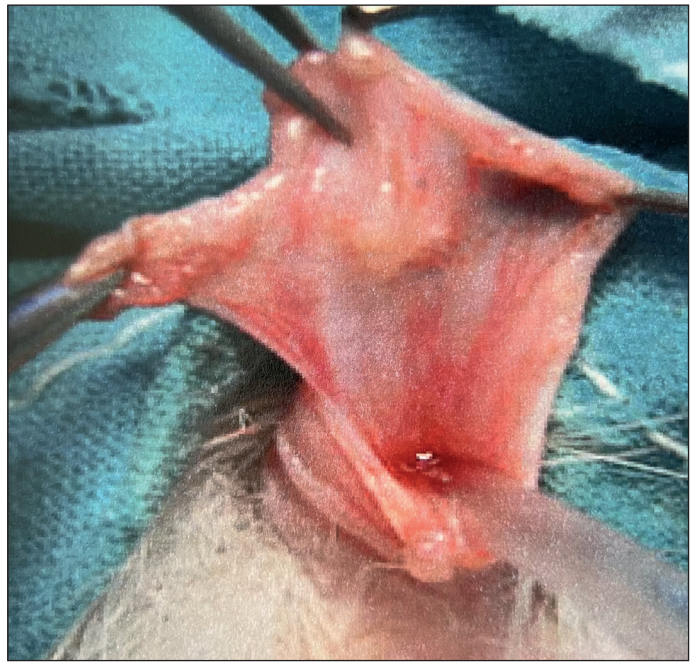

Figure 4: Area of approximately 1 cm from the midline of the urethral plate was excised, and the buccal tissue graft was anastomosed.

prophylactic antibiotic therapy was administered with 0.1 cc/kg intramuscular Terramycin (oxytetracycline, 100 ml vial, 30 mg/ml, Pfizer) once a day.

Twenty-one days after graft anastomosis, rabbits underwent partial penectomy. The penis was tied with 2/0 silk from the proximal of the graft anastomosis line and the distal penis was excised with the help of a scalpel along with the grafted area. Bleeding control was achieved with bipolar cautery. The tissue was fixed on hard plastic by stretching it from its four corners with the help of an insulin injector needles and left in formalin solution.

Tissue samples were investigated comparatively for histopathological changes. Tissue samples fixed in ten percent buffered formalin solution were then sampled in longitudinal sections, showing the urethral mucosa. After standard tissue follow-up, paraffin blocks were prepared and 4 micron thick sections were obtained from each block and stained with Hematoxylin and Eosin. The distance between the basal layer

of the epithelium and the tunica albuginea was measured visually with the help of a millimeter scale ruler on the carriage of the microscope and measured from the thickest point. Results are recorded in millimeters. Evaluation of the data was done in SPSS - 22 package program and Mann-Whitney U test was used. Values with p<0.050 were considered statistically significant.

RESULTS

All rabbits were followed up in a cage life for three weeks. When the cage bottoms were examined, no obstruction findings were found during voiding. Three weeks later, in the macroscopic examination performed during penectomy, it was observed that there was no polyp in the urethra and stenosis in the anastomosis line in both groups. It was observed that the vein grafts did not differ from the normal urethra in macroscopic view, and their location was determined only with the help of sutures. It was observed that the buccal mucosa was slightly lighter in color and was noticeable from the surrounding tissue. In the histopathological examination, differences in epithelialization, fibrosis and inflammation between and within groups were evaluated. In the histopathological examination of the jugular vein graft group (Group 1), it was observed that uroepithelialization was complete or nearly complete, fibrotic activity (+) and inflammation were minimal (Table I) (Figure 5).

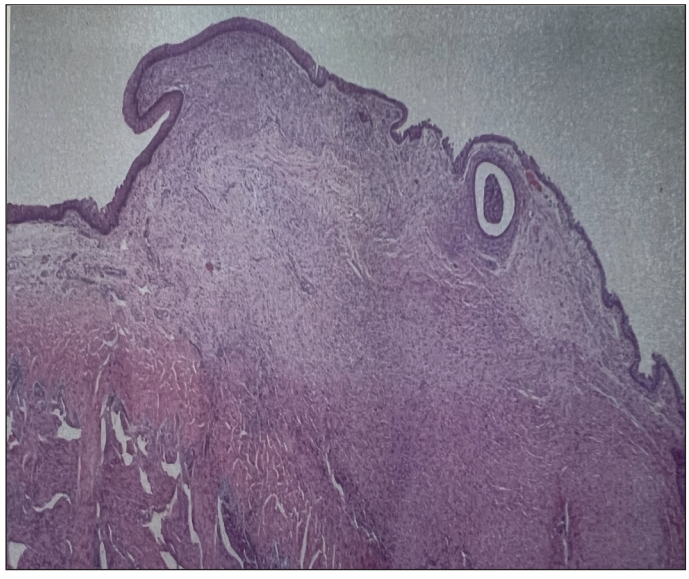

Figure 5: Uroepithelialization was complete or nearly complete.

Table I: Experimental Group 1 (Jugular vein graft).

Rabbit No:	Inflammation	Fibrosis	Fibrosis mm width	Uroepithelialization
1	-	++	1.20	%100
2	-	++	1.00	%80
3	-	++	1.40	%80
4	minimal	+	1.00	%100
5	-	+	0.60	%80
6	-	+	1.10	%100
7	-	+	0.80	%100
Mean			1.01	%91.4

Table II: Experimental group 2 (Buccal mucosal graft)

Rabbit No:	Inflammation	Fibrosis	Fibrosis mm width	Uroepithelialization
1	Minimal	++	1.50	%20
2	Minimal	+	1.10	%20
3	Medium	+++ (nodular)	1.60	%60
4	Minimal	++ (nodular)	1.40	%50
5	-	+	1.00	%30
6	Minimal	++	1.20	%30
7	Minimal	++	1.20	%20
Average			1.28	%32.8

In the buccal mucosa graft group (Group 2), it was observed that uroepithelialization was around 30% on the graft at the end of the twenty-first day, fibrotic activity was nodular (++) and inflammation was minimal (Table II).

There was no statistically significant difference between the two groups in terms of fibrotic activity (p=0.061), but a significant difference was found in terms of uroepithelialization (p=0.001)

DISCUSSION

For many surgeons dealing with hypospadias surgery, the first option is usually one-session repair methods using urethral plates or skin flaps with natural blood supply (9). Various grafts such as buccal mucosa, bladder mucosa and genital skin are used for urethral defect repair in cases of cripple hypospadias whose penile skin and urethral plate are not suitable for use due to previous unsuccessful operations. Skin grafts are not preferred as the first choice due to complications such as infection, hair growth, fistula and stone formation, development of diverticula and stenosis (10). The most important problem encountered when the bladder mucosa is used as a replacement material in urethral defect repair is the hypertrophy and stickiness of the mucosa in the part of the newly formed urethra exposed to air at the tip of the penis as seen in bladder exstrophy (11). Prolapsed bladder mucosa at the newly formed meatus level often requires revision. In addition, due to the tendency of the bladder mucosa graft to shrink, larger tissue should be removed by open surgery compared to the defect. Fistula, meatal stenosis, urethral stenosis and graft loss are also common problems in cases where buccal mucosal grafts are used (12,13). When the buccal mucosal graft used for urethroplasty as a ventral flap, it contracts due to insufficient

blood supply and the soft support tissue of the ventral flap (14). As a result of this, in the neourethra, fistula, stenosis, and the development of megalourethra due to the stenosis is inevitable (15). If the dorsal urethroplasty is performed as the buccal mucosal graft forms the dorsal half of the urethra, the viability of the graft will increase and related complications will be reduced because of a solid ground with sufficient blood support such as the tunica albuginea dorsally (16). In this case, the ventral surface of the urethra will be closed with local flaps.

The staged buccal mucosal grafting method, in which the urethral plate is excised in cases where the urethral plate is excessively scarred, as described by Bracka (17), was the basis for the above mentioned method. In this method, the unhealthy urethral plate, which has been damaged to such an extent that it cannot be used in urethral repair with dense scar tissue, is excised and removed, and the buccal mucosal graft is anastomosed to the corpus cavernosum, from the proximal urethrostomy to the tip of the glans, and tubularization is performed in the second session six months later (18). In cases where the urethral plate or midline ventral skin is extensively scarred after hypospadias surgery, a free tissue graft can be provided for urethroplasty by using a vein graft, which is easily accessible, has minimal complications that may occur during graft retrieval, and minimizes patient comfort. Veins are easily accessible tissues.

For the last hundred years, vein grafting has been experimentally tested in animal studies. Saphenous vein was used by many researchers which resulted in fistula and graft rejection (19). The first successful study with a vein graft was in 1982 by Frang et al. (20) made by the use of autologous everted vein graft for urethral defect in dogs and then lately by Xu et al in rabbits in 2017 (21). Both the jugular vein graft and the buccal mucosal graft are superior to other grafts in urethral reconstruction because they are wet and thin. It was observed that the removal of the jugular vein graft in rabbits did not cause any complications and deterioration of comfort. Access to the jugular vein was very easy between the muscles and the vein was easily dissected from the surrounding tissue. Both the tight adhesion of the buccinator muscle and the difficulty in reaching the oral mucosa while obtaining the buccal mucosal graft seems to be the disadvantage of this technique. While there was no difficulty in the anastomosis stage of both tissues, in the light of the data we obtained as a result of our study, it was decided that the free jugular vein graft in the repair of defective urethral plate is more advantageous than the buccal mucosa graft because it is easy to obtain and can be easily prepared from the surrounding tissue.

Macrophages and lymphocytes in the lamina propria of the buccal mucosa play a role in fibrosis, especially in the nodular form in our study. Although there was no statistically significant difference between them (p=0061), significantly more fibrosis was observed in buccal mucosa grafts than in jugular vein free grafts. Many authors have pointed out that after buccal mucosal grafting, a second operation is required for small fistula and meatal revision (22). In our study, in order to protect the endothelial surface of the jugular free vein graft, the graft was anastomosed reversely to the defective urethral plate. Thus, the jugular vein graft was better supplied with the endothelial tissue by passive diffusion from the adjacent tunica albuginea. Hubner et al. (23), who studied the reverse use of the jugular vein and the longer survival of the graft, also reached the same conclusion. Inflammation was minimal in both graft tissues. The most important difference we obtained in our study is the degree of uroepithelialization on both grafts, which was evaluated at twenty-one postoperative day. In the histopathological examination of the jugular vein graft, uroepithelialization was observed as complete or almost complete in all grafts, while it was observed as a patchy and small amount in the buccal mucosa. It has been determined that the uroepithelium covers on the graft surface starting from the edges and epithelialization is completed on the twenty-first day. In a different experimental study performed by Kahveci et al. (24) on 44 rabbits, have shown that, similar findings as in our study on the postoperative 3, 6, 9, 15 and 22 days, the transitional epithelium began to progress from the corners on the 6th day, turned into stratified on the 15th day, and the transitional epithelium appearance seen on the 22nd day.

Recently two adult man studies who have chronic tobacco exposed oral mucosa have been reported in the literature, urethroplasty using an everted saphenous vein graft (SVG). Substitution urethroplasty using an SVG was performed using a dorsolateral onlay technique in 20 male patients reported by Akhtar et al. The result of the report, an autologous SVG is a viable option in long-segment urethral strictures, with minimum morbidity (25). Swatantra Nagendra Rao et al reported that, thirty male patients were taken up for urethroplasty, to compare the initial outcomes of everted SVG and buccal mucosal graft as dorsolateral onlay urethroplasty. They found that outcomes of everted saphenous vein graft urethroplasty are comparable to oral mucosa graft urethroplasty (26).

CONCLUSION and SUGGESTIONS

In hypospadias surgery, the urethral plate has been the most preferred tissue for urethral reconstruction in recent years, due to its good vascularity, rich nerve support, and extensive muscle and connective layer. However, almost complete uroepithelialization of the free jugular vein graft in cases with defective urethral plate due to excessive scary tissue increases the chance of success in the next step of tubularization and also thought that reverse free vein graft could be an effective replacement material for the reconstruction of the defective urethral plate in cases of cripple hypospadias as a result of studies.

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