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Augmentation Cystoplasty Using Autograft of Thigh Fascia Lata: Experimental Study in Canine Model

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Abstract

Objective: The aim of this study was to evaluate the compatibility, enhancement of healing and late complications of autogenous fascia lata graft in the treatment ofexperimentally induced defects on the urinary bladder in dogs.

Method: Twelve adult healthy local breed male dogs were used. Animal was anthesized by atropine sulphate 0.03 mg/kg. B.W. as a premedication and 10 min later a mixture of ketamine hydrochloride and xylazine hydrochloride injected I\M at dose of 15 and 5 mg/k.g B.W. respectively. Under aseptic technique fascia lata was harvest from lateral surface of thigh, then celiotomy performed to exposed urinary bladder (UB) and induce 2×3 cm defect in ventral surface of it then substituted by 3×4cm patch of fascia lata and fixing by simple continuous suture pattern 3\0 polydioxanone.

Results: There were no death related to the procedure, all dogs voided spontaneously after surgery, urinary frequency and animal behavior changed observed through the first threedayspost-grafting includingoliguria and increase urinefrequency,loss appetite and less depress also noticed. Macroscopically good tissue integration was observed between graft and surrounded bladder tissue with gradual decrease in graft sizewith progress of study, bladder intraluminal stone was not recognized in this study. Histoopathological picture at 2nd week post graftingshowed well incorporation between fascia lata graft and host tissue throughgood infiltration of inflammatory cell, fibroblast and myofibroblast, as well as active neovascularization with complete mucosal urothelium comprised of transitional cells, while at 4thweek showed deposition of dense collagen that replaced the fascia late in several section that attached mostly with host muscle fibers,thickening in epithelia was showed at 8th week and underlying well developedby newly muscle regeneration within fasciatissue, smallfragmentof fascia lata still showed at this time.

Conclusion: Autograftfascia lata can be successfully used for replacementsmall defect of (UB) without any local or system complications in dogs.defect in

Key words:urinary bladder, autograft, fascia lata, cystoplasty, dogs.

Fascia lata=FL.

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Autograft fascia lata graft =AFLG
Post operation=P.O
Mononuclear cells=MNCs
Urinary bladder=UB

Introduction

Urinary bladder (UB) is an elastic reservoir of urine to prevent renal damage and voiding of urine (Shakeri*et al.*, 2008). Numerous conditions disrupt the anatomy and physiology of the (UB) leads to insufficient and restrict evacuation of urine (Walters and Weber, 2000).Augmentation cystoplasty (AC) aims to change the high pressure low capacity bladder into a stable environment for urine storage and emptying, so that it would prevent renal function deterioration and preserve continence (Salehipour*et al*., 2016). Varity of autologous, homologous, heterologous tissues and artificial (alloplastic) biomaterials were used for bladder wall substitution in human and animalsto keep bladder wall integrity, increase its capacity and decrease intravesical pressure (Breen *et al*., 2015).

Fascia lata is the deep fascia of the thigh originates from the tensor fascia lata muscle (Rubel*et al*., 2004;Buschmannand Bürgisser,2017), it is a strong three-dimensional network of connective tissue made up of great collagen fibers arranged in a three-dimensional network with less amount of elastic fibers give it adequate strength and flexibility against stretching, therefore commonly employed in the reconstruction of several damaged tissues (Pavletic, 2007; Maksymowicz, 2012), in addition to,it has low immunologic and inflammatory effects, non-toxic effects, low cost, more resistance to bacterial contaminations and harvestwith easy (Yonez, *et al.*, 2019).It was used in animal'sabdominal wall reconstruction by Disa*et al*., (1996), as substitution of mucosa defects in oral cavity in dogs by Uğurlu*et al.*,(2004), as well as, in repair of canine perineum and diaphragmatic hernias (Suzuki,*et al*. 2002; Bongartz*et al.*, 2005). Fascia lata also givessuccessful results when employed for reconstruction of urethral defect and urethral fistulain dogs by Atalan*et al*., (2005)and(Ayyildiz *et al.* 2006), in addition to, treatment the glottal insufficiency in the rabbit voice muscle (Pinna *et al*., 2011).

Materials and Methods

Experimental animals:

The present experimental study was carried out on twelve apparently healthy adult local breed of male dogs aging 1-3years and weighing 15-20 kg. Animals were divided into three subgroups according to specimen collection of graft site and surrounded tissue of bladder at 2^{nd}, 4^{th}, and 8^{th} weeks post operation. All experimental techniques were reviewed and approved by the Institution Animal Use and Care committee at Baghdad of veterinary medicine, Baghdad University. Iraq.

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Animal preparation and anesthesia:

All dogs were kept under observation for two weeks before the study and Ivermectin 0.2mg/kg was injected SC twice dose ofinterval 14 days. Animals received IM Cefotaxime antibiotic as 22 mg/kg, 24 hrs. before surgery.The animal was fasted for 12 hours before surgery, dogs were pre-medicated with atropine sulfate at dose rate 0.03 mg/kg, later 10 mint animals injected IM with a mixture of 2% XylazineHcl (2%, Holand) as 5 mg/kg and Ketamine Hcl (10%, Holand) as 15 mg/kg (AL-Asadi and Al-Marashdi, 1990). Both abdominal region and surface of the left thigh was prepared for surgical aseptic, then intra urethral catheter introduce and fixed in place.

Surgical procedure:

Autograftfascia lata harvest:

The dogs were position in right lateral recumbency,surgeon facing the lateral surface of left thigh,lateral longitudinal skin incision was placed at the line that connects the lateral tibial epicondyle and the femoral greater trochanter approximately 5 cm above lateral tibial epicondyle. Afterwards, a careful dissection was performed with special attention paid to avoid the injury of the iliotibial tractthen 3×4 cmfascia lata patch washarvest and put in sterile phosphate buffer saline for used later.

Partial cystectomy:

Theanimals position in dorsal recumbencythen(UB) was approach through mid-line celiotomy started lateral to prepuce and extend caudally. Once bladder exposure and emptied, it isolated from the abdominal viscera with moistened sterile laparotomy towels, then stay suture was placedat apex of bladder for handling,a stab incision by scalpel was made on ventral surface of bladder for resect 2×3cm of bladder wall(figure:1A). Sucker device was used to drain all urine or blood along the time of surgical procedure.

Autograftfscia lata cystoplasty:

The defect was induced in (UB) substitutes by 3×4 cm autograftfascia lata graft, it was suturing to bladder by simple continues suture pattern using 3/0 polydioxanon suture(figure:1B)**,**later on, the augmented bladder was repositioned after it was checked for leakage, and then abdominal incision was routinely sutured.

Post-operative care:

Each animal injected IM with Metalgen 0.2ml/ kg.once\day for three days post-operation and injected IM Cefotaxime 20mg/kg, 24hrs.for seven days. Surgical site and stich were checked daily for any complication until suture had been removed 10-14 days postoperatively.

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Evaluation:

Dogs in the current study were observed post operatively for general health status, food intake and urination behavior. Animals were euthanized at 2nd, 4th, and 8th weeks post operatively 4 animal\period for macroscopically and microscopically examine of grafting site. Thetissue specimens the graft site and surrounded tissuewere preserved in 10% natural buffer formalin solutionto prepare 5-7 µm thickness sections and stained by hematoxylin-eosin (H&E), MassoneTrichrome stains (Bancroft, 2008).

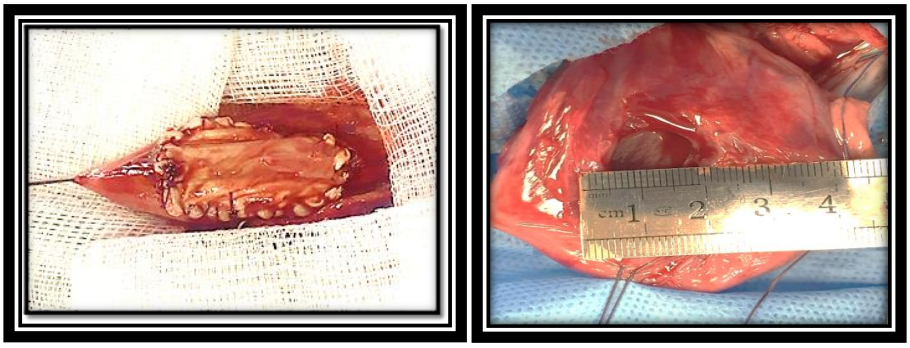

Figure:1-A shows cystectomy byresect 2×3 cm of bladder wall on ventral surface of bladder**B-** showsautogenous fascia lata graft secured on bladder defect by simple continuous suture patterns 3\0 polydioxanon suture.

Results

All dogs were survived a long the period of study with good general healthcondition. The urination process during the first third day post operation manifested by urine in continence, stranguries associated with arching of the back during urination then return to normal state gradually. Seroma was detected in two animals after 48 hours post operation ventral to the incision of the thigh that associated with severe pain and treated with draing and dressing under aseptic condition. Not recorded any stich abscess or peritonitis due to this procedure in all animal along studied period. Macroscopic examination of grafting site and lumen of bladder revealed absence of leakage at anastomosis site in addition to lack of intraluminal stone formation. Gross examination showed gradual decrease in the size of fascia lata graft with progress of time with good incorporation(figure.2 A and B), while luminal surface of graft site showed it is more progress in replacement by mucosal layer of bladder and appear as a constant surface (flat) and well incorporated with surrounding tissue without bulging surface in addition it was difficult to distinguish fascia lata graft at last period and become as small spot (figure.3 A and B).

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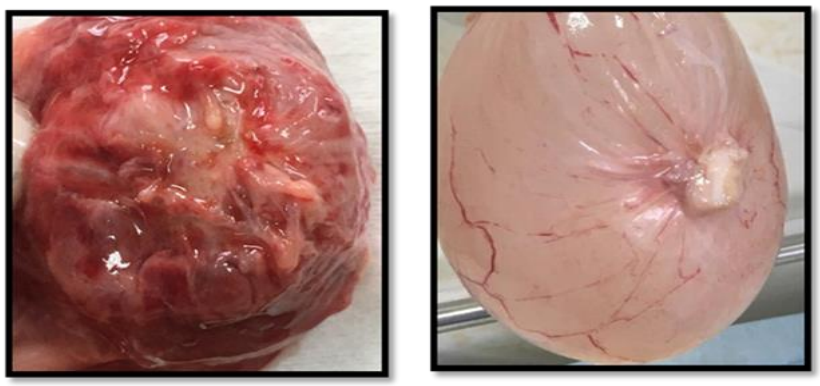

Fig. (2). A-Shows size of FL graft at 4th weeks P.O (left) which decrease in size gradually with time therefore it become less size at 8th weeks P.O as show in **fig B (right).**

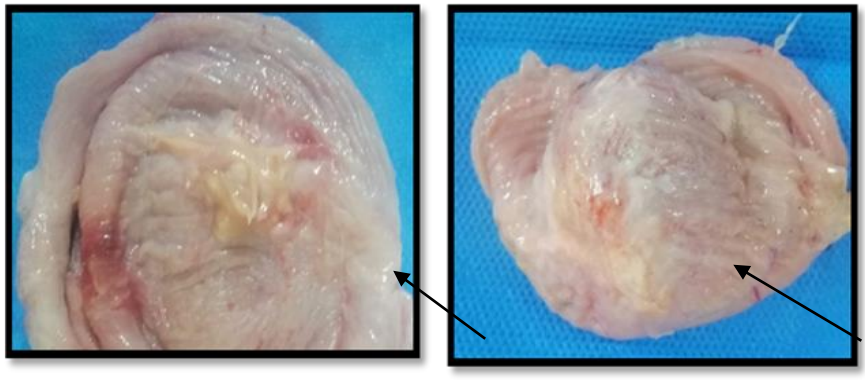

Fig. (3).A- Shows the luminal surface of FL graft at 4th weeks P.O (left). it appears more incorporated with mucosal layer of bladder (arrow) and as a constant surface (flat). **B-** Show FL grafting site at 8th weeks P.O, it is difficult to distinguish and become as small spot (arrow).

Histopathological results of the current study at 2nd week P.O showed the fragments of AFLG were infiltrated with mononuclear cells mainly plasma cells and macrophages (figure.4A) and surrounded by large amount of collagen- rich granulation tissue that extended and invaded the AFL graft. Also, early fibrous encapsulation of the AFL graft was obvious with peripheral myofibroblasts infiltration (figure.4B. Early epithelization near the surface of graft with thickened uroepithelia layer was seen that mostly associated with collagen-rich granulation tissue as well as squamous cells like appearance (figure: 4C). The results of histopathological sections at 4th week P.O demonstrated excessive fibrovascular tissue proliferation with fibrous encapsulation. as well as deposition of dense collagen that replaced the fascia late that attached mostly with host muscle fibers (figure:4D). While the other section showed fascia late- bladder were fused and the intramuscular tissue was still recognized with prominence nerve vesicles or bundles (figure:4E). Collagen fibers deposition in the submucosal layer was confirmed by trichrome stain, with highly vascular lamina propria as well as the presence of small fragments of fascia late (figure:4F).

At 8th week post grafting reveled the main characteristic of histological finding moderate newly formed smooth muscle bundle surrounded by numerous

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myofibroblast infiltration (figure:4G) with number of immature myocyte with well-developed of nerve bundle adjacent to regenerated muscle (figure:4H). In other section show that may appear epithelia thickening were noticed with underlying well developed (highly vascular) fibrous connective tissue with collagen accentuation mainly on the upper part of submucosal layer and start of new muscle regeneration within fascia section (figure:4I).

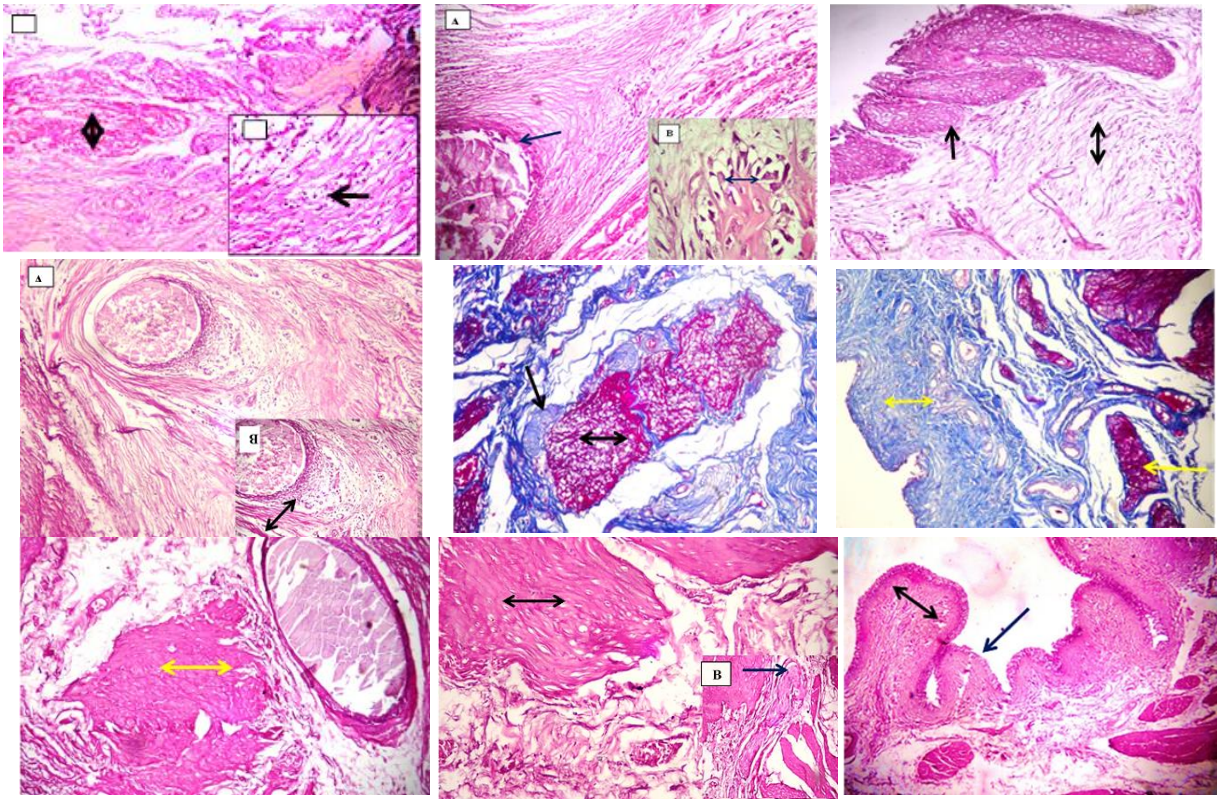

Fig.4.Histopathological findingpost cystoplasty with AFLGat week 2 (A,B,C), (D, E, F) at week 4 and (G, H, I) at week 8 P.O show **A-** (A), Fragment of FL. (H&E.) 10X. (B)- Infiltrated mononuclear cell. (H&E.). 40 X.**B-**(A)Fibrous encapsulation of FLG (H&E)10X(B)Myofibroblastinfiltration(H&E).**C-** Early epithelization at surface of implant like squamousappearancecollagen rich granulation tissue. (H&E.) 10X**D-** (A). Fibrous encapsulation around graft. (H&E) 10X (B). Dense collagen depositions replace graft. (H.&E.) 40X**.E-** Deep penetration of AFL in the bladder tissue (two-headed arrow black), prominence nerve vesicles (black arrow) (masson trichome stain).10X.**F-** deposition of collaggn in submucosal (Two-head arrow yellow). Fragment of AFL (arrow yellow) (masson trichome) stain 10X.**G-** Newly formed smooth muscle (H&E.) 10X .**H-**(A)Immature myocyte. B)well –developed of nerve bundle. (H&E.) 10X**I-**Epithelia thickening (black arrow). highly fibrous connective tissue with collagen in submucosal layer (two- head black arrow) (H&E.) 10X

Discussion

Congenital or acquired disorders of the (UB) can make it a non–compliant environment for storage and emptying of urine, causing complications such as recurrent urinary tract infections, irritation urinary symptoms, urinary incontinence, vesicoureteral reflux, or even chronic renal failure(Salehipour e*t al.*, 2016).In the current study, the clinical follow up reflect thegood general health condition of dogs along the eight weeks post augmentation of (UB) by AFL.The signs of urine incontinence and arching of the back during urination in the first three days P.O may

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be related to the feeling of pain at surgical site of cystoplasty and celiotomy through contract abdominal muscle for urination. Dewangan*et al.,* (2013) report in, it was not an abnormal manifestation during the first 10 days following cystoplasty, and it might be attributed to the diminished tone of bladder in the early P.O period and signs missing later give an indicates about the satisfactory healing at the host graft junction.

The incidence of seromadistal to the thigh incision in two animals after 48 hours P.O may be due to surgical trauma while dissections of FL from underlying muscle. Bendavid and Kux, (2001), was referred thatseroma can occurs as a result of local inflammatory response to a mechanical injury.Westphalen*et al.,* (2015), also explain the mechanisms for seroma formation is blood and lymphatic vessel injury during dissection and the release of inflammatory mediators.

Leakage of urineand stone formation were not recorded in this study. Suture type and pattern as well as tissue of F.L together may play a role in prevent occurs of these complications. Thesethought also agree with many studies Abass*et al.,* (2011); Al-Asadi and Khwaf, (2014) and Xiao *et al*., (2017),who recommended of utilizing running technique with single layer in bladder augmentation and cystotomyprocedures due to it is more strong, less time and suture remain in tissue of bladder without remark any leakage. Furthermore,Kosan *et al.,* (2008); Al-Asadi and Khwaf,(2014)andYonezand Atalan (2018) not recorded any complication when used Polydixenon suture material in cystotomy or with cystoplasty procedure when use homologues FLG in rabbitand confirmed absence of stone formation through using of FLGfor cystoplasty in rabbit and refer tissue of FL not possess features such as secretion of mucus as stomach or ileum to induce stone formation.

The gross examination of grafting site of the current study indicates the well incorporation of FLG with host tissue with gradual decrease in size of graft agreement with the histopathological results thatinclude well infiltration of FLG with inflammatory cells, fibroblast and myofibroblast, it was result in deposition of dense collagen fiber and showed partial reepithelization on the surface of FLG, all that reflect the successful role of FLG in induce acceleration of its incorporation with host tissue. it may be duo to their specific fibroblast components and collagenous network arrangement,as well as FL may reserve growth factors as it considers an extracellular matrix. The authors(Peabody and Bordoni, 2021)refer the tissue of FL is mainly composed of collagen fibers, a lower percentage of elastin within the groundsubstance compared to other brands of the body, as well as found its extracellular matrix have greatest presence fibroblasts, mast cells, myofibroblast and tenocyte, also (Gould, 2016),mentionedall ECM implants allowed and providing initial support for cell migration and adherence and facilitates ECM turnover and remodeling. The current results agree with (Shakeri*et al.,* 2008) when confirm FL scaffold acts as a bridge, not only to connect between the two edges of the cystectomies bladder, but also to facilitate cellular creeping over it for the purpose of a new bladder wall regenerative substitution.

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The results of current study at 4th week showed that FLG was moderately replaced by fibrous connective tissue and fused with host tissue with prominence nerve. While at 8th weeks showed moderate newly formed smooth muscle bundle surrounded bymyofibroblast and immature myocytes with newly muscle regeneration within fascia,as well as prominence thickening of epithelia surfaceand still the presence of small fragments of FLG.This results reflect the progress regeneration of bladder tissue at grafting site and this agree with the gross examination about decrease in graft size with time and loss of stranguries. Because the most components of FL tissue are fibroblast and myofibroblast,(Zhang *et al*., 2012) refer that autologous myofibroblast have superior potential for facilitating the repair of hollow smooth muscle organs, such as the uterus, arteries, the vas deferens, the bladder, and urethra. Also (Yonez*et al.*,2019) mention about the fused of FLG with the UB at the end of study 12th week P.O.while notable to observe the complete conversion of the fascia tissue to smooth muscle cells ended of their study at the 12thweek. (De-Eun Lee *et al.*, 2019)mention about the role of FLG in encourage the moving of urethral epithelium towards the graft and formed a single layer of epithelium on at 10th P.O day while at 30th O.P day found transitional epithelium completely covered the urethra.In addition, Sade *et al.*, (2007) was mention FLG has rapid epithelization capacity and low complication rate in animals when used in urogenital system replacement.(Yonez*et al* .,2019) referred about the newly formed urothelium in the region of the had covered the entire lumen of AFL as a whole with the normal UB after cystoplasty in rabbit.

Conclusions

According to clinical and histopathological results AFLGwere found to be effective, durable and well improved quality of life specially when used in patients with urinary bladder tumor, stress urinary incontinence, bladder tissue loss due to accidently but long-term results are awaited. Further study is required for measuring intravesical pressure, and using of allogenic FLG after process and compared itwith AFLG in canine.

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