

REPAIRING OF TRANSVERSELY CUTTING ACHILLES TENDON WITH AUTOGENOUS SMALL INTESTINAL SUBMUCOSAL LAYER IN DOGS

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Key words: Achilles tendon rupture ,intestinal submusosa ,dogs

(Received 16 September 2013, Accepted 12 October 2013)

ABSTRACT

The current trial designed to evaluate the effectiveness of using small intestinal submucosa (SIS) to accelerate healing of Achilles tendon in the stray local breed dog . Ten healthy dogs of both sexes were used in the present study which randomly divided into two equal groups. The first group; Achilles tendon acute transversely transected and sutured via cross mattress technique with silk suture material with supporting of gypsum. The second group acute transected tendon sutured and treated with the small intestinal submucosal patch . All operative dogs inspected clinically, grossly as well as histopathologically during different periods. Results obtained in the first group showed no contamination, no complications and there was no anatomical distortions were detected at operative site except there is adhesion between tendon and neighboring tissues in the first group healing was uncompleted, suture material still presence up to 90 day. At treatment group clinically animals lame and sign of mild stiffness appeared for three days, no serious complications, grossly was no adhesion detected, the submucosa subsided completely and complete healing occurs at 90 days postoperatively. Histologically at control group 30 days post operatively uncompleted healing, moderate fibroblast proliferation and inflammatory cells were presence, but at 90 days presence of dense connective at the site of operation. In treatment group healing progress, autograft is mostly atrophied and organized with granulation at 90 days, hence healing completed perfectly at this period.

In conclusion The repairing of acute transverse cutting of Achilles tendon was significantly accelerated with small intestinal submucosal layer which was absorbed and improves the tissue quality render the native one .

INTRODUCTION

The main objective of surgery for tendon injuries in the dog is to restore an adequate tensile strength to support body weight(1).As with other tissues its required to cell infiltration from the blood system to provide the necessary reparative factors for tissue healing.(2).It is usually difficult and uncertain because the prognosis varies widely depending on the amount of trauma, vascularization, create adhesions and tension, active loads (3).Occurrence of massive adhesions post-operatively between tendon and surrounding soft tissue may delay normal healing.(4), therefore different materials have been used for prevent adhesion such as small intestinal submucosa (SIS) has been used as a biological scaffold for the repair different tissue including those of the musculoskeletal systems and for repairing of defective urinary bladder in dog subcutaneous fascia in dogs (5-7).On other side ovine submucosa was used to repair superficial digital flexor tendon in goat(8). It has been also used experimentally in veterinary medicine as vascular grafts ,for reconstruction esophagus) and for accelerate ligaments and tendons healing(8-11).the aim of present study to evaluate the efficiency of SIS to improve tendon healing.

MATERIALS AND METHODS

Ten adult stray dogs of both sexes were used in this study to perform current study . The experiments were carried out with license of veterinary medicine college in university of Mosul at animals house. Operative animals randomly divided in two equal groups 5 for each. A protocol of anesthesia included xylazine \ketamine mixture 5mg \15 mg intramuscularly premedicated with atropine sulfate at dose of 0.04 mg \B.wt. In both groups site of operation in left leg at Achilles tendon was prepared aseptically, skin incised longitudinally about 5 cm over the tendon that exteriorized from sheet

and resected transversely with surgical blade. Group 1 (control): tendon was sutured with cross mattress then subcutaneous tissue closed with simple continuous suture technique then the skin incision was closed, gypsum applied to provide proper fixation. Group 2: The animals were prepared to perform laparotomy, loop from proximal jejunum exteriorized and 5 cm resected to prepare the submucosal layer. The tunica mucosa was mechanically removed by using a longitudinal wiping motion with a scalpel handle and moistened gauze. The segment was returned to original orientation and the tunica serosa and tunica muscularis were removed from the intestine by similar method. The remaining thin, whitish, translucent tube consisted of the tunica submucosa with attached stratum compactum and muscularis mucosa of the tunica mucosa (12). The SIS was then disinfected and decellularized in a 0.1% peracetic acid for 2h and sterilized with ethyl alcohol 70% for 18h and stored in distilled water at 4°C (hydrated form) and terminally re-hydrated in phosphate buffered saline for 20 min prior to use. In treatment group the Achilles tendon was transected then sutured as group 1 the treated intestinal submucosa was fixed firmly with non-absorbable suture material (Figure 1) then operation was completed as in group 1. The animals were inspected clinically then gypsum removed after 30 days postoperatively, gross and histopathological examination were taken at 30 and 90 days post operatively.



Figure 1: Intestinal submucosa was fixed firmly with non-absorbable suture material

RESULTS

Clinical results was showed no distortion in animal behavior and condition so all operative animals seem active, gain weight during over the study period no local infection or wound dehiscence were evidence at the site of operation. At control group animals were lame severely for few days, this sign was decrease and subside later. At treatment group animals underwent some undesired signs as lameness, restlessness swelling at the site of operation, the operative animals cannot use affected leg perfectly, stiffness, flexion and dragging with difficulties these signs gradually subside and the condition improved positively.

Macroscopic results

At first month in control group there were massive adhesions between Achilles tendon and neighboring tissues, presence of suture material and there was incomplete healing at the site of transection between two ends of tendon. At third month there were severe adhesion complete healing occur and presence of fibrous tissue at the site of operation. (Figure 2). In the treatment group at first month there was mild adhesion between Achilles tendon and neighboring tissues, presence of suture material and the two ends of tendon were united firmly at the site of operation (transection) (Figure 3). At the third months postoperatively the signs were observed in addition to the intestinal submucosal layer completely subsided. (Figure 4)

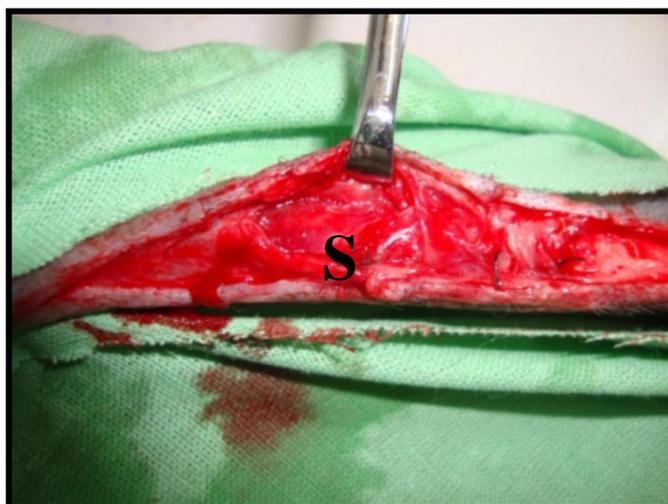


Figure 2 Canine Achilles tendon after 90 days of operation in control group there were sever adhesion (A) between Achilles tendon and surrounding tissue ,presence of suture material(S).

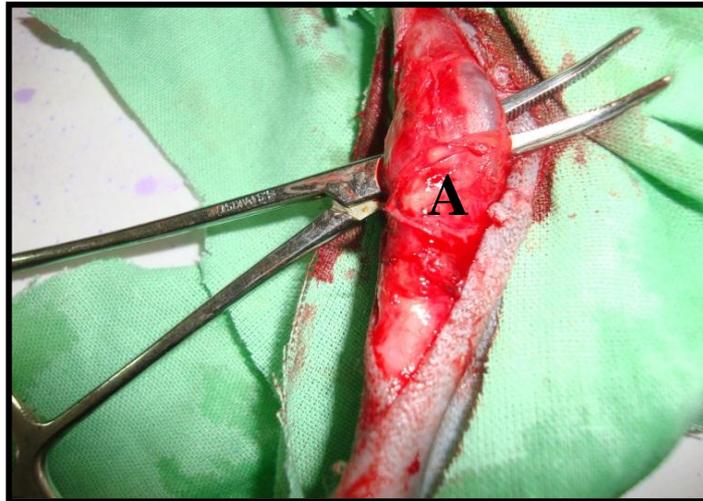


Figure 3 Canine Achilles tendon 30 days post operatively showed mild adhesion (A) at treatment group.

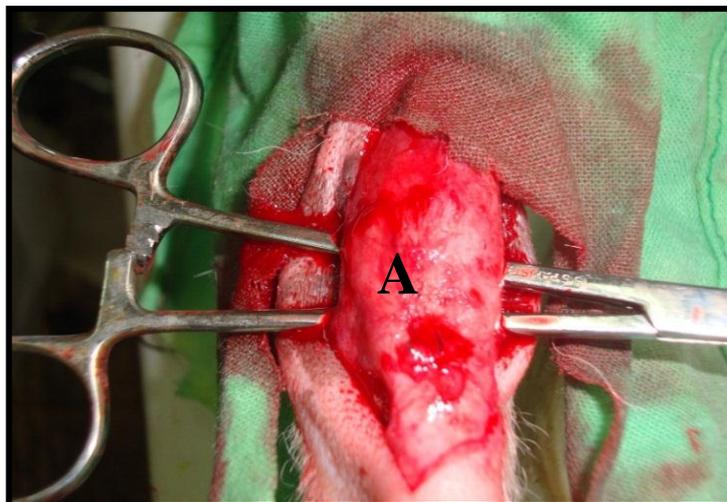


Figure 4 Canine Achilles tendon 90 days post operatively showed mild adhesion (A) SIS completely subsided. At treatment group.

Microscopical results :

At control group 30 days post operatively healing progressing with maturity of collagen bundles and moderate fibroblast proliferation, few inflammatory cells and new capillary construction (Figure 5). At 90 days post operatively showing mature fibrous tissue at site of obliteration containing regular tensile collagen bundles, well circulated blood vessels and capillaries, few proliferating fibroblast and minimal focal inflammatory infiltration (Figure 6). In the treatment group histopathological findings at 30 days postoperatively showed developing of healing reaction at both site of tendon sections, longitudinal collagen fibers were obvious in between two tendon parts, between tendon and auto graft showing transverse collagen fibers, Healing areas contains newly formed delicate woven collagen bundles, newly formed transverse and longitudinal capillaries moderate to mild fibroblasts, mild infiltrations of lymphocytes and macrophages (Figure 7,8). At 90 days post operatively two site of healing demonstrated but with much less diameter showing dense longitudinal collagen bundles with few fibroblasts and well-formed longitudinal blood vessels and capillaries, the autograft is mostly atrophied and organized with granulation tissue (fibrosed). (Image 9,10)

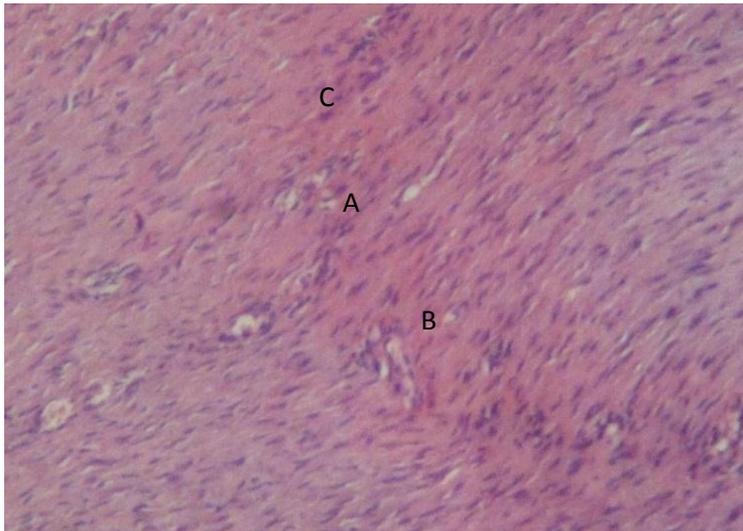


Figure 5 Photomicrograph of non grafted canine Achilles tendon at 30 days postoperatively showing site of healing progressing with maturity of collagen bundles (A) and moderate fibroblast proliferation (B), few inflammatory cells (C) and new capillary construction H&E 256X

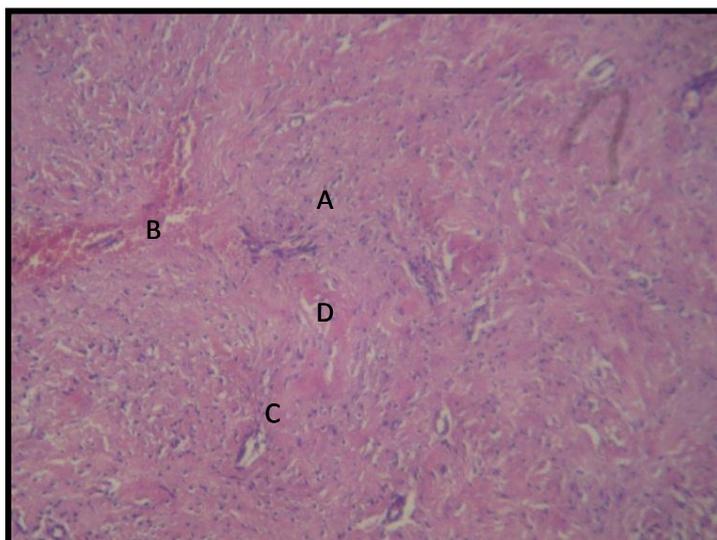


Figure 6 Photomicrograph of non grafted canine Achilles tendon at 90 days postoperatively showing mature fibrous tissue at site of obliteration

containing regular tensile collagen bundles(A) , well circulated blood vessels and capillaries(B), few proliferating fibroblast (C)and minimal focal inflammatory infiltration (D)H&E 90X

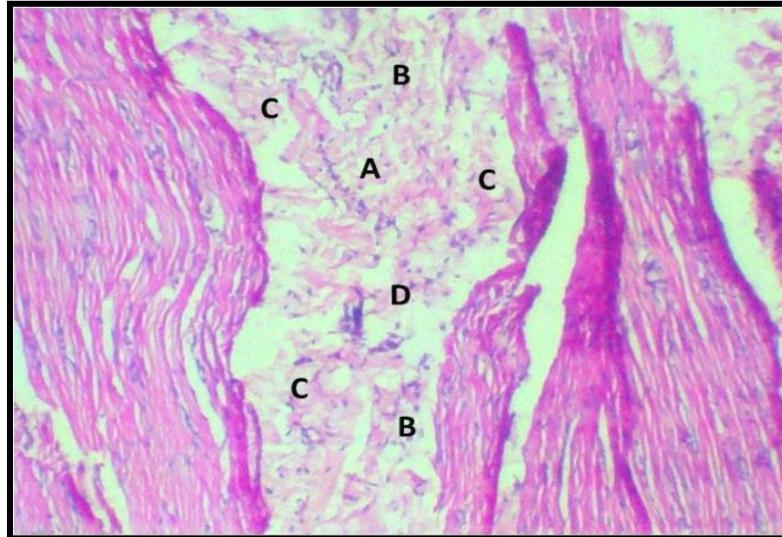


Figure 7: Photomicrograph of site of reaction between two tendon edge after 30 days in treatment group showing newly formed granulation tissue (A) , composed of moderate number of fibroblast (B), immature woven bundles of collagen fibers (C),with few inflammatory cells infiltration (D). Stain H & E,Magnification 142X

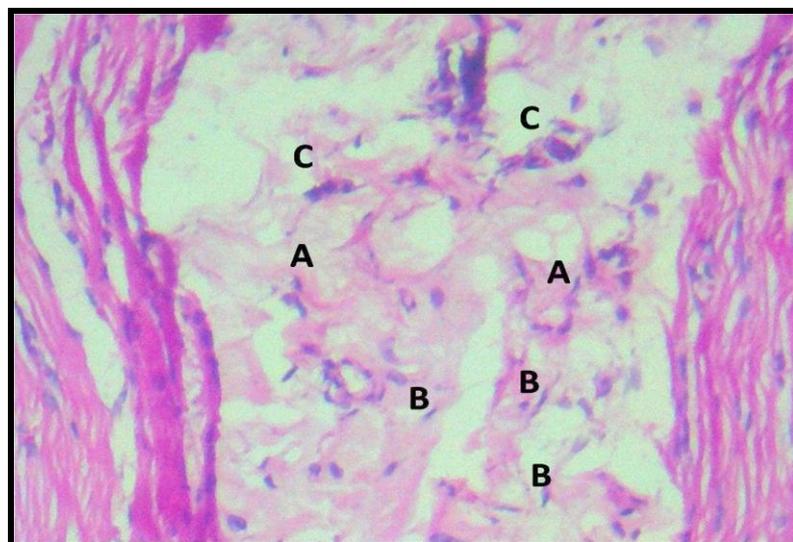
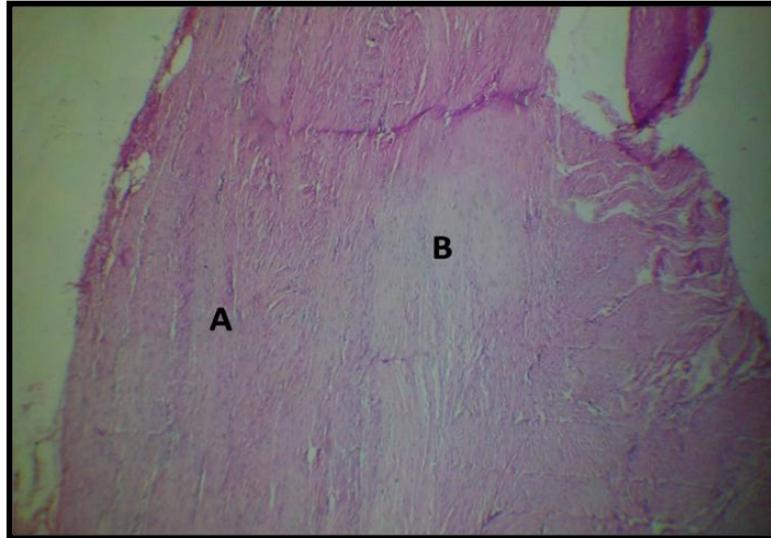


Figure 8:Photomicrograph Magnified from the previous image the site of reaction between two tendon edge after 30 days in treatment group showing collagen fibers immature (A), with moderate number of fibroblast (B) mildinfiltration of inflammatory cells (C) . Stain H& E , Magnification 350X



**Figure 9:Photomicrograph after 90 days of operationin treatment group showing two sites of reaction , between two tendon edge (A) and between tendon and autograft (B) , with mature connective tissue (fibrous tissue) , mature collagen bundles and no inflammatory cells ,few fibroblast and shrunken in size .
.Stain H& E, Magnification 105 X**

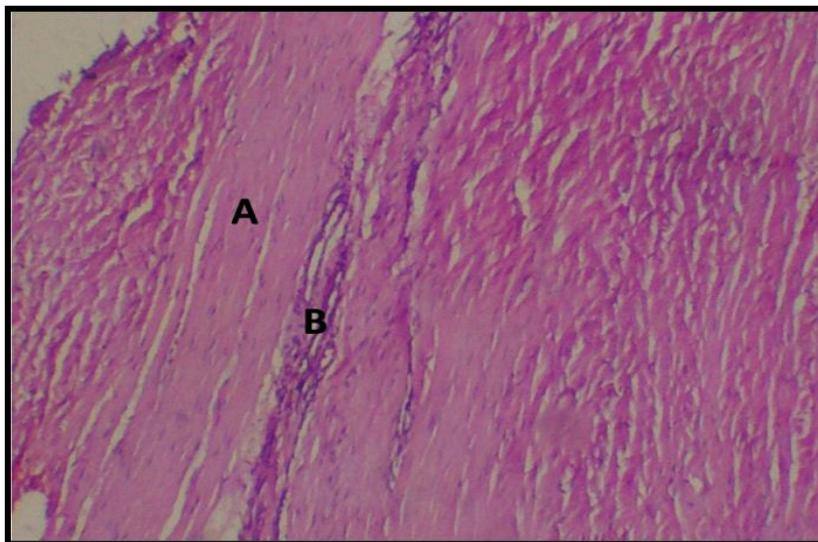


Figure 10:photomicrograph of 90 daysafter operationshowing site of reaction between two tendon part composed from mature dense connective tissue (A)with .presence of longitudinal capillaries(B) . Stain H&E. Magnification 350 X

DISCUSSION

Submucosal intestinal layer SIS has been used to repair musculoskeletal tissues and has been shown to promote cell migration into the healing region to accelerate revascularization and repair of defective tendon which still with acceptable tensile strength (13). Furthermore, animal studies have exhibited that the SIS contribute in resist infection (14). This substance contain more than the 90 protein as collagen as main structure and 10% fat and contain growth factors which promote healing and this substance easily absorbed so, it used freely as autograph this consider with (13). In present study signs of lameness mild stiffness were exhibited this due to manipulation of tendon and acute inflammation occur these signs subsided later and indicated in another studies (15). Grossly there were milds adhesion as compare to the control group and there was complete healing. The adhesions between tendon and adjacent tissue consider with (4). The Increased vascularity at the tendon following tendon division and suture has also been shown in the flexor tendon of the canine forefoot (17). The SIS occupied the site of operation and seem as native tendon tissue because this tissue act as temporary scaffold which allow to migration cells to the cutting end and accelerate the healing and reconstruction of new tissue this agree with (4). Many workers have demonstrated that the implantation of intestinal submucosa promote repairing of various connective tissues, hence helping the host tissue in growth and accelerate the cellular differentiation so new tissue seem structurally and functionally as native one this agree with (7,13). There was no indication for any remaining submucosa scaffold material this due to the ability of this layer to absorb completely (4). There was no evidence of any active inflammatory reaction present. Vascular supply was the slightly greater than the vascularity noted within the non-treated control specimens, this due to ability of this collagenous material to absorb rapidly from the hosted tissue (10).

. Histologically there was inflammatory response after one month this due to profuse blood supply to the treatment site in operative animals this permit to bringing oxygen and nutrition to the operative site and prevent rejection this agree with (12,16).Ninety days postoperatively, There was organized and collagenfew proliferating fibroblastthis due to biological nature of intestinal submucosa that induce the healing in various connective tissue and proliferate the tissue regeneration similar thenative tendon tissue in histological structure and function(10)

We can concluded,The repairing of acute transverse injury of Achills tendon was significantly accelerated with intestinal submucosal layer which was absorbed andimproves the tissue quality render the native one

إصلاح القطع المستعرض لوتر أكيل باستخدام طبقة التحت المخاطية للأمعاء في الكلاب

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اصلاح القطع المستعرض لوتر اكيل صممت الدراسة الحاليه لتقييم تاثير استخدام الطبقة التحت المخاطية لامعاء الكلاب الصغيرة لتعجيل التئام وتر اكيل في الكلاب المحلية السائبة عشرة كلاب سليمة من كلا الجنسين قسمت عشوائيا الى مجموعتين متساويتين . في المجموعة الاولى تم احداث قطع حاد مستعرض وتم خياطته بطريقة الخياطة المتقاطعة ويخيط الحرير الغير ممتص ثم تم تدعيمه بواسطة جبيرة من الجبس .في المجموعة الثانية قطع مستعرض حاد للوتر تم خياطته وتثبيت رقع من الطبقة التحت المخاطية لامعاء نفس الحيوان عليه . تم مشاهدة الحيوانات سريريا وعيانيا ونسجيا خلال فترات مختلفة بينت النتائج عدم وجود تلوث لمكان العملية وعدم وجود اية مضاعفات او تشويه في التركيب التشريحي لمكان العملية ووجود التصاق بين الوتر والانسجة المجاورة وعدم اكتمال الالتئام ووجود الخيط الجراحي لغاية 90 يوم من اجراء العملية في مجموعة السيطرة . في مجموعة المعالجة سريريا تم ملاحظة العرج وتصلب بسيط بالقوائم استمر لثلاثة ايام ولايوجد مضاعفات شديدة عيانيا لم يلاحظ وجود التصاقات مع اختفاء تام للطبقة التحت المخاطيه واكمال الالتئام بعد 90 يوم من اجراء العملية نسجيا في مجموعة السيطرة بعد 30 يوم لوحظ عدم اكتمال الالتئام ووجود ارومات ليفية بشكل متوسط مع خلايا التهابية لكن بعد 90 يوم لوحظ وجود نسيج رابط كثيف في مكان العملية . في مجموعة المعالجة لوحظ تقدم الالتئام وضمور نسيج الرقعة الذاتيه مع تعضيه ووجود النسيج الحبيبي في اليوم 90 لذلك تم الالتئام في هذه الفترة .

نستنتج من هذه الدراسة ان اصلاح القطع المستعرض الحاد لوتر اكيل سرع معنويا بواسطة الطبقة التحت المخاطية للامعاء والتي امتصت بالكامل وحسنت من نوعية النسيج وجعلته مقاربا للنسيج الام .باستخدام طبقة التحت المخاطيه للامعاء في الكلاب

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