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Palatoplasty using skin autograft and bovine skin xenograft for reconstituting an induced cleft in the hard palate of puppies: A comparative study

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Abstract

This study aims at evaluating the role of skin autograft and bovine skin xenograft to reconstitute an induced cleft in the hard palate of puppies. The number of animals in this study are eighteen puppies. The puppies are divided into two equal groups: group one (skin autograph) and group two (bovine skin xenograft). The protocol of general anesthesia used includes a mixture of 2% xylazine at dose 2mg/kg and 10% ketamine HCL at dose 10mg/kg. A cleft about (1x 2 cm) in diameter is established in the hard palate of all animals. In group one, the cleft is reconstituted using a piece of skin taken from the same animal as autograft, while in group two, a piece of bovine skin as xenograft is used. The results are evaluated through recording postoperative clinical signs with gross and histopathological changes at the 15th, 30th, 45th postoperative days. Also, the statistical analysis of histological scoring is studied. No sign of respiratory distress, especially aspiration pneumonia is observed postoperatively for all animals. Grossly, in both groups the induced cleft in the hard palate disappeared at the end of the experimental study and the implanted skin infused with the host tissue. In both groups, the histopathological changes are represented by a new granulation tissue formation and infiltrations of inflammatory cells, especially in group two than group one at early stages of the healing process, then subsides at the end of the study with a good mucosal regeneration. It is concluded that we could use skin autograft and xenograft for repairing the cleft in the hard palate of puppies.

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Introduction

Palatoschisis or cleft palate is defined a slit between the oral and nasal cavity due to failure apposition or connection of the lateral palatine process (1). Cleft palate appears or develops commonly at birth as a congenital defect in different species of animals (2-6), resulting in difficulties in swallowing with malnutrition and development of aspiration pneumonia unless excellent management is provided until surgical correction (1,7,8). Also, the defects of the maxillary and palatine bones may lead to cleft development because of acquired causes such as trauma, treatment of tumor mass, periodontal disease and erosive lesions (9-12). Surgical correction of the cleft is very necessary to preserve the life and prevent the occurrence of major complications. The main sequelae such as malnutrition, stopping or decrease of the growth, infection or inflammation of respiratory system, dental and hearing problems and death may occur (13-17). Therefore, different techniques are used to reconstitute cleft palate, including surgical and nonsurgical. However, the permeant surgical correction is the best treatment. Some techniques such as mucosal flaps, auricular cartilage grafts and artificial obturation are used to close the communication between nasal and oral cavity (18-20). Skin graft as a pedicel is used to repair palatal defects in dogs (21). Histoacryl (n-2-butyl-cyanoacrylate) is an adhesive tissue glue used in

medical applications to close tissue wounds rapidly with a minimum tissue deformation such as in bronchopleural fistula and lung wounds (22-24). Palatoplasty is a challenge to surgeons, because most techniques have undesirable results due to some affecting factors that lead to failure of the cleft palate reparation such as maxilla growth, age of animal, suture or tissue dehiscence and the cost of intensive care required (25-27).

The goal of this work is to evaluate the effect of skin autograft and bovine skin xenograft with the use of mini screws instead of suturing to close the created cleft in the hard palate of puppies.

Materials and methods

Ethical approve

The research is approved by the Ethics Committee of College of Veterinary Medicine, University of Mosul. Reference no. UM. VET.2022.027.

Experimental animals

In this experimental study, eighteen puppies of both sexes are used. The age of puppy's range 2 -3 months old, relatively. The animals are divided into two equal groups, first and second. The animals are examined clinically to ensure their safety from any diseases or oral congenital defect. All animals are kept in specific cages in the animal house at College of Veterinary Medicine, University of Mosul.

Anesthesia

All animals are fasted from food and water before the surgical operation. The animals are placed on a dorsal after the induction of general anesthesia using a mixture of 10 % ketamine HCL at dose 10 mg/kg (28) and 2% xylazine at dose 2mg/kg., intramuscularly with an injection of Atropine Sulphate as a premedication at dose 0.05 mg/kg., subcutaneously (29).

Surgical operation

After general anesthesia, an endotracheal tube is inserted into trachea to prevent any foreign materials or blood from entering to the respiratory tract during the operation. A cleft about (1x2 cm) in diameter is created in the hard palate of all animals, leaving the nasal septum intact (Figures 1 and 2). In group one, the induced cleft in the hard palate is closed by the application of a piece of skin tacked from the forehead of the same animal. The piece of skin used for palatoplasty is fixed to the hard palate by mini screws (Figure 3). While in group two, the subject used for palatoplasty is a piece of bovine skin as a xenograft after the slaughter of the animal in the abattoir. The piece of skin is cleaned mechanically to rid of any undesirable tissue such as subcutaneous and fat tissues. Then, the piece of skin is fixed like group one. Histoacryl (n-2-butyl-cyanoacrylate) as adhesive tissue glue is applied along the length of incised palatal mucosa to prevent any material from accumulation at the grafted site.

Figure 1: Showing palatal mucosal resection.

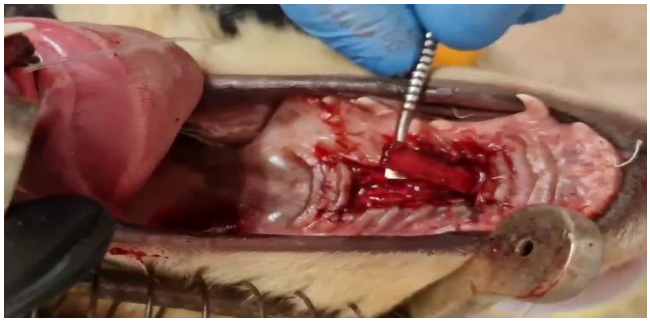

Figure 2: Showing induced cleft hard palate.

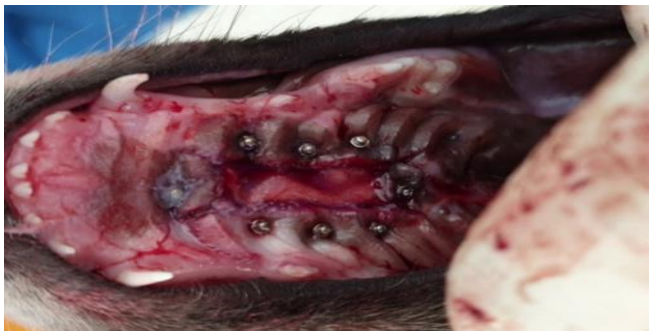

Figure 3: Showing fixation of skin piece by screws.

Post-operative care

After the operation the puppies were injected with antibiotics and antipyretics for five days, postoperatively. The animals are recovered after the surgical operation with a soft diet and water for about 15 days. The mini screws are removed at the 15th postoperative days.

Assessment of healing

The results are evaluated after euthanizing all animals using a high dose of general anesthesia using a mixture of ketamine and xylazine for biopsy collection, depending on

the monitoring of clinical signs postoperatively along with the study of the gross and histopathological changes of the grafted site at the 15th, 30th, 45th postoperative days. The statistical analysis for histological scoring is depended also.

Histological scoring

The histological sections were scored according to the following criteria (30,31): 1-Intensity of granulation tissue, 2-Degree of reepithelization, 3-Intensity of inflammation.

Table 1: Scoring of histological criteria

Criteria	0	1	2	3	4
Granulation tissue	Absent	Discrete	Moderate	Intense	Complete
reepithelization	Absent	Discrete	Moderate	Intense	Complete
Severity of inflammation	Severe	Moderate	Few	Few	Absent

Statistical analysis

The scores of histological data was analyzed statistically by RM ANOVA for the ranks test was used in the comparison of groups and periods with the utilized post hoc Duncan's multiple comparison in the Sigma Plot software program at $P \leq 0.05$ for statistical analysis.

Results

Clinical signs

The animals suffered relatively from mild degrees of anorexia that subsided gradually at the post-surgery 7th day. No signs of respiratory distress are shown, postoperatively. Also, the water and diet did not leak through nostrils during the feeding process.

Gross changes

In group one at the 15th P.O.Ds., there are some areas of palatal mucosal damage at the site of fixation with the screws and edges of oral mucosa. The connection between the host tissue and the implanted skin piece is relatively accepted at the 15th postoperative days (Figure 4). At the 30th postoperative days, there is increasing in binding between the piece of skin and tissues of the hard palate (Figure 5). At the 45th postoperative days, there is complete closure of the cleft palate (Figure 6).

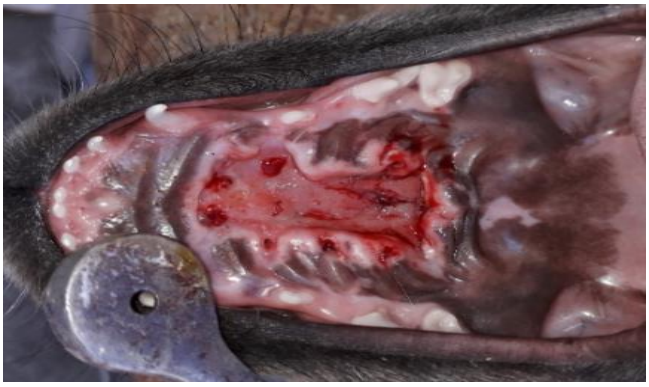

Figure 4: Showing the site of cleft palate in one of animals in G1at 15th postoperative days.

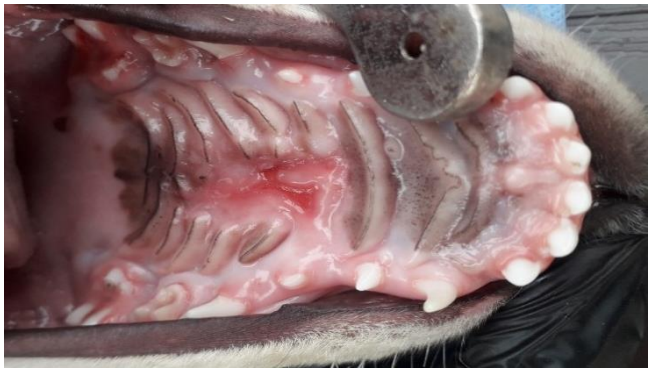

Figure 5: Showing the site of cleft palate in one of animals in G1 at 30th postoperative days.

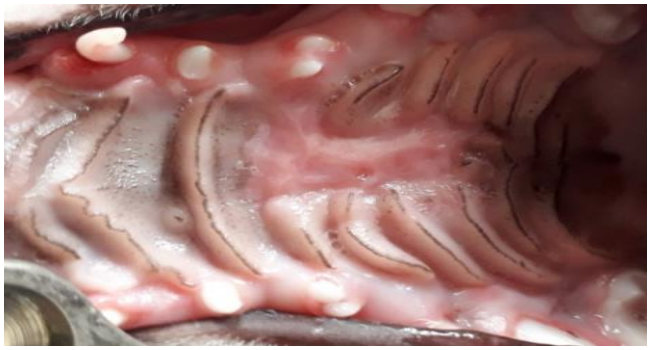

Figure 6: Showing the site of cleft palate in one of animals in G1 at 45th postoperative days.

In group two, also there are several areas of palatal mucosal damage with more redness at the site of repairing at the 15th postoperative days (Figure 7). At the 30th postoperative days, there is increasing in binding between the piece of bovine skin xenograft and the tissues of the hard palate along with a decrease in congestion of the grafted area (Figure 8). At the 45th postoperative days, there is complete closure of the cleft palate (Figure 9).

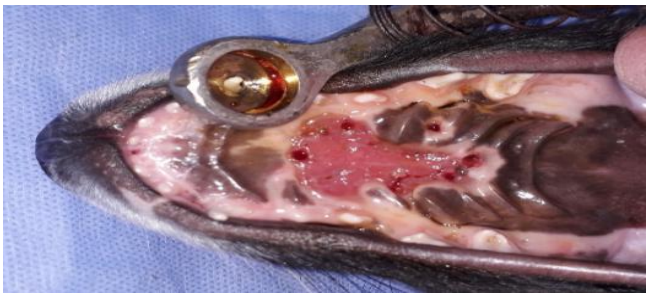

Figure 7: Showing the site of cleft palate in one of animals in G2 at 15th postoperative days.

Figure 8: Showing the site of cleft palate in one of animals in G2 at 30th postoperative days.

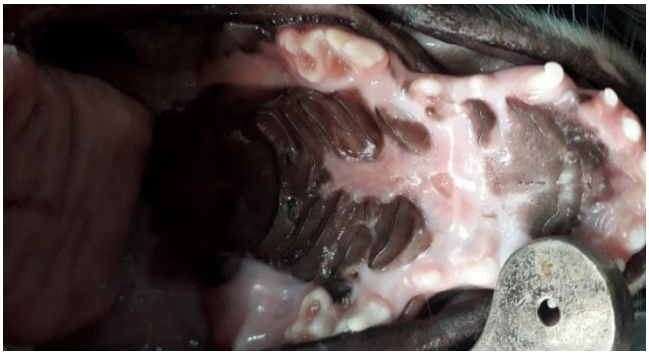

Figure 9: Showing the site of cleft palate in one of animals in G2 at 45th postoperative days.

Histopathological changes

In group one, the histopathological sections at the 15th postoperative days revealed the presence of new formation of granulation tissue with the infiltration of inflammatory cells and congestion of blood vessels with the starting of reepithelization (Figures 10 and 11). At the 30th postoperative days, the histological sections are characterized by increasing of the granulation tissue, infiltration of inflammatory cells, angiogenesis with increasing in the regeneration of mucosa. At the 45th postoperative days, the sections are characterized by more maturation of the granulation tissue and a pronounced good binding between the piece of skin autografted with the surrounding tissue at the site of the cleft reparation, in addition to the continuous regeneration of the palatal mucosa and few infiltrations of inflammatory cells (Figure 12 and 13).

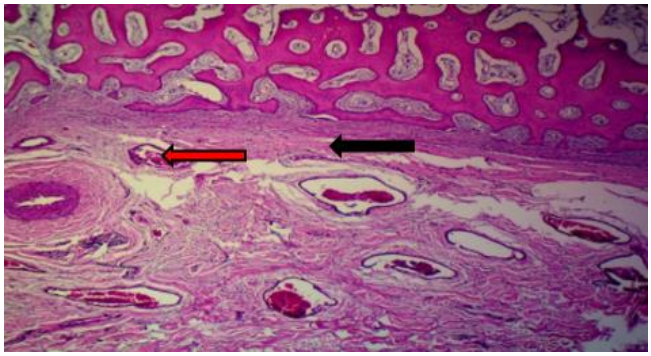

Figure 10: Micrograph at 15th postoperative days in G1 show granulation tissue formation between hard palate and skin piece (black arrow) and congestion of blood vessels (red arrow). H&E, 40X.

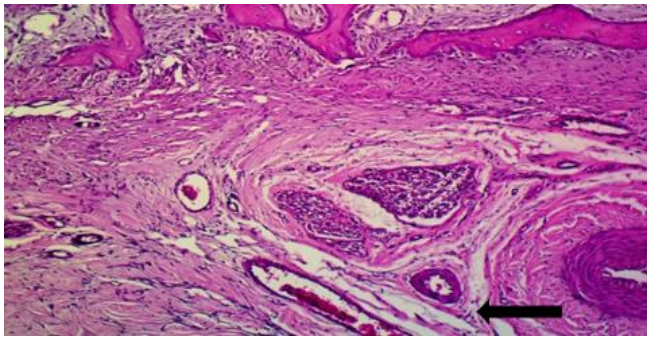

Figure 11: Micrograph at 15th postoperative days in G1 show infiltration of inflammatory cells (black arrow). H&E, 100X.

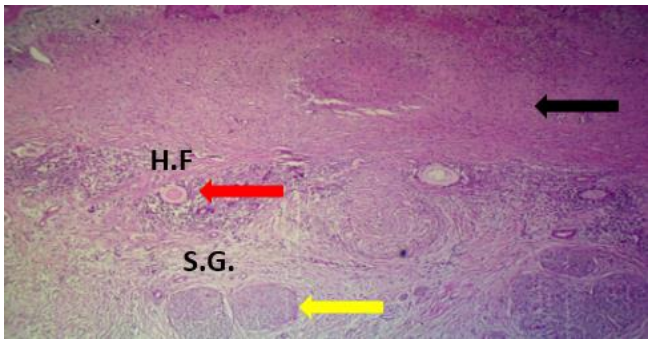

Figure 12: Micrograph at 45th postoperative days in G1show maturation of granulation tissue (black arrow), hair follicle (red arrow) and sebaceous gland (yellow arrow). H&E, 40X.

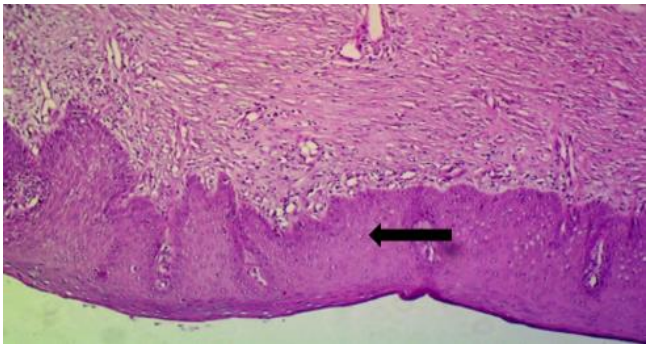

Figure 13: Micrograph at 45th postoperative days in G1 show mucosal regeneration (black arrow). H&E, 100X.

In group two, the histopathological features at the 15th postoperative days are characterized by granulation tissue formation also with a pronounced infiltration of inflammatory cells and the beginning of reepithelization like group one (Figures 14-16). The histological changes at the 30th postoperative days are similar to these shown at the previous period, but with increasing in the maturity of the granulation tissue and the degree of reepithelization with a decrease in the infiltration of inflammatory cells. At the 45th postoperative days, the histological sections are characterized by continuous reduction in the infiltration of inflammatory cells with increasing in the maturity of the granulation tissue and binding between the piece of bovine skin xenograft and the hard palate tissue, in addition to a pronounced progress in the regeneration of palatal mucosa (Figure 17).

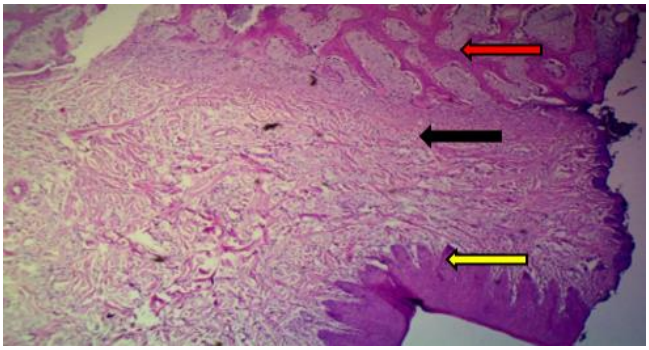

Figure 14: Micrograph at 15th postoperative days in G2 show granulation tissue formation (black arrow) between hard palate (red arrow) and mucosa (yellow arrow). H&E, 40X.

Histological scoring

The statistical analysis of histopathological sections in both groups revealed no significant difference at P≤0.05 in the formation of the granulation tissue, inflammation and reepithelization, while the statistical analysis of histopathological sections for all periods of the study, for each group revealed a significant difference at P≤0.05 in the reduction of inflammation and increasing of reepithelization rate and there is no significant difference at P≤0.05 in the formation of the granulation tissue (Tables 2-4).

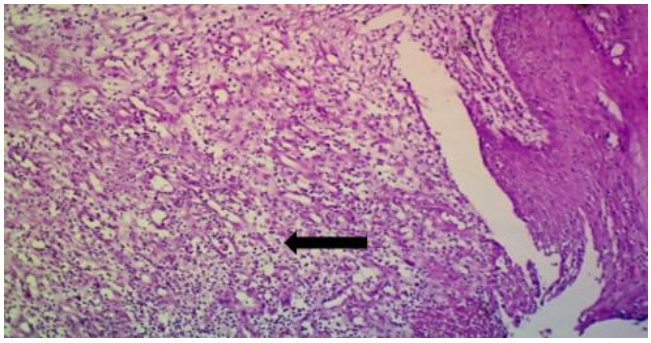

Figure 15: Micrograph at 15th postoperative days in G2 show infiltration of inflammatory cells (black arrow). H&E, 100X.

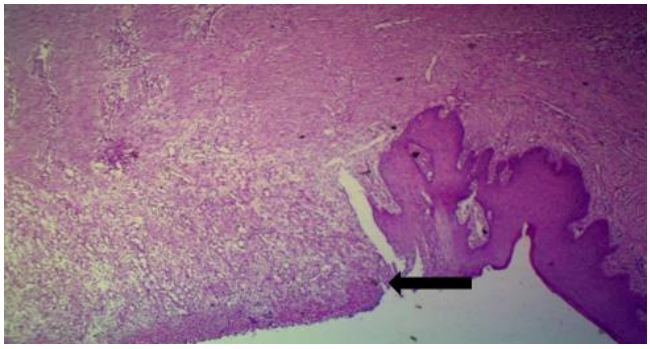

Figure 16: Micrograph at 15th postoperative days in G2 show beginning of reepithelization (black arrow). H&E, 40X.

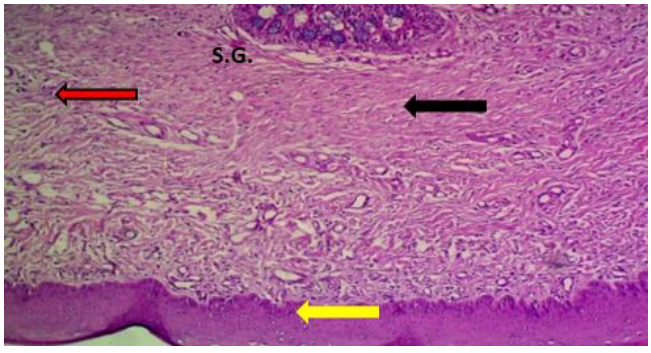

Figure 17: Micrograph at 45th postoperative days in G2 show maturation of granulation tissue (black arrow) and few infiltrations of inflammatory cells (red arrow) and Palatal mucosal regeneration (yellow arrow). H&E, 100X.

Table 2: The scores of granulation tissue

Group	15th P.O.Ds.	30th P.O.Ds.	45th P.O.Ds.
G1	2.33 ± 0.33 ^{Ab}	3.66 ± 0.33 ^{Aa}	3.66 ± 0.33 ^{Aa}
G2	3.66 ± 0.33 ^{Aa}	3.33 ± 0.33 ^{Aa}	4.00 ± 0.00 ^{Aa}

Total specimens (dogs) = 3. Data expressed as Mean ± Stander error SE. The Capital letter mean there are significant differences between groups at P≤0.05. The Small letter mean there are significant differences between periods at P≤0.05.

Table 3: The scores of reepithelization

Group	15th P.O.Ds.	30th P.O.Ds.	45th P.O.Ds.
G1	1.00 ± 0.00 ^{Ab}	2.66 ± 0.33 ^{Aa}	3.00 ± 0.57 ^{Aa}
G2	0.66 ± 0.33 ^{Ab}	3.00 ± 0.57 ^{Aa}	3.33 ± 0.33 ^{Aa}

Total specimens (dogs) = 3. Data expressed as Mean ± Stander error SE. The Capital letter mean there are significant differences between groups at P≤0.05. The Small letter mean there are significant differences between periods at P≤0.05

Table 4: The scores of inflammations

Group	15th P.O.Ds.	30th P.O.Ds.	45th P.O.Ds.
G1	1.00 ± 0.00 ^{Aa}	0.66 ± 0.33 ^{Aa}	0.00 ± 0.00 ^{Aa}
G2	1.66 ± 0.33 ^{Aa}	1.33 ± 0.33 ^{Aa}	0.33 ± 0.00 ^{Ab}

Total specimens (dogs) = 3. Data expressed as Mean ± Stander error SE. The Capital letter mean there are significant differences between groups at P≤0.05. The Small letter mean there are significant differences between periods at P≤0.05

Discussion

The surgical correction of the cleft palate is very difficult and challenging to surgeons specially in newborn animals due to several factors such as small size of oral cavity and fragility of palatal mucosa. In addition, the surgical correction may fail because of the occurrence of dehiscence after repairing the cleft surgically (32,33) and the formation of oronasal fistula (34). Therefore, some special intensive care must be provided for these animals until they become older, while the treatment at early stages is necessary to produce successful results (35). However, according to the results, the use of skin piece as auto or xenograft gives good results, where the piece of skin act as a barrier that prevents any particle of food or water to pass from oral to nasal cavity until healing is complete. The mucosal tissue damage that is seen at the site of the screw application may be due to the tightening of the screw that causes pressure on the tissue and subsequent local tissue ischemia. This feature disappeared after the removal of screws, where the local vascular circulation returned normal. In addition, the elevation of mucosa during the surgical work may lead to vascular destruction that led to the appearance of some area of tissue damage, as well. Regardless of the cause of the wound, the preservation of vascular innervation is very important to heal the wound, where any disruption in blood supply innervation may impair the processes of collagen deposition, angiogenesis and reepithelization (36-40). However, the principles of hard palate surgery such as avoiding tension on the line of suturing, preservation of blood supply, suture the fresh edges only and good apposition between both edges of palatal mucosa (41) were discarded completely through the use of mini screws instead of suturing, where the mini screws provide a strong and a good fixation to the piece of the skin in both groups with the hard palate of the animals. In both groups there is a pronounced newly granulation tissue formation, which is characterized by collagen fiber formation, fibroblast and new blood vessels with high degree of reepithelization. This agrees with Okazaki *et.al.* (42) where the healing process of wounds inside the oral cavity was faster than the wounds in the skin with a less degree of scar tissue formation. As result, the intraoral wounds are similar relatively to the fetal wounds. The presence of saliva that contains several growth factors such as the epidermal growth factor (EGF) may play an important role in enhancing the healing process of intraoral wounds (43). The production of collagen is decreased until the granulation tissue files the wound bed. In addition, the keratinocytes of oral cavity express high levels of hepatocyte and keratinocyte growth factor (42). Infiltration of inflammatory cells occurs as a result of tissue injury, where the cytokines that are released from platelets lead to attract the inflammatory cells (44). The infiltration of inflammatory cells in the second group was relatively more than group one at the early stages of the healing process and this may be due to the presence of a xenograft tissue that is considered a foreign material (bovine skin). However, at the end of the study, the infiltration of inflammatory cells decreased in both groups and this may be due to the fact that the wounds of the oral mucosa have lower levels of pro-inflammatory cytokines (45). Generally, the piece of skin in both groups acts as a scaffold to the passage and regeneration of the palatal tissue.

Conclusion

It is concluded that both, skin autograft and bovine skin xenograft could be used successfully to close the cleft hard palate in, in addition to the use of mini screws instead of suturing during the reparation of the cleft.

Acknowledgments

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Conflict of interest

The authors declare that there is no conflict of interest.

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رأب الحنك باستخدام الترقيع الذاتي بالجلد والترقيع المغاير بجلد الأبقار لترميم الشق الحنكي المستحدث في الجراء: دراسة مقارنة

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الخلاصة

تهدف هذه الدراسة الى تقييم تأثير الترقيع الذاتي بالجلد والترقيع المغاير بجلد الأبقار على إصلاح الشق المستحدث في الحنك الصلب للجراء. تستخدم في هذه الدراسة ثمانية عشر من الجراء التي تقسم الى مجموعتين متساويتين. يستخدم التخدير العام في هذه الدراسة بحقن مزيج من الكيتامين بتركيز ١٠٪ وبجرعة ١٠ملغم/كغم مع الزايلازين بتركيز ٢٪ وبجرعة ٢ ملغم / كغم. يستحدث شق بحجم ١ -٢ سم في الحنك الصلب في كل الحيوانات. يغلق الشق المستحدث في المجموعة الأولى باستخدام قطعة جلد تؤخذ من نفس الحيوان في حين يغلق الشق في المجموعة الثانية باستخدام قطعة جلد مغايرة مأخوذة من الأبقار. تقيم النتائج بتسجيل العلامات السريرية ما بعد العملية، إضافة الى دراسة التغيرات العيانية والنسيجية كل ١٥، ٣٠، ٤٥ يوم فيما بعد العملية وكذلك يعمل تحليل إحصائي للمقاطع النسيجية. لم يشاهد حدوث أي علامة سريرية تنفسية، خصوصاً ذات الرئة الاستنشاقي بعد إجراء العملية في كل الحيوانات. تظهر النتائج العيانية في كلا المجموعتين اختفاء الشق الحنكي المستحدث في نهاية التجربة مع اندماج قطعة الجلد المزروعة مع نسيج الحنك الصلب. أما نسيجياً، فتظهر النتائج لكلا المجموعتين تكون نسيج حُبيبي جديد مع ارتشاح للخلايا الالتهابية خصوصاً في المجموعة الثانية في المراحل الأولى من عملية الالتئام والتي تبدأ بالانخفاض في نهاية التجربة مع تجدد الظهارة بشكل جيد. يُستنتج من ذلك انه من الممكن استخدام الترقيع الذاتي والمغاير بالجلد في إصلاح شق الحنك الصلب للجراء.

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