

 <p>ISSN NO. 2320-5407</p>	<p>Journal Homepage: -www.journalijar.com</p> <h2 style="text-align: center;">INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)</h2> <p style="text-align: center;">Article DOI:10.21474/IJAR01/8333 DOI URL: http://dx.doi.org/10.21474/IJAR01/8333</p>	 <p>INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR) ISSN 2320-5407 Journal Homepage: http://www.journalijar.com Journal DOI:10.21474/IJAR01</p>
-------------------------------------------------------------------------------------------------------------	----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------	-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------

RESEARCH ARTICLE

VERSATILITY OF HUMAN AMNIOTIC MEMBRANE IN ORAL AND MAXILLOFACIAL SURGERY.

**ShrikantChakrawarti, Jitender Kumar Aurora, Ravinder Singh Bedi, Shiva Mani,
AmartyaPrakashSrivastava and SupriyaShakya.**

Manuscript Info

Manuscript History

Received: 08 November 2018

Final Accepted: 10 December 2018

Published: January 2019

Key words:-

Low immunogenicity, Allograft, Wound
Healing

Abstract

Objective: The objective of the study is to evaluate the versatility of Human Amniotic Membrane (HAM) in Oral and Maxillofacial Surgery.

Method: This prospective study included 15 patients having post surgical soft tissue defect requiring primary wound coverage with HAM. Patients were evaluated on subjective (pain and sensory response) and objective parameters (swelling, mouth opening, epithelization, mucosal suppleness & scar contracture) over a period of more than 6 months.

Result: Results showed significant improvement in pain. In OSMF cases, mouth opening was significantly increased when compared to pre-op mouth opening. Swelling was found to decrease on 7th post op day. The entire patient showed good sensory response (100%). Epithelization was good while one patient presented with reoccurrence. Mucosal suppleness and scar contracture was good in 93.3% patients while only 6.7% patient presented with poor mucosal suppleness and scar contracture.

Conclusion: Clinical acceptability and applicability of HAM with diverse properties makes it a versatile allograft material in maxillofacial reconstruction of soft tissue defects.

Copy Right, IJAR, 2018,. All rights reserved.

Introduction:-

Reconstruction of maxillofacial soft tissue continuity defects has always been a challenging task for the scientists, clinicians and surgeons over the years. The main goal of the reconstruction is to restore facial form, function and esthetics. Various types of grafts are available, from mucosal autografts to allogenic collagen membranes with following limitations such as donor site morbidity, intensity of pain, difficulty in harvesting, infection, rejection of grafts. Thus a need arises for an alternative graft material.

One of the new materials which have also been tried recently includes placental membranes. Human amniotic membrane (HAM) has been used successfully for a wide range of applications for over 70 years. HAM was first reported by Davis in 1910 in skin transplantation [1]. In 1913 Stern and Sabella described its use on burned and ulcerated skin surfaces [2]. In 1985, Lawson first used HAM in maxillofacial region with pectoralis major myocutaneous flap in intraoral lining [3]. Later, Lai et al. used HAM in case of oral submucous fibrosis in 1995[4]. Other uses of HAM were reported in cases of vestibuloplasty (Guler et al. 1997) [5], gingival recession (Gurinsky

Corresponding Author:-ShrikantChakrawarti.

2009) [6], mid palate oronasal fistulas (Kesting& Denys 2010) [7]. More recently, Tuncel and Ozgenel studied efficacy of HAM as an interpositioning material to prevent TMJ reankylosis in rabbit model [8].

The present study evaluates the efficacy of HAM as an alternative material for soft tissue reconstruction in maxillofacial region.

Material and Methods:-

Material:

Fetal membranes are composed of an outer layer (chorion), which contacts maternal cells and an inner layer (amniotic membrane; AM). AM or amnion is a thin membrane on the inner side of the placenta; it completely surrounds the embryo/fetus and delimits the amniotic cavity, filled with amniotic fluid. The amniotic membrane was procured from the placenta of healthy pregnant women undergoing elective caesarean sections. AM was washed with sterile phosphate-buffered saline (PBS) containing antibiotic-antimycotic liquid (penicillin, 10,000 U/mL; streptomycin, 10,000 g/mL; and amphotericin B, 25 g/mL. The AM was then deprived of amniotic epithelial cells by incubation with 0.02% ethylene diaminetetraacetic acid (EDTA) at 37°C for 2 hours. Denuded AM was freeze dried under vacuum conditions and vacuum packed at room temperature as soon as possible. Finally irradiation (25 kGy) was used to sterilize the resultant FD-AM. Bacteriologic tests were performed on the cultures to confirm sterilization

Method:

This prospective study included 15 patients (12 males and 3 females, aged 23 to 65 years; mean age: 39.80±14.47 years) having postsurgical soft tissue defects requiring primary closure. All selected patients underwent thorough clinical, routine blood and radiological investigations. The written informed consent was obtained from all patients. Institutional ethical committee clearance was obtained prior to study commencement. Patients having systemic diseases, allergic to any drug, pregnancy and lactating mother, an immune compromised state and infection locally in the area of graft were excluded from the study.

All patients underwent soft tissue reconstruction at various maxillofacial sites using freeze dried HAM procured from Department of Tissue Bank Tata Memorial Hospital, Mumbai in different sizes.

The clinical parameter evaluations were divided into two groups: Subjective parameters (pain and sensory response) objective parameters (swelling, epithelialization, mouth opening, mucosal suppleness and scar contracture).

Pain was recorded using visual analogue scale (VAS) on postoperative days 1st, 3rd, 5th and 14th day. We divided the VAS scale into four levels, as per the criteria of Izumi et al [9]:

None (0) = 0
Mild (1-3) = 1
Moderate (4-6) = 2
Severe (7-10) = 3

Sensory response was recorded by two point discrimination method at 1st, 3rd, and 6th months intervals , postoperatively, using a scoring system:

Normal sensation = 0
Altered sensation = 1

In case of bilateral involvement, the adjacent normal areas were compared.

Swelling was recorded on postoperative 1st, 3rd, 7th and 14th day. In Oral Submucous Fibrosis cases, swelling was expected on the buccal soft tissue so following landmarks were taken as reference point-

C. Lateral canthus of the eye
G. Soft tissue gonion
P. Soft tissue pogonion
T. Tragus

When the sites to be operated was tongue or lip the swelling was recorded by visualizing the swelling clinically and evaluated by using the scoring criteria of Siddique et al [10]:

None (absent) = 0
Mild (just visible and palpable) = 1
Moderate (obvious) = 2
Severe = 3

Assessment of epithelization was done at 1st week, 2nd weeks, 1st month and 3rd months postoperative. It was recorded using the scoring criteria of Arai et al [11]:

Poor (inadequate) = 1
Fair (nearly the entire wound) = 2
Good (entire wound) = 3

Assessment of mouth opening in soft tissue or pre-cancerous lesion cases was done by measuring inter-incisal distance at 1st day, 3rd day, 7th day and 14th day postoperative. It was recorded using a scoring system:

Poor (decreased) = 1
Fair (same or slightly increased < 5mm) = 2
Good (increased > 5mm) = 3

Assessment of mouth opening in oral submucous fibrosis cases was done at 1st day, 7th day, 14th day, 1 month, 3 month, 6 month and 1 year postoperative.

Assessment of mucosal suppleness was done at 1 month, 3 months and 6 months postoperative and was recorded using a scoring system:

Poor (contracture) = 1
Fair (slightly altered) = 2
Good (similar on both sides) = 3

Assessment of scar contracture of the wound was assessed at 1st month and 3rd month after surgery and categorized as:

Absent- 0
Present- 1

Following Cases were taken up in this study:

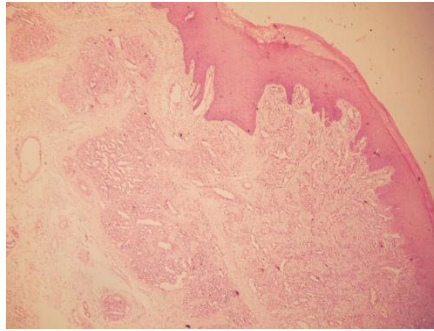
Case 1:-Capillary hemangioma

Pre-op



Pre-op Lesion





Histopathology of the lesion



Excision of lesion with HAM placement



Post-op after 6months.

Case 2:-Oral Submucous Fibrosis.



Pre-op



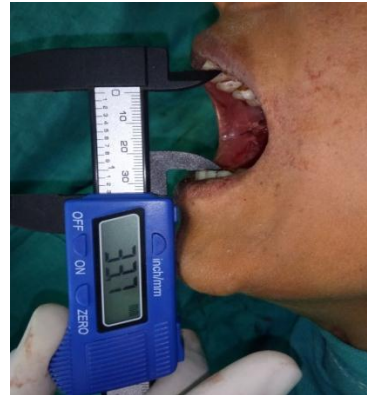
Pre-op Mouth Opening



HAM Placement



Intra-op Mouth Opening



Post-op after 6 Months

Case 3:-Tongue leukoplakia



Pre-op



Pre-op Lesion



Excision of Lesion



Excised Lesion



HAM Placement



Post-op

Case 4:-Tongue Leukoplakia.



Pre-op



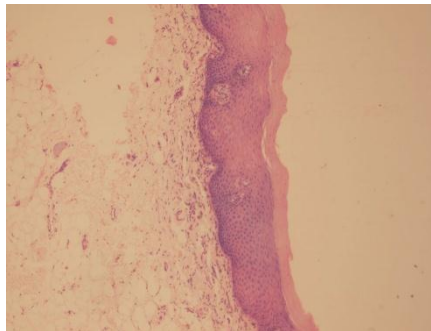
Pre- op Lesion



Excision of Lesion



Excised Lesion



Histopathology of the lesion

**HAM Placement****Reoccurrence of the Lesion****Table 1:-**Brief overview of the patient

S.No	Pathology/Diagnosis	Age/ Gender	Surgical Procedure	Site reconstruction of	Follow up
1.	Oral submucous fibrosis	26/F 39/M 28/F 28/M 34/M 42/M 28/M	Fibrous band removal+ coronoidectomy +HAM	Buccal mucosa	>6month
2.	Pre-cancerous/ Oral mucosal lesions	24/M 50/F 23/M 62/M 40/F 65/M 61/M 48/M	Excision biopsy+ HAM	Labial mucosa, buccal mucosa	6 mont h

Statistical Method:-

The results were analyzed using descriptive statistics and making comparisons between the baseline and various follow up values.

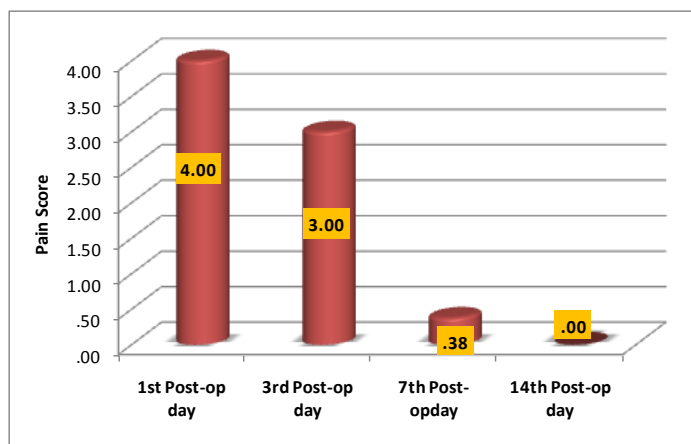
Data was analysed using the softwares MS Office Excel software & SPSS 17 for Windows.

The following statistics were calculated in the present analysis: Mean and standard deviation (SD) , Chi Square Test, Paired t-test, Wilcoxon z test (Wilcoxon signed rank test).

Statistics and Result:-

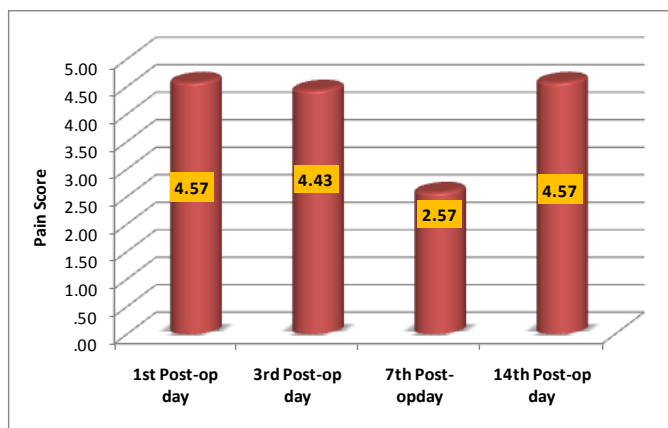
Fifteen patients were recruited into the study; 12 were males and 3 were females. The average age of the patients at the time of surgery was 39.80 ± 14.47 years (range 23-65 years). Oral submucous fibrosis, soft tissue and precancerous lesions such as leukoplakia, erythroplakia, affecting various areas of the oral cavity, such as the buccal mucosa, tongue, alveolar mucosa and both buccal and alveolar mucosa were included (gingival lesions were included in the alveolar mucosa).

The results indicated that pain relief was good in all the patients having soft tissue and pre cancerous lesion. Improvement in pain was seen on 7th post-op day which reduced to nil on 14th post-op day. There was significant improvement in the scores achieved from the first to the seventh day(Graph 1).



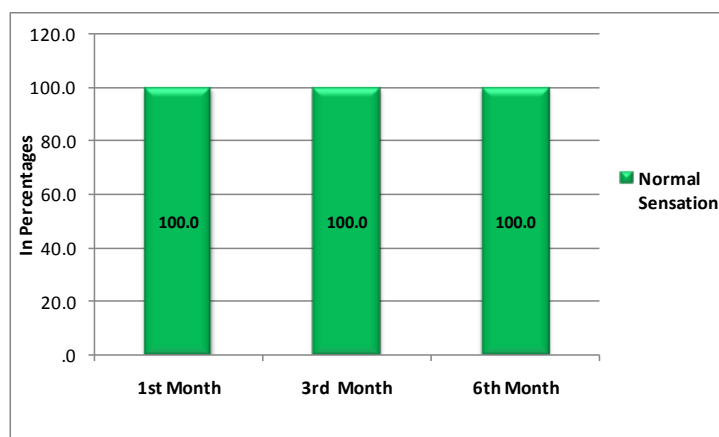
Graph 1:-Pain Score Changes with time

On the other hand, patients treated with oral submucous fibrosis had a mean pain score of 4.57 ± 0.54 on 1st post-op day which showed gradual reduction to 4.43 ± 0.54 on 3rd post-op day ($p=0.317$) and 2.57 ± 0.54 on 7th post-op day ($p=0.014$) but again increased to 4.57 ± 0.54 on 14th post-op day ($p=1.000$) as they were started with the active physiotherapy (Graph 2).



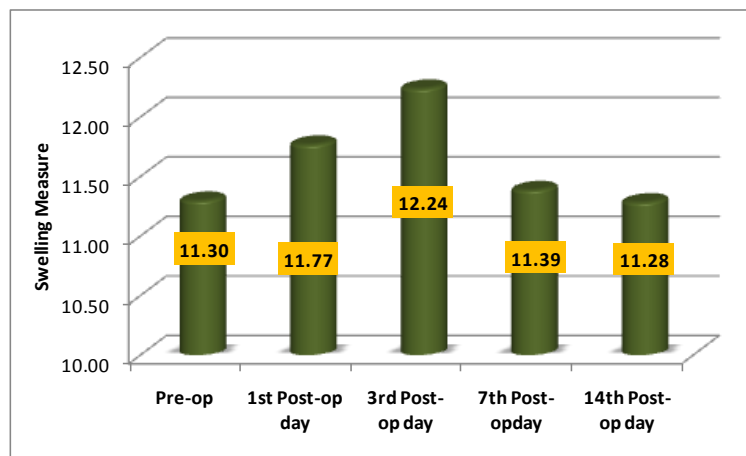
Graph 2:-Pain Score Changes with time

In the present study the sensory response status of patients was recorded at 1st month, 3rd month and 6th month. Sensory response was observed to be normal in all the cases (100%) at the follow up of 6 months (Graph 3).



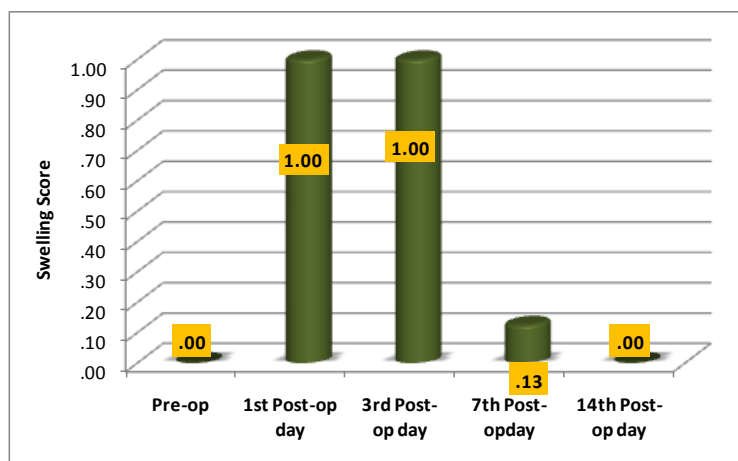
Graph 3:-Sensory Response Status of Patients

In our study, swelling in OSMF cases measured pre operatively which increased significantly on 1st post-op day and 3rd post-op day. Swelling was found to decrease on 7th post op day which further reduced on 14th post-op day which was not significantly different from the baseline.(Graph 4)



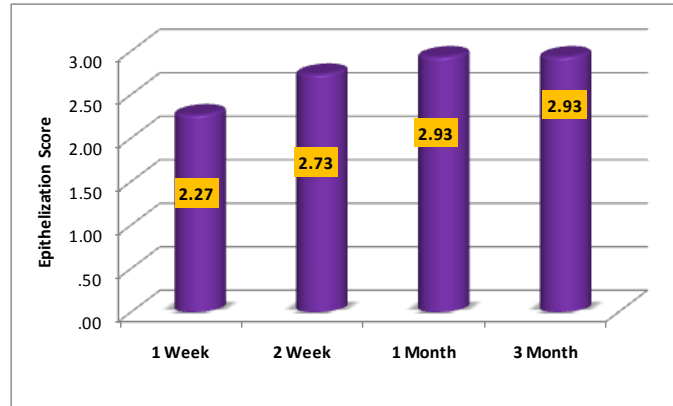
Graph 4:–Swelling Measure Changes with time

However, swelling in pre-cancerous lesion swelling was found to subside on 7th day itself. Only 12.5% patients suffered with mild swelling while rest 87.5% patients presented with no swelling on 7th post op day. Swelling was completely eliminated from all the patients on 14th day. (Graph 5)



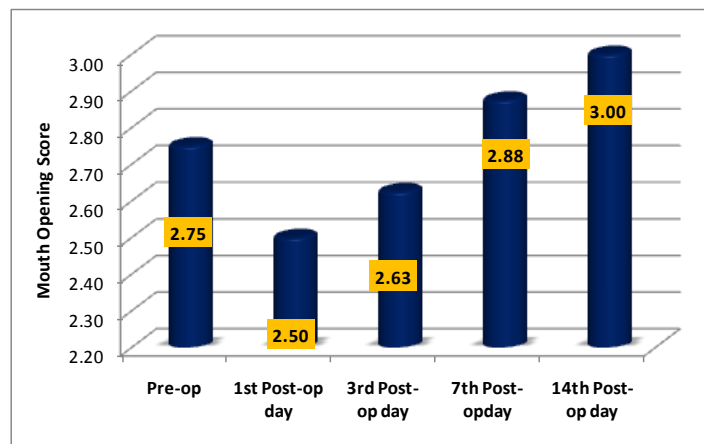
Graph 5:–Swelling Score Changes with time

After first week, in all the patients an unepithelialized mucosa was present over the graft area. At the end of the second week, a slight hyperaemic mucosal tissue was present, and a completely epithelialized wound was seen after 1 month. By the third month, the graft area could not be distinguished from the normal mucosa. Epithelization was good in 93.3% patients. Only 6.7% patients (one patient) presented with reoccurrence (Graph 6). This patient was the oldest and had the largest wound among the patients and had poor oral hygiene.



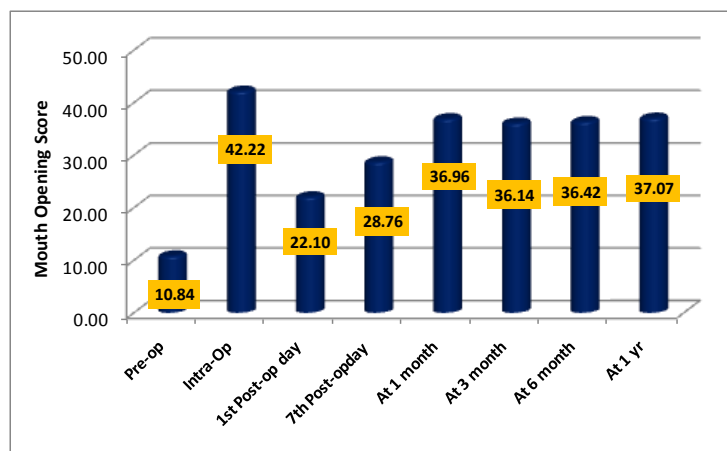
Graph 6:-Epithelization Score Changes with time

With regard to mouth opening, in pre-cancerous lesion cases where lesion was present on tongue or lip. Good mouth opening was found in 87.5% patients while 12.5% of the patients had slightly reduced mouth opening status on 7th post op day. All the patients showed good mouth opening (100%) at 14th day (Graph 7).



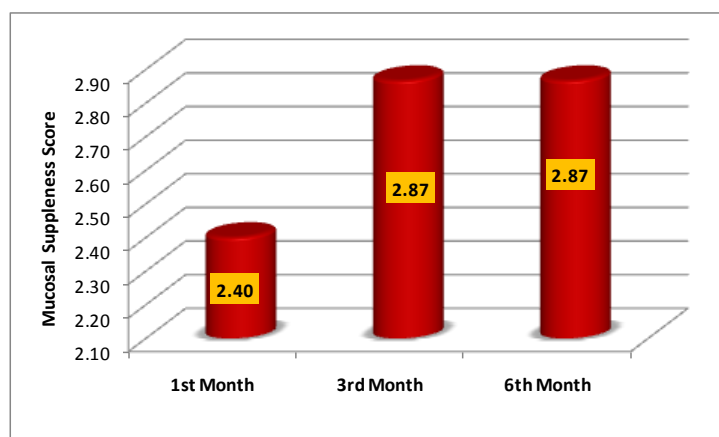
Graph 7:-Mouth Opening Score Changes with time

While mouth opening in OSMF cases when compared to pre-operative mouth opening there was significant increase on 7th post op day, 1 month, 3 month and 6 month respectively. Only 2 patients gave a follow up of 1 year and the mean mouth opening was significantly increased. (Graph 8). However, when compared to intra-operative mouth opening there was significant decrease on 7th post op day ($p < 0.001$), 1 month ($p < 0.013$), 3month ($p = 0.001$) and 6 month ($p = 0.001$).



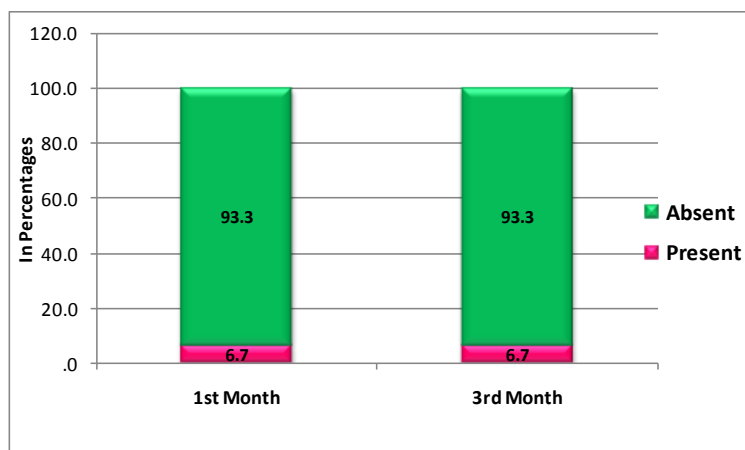
Graph 8:-Mouth Opening Measure Changes with time

After 6th months, mucosal suppleness was poor only in 6.7% patients (one patient) while remaining 93.3% patients had good mucosal suppleness (Graph 9).



Graph 9:-Mucosal Suppleness Score Changes with time

And only 6.7% patient (one patient) had the scar contracture. This may be due to the large size of the lesion (Graph 10).



Graph 10:Scar Contracture Status of Patients

Discussion:-

HAM is a thin, tough, transparent, avascular composite membrane composed of three major layers: an epithelial monolayer, a thick basement membrane, and underlying stroma. Nutrients are supplied directly by diffusion, from the amniotic fluid and/or from the underlining decidua. The amniotic epithelial cell layer is a single layer of flat, cuboidal and columnar cells that are in direct contact with the amniotic fluid [12]. Normal AM is 0.02-0.5 mm thick, which is equivalent to 6-8 cells [13].

HAM has gained importance because of following factors:[14]

1. Reduces scarring and inflammation and enhances wound healing.
2. Serves as a scaffold for differentiation and proliferation of cells.
3. Due to the presence of growth factors in it's extracellular matrix, it is an excellent biomaterial for tissue engineering.
4. It can be easily procured, processed and transported.

Human amniotic membrane is mostly used as dried, frozen, irradiated and lyophilized form.

In the present study we have evaluated the versatility of Deep Freeze-Dried HAM in oral and maxillofacial surgery. Samandari et al [15] studied the use of amnion as a graft material in vestibuloplasty and found that graft was completely epithelialized by the third month. In the study of I.B Karet al [16], the patients showed good epithelialization after 3 months. Lawson in his study, suggested that amnion enhances re-epithelialization of the oral cavity and reduces the contracture of moderate sized defect [3]. In our study Epithelialization was good in 93.3% patients. Only 6.7% patients (one patient) presented with reoccurrence. This patient was the oldest and had the largest wound among the patients and had poor oral hygiene.

Unlike other organs such as the skin, grafts to oral wounds have some special problems. First, the oral environment is always wet by salivary secretion. Second, there are constant movements of the cheek and tongue. These factors may interfere with the adherence and retention of the graft materials, resulting in the failure of epithelialization and in the formation of scar tissues.

Lai et al [4] studied various modalities in the treatment of oral submucous fibrosis, one of which was the application of a fresh amnion graft over the buccal mucosa after excision of the fibrous tissue. They found that a decrease in inter-incisal distance in the range of 5– 10 mm after 2 years of follow-up occurred in 62% of cases with a fresh amnion graft as compared to 50% with a split thickness skin graft and 38% with a buccal fat pad graft. They suggested that fresh amnion grafts were not effective in a single layer over deep buccal defects. Of note, however, we found no case with a decrease in oral opening during 6 months of follow-up in the present study.

Mucosal Suppleness was poor only in 6.7% patients (one patient) while remaining 93.3% patients had good mucosal suppleness. And only 6.7% patient (one patient) had the scar contracture. This may be due to the large size of the lesion. In the study of I.B. Kar et al [16] after 6 months, 20 patients (59%) had good mucosal suppleness, 12 patients (35%) had fair suppleness and 2 patients (6%) had scar contracture at the operated site.

Borle and Borle [17] reported disappointing results with split skin grafts in covering the mucosal defect after excision of fibrotic bands in the management of oral submucous fibrosis. Khanna and Andrade [18] reported the incidence of shrinkage, contraction, and rejection of split skin grafts to be very high, leading to poor oral conditions and recurrence in 12 cases of oral submucous fibrosis. Shi et al [19] reported a 94% success rate after the application of acellular dermal matrix in the reconstruction of oral mucosal defects. Hao [20] reported one (5%) failure and one (5%) complication in 21 patients after applying a pedicled buccal fat pad flap in the reconstruction of oral defects. Similar results were reported by Dean et al [21]; only one (3%) out of 32 patients showed partial loss of the buccal fat pad flap. This suggests that in comparison to the split skin graft, AlloDerm, and buccal fat pad flap, the HAM graft is a better option for oral reconstruction in terms of infection and graft failure.

Conclusion:-

1. In our study we found that Human Amniotic Membrane is a biological graft material having diverse properties like anti-inflammatory, anti-fibrotic, anti-adhesive, anti-angiogenic, anti-scarring, antimicrobial and low

immunogenicity with reasonable mechanical properties, thus making it a versatile allograft in maxillofacial reconstruction of soft tissue defects.

2. Clinical acceptability and applicability of HAM as a good grafting material for oral cavity reconstruction above all other graft materials will ensure a reliable, available and easily affordable option for surgeons and patients.

Conflict of interest

None

Funding

None

Ethical Approval

Approved by ethic committee of Saraswati Dental College and Hospital, Lucknow, India (SDC/IRDC/03/11.01.2016).

Patient consent

The patients provided written informed consent.

References:-

1. J. W. Davis, "Skin transplantation with a review of 550 cases at the Johns Hopkins Hospital," Johns Hopkins Medical Journal, vol. 15, pp. 307–396, 1910.
2. Trelford JD, Trelford SM. The amnion in surgery, past and present. Am J Obstet Gyne-col;134:833–45. 1979.
3. Lawson VG. Oral cavity reconstruction using pectoralis major muscle and amnion. Arch Otolaryngol;111:230–3;1985.
4. Lai DR, Chen HR, Lin LM, Huang YL, Tsai CC. Clinical evaluation of different treatment methods for oral submucous fibrosis. A 10 year experience with 150 cases. J Oral Pathol Med;24:402–6;1995.
5. Guler R, Ercan MT, Ulutuncel N, Devrim H, Uran N. Measurement of blood flow by the ¹³³Xe clearance technique to grafts of amnion used in vestibuloplasty. Br J Oral Maxillofac Surg;35:280–3;1997.
6. Gurinsky B. A novel dehydrated amnion allograft for use in the treatment of gingival recession: an observational case series. J Implant Adv Clin Dent;1:124–30;2009.
7. Kesting MR, Denys JL. Repair of oronasal fistulas with human amniotic membrane in minipigs. Br J Oral Maxillofac Surg;48:131–5;2010.
8. Tuncel U, Ozgenel GY. Use of human amniotic membrane as an interpositional material in treatment of temporomandibular joint ankylosis. J Oral Maxillofac Surg;69:e58–66;2011.
9. Izumi K, Feinberg SE, Iida A, Yoshizawa M. Intraoral grafting of an ex vivo produced oral mucosa equivalent: a preliminary report. Int J Oral Maxillofac Surg 2003;32:188–97.
10. Siddiqui A, Morkel JA, Zafar S. Antibiotic prophylaxis in third molar surgery: a randomized double blind placebo controlled clinical trial using split mouth technique. Int J Oral Maxillofac Surg 2010;39:107–14.
11. Arai N, Tsuno H, Okabe M, Yoshida T, Koike C, Noguchi M, et al. Clinical application of a hyperdry amniotic membrane on surgical defects of the oral mucosa. J Oral Maxillofac Surg 2012;70:2221–8.
12. Gordon Bourne, The Foetal Membranes A Review Of The Anatomy Of Normal Amnion And Chorion And Some Aspects Of Their Function Postgrad. Med. J. (1962), 38, 193.
13. Sikder MA, Alam Khan ASMD, Ferdousi F, P Leeza, BN HasanTare. Reconstruction of oral mucosal defect with Oven Dried Human Amniotic Membrane graft: A case report, Bangladesh Journal of Medical Science Vol.09 No.3 Jul'10.
14. A. Gupta et al. "Amnion and chorion membranes: Potential stem cell reservoir with wide applications in periodontics" International Journal of Biomaterials;274083;1-9;2015.
15. Samandari MH, Yaghmaei M, Eijali M, Moshref M, Saffar AS. Use of amnion as a graft material in vestibuloplasty: a preliminary report. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2004;97:574–8.
16. I. B. Kar, A. K. Singh, P. C. Mohapatra, P. K. Mohanty, S. Misra: Repair of oral mucosal defects with cryopreserved human amniotic membrane grafts: prospective clinical study. Int. J. Oral Maxillofac. Surg. 2014; 43: 1339–1344; 2014.
17. Borle RM, Borle SR. Management of oral submucous fibrosis: a conservative approach. J Oral Maxillofac Surg 1991;49:788–91.
18. Khanna JN, Andrade NN. Oral submucous fibrosis: a new concept in surgical management. Report of 100 cases. Int J Oral Maxillofac Surg 1995;24:433–9.

19. Shi LJ, Wang Y, Yang C, Jiang WW. Application of acellular dermal matrix in reconstruction of oral mucosal defects in 36 cases. *J Oral Maxillofac Surg* 2012;70:e586–91
20. Hao SP. Reconstruction of oral defects with the pedicled buccal fat pad flap. *Otolaryngol Head Neck Surg* 2000; 122:863–7.
21. Dean A, Alamillos F, García-Lopez A, Sañchez J, Pen˜alva M. The buccal fat pad flap in oral reconstruction. *Head Neck* 2001; 23: 383–8.