

Fresh Frozen Amniotic Membrane and Buccal Pad of Fat for Reconstruction of Oral Mucosal Defect After Surgical Excision of Leukoplakia


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Abstract

Objective: To compare the use of Fresh Frozen Amniotic Membrane (FFAM) and Buccal Pad of Fat (BPF) for reconstruction of oral mucosal defect after surgical excision of leukoplakia.

Material and Methods: Twenty patients were randomly selected and divided into two groups. Group 1 use amniotic membrane graft and Group 2 BPF. Both groups were evaluated preoperatively and postoperatively. Incisal opening, epithelialization and fibrosis were evaluated after one month of surgery. Chi square and Student t tests were used. **Results:** According to the presence of smoking habits, the highest frequencies were for smoking (30%) and betel leaf areca nut with tobacco (30%). Regarding the diameter of oral leukoplakia, in 40% of the participants it was 2x3 cm². In Group 1, after one month of surgery preoperative and postoperative inter-incisal opening values were 44.20 ± 3.37 and 42.05 ± 3.47 (p<0.001). In Group 2, preoperative and postoperative inter-incisal opening values were 44.09 ± 3.32 and 43.01±3.38 (p>0.05). When FFAM was used complete epithelialization in 70% and incomplete epithelialization in 30% patients. When BPF was used the results were almost similar. Fibrosis occurred in 30% in Group 1. There were no complications like flap necrosis, infection and hematoma formation. **Conclusion:** Incisal opening was significantly better in Fresh Frozen Amniotic Membrane Group, epithelialization and fibrosis were almost same in both groups after surgical excision of oral leukoplakia.

Keywords: Extraembryonic Membranes; Amnion; Leukoplakia, Oral; Mouth Neoplasms.

Introduction

In 1978 the World Health Organization (WHO) defined leukoplakia (LP) as “a white patch or plaque that cannot be characterized clinically or pathologically as any other disease” [1]. The current definition is that of “a white plaque of questionable risk having excluded (other) known diseases or disorders that carry no increased risk for cancer” [2]. It is considered as one of the most common premalignant lesion or Potentially Malignant Disorder [3]. It is a clinicopathological diagnosis that can only be made after histological examination of the tissue. It represents the most common premalignant disease of the oral mucosa.

The prevalence varies geographically but also reflects differences in study design and populations studies [4]. The presence of dysplasia in LP is believed to be associated with a higher probability to transform into oral cancer, which increases with the grade [5,6].

The reason to treat LP may be the presence of symptoms and an attempt to prevent malignant transformation. The gold standard for the diagnosis and management remains histopathologic assessment from a suspicious lesion. This depends on the quality of the biopsy, patient clinical information, interpretation of biopsy by a pathologist and the correct action by the clinician. The pathologist can only confirm that no other pathology is present and comment on the presence and degree of dysplasia or infiltration [4]. It is usually treated by surgical excision with a healthy margin. In case of a large mucosal defect after excision of any benign or malignant soft tissue lesion, reconstruction is mandatory.

Human amniotic membrane has been used successfully over 70 years for a wide range of surgical application. The use of fetal membrane in skin transplantation was first reported in 1910 [7]. In 1913, it was described the use of human amniotic membrane for burned and ulcerated skin surfaces [8].

The use of human amniotic membrane as a surgical wound dressing, treatment of leg ulcers, skin loss in Stevens-Johnsons diseases, reconstruction of the pelvic floor, vaginal epithelialization, replacement of normal mucosa in Rendu Osler-weber diseases and ear surgery has been described earlier [9]. Subsequently, it has been widely used as a surgical dressing in management of burns, surgical reconstruction of the bladder, vagina and in the prevention of surgical adhesions [10-14].

In a previous study, mandibular vestibuloplasty using Clarks's technique and amnion as graft material was analyzed. Fresh amniotic membrane was placed in the area and an acrylic splint was used with soft liner and 0.4mm wires to cover the surgical site. The area was reexamined after 1 week, 2 weeks, 4 weeks, 3 months and 6 months. The authors concluded that the amniotic membrane might be a favorable graft material for vestibuloplasty prompting healing and preventing relapse [15].

The prime purpose of this study was to compare the tissue engineering potential of Fresh Frozen Amniotic Membrane (FFAM) and Buccal Pad of Fat (BPF) for reconstruction of oral mucosal defect after surgical excision of leukoplakia.

Material and Methods

Study Design and Data Collection

The study comprising 20 subjects was carried out in the Department of Oral & Maxillofacial Surgery, Bangabandhu Sheikh Mujib Medical University. These 20 patients were randomly divided in 10 patients in groups 1 and 2. In Group 1, patient received FFAM while in Group 2 patient received BPF after excision of LP from the buccal mucosa.

A detailed history was taken from the patients with their habits and duration and the patient were asked to discontinue the habit before the procedure started. Routine investigation and clinical examination were done to rule out any associated systemic diseases. Local examination included distribution of sites, size and inter-incisal opening between the incisal edges of the maxillary and mandibular central incisors, which were measured using a simple ruler and expressed in millimeters. Patients were clinically followed up preoperatively and postoperatively. Postoperative clinical evaluation was done on the 3rd postoperative day, 1st week, 3rd week and 1st month. At above-mentioned various intervals, mouth opening measured visually with metal scale from incisal edges. The values for the reduction of inter-incisal opening were categorized in little (1-2 mm), slight (3-4 mm) and serious (>5 mm). Epithelialization was assessed visually regularly till complete epithelialization had occurred. Fibrosis was assessed after one month of surgery histologically.

Processing and Preservation of Fresh Frozen (-80°C) Amniotic Membrane Allograft

Human placenta was collected from healthy and seronegative donor during elective caesarean or vaginal deliveries. Under a laminar flow cabinet amnion membrane was separated from chorion and cleaned blood clots & other debris from amnion membrane. Then the membrane washed $\frac{3}{4}$ times (30 minutes per cycle) using Balanced Salt Solution (BSS) containing antibiotics mixture (penicillin 50 µg/ml, Streptomycin 50 µg/ml, gentamycin 25 µg/ml and amphotericin B 2.5 µg/ml).

After washing the amnion membrane was then flattened onto a nitrocellulose paper with a size of 0.22/0.45 µ with the epithelium/basement membrane surface up. The membrane with nitrocellulose paper was then cut into 5x5 cm² pieces and kept into a sterile vial containing the DMEM (Dublecco's Eagle Modified Medium, Merck KGaA, Darmstadt, Germany) and glycerol at the ratio of 1:1 (V/V). Then the vials containing amnion membrane was stored in -80°C deep freezer [16]. Before clinical applications in tissue banking and biomaterial research unit of Bangladesh atomic energy commission and they supply us before operation.

Operative Procedure

Isolation of the operative field (oral cavity) was done by sterile drape. Scrubbing and painting the facial skin with 5% povidone iodine and oral mucosa with 1% povidone iodine maintained aseptis. With all aseptic preparation infiltration was given.

- Group 1: After completing examination decision was taken to excise the LP under local anesthesia. Marginal resection of LP was done and was washed the surgical wound by 0.9 %

normal saline and proper hemostasis was achieved. FFAM was prepared into a suitable shape that was larger than the actual defect with proper precaution and measure. After thorough cleaning and debridement of the defect under sterile condition FFAM (four-fold) was placed directly on the defect and stabilized with suture by 4/0 vicryl.

- Group 2: After completing examination decision was taken to excise the LP under local anesthesia. Marginal resection of LP was done and was washed the surgical wound by 0.9 % normal saline and proper hemostasis was achieved. Now buccal fat pad was harvested by exposing the underline buccal mucosa and blind dissecting the area until yellow colour buccal fat pad was visible. Now non-toothed forcep was used to grasp the buccal fat pad. It was gently teased and pulled to the respective wound. Fibrous buccal fat pad is supplied by branch of facial artery. The buccal fat pad was sutured to the underlying wound using 4-0 vicryl suture with round body needle. After obtaining proper hemostasis the flap was repositioned and closed by means of interrupted sutures using 4/0 vicryl.

Data Analysis

Descriptive and comparative analysis has been done using IBM SPSS Statistics for Windows Software, version 14 (IBM Corp., Chicago, IL, USA). Chi-square and Student t tests were used. Significance level set at 5%.

Ethical Aspects

Ethical approval for this study obtained from Institutional review board of Bangabandhu Sheikh Mujib Medical University (I.R.B/BSMMU/2013/0944).

Results

Figure 1 shows the distribution of subjects according to the presence of smoking habits. The highest frequencies were for smoking (30%) and betel leaf areca nut with tobacco (30%).

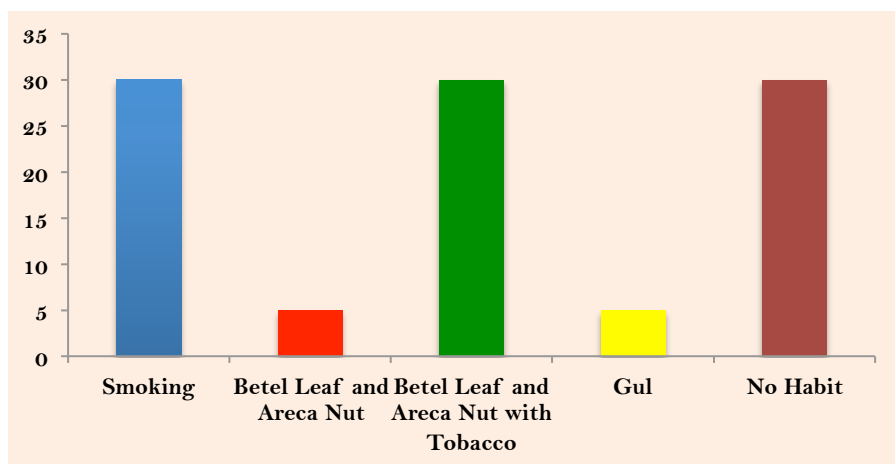


Figure 1. Distribution of patients according to the presence of smoking habits.

Regarding the diameter of oral leukoplakia, in 40% (n = 8) of the participants it was 2x3 cm², in 35% (n = 7) it was 1x2 cm² and in 25% (n = 5) it was 3x4 cm².

Table 1 shows, in Group 1 after one month of surgery preoperative and postoperative inter-incisal opening values were 44.20 ± 3.37 and 42.05 ± 3.47 (p<0.001). In Group 2, preoperative and postoperative inter-incisal opening values were 44.09 ± 3.32 and 43.01 ± 3.38 (p = 0.46).

Table 1. Assessment of inter-incisal opening range.

Group	Inter-incisal Opening Range (mm)				p-value
	Preoperative		Postoperative		
	Mean (SD)	Range (mm)	Mean (SD)	Range (mm)	
Fresh Frozen Amniotic Membrane	44.20 ± 3.37	38-52	42.05 ± 3.47	37-50	<0.001
Buccal Pad of Fat	44.09 ± 3.32	37-52	43.01 ± 3.38	36-50	0.46

Table 2 shows, when FFAM was used (Group 1), it resulted little in 70% and serious in 10%. When BPF was used (Group 2) than little reduction in 80% and serious in 0% (Table 2).

Table 2. Assessment of reduced inter-incisal opening.

Reduced Inter-incisal Opening (mm)	Type of Reconstruction Material			p-value
	FFAM (n=10) N (%)	BPF (n=10) N (%)	Total	
Little (1-2)	7 (70.0)	8 (80.0)	15 (75.0)	0.58
Slight (3-4)	2 (20.0)	2 (20.0)	4 (20.0)	
Serious (> 5)	1 (10.0)	0 (0.0)	1 (10.0)	
Total	10 (100.0)	10 (100.0)	20 (100.0)	

When Fresh Frozen Amniotic Membrane (FFAM) was used then fibrosis occurred in 30%, while Buccal Pad of Fat (BPF) was 20% (Table 3).

Table 3. Presence of fibrous tissue when use FFAM and BPF.

Fibrous Tissue	FFAM	BPF
	N (%)	N (%)
Present	3 (30.0)	2 (20.0)
Absent	7 (70.0)	8 (80.0)

Epithelialization was evaluated after one month of surgery clinically (Table 4). When FFAM was used complete epithelialization occurred in 70% while when BPF was used complete epithelialization occurred in 80% (Table 4).

Table 4. Clinically assessment of epithelialization after 1 month.

Epithelialization	Type of Reconstruction Material			p-value
	FFAM N (%)	BPF N (%)	Total N (%)	
Complete Epithelialization	7 (70.0)	8 (80.0)	15 (75.0)	0.86
Incomplete Epithelialization	3 (30.0)	2 (20.0)	5 (25.0)	

Clinical photographs of a subject treated with frozen amniotic membrane and BPF were shown in Figures 2 and 3, respectively.



Figure 2. Preoperative (A), Operative (B1, B2, B3) and Postoperative (C1, C2) images. Reconstruction done by FFAM.



Figure 3. Preoperative (A) and postoperative (B, C) images. Reconstruction done by BPF.

Discussion

A number of surgical procedures have been advocated for the reconstruction of oral cavity defects after surgery, including primary closure, buccal mucosal graft, split thickness skin graft, BPF, allogeneic graft, regional rotational flap and distant flap [17].

In this study, for the first time based on literature search, between FFAM and BPF, three parameters were evaluated: inter-incisal opening, presence of fibrous tissue and epithelialization. The presence of fibrous tissue was evaluated after one month histologically while epithelialization was evaluated by clinically.

Incisal opening reduced maximum when reconstruction was done by FFAM (Table 1 and Figure 2). A similar type of study was done which consisting of twenty-eight cases of clinically and histologically diagnosed oral sub mucosal fibrosis which were divided into 2 groups: group I (n = 15) and group II (n = 13), corresponding to clinical stage III and stage IV, respectively. Pedicled BPF was used in buccal defect in all patients. Both groups were analyzed separately for mouth opening (inter-incisal distance in millimeters) preoperatively and 1 year postoperatively, time taken for epithelialization of BPF, time taken for establishment of normal contour, and changes in symptoms 1 year after grafting [18].

Fibrosis was assessed histologically after one month of surgery. Fibrosis was more when reconstruction done by FFAM. However, in another study, FFAM showed unique properties that can be helpful to treat different ocular surface diseases. It is useful in promoting normal epithelialization of cornea and conjunctiva. It is also effective in preventing excessive fibrosis during ocular surface reconstruction [19].

In this study epithelialization was evaluated after one month of surgery clinically. Based on results, BPF and FFAM gave almost the same result in post-surgical reconstruction of LP.

Conclusion

Fresh frozen amniotic membrane was not a suitable allograft material over buccal pad of fat during reconstruction of oral mucosal defect after surgical excision of leukoplakia because of epithelialization, fibrosis and wound contracture were almost higher in fresh frozen amniotic membrane than buccal pad of fat.

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Conflict of Interest: The authors declare no conflicts of interest.

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