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Evaluation of Autologous Platelet Rich Fibrin in Cleft Palate Repair

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ABSTRACT

Background: Clefts of the lip and/or palate (CLP) are common birth defects of complex etiology. Management of this problem is somehow challenging depending on many factors. The main goal of treatment is mostly functional for the sake of creating normal feeding pattern, acquaintance of normal hearing and hence normal speech development. These goals carry a great impact on social and psychological status of the patient and his family. The repair of cleft palates ideally involves an interdisciplinary team contributing together to achieve this mission.

Objective: Evaluation of the role of platelet-rich Fibrin (PRF) on wound healing following primary cleft palate repair. **Patients and Methods:** It was a prospective study done at the Department of Pediatric Surgery, Al-Azhar University, Cairo, Egypt. A total of forty patients (both sexes) were selected randomly and performed surgery using PRF which was placed during surgical procedure using two flap palatoplasty between the nasal and the oral mucosa. Then, the patients were followed up for six months to evaluate its efficacy in wound healing by monitoring incidence of fistula. All cases in the study group showed complete healing with no fistula or wound dehiscence all over the follow up period except one patient developed oronasal fistula.

Results: In this study, post-operative bleeding tendency decreased in patients with preoperative injection of local anesthetic with vasoconstrictor agent and the extent of the surgical field. We measured the amount of bleeding with number of gauzes used by the parents to collect blood from the patients' mouth. Actually, it was blood mixed with saliva in addition to the remaining serum from the PRF.

Conclusion: The use of PRF showed satisfying healing for most of the cases under study along the whole follow up period.

Keywords: Autologous Platelet Rich Fibrin, Cleft Palate.

INTRODUCTION

Cleft lip and palate (CLP) are considered as one of the most common congenital anomalies. Regardless of the race, cleft palate affects about 1:2.000 live births worldwide **(1)**.

There is no specific etiology identified for CLP. It is a multifactorial anomaly with other contributing factors that may include radiation, maternal hypoxia, teratogenic drugs, nutritional deficiency, chemical exposures **(2)**.

The pathological sequalae of cleft palate include feeding and nutritional difficulties, recurrent ear infections, hearing problems, abnormal speech development and distortion of facial growth **(3)**.

One of the challenging problems in wound healing that occur after surgical repair of cleft palate is cleft recurrence or fistula development. It occurs due to local wound breakage because of tension or compromise in the vascularity of the flap. The recurrence of primary cleft palate fistula is reported as high as up to 76 % **(4)**.

Cleft demands a long term treatment plan and a multidisciplinary management by qualified cleft team. Specialists from the major areas of cleft care should collaborate to give the child pleasant outcome and self-confidence that comes out from intelligible speech, healthy teeth and pleasant facial appearance **(5)**.

The concept of this thesis came from utilizing recent tissue engineering techniques to overcome the previously mentioned challenges.

Platelet rich fibrin (PRF) is a new generation of platelet concentrate that is very easy to prepare and handle with no need for biochemical materials. Its production depends on accumulation of platelets that release cytokines and growth factors.[6] Fibrin adhesives have been already used in cardiothoracic and vascular surgery for sealing of diffuse microvascular bleeding. Also, they were used for sealing of wound borders in general and plastic surgery **(6)**.

AIM OF THE WORK

Evaluation of the role of platelet-rich Fibrin (PRF) on wound healing following primary cleft palate repair.

PATIENTS AND METHODS

Study design: prospective study.

Study settings: Forty patients with cleft palate were selected from the outpatient clinic, Department of Pediatric Surgery, the Al Hussein and Sayed Galal University Hospitals, Al-Azhar University, Cairo, Egypt. After repair they followed for 6months started 1 June till 30 November 20119.

Ethical approval:

Received:18/08/2019
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The study was approved by the medical ethics committee of Al-Azhar University Hospitals and a written informed consent is obtained from all patients.

Patient selection: All patients were selected with primary non-syndromic cleft palate. They were 28 males and 12 females within age range from 10 to 20 months. Patients were evaluated for type of cleft according to Veau's classification (Class I, II, III or IV).

Allocation: The participants were allocated in one group.

Allocation concealment mechanism: All patients were selected randomly, and family history as well as history of drug intake were evaluated. General condition was evaluated by a pediatrician to ensure that all cases were medically free with no other systemic diseases.

The following preoperative investigations were done: Complete blood picture. Coagulation profile (PT, PTT, and INR). Echocardiography. Chest condition was examined by the pediatrician or anesthesiologist.

Inclusion criteria: Patients with non-syndromic cleft palate. Patients with Venn class I, II, III.IV Patients' age mimes between 10 and 20 months.

Exclusion criteria: Patients with systemic disease or bleeding disorders. Patients with syndromic cleft palate.

Parents were informed about the procedure and the possible postoperative complications.

All patients underwent two flap palatoplasty surgical technique for repair using PRF preparation.

Bias: Only the colleague surgeon who participated in assessing the outcome was blind since the researcher was a part of the surgical procedure.

Operative procedures: In the operating room, the patient put in a supine position on the operating table; the head supported by head ring, and shoulder roll below the shoulders. General anesthesia was induced by Sevoflurane maintained with muscle relaxant atracurium besylate and isoflurane. A right angled endotracheal tube (RAET) was placed and secured in the midline to the chin with tape. The tube was also secured with oral pack. Sterile tapes were placed over the closed eyelids.

For preparation of PRF, the aneastheologist was asked to withdraw 10 mL of venous blood in 10 cm sterile plastic syringe.

In aseptic conditions, the collected blood was immediately transferred into 10 ml plain tube which is a tube with no addition of anticoagulants.

The centrifuge adjusted at 3000 rpm and time of spin for 10 minutes for preparation of PRP and after that we add 1cm distilled water to the fibrin and 1cm distilled water to the thrombin to dissolve them then we mix them double way mixing pump then 2cm of PRP added to the previous mixed solution to form the PRF. Surgical instruments were prepared for palatal repair and surgery started.

The blood was centrifuged simultaneously while the surgeon had already started to perform surgery after infiltration of 3 ml of 2% lidocaiue with 1:2000GO epinephrine into the palate to achieve hemostasis.

The two flap palatoplasty was performed through medial incisions along soft and hard palate between oral and nasal mucosa extending anteriorly. Another incision lines at the lateral border of the soft and hard palate.

Then palatal flaps were raised, the nasal mucosa was also raised then approximated towards the midline. The nasal layer was sutured starting anteriorly towards the soft palate and uvula using vieryl suture with the knot buried inside.

The PRF, which had already been prepared was applied over wet gauze to drive out serum. It ended up in the form of a physically stable membrane. The PRF membrane was fixed into (the surgical wound by lying over the nasal layer and stabilized with 2 or 3 stitches into the nasal mucosa.

Then the palatal mucosal flap was approximated and sutured.

Post-operative care: Arm restrains were fixed to the patient's body to inhibit trauma of the wound by the child's fingers. Patient's feeding was done only through plastic syringe, breast or bottle feeding were inhibited to avoid trauma by suction at first week.

Postoperative medications: Oral antibiotics were given for 7 days. Cephalosporin antibiotic 125mg orally every 12 hours for 5 days. Nystatin antifungal drops every 8 hours for 5 days. Otrivin baby nasal drops for 5 days. Paracetamol drops 12 drops every 6 hours.

Follow up visits: 1st visit: at the 1st postoperative week to assess incidence of dehiscence or wound infection and to confirm feeding instructions and postoperative care protocol for the guardians. 2nd visit: at the 3rd postoperative weeks to detect any formation of fistula. Assessment of fistula incidence was performed subjectively; we evaluated each patient if there was passage of solid or liquid food into the nostrils. 3rd visit: after 6 months postoperatively to confirm if a late postoperative fistula had developed.

Assessment of variables: Factors included were age (10-20 months), type of cleft (Veau class I, II, III, IV gender (male/female), fistula development and amount of postoperative bleeding measured by "number of gauzes".

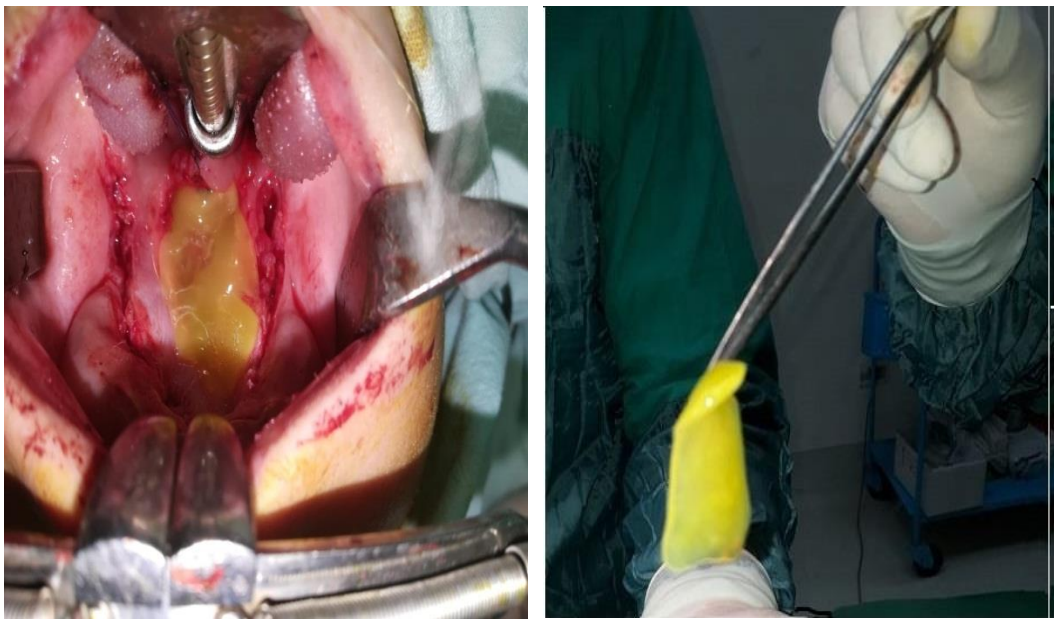

Figure (1): show platelet rich fibrin and application in repair of cleft palate

Statistical analysis

Recorded data were analyzed using the statistical package for social sciences, version 20.0 (SPSS Inc., Chicago, Illinois, USA). Quantitative data were expressed as mean± standard deviation (SD). Qualitative data were expressed as frequency and percentage.

The following tests were done: Chi-square (x2) test of significance was used in order to compare proportions between qualitative parameters. The confidence interval was set to 95% and the margin of error accepted was set to 5%. So, the p-value was considered significant as the following: Probability (P-value): P-value <0.05 was considered significant. P-value <0.001 was considered as highly significant. P-value >0.05 was considered insignificant.

RESULTS

Table (1): Distribution of procedure for palate closure cases according to their demographic data regarding gender, age, veau classification, cleft alveolus and cleft lip (n=40).

	Demographic data	**Total (n=40)**
Gender	Females	12 (30.0%)
	Males	28 (70.0%)
Age (Month)	10-15 months	19 (47.5%)
	>15-20 months	21 (52.5%)
	Range [Mean ±SD]	10-20 [15.75±3.36]
Veau classification	I	7 (17.5%)
	II	27 (67.5%)
	III	3 (7.5%)
	IV	3 (7.5%)
Cleft alveolus	Left	3 (7.5%)
	None	34 (85.0%)
	Right	3 (7.5%)
Cleft lip	Bilateral	6 (15.0%)
	Rt	12 (30.0%)
	Lt	12 (30.0%)
	None	10 (25.0%)

This table shows that the number of females was 25 (62.5%), while males reached 15 (37.5%). Their age ranged between 10-20 months with mean 15.75±3.36 according to the demographic data.

Table (2): Distribution of procedure for palate closure cases according to their complications regarding oronasal fistula, wound infection, postoperative bleeding, recurrence, VPI and outcome complications (n=40).

Complications	Total (n=40)
Oronasal fistula	2 (5.0%)
Wound infection	4 (10.0%)
Postoperative bleeding	2 (5.0%)
Recurrence	0 (0.0%)
VPI	0 (0.0%)
Outcome complications	6 (15.0%)

This table shows that the complicated patients after repair were oronasal fistula (5%), wound infection (10%), postoperative bleeding (5%), recurrence (0%), VPI (0%) and outcome complications (15%).

Table (3): Comparison between non-complicated and complicated patients according to age

Age (Month)	No complications		Complications		Chi-square test	
	No.	%	No.	%	x2	p-value
10-15 months	16	47.1%	3	50.0%	0.018	0.894
>15-20 months	18	52.9%	3	50.0%		
Total	34	100.0%	6	100.0%		

x^2: Chi-square test; p-value >0.05 NS

This table shows no statistically significant difference between non-complicated and complicated patients according to age.

Table (4): Comparison between non-complicated and complicated patients according to gender.

Gender	No complications		Complications		Chi-square test	
	No.	%	No.	%	x2	p-value
Female	10	29.4%	2	33.3%	0.037	0.847
Male	24	70.6%	4	66.7%		
Total	34	100.0%	6	100.0%		

x^2: Chi-square test; p-value >0.05 NS

This table shows no statistically significant difference between non-complicated and complicated patients according to gender.

Table (5): Comparison between non-complicated and complicated patients according to veau classification.

Veau classification	No complications		Complications		Chi-square test	
	No.	%	No.	%	x2	p-value
I	7	20.6%	0	0.0%	15.018	0.002*
II	25	73.5%	2	33.3%		
III	1	2.9%	2	33.3%		
IV	1	2.9%	2	33.3%		
Total	34	100.0%	6	100.0%		

*x^2: Chi-square test; *p-value <0.05 S*

This table shows statistically significant difference between non-complicated and complicated patients according to veau classification.

Table (6): Comparison between non-complicated and complicated patients according to cleft alveolus.

Cleft alveolus	No complications		Complications		Chi-square test	
	No.	%	No.	%	x2	p-value
Lt	1	2.9%	2	33.3%	1.683	0.129
Rt	3	8.8%	0	0.0%		
None	30	88.2%	4	66.7%		
Total	34	100.0%	6	100.0%		

x^2: Chi-square test; p-value >0.05 NS

This table shows no statistically significant difference between non-complicated and complicated patients according to cleft alveolus.

Table (7): Comparison between non-complicated and complicated patients according to cleft lip.

Cleft lip	No complications		Complications		Chi-square test	
	No.	%	No.	%	x2	p-value
Bilateral	4	11.8%	2	33.3%	3.399	0.334
Lt	10	29.4%	2	33.3%		
Rt	10	29.4%	2	33.3%		
None	10	29.4%	0	0.0%		
Total	34	100.0%	6	100.0%		

x^2: Chi-square test; p-value >0.05 N

This table shows no statistically significant difference between non-complicated and complicated patients according to cleft lip.

DISCUSSION

The optimal goals for cleft palate repair are to achieve a satisfactory anatomical and functional closure of the defect with normal speech, no fluid or food regurgitation into the nasal cavity, limitation of maxillary growth disturbance, and minimization of potential hearing loss [(7)].

During registration of the protocol for this study, it was launched as a prospective study as after searching the literature, we didn't find that PRF membrane has been used before in primary surgical repair of cleft palates.

In this study we tried to unify the surgical technique used as the two flap palatoplasty to rule out fistula incidence due to technical difference. Fistula rates for primary palatoplasty vary widely in the literature, between 3and 45 percent of cases [(8)].

Another study conducted by the University of Pittsburgh introduced an algorithm to be applied during palatal surgery to limit fistula incidence; this algorithm included relaxing incisions with complete intravelar veloplasty, total release of the tensor tendon and dissection of the neurovascular bundle with optional osteotomy of the foramen, then incorporation of acellular dermal matrix to achieve complete nasal lining reconstruction. The authors used the Furlow palatoplasty technique and reported a fistula rate of 3 % [(9)].

A study performed by **Kalzel *el al.*** [(10)] showed no significant difference in fistula rates between the Bardach technique and Furlow palatoplasty in the hands of competent surgeons.

Another surgical approach for palatal cleft repair is utilizing buccal fat pad for clefts less than 20 mm in length. Located in the posterior two-thirds of the palate [(11)]. Use of buccal fat pad may result in a reduction of palatal scarring [(12)].

Another method of cleft palate repair is by expansion of tissue using osmotic expanders implanted in the first stage of treatment. Self-expanding expanders manufactured by OSMED (ilmenau, Germany) were implanted under the mucoperiosteal layer of the hard palate to generate more tissue to facilitate cleft palate repair supposed to be performed 24-48 h later. Despite some technical problems related to optimal filling phase, tissue expansion makes palatal repair easier, probably without need to perform relaxing incisions and bone denudation [(13)].

Proper wound healing after surgical repair of cleft palate has always been the chief demand for cleft surgeons. Secondary repair of impaired wound is more challenging because of tissue scarring that yields more difficulty for closure of oronasal communication due to lack of tissue laxity depending on size of the defect. Once the closure of primary defect has failed, it is more probable for fistula to recur due to the fibrosis and decreased vascularization that occur with each surgery [(4)].

The prevalence of these complications are related to other factors such as surgeon's experience, local tissue injury, the surgical technique used and timing for repair, but the most relevant factor is the width of the original defect[(14)]. Based on Veau classification, the chance increases in proportion to the cleft extent [(15)].

These previously mentioned complications carried an extra psychological and social impact on the patient [(16)].

Many materials have been used as tissue engineering scaffolds; hyaluronic acid, hydroxyapatite, PRP and PRF. They stimulate bone regeneration from undifferentiated mesenchymal cells [(17)].

Other different synthetic materials had also been used in palatal repair as interpositional grafts such as alloderm and collagen membrane [(18)]. They are used in a multilayer repair represented by the nasal mucosa, the inter-positional graft and oral mucosa. These provide a scaffold for tissue growth and mucosal epithelialization [(19)].

A 5-year retrospective review by **Hudson *et al.*** [(20)] for cases underwent Primary Palatoplasty utilizing an acellular collagen membrane that was placed between the oral mucosa and the muscular layer of soft palate after reorientation of the levator and tensor veli muscles across the midline. At 1 year follow up, no oronasal fistulas had developed where the acellular collagen membrane was used.

Use of acellularized dermal matrix (AlloDerm) [(21)] to facilitate closure has been reported

in the literature as well. It has been applied for repair of cleft palate and palatal fistulas [22].

In a study performed by **Clark *et al.*** [23] they retrospectively reviewed patients underwent repair of wide cleft palates using decellularized dermal allograft. It was proven to be safe and effective for use in primary closure of wide clefts involving the hard and soft palates. Its use in repair of an existing fistula is promising.

In an evidence-based review conducted by **Aldekhayel *et al***. [22] in 2012, four studies examined the use of acellular dermal matrix in primary palatoplasty (n = 92) with a mean cleft width of 14.2 mm. The overall fistula rate was 5.4 percent compared with 10.6 percent in the control group. Five studies used acellular dermal matrix in palatal fistula repair (n = 74). The overall recurrent fistula rate was 8.1 percent compared with 12.9 percent in the control group.

An experimental, prospective, longitudinal study performed by **Glicerio *et al.*** [4] from April 2008 to July 2010 on 11 recurrent cleft palate fistulas using local mucoperiosteal flap with addition of PRGF gel mixed with autologous bone graft and placed between two sheets of solid collagen filling the bone defect between the palatal and nasal mucosa complete closure of palate fistulas was achieved in 90.9% (follow-up of 6-24M months), decreasing the reported incidence for the recurrence by other authors with other techniques.

They concluded that the use of PRGF mixed with autologous bone graft seems to be an effective, safe and low-cost for the closure of recurrent cleft palate fistulas.

So, we tried to use autologous blood product" the PRF" recently used in many tissue engineering protocols and evaluate its therapeutic value in promoting wound healing after palatoplasty. Fibrin acts as a vehicle for migration of fibroblasts and endothelial cells that aids in tissue regeneration [24].

PRF has been used recently in maxillofacial and plastic surgery to take advantage of persistent release of growth factors that enhance wound healing over a significant period of time without provoking inflammatory reactions. It has been combined with bone grafts in vitro and showed great potential for cell attachment, proliferation and differentiation of osteoblasts [5].

PRF preparation protocol that was first conducted by **Choukroun *et al.*** [25] is an easy, rapid and cheap method which also provides autogenous blood derived product without addition of chemicals, minimizing risk of cross infection or immune reaction. Also, it delivers cytokines and growth factors which is a great benefit for healing process[26].

PRF has been used in combination with bone grafts in alveolar cleft reconstruction surgery and maxillary sinus lift. it acts as a biologic connector that attracts stem cells, osteoprogenitor cells and provides neo angiogenesis, radiographically and histologically, it showed higher bone maturity and density [27].

Choukroun *et al.* [28]**,** conducted a study in which they wanted to see the efficacy of using PRF combined with freeze-dried bone allograft (FDBA) to enhance bone regeneration in a maxillary sinus lift surgery. The results showed a decreased healing time before implant placement. The healing time was reduced by half.

Simonpieri *et al*. [29], mixed PRF with a bone graft and inserted in bone defects and with immediate dental implants noting good clinical results in natural bone regeneration.

Yilmaz *et al.* [30] compared the healing effects of *B*-TCP and PRF, alone and in combination, in standardized bone defects in pig's tibiae. The results showed that when *B*-TCP and PRF were used together, the newly formed bone was significantly greater than when used both separately.

In our study, PRF was immediately prepared during surgery, easily handled and inserted into the surgical site in a sandwich like manner, between the nasal and the oral layer.

In this study, post-operative bleeding tendency decreased in patients with preoperative injection of local anesthetic with vasoconstrictor agent and the extent of the surgical field.

We measured the amount of bleeding with number of gauzes used by the parents to collect blood from the patients' mouth. Actually, it was blood mixed with saliva in addition to the remaining serum from the PRF.

CONCLUSION

The use of PRF showed satisfying healing for most of the cases under study along the whole follow up period.

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