

Efficacy Of Autologous Platelet Rich Fibrin In Myringoplasty In Dogs

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ABSTRACT

Objectives: To assess the effect of local application of platelet rich fibrin in myringoplasty for perforated dogs' tympanic membranes.

Material and methods: forty four dogs were subjected to large central tympanic perforation (at least two quadrant) in right ears and Follow up for 8 weeks and further were divided into two equal groups randomly by lottery. Group (A): study group: PRF was been used in repairing dry chronic tympanic perforation in dogs in their right ears. Group (B): control group: PRF was not been used only trimming of the edges of tympanic membrane perforation. Closure of perforated tympanic membrane with platelet-rich fibrin membrane was assessed by healing.

Results: Percentage of healing of study group was 47.5% versus 25% among control group. There were statistically significant difference between Study Group and Control Group regarding Healing at 8 weeks. Percentage of healing of study group was 90% versus 75% among control group.

Conclusion: PRF membrane could be used successfully in the repair of tympanic membrane perforation and wound healing. This method could be more frequently preferred in the future in various areas as it is an autogenous material which is safe and effective, easy to use, and has low costs, while it does not prolong operating time.

keywords: Myringoplasty, perforated tympanic membrane, PRF.

Introduction

The repair of tympanic membrane (TM) dates back more than a century. Berthold successfully closed a perforation with full thickness skin graft. The term myringoplasty refers to reconstructive surgery that is limited to the TM only. Tympanic membrane perforation is one of the most common

causes of hearing impairment. Among the many causes, infection is the principle cause of TM perforation. Infection may be bacterial or mycobacterial, acute or chronic. Perforation of TM may be caused by various types of trauma—blunt trauma, penetrating trauma, surgical trauma etc. Perforations due to trauma and acute infection usually heal if treated timely. A simple perforation of pars tensa with intact annulus (central perforation) with no additional lesion of the middle ear is indicated for myringoplasty (1, 2).

Several investigations have shown that various medicines, such as hyaluronic acid, pentoxifylline, and fibroblast growth factors, can be used experimentally to cure tympanic membrane perforation (3).

Platelet-rich fibrin (PRF), a second-generation platelet concentrate generated from the patient's own blood, containing growth factors such as platelet derived growth factor (PDGF), vascular endothelial growth factor (VEGF), and transforming growth factor-B are all present (TGF-B). PRF protects the tympanic graft mechanically as well as accelerating cell proliferation and matrix remodeling. This autologous biomaterial is simple, quick, and inexpensive to manufacture, and it is biocompatible, safe, and easy to handle during surgical procedures (5).

PRF is a natural concentrate, hence eliminating the danger associated with the use of bovine thrombin (6, 7). Platelet-rich fibrin has an impact on cellular functions at the cellular levels. One significant advantage of PRF is that it has a simple preparation protocol. Hence the present experimental study was conducted to determine the healing of perforated tympanic membrane following the use of autologous platelet rich fibrin.

Subjects and methods

Type of the study: experimental study

This is animal study to assess the effect of local application of platelet-rich fibrin to perforated tympanic membranes of dogs during myringoplasty. This study was carried out in Otorhinolaryngology Department, Faculty of Medicine and Experimental labs of the veterinary Medicine school. Suez Canal University With approval of the institutional review board (IRB).

Animal model: Dogs.

Inclusion criteria: All dogs (male and female). Dogs less than 9 months. Healthy dogs. Wide and healthy canal.

Exclusion criteria: Dogs with total or subtotal or marginal or large perforation of the tympanic membrane. Infection in ear (like otitis media or otitis external). Congenital abnormalities in ear (like stenosed canal or atresia). Unhealthy dogs and Old dogs (more than 9 months).

Sample size calculations:

Sample size was calculated according to the following equation: $n = [(Z_{\alpha/2} + Z_{\beta}) / (P_1 + P_2)]^2 (p_1 q_1 + p_2 q_2)$ Where: $Z_{\alpha/2} = 1.96$, $Z_{\beta} = 0.84$. $P_1 =$ percentages of total closure of the tympanic membrane of dogs in the study group (64.3%). $P_2 =$ percentages of total closure of the tympanic membrane of dogs in the control group (22.2%) (Habesoglu et al., 2014) According to above equation the sample size was 44 dogs.

Methods:

All dogs were subjected to large central tympanic perforation (at least two quadrant) in right ears and Follow up for 8 weeks and further were divided into two equal groups randomly by lottery. Group (A): study group: PRF was been used in repairing dry chronic tympanic perforation in dogs in their right ears. Group (B): control group: PRF was not been used only trimming of the edges of tympanic membrane perforation.

The surgical procedure:

The surgical procedure was carried out under general anesthesia and utilizing of an endoscopic zero lens telescope 9 cm. long 2.3 mm wide and endoscopic camera. The anesthesia was intramuscular administration of 7 mg/kg body weight xylazine and 50 mg kg body weight of 5% ketamine. The perforation in tympanic membrane of dogs were done by endoscopic technique through the canal and the perforation was done by the needle. Follow up done at 1week then every 2weeks till 8 weeks till the transforming the induced perforation to dry chronic perforation (any healed perforation was been reopening). Giving antibiotic to all animals for one week and any animal developed infection was been treated by antibiotic 0.2 ml/kg trimethoprim and sulfamethoxazole (16 +80 mg) subcutaneously till curing. The eardrum findings were documented with photographs with a endoscopic camera. From 44 dogs we have excluded 3 dogs with tympanic membrane discharging and one dog with subtotal perforation (the drop out cases). So, we randomly divide the remaining 40 dogs into two groups each one 20 animals , Randomization was done by numbering the dogs from 1 to 40, then the dogs with odds numbers “1, 3, 5, 7, 9 till dog number 39” was considered as group “A” and the dogs with even numbers “2, 4, 6, 8 till the dog number 40” was considered as group “B”) the two groups “A & B” were randomly allocated by lottery as study and control groups. the dogs had been prepared for myringoplasty with PRF for study group (group 1) while PRF not used in control group (group 2). Observing of healing process every 2 weeks for 8 weeks. Giving antibiotic to all animals for one week and any animal developed infection was been treated by antibiotic 0.2 ml/kg trimethoprim and sulfamethoxazole (16 +80 mg) subcutaneously till curing. The eardrum findings were documented with photographs with a endoscopic camera. Finally, comparison of the data between the groups were made for statistical analysis

Preparation and application of Choukroun's platelet-rich fibrin:

Ten cc blood were obtained and drawn into 10-ml test tubes without an anticoagulant; and centrifuged without a delay for 12 min at 2.700 rpm. The resultant material consisted of the following three layers: a superficial layer consisting of a cellular serum, a PRF clot in the middle and RBCs at the bottom. This PRF clot could be shaped out as a thin membrane between two sterile sponges. A large piece of PRF clot bigger than the perforation size was inserted into the perforation in a dumbbell fashion.

Statistical analysis: Chi square test (X^2), or Fisher's exact test (FET) were used to analyze categorical variables. Student "t" test was used to analyze normally distributed variables among 2 independent groups, or Man Whitney U test for nonparametric ones. The accepted level of significance in this work was stated at 0.05 (P <0.05 was significant).

RESULTS

This study was carried out on 40 right ears of dogs with variable size of dry chronic tympanic membrane divided into two groups: There was statistically significant difference between Study Group and Control Group regarding healing after 8 weeks. **Table (1)**.

DISCUSSION

The present study involved 40 dogs with varying sizes of dry chronic tympanic membranes in their right ears. Tympanic membranes of dogs and humans have similar histological structure. Especially the similarity in the sequence pattern of the radial and circular fibres that constitute lamina propria is salient. Because of this reason, with the consideration that similar results can be obtained in the humans, dogs are frequently preferred in the experimental studies on tympanic membrane.

Blood products, primarily fibrin glue (adhesives), have been used for many years in wound healing. Biomaterials obtained from blood provide more rapid and effective graft migration as they contain thrombocyte growth factors. However, because of the costs entailed and complicated protocols applied in the preparation, it is difficult to obtain autologous fibrin glue (5).

PRF provides both mechanical and inflammatory protection to the tympanic graft and accelerates cell proliferation and matrix remodelling. This autologous biomaterial is easy, quick and cheap in preparing and is biocompatible, safe and easily manipulated during surgical procedure (5, 6). The aim of this study was to assess the effect of local application of platelet rich fibrin to perforated dogs tympanic membranes in myringoplasty.

The process of healing of the tympanic membrane is not the same as other cutaneous tissues. During the healing of tympanic membrane; proliferation of epithelial cell and formation of granulation tissue do not take place simultaneously. In the first phase perforation is sealed by epithelial cells and then in the second phase the middle fibrous layer forms between the edges of perforation. (9, 10).

PRF is used in several areas in the graft and wound treatment due to its accelerating effect on the proliferation of angiogenesis, chemotaxis, mitosis, and stem cells (4).

In the current study, we used autologous PRF membrane. In the present work, there were statistically significant difference between Study Group and Control Group regarding Healing at 8 weeks. Percentage of healing of study group was 90% versus 75% among control group. This is in agreement with Sankaranarayanan et al. (11) who found that, 96% closure were seen in case group with the use of PRF and 80% closure in control group seen after two month in tympanic membrane perforation during myringoplasty.

This agrees with Nair et al. (2) who found that, The success rate was found to be 97.7% in the study group as compared with 81% in control group ($p=0.012$). The results were found to be statistically significant. The healing time of the tympanic membrane perforation was determined as a mean of 10 days in the group applied with PRF. PRF provided more rapid healing. In addition to the healing effect of growth factors which are dense in the content of platelet concentrations, many products such as leukocytes, fibrin matrix, and circulating progenitor cells in the content also

demonstrate a synergistic. The reasons for selection of PRF rather than PRP are that no synthetic material or anticoagulant is added, it can be obtained easily in a short time and has low costs. It is noteworthy that, to date, this technique has been the easiest and cheapest method of obtaining blood products (4).

In the current study, There were statistically significant difference between Study Group and Control Group regarding Healing at 4 and 8 weeks. Navarrete Álvaro et al. (12). reported successful healing after the use of PRP in three patients with eardrum perforations that had not healed spontaneously. El-Anwar et al. (13). also reported successful results with the use of topical autologous PRP on the lateral surface of a cartilage graft and TM remnant during myringoplasty. In the current study we have used PRF membrane in the treatment of acute and small perforations; however, we think that in the future it will have an area of use as graft or support for graft in the treatment of chronic tympanic perforations.

Gun et al. (16) suggested the perforation closure rate after fat graft myringoplasty for patients with small perforation was 86.4%. However, getting the fat graft is a surgical operation, even if a small one. The preparation of PRF membrane does not require any surgical operation and just a blood sample from the patient is sufficient.

Additionally, PRF has a protective effect against infections. Biomaterials containing various growth factors can be used to obtain better results in tympanic membrane repair, especially in small perforations. An increase in successful outcomes is expected with the use of this method, which is easy, cheap, and has lower patient morbidity. In summary, According to the data obtained in this study, it can be said that PRF membrane could be used successfully in the repair of tympanic membrane perforation and wound healing. It offers the possibility of application per patient. It can be easily adjusted by cutting to size. One of the main advantages is that it can be easily adapted as graft material during use, as it is elastic. This method could be more frequently preferred in the future in various areas as it is an autogenous material which is safe and effective, easy to use, and has low costs, while it does not prolong operating time.

CONCLUSION

According to the data obtained in this study, it can be said that PRF membrane could be used successfully in the repair of tympanic membrane perforation and wound healing. One of the main advantages is that it can be easily adapted as graft material during use, as it is elastic. This method could be more frequently preferred in the future in various areas as it is an autogenous material which is safe and effective, easy to use, and has low costs, while it does not prolong operating time.

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Table (1): Comparison between Study Group and Control Group regarding Healing at 2 weeks, 4 weeks, and 8 weeks

				Study Group (No.= 20)	Control Group (No.= 20)	P. value
Healing	2 weeks	healed	No.	5	2	0.07
			%	25%	10%	
		not healed	No.	15	18	
			%	75%	90%	
	4 weeks	Healed	No.	9	5	0.03*
			%	47.5 %	25%	
		Not healed	No.	11	15	
			%	52.5 %	75%	
	8 weeks	healed	No.	18	15	0.003*
			%	90 %	75%	
not healed		No.	2	5		
		%	10%	25%		



Figure (1): Preparation of platelet-rich fibrin