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Platelet Rich Fibrin Tympanic Membrane Regeneration- An Alternative to Conventional Surgical Myringoplasty

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ABSTRACT :Hearing loss has become a major concern. In 2018 WHO reported that approximately 466 million people live with disabling hearing loss. Majority of them reside in low- and middle-income countries and commonly lack access to the required services and interventions. The most common disease involving the tympanic membrane is Otitis Media which in severe and chronic cases leads to a perforation of the tympanic membrane. Although tympanic membrane perforation often are self-healing, a patch or surgery may be needed to close the perforation. Failure of membrane to heal can result in varying degrees of hearing loss, tinnitus and increased susceptibility to recurrent Otitis media and sometimes cholesteatomas. Patients would thus benefit from the ability to repair tympanic membrane perforation as soon as possible. At present tympanic membrane repair is done by conventional surgical procedures-Myringoplasty and tympanoplasty. In this clinical trial, our approach is to provide a cost effective, nonsurgical, minimal invasive OPD procedure for regeneration of tympanic membrane using Platelet rich fibrin membrane.

Keywords: Platelet rich fibrin, myringoplasty, OPD procedure

I. INTRODUCTION

The tympanic membrane is a thin layer of tissue that is made of three layers- the ectoderm which is an outer layer of stratified squamous epithelium composed of keratocytes; mesoderm-middle layer consisting of fibroblast and type II and III collagen whose function is to provide mechanical strength, consistency and plasticity; and endoderm-the inner nonkeartinised mucsal epithelium.[1,2] Its function is to receive sound vibrations from the outer ear and transmits them to the auditory ossicles from where through the sensory neural pathway are carried to the brain and hence hearing is perceived. Any perforation in the tympanic membrane decreases the hearing to a few decibels depending on the size of the perforation. Almost all acute and small tympanic membrane perforations such as those caused by injury will naturally heal themselves, however Chronic and large tympanic membrane perforations fail to heal spontaneously. Considering the innate potential of the tympanic membrane to repair itself, it is possible that chronic and large perforations can regenerate if adequate conditions for growth are made available. We know that the myringoplasty and tympanoplasty works on the principle of regeneration of the tympanic membrane where the graft works as a scaffold. Patients own tissue is used as graft material on which a neomembrane grows. Platelet rich fibrin membrane (PRF) is an alternative for tympanic membrane repair as it aims to generate the membrane that reproduces the structure, mechanical properties and function of the native membrane. There are basically three elements required for the regeneration of the tissue-cells, scaffold and regulatory factors (growth factors).

Platelet rich fibrin is described as a second-generation platelet concentrate.[3] It is an autologous leucocyte and platelet rich fibrin biomaterial. Platelet rich fibrin contains physiologically available thrombin along with many growth factor.[3] The thrombin in PRF results in slow polymerization of fibrinogen into fibrin with increased incorporation of the circulating cytokines in the fibrin meshes which results in a physiologic architecture that favours wound healing.[4,5] The structural configuration of PRF with respect to cytokine incorporation in fibrin meshes is different from the platelet rich plasma(PRP).[6] There are five main groups of growth factors-epithelial growth factor(EGF), fibroblast growth factor(FGF), platelet derived growth factor,

transforming growth factor(TGF) and insulin like growth factor.[7] Perforation closure occurs by epithelial proliferation and migration and hence researchers have focused on the first two groups mainly.

EGF stmulates the synthesis of DNA, RNA, proteins and hyaluronic acid[8]. Moreover, there is a high affinity for EGF receptors in tympanic epithelial and stromal cells and after injury to the tympanic membrane, the EGF expression is parallel to the reparative process[8].

Within the FGF family, basic fibroblast growth factor has been most investigated. This factor is produced after TM injury and it facilitates the perforation closure through various mechanisms. bFGF acts mainly in the epithelial layer, where there are more specific receptors for it, although these receptors are also present in the mucosal layer, the beneficial effect of bFGF also lies in the induction of rapid proliferation of the subepithelial connective tissue.

So far, only the PDGF has been approved by the United States Food and Drug Administration (FDA) and by the European Authorities (EMEA) for clinical application in patients[9]. Growth factors are a group of soluble and diffusible polypeptide substances[9]. They promote endothelial and epithelial regeneration, stimulate angiogenesis, collagen synthesis, soft tissue healing, and hemostasis.

There are different Preparation methods. Few are listed below-

1. PRGF-System® (BTI Biotechnology Institute, Vitoria, Spain): At least 1 million platelets per microliter are available in PRP. It must have a platelet concentration of approximately 1.5 times the concentration in whole blood. It has bacteriostatic effecst and is used therapeutically having no side effects. One centrifugation at 460× g during 8 min, is used for preparation of PRP[10].
2. Platelet Concentrate Collection System (PCCS®Kit) (3i–Implant Innovations, Palm Beach Gardens, FL, USA): Two centrifugations are used the first at 3000rpm for 3 minutes and 45 seconds and second 13 minutes at 3000. This technique allows a platelet concentration between 1,100,000 and 2,200,000/µL, and includes leukocytes, with a concentration between 5500 and 14,800/µL. As an anticoagulant, it uses ACD-A9 and is subjected to two centrifugations: the first 3 min and 45 s at 3000 rpm and the second 13 min at 3000 rpm.
3. Gravitational Platelet Separation (GPS® System) (Biomet Merck Biomaterials, Darmstadt, Germany): With this method a platelet concentration of 1,600,000/µL and leukocyte levels of 31,100/µL are obtained.
4. Smart PReP® System (Harvest Technologies Corporation, Munich, Germany)[13]: 52 mL in women and 48 mL in men, the amount of blood needed. A double centrifugation of the blood is performed for 12 min, obtaining a PRP with a close platelet concentration to 1,250,000/µL and a leukocyte concentration of 19,261/µL].
5. Plateltex® (Plateltex, Bratislava, Slovakia):The platelet concentration obtained with this method is approximately 1,600,000/µL and is carried out by means of a double centrifugation. The blood is obtained is 8.0 mL in test tubes, which contain ACD-A as an anticoagulant, and are subjected to a gentle first centrifugation at 180× g for 10 min. After aspirating the supernatant plasma, a second centrifugation is performed at 1000× g for 10 min[13].

However, to succeed several challenges have to be completed, such as a complete characterization of the platelet released growth factors and proteins, evaluating individual reasons that make some products more effective than others and designing new associations with biomaterials or other regenerative therapies, such as stem cells.

The most critical issues about PRP are:

1. The variety of preparation methods (concentrations and centrifugation) and platelet activation techniques;
2. The choice of autologous plasma against homologous or allogeneic: the choice to use autologous plasma is strongly recommended in order to avoid problem of contagious disease and immune response;
3. The presence of leucocytes: however it hasn't been proved if they add an immune response;
4. The timing and cost of preparation;
5. The long-term safety assessment;

Therefore, it is necessary to continue the research, the healing process depends not only on growth factors but also on appropriate wound care, infection control and nutrition (global takeover).

PRP may be prepared by, single centrifugation, double centrifugation, or blood selective filtration procedures, and on manual or automatic systems operated in open or closed circuits. Ex vivo, platelet activation can be triggered mechanically with freeze–thawing cycles, chemically with thrombin or calcium chloride, or endogenously.

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Mishra and colleagues proposed to classify PRPs with two parameters[11]:
Firstly, 'type' of PRP:
1. Increased WBCs and no activation;
2. Increased WBCs and activated;
3. Minimal/no WBCs and no activation;
4. Minimal/no WBCs and activated;

And secondly, its platelet enrichment factor, A if the PRP contains a platelet concentration at or above five times the baseline, or B if platelet concentration is less than five times the baseline[11].

II. MATERIALS AND METHODS

We conducted a prospective study of 12 patients with tympanic membrane perforations of varying sizes in both men and women in age group of 20 to 40 years, during the period from December 21018 till December 2019. All the patients were cases of chronic otitis media having a central perforation in pars tensa. All patients were divided into two groups: Group A (study group) includes patients who underwent PRF membrane procedure and Group B (control Group) who underwent the normal Conventional Myringoplasty procedure using cartilage as Graft material. Both Groups were followed at 1wk, 3wk and 3month for Otoscopic examination and pure tone audiometry was done along with the otoscopic examination. Informed written consent was obtained from all patients studied.

The aim of the study was to regenerate the tympanic membrane using PRF membrane to produce a neomembrane thereby providing a minimal invasive OPD procedure obviating the need for conventional surgical procedure.

In Group A (Study Group) 6patients irrespective of the size of perforation were treated using PRF membrane as Scaffold and in Group B (control Group) other 6 patients underwent conventional myringoplasty using cartilage as graft
Our inclusion criteria required that
1. Tympanic membrane perforation is dry for atleast one month before the procedure
2. The middle ear mucosa was healthy
3. Central perforations of the pars tensa part of the tympanic membrane were considered irrespective of the size.

Exclusion criteria were
1. Total, marginal and attic perforations.
2. Patients with cholesteatomas, poor Eustachian tube function and ossicular involvement were excluded from the study.

A complete history and otoscopic examination were done. Pure tone audiometry was done for all patients and a preoperative examination under microscope was conducted. Routine complete blood count and coagulation parameters were recorded.

PRF membrane preparation

Blood sample is collected from patient in a tube without anticoagulant using an 18No. needle. 12ml blood is collected to obtain 1ml PRF. Collected blood was sent to lab where it was centrifuged at 3000RPM for 15 minutes. During centrifugation technique when the blood gets in contact with the test tube wall the platelets get activated leading to the initiation of coagulation cascade. The centrifugation caused the blood to separate into three layers of different density. The topmost layer consists of an acellular platelet poor plasma, PRF clot in the middle and RBCs at the bottom of the tube. The fibrin clot obtained is removed from the tube and attached RBCs scraped off from it and discarded. PRF membrane is formed by squeezing out the fluids present in the fibrin clot.

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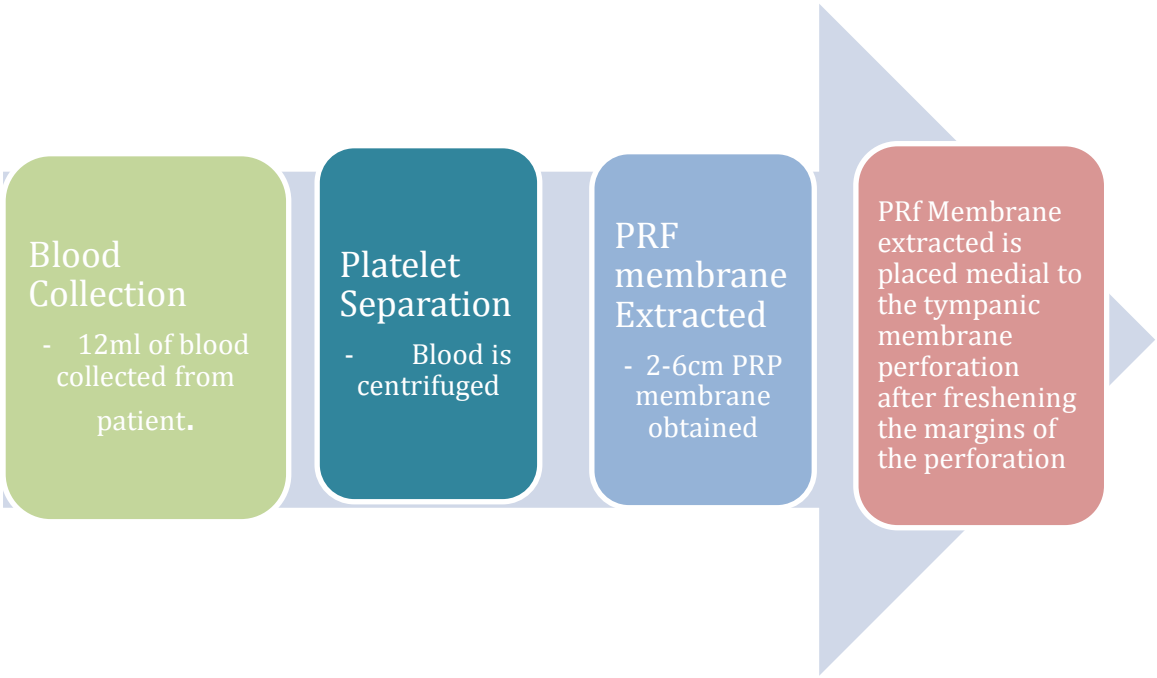

Figure 1: PRF Membrane Preparation Process

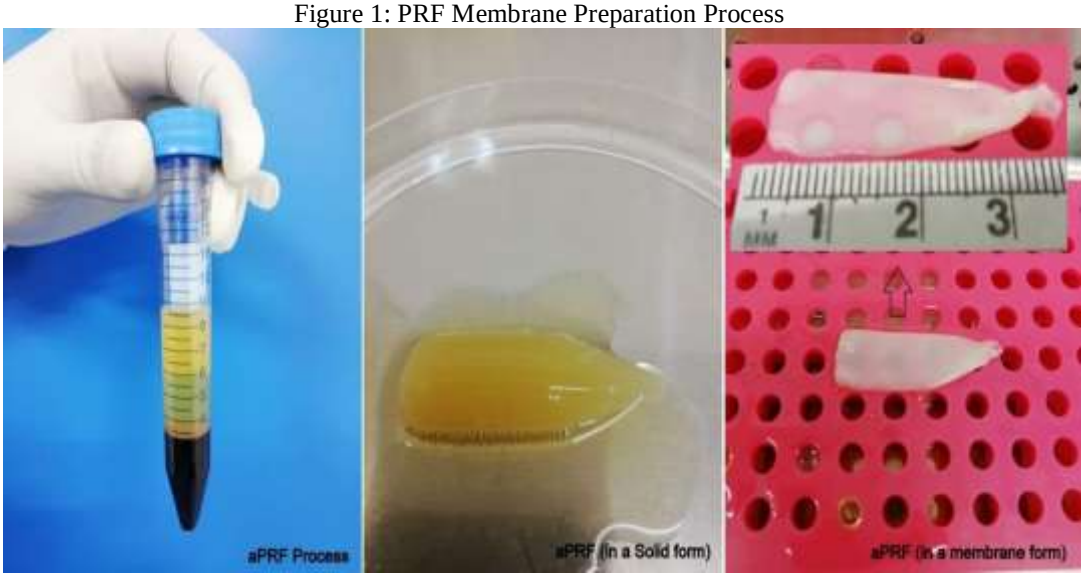

Figure 2: Extracted PRF membrane

Method for tympanic membrane repair

Topical anesthesia was achieved by placing a cotton ball soaked in 4%lox with adrenaline in the external auditory canal for 15 minutes. The margins of the perforation were freshened using a rosen sickle knife. The inner surface of the tympanic membrane was also scraped slightly. Using the transcanal approach PRF membrane was placed through the perforation medial to the tympanic membrane. A second larger piece was also placed in the external auditory canal. The canal was further packed with medicated gelfoam. The tympanic membrane was examined after 1week and followed every 3weeks for 3 months till complete closure of membrane was achieved.

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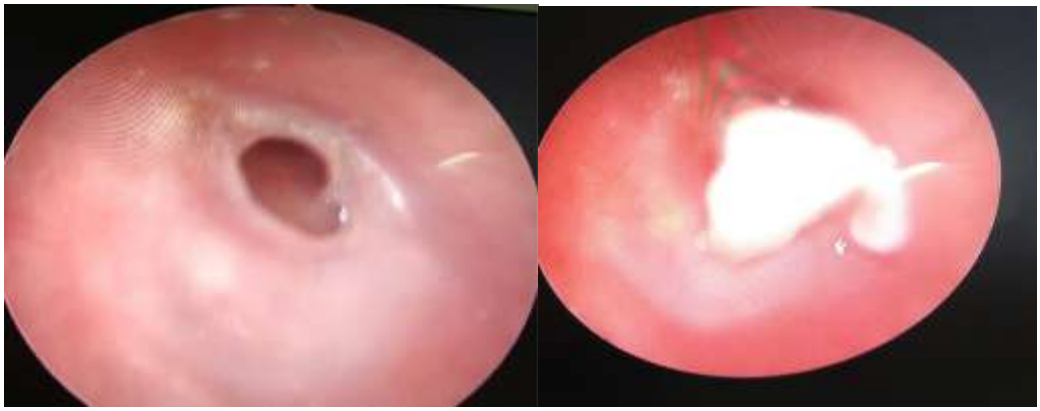

Figure 3. PRF membrane positioned on tympanic membrane perforation

OUTCOME OF THE STUDY- was evaluated as

1. Complete Improvement- was defined as complete healing of tympanic membrane on Otoscopic examination with improvement in hearing on pure tone audiometry.
2. Partial improvement was defined as when the tympanic membrane perforation decresed in size but a smaller perforation persisted (residual perforation) with partial improvement in hearing on pure tone audiometry
3. No improvement was defined as no change in the size of perforation and hearing.

DATA COLLECTION

1. Distribution of tympanic membrane rupture among study individuals

S.No.	Group	Ear	Size of perforation		
			small	medium	large
1.	Study group	Right	1	1	0
		Left	1	2	1
2.	Control group	Right	1	1	0
		Left	0	3	1

2. Basic parameters in study and control group

S.No.	Parameter	Treatment with PRF Membrane	Conventional myringoplasty using cartilage as graft	P value	Result
1.	Age	20-40yrs	20-40years		
2.	Gender Male Female	 2 patients 4 patients	 3 patients 3 patients	0.56	Not Sig.
3.	Ear side Right Left	 2 4	 2 4	1.0	Not Sig
4	Size of perforation Small Medium Large	 2 3 1	 1 4 1	0.79	Not Sig
5.	Healing of perforation Complete	 5 1	 5 0	1.0	Not Sig

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	healing Partial healing No healing	0	1		
6.	Time for healing of perforation 1week 3weeks 3months	 3 2	 3 3	0.74	Not Sig
7.	Improvement of hearing on audiometry Complete improvement Partial improvement No improvement	 5 1 0	 5 0 1	P value is 1.0, Not Sig	

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3. Comparison of postoperative infection among study and control group

S.no.	Group	Post-operative infection	No post-operative infection	Total	P Value	Result
1.	Group 1 PRF membrane (study group)	0	6	6	1	Not Sig
2.	Group 2. Conventional myringoplasty (control Group)	1	5	6		

III. RESULTS

In Group A –Successful complete closure was achieved in 5 patients in whom PRF membrane was used. One patient showed incomplete closure. However all patients showed improvement in hearing of 20-35dB on pure tone audiometry.

In Group B- 5 patients showed complete closure of tympanic membrane except one patient who developed postoperative infection due to which graft was rejected and no improvement was seen in size of perforation as well as in hearing.

In Group A- All patients tolerated the procedure in topical anaesthesia and no sedation was required was required for PRF membrane procedure. No patient was required to stay in the hospital. The procedure was done in the Minor OT.

In Group B- all patients surgery was done in Major OT and the patient were required to stay in the hospital for 3 days.

IV. DISCUSSION

In this study, we found that in Group A , application of PRF membrane in chronic cases of tympanic membrane perforation without complications healed efficiently without the need for an autologous graft.

Irrespective of the size of the perforation the perforation healed. Two perforations of 2mm healed within 3 weeks' time using the PRF membrane while perforation of more than 2mm required approximately 2 to 3 months to show complete healing. No Patient developed post operative infection. In one patient incomplete improvement was seen. As the procedure was done in minor OT and no sedation was give, hospital stay was zero in PRF membrane myringoplasty.

In Group B, conventional myringoplasty using cartilage as graft also showed complete improvement in 5 patients however one patient developed post operative infection as a result the graft was rejected and no improvement in the size as well as hearing was noted. Moreover patients were required to stay in hospital for 3 days,

sedation was required as a result expenditure was increased.

In Kartush et al[12] study it was determined that patching does not heal perforations with a size of more than 5mm. however in our study we saw perforation of 6mm being healed if adequate conditions (Growth factors and scaffold) are provided for the repair of tympanic membrane.

In a study by Habesogolu et al[13] in 2014, 32 patients with acute traumatic TM perforation underwent repair with platelet rich fibrin. He concluded that the use of platelet-rich fibrin accelerated the tympanic membrane closure in 64% patients. In our study chronic cases of TM perforation who were treated with PRF Membrane irrespective of the size also showed complete healing of the tympanic membrane if adequate conditions (Growth factor and scaffold) are provided for regeneration of the tympanic membrane.

In a similar study conducted by Nair et al[14] in 2018, 43 patients underwent myringoplasty aided with platelet rich fibrin showed a graft uptake of 97.7% in study group as compared to 81% in control group. In our study, the PRF membrane was used as graft which gradually was absorbed providing adequate conditions for regeneration of tympanic membrane.

Chauvin et al[15] proved in their study that growth accelerators are the most promising agents. Our study also proved that PRF membrane which had growth factors has shown promising results.

V. CONCLUSION

From our study we have concluded that using PRF membrane for TM perforation repair increases the regeneration rate of the perforation margins to a significant extent without the need for a surgical myringoplasty as well as a graft.

Also, it proved to be a cost effective noninvasive OPD procedure which could be performed in the OPD also avoiding the need for sedation and postoperative complications.

However, our present study has a relatively low sample size, so further studies using larger sample size are required to be conducted to study the effects of PRF membrane on TM repair.

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