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Role of platelet rich plasma in Type I tympanoplasty: A prospective study to assess the effectiveness of a platelet rich plasma soaked graft in the repair of tympanic membrane perforation

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Abstract:

Introduction: Chronic tympanic membrane perforation requires surgical intervention, typically Type I Tympanoplasty, to restore hearing and prevent recurrent infection. Conventional repair methods, such as using an autologous temporalis fascia graft, can be limited by variables including non-uptake, infection, and suboptimal healing. Platelet-Rich Plasma, a source of concentrated growth factors, has shown promise in tissue engineering to stimulate and strengthen the natural healing process.

Objective: This prospective, double-blind, interventional study aimed to evaluate the effectiveness of a Platelet-Rich Plasma soaked temporalis fascia graft in Type I Tympanoplasty compared to conventional tympanoplasty in repairing chronic tympanic membrane perforation, specifically assessing graft uptake rate, infection rate, and audiometric improvement.

Methods: A total of 80 patients with dry chronic TM perforation were enrolled and divided into two groups of 40: the Tympanoplasty + PRP group and the Tympanoplasty group. In the PRP group, autologous PRP was prepared using a double-spin method and applied to a temporal fascia graft, which was then placed medial to the malleus. Follow-up audiometric evaluation and otoscopic examination were conducted at 3 months and 6 months post-surgery and statistical analysis was performed.

Results: The use of PRP demonstrated significantly superior outcomes with respect to graft uptake rate, infection rate and hearing improvement.

Conclusion: The application of Platelet-Rich Plasma (PRP) to the temporalis fascia graft in Type I Tympanoplasty significantly enhances the complete healing rate, minimizes infection, and provides superior hearing improvement compared to conventional techniques.

Keywords: Tympanoplasty, Platelet-Rich Plasma, Tympanic Membrane Perforation

1. Introduction

Tympanic membrane perforation is one of the most common presentation came across in otorhinolaryngology clinical settings, which could be completely asymptomatic or be just found incidentally. If healing does not occur within three months, it is referred as chronic tympanic membrane perforation and needs surgical intervention such as tympanoplasty.[1] The three principal

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indications for tympanoplasty are recurrent otorrhoea, hearing loss due to a chronic perforation, and the desire to swim without having to waterproof the ear.[2] Tympanoplasty is performed to provide a scaffold over the perforated membrane in order to support the surrounding tympanic membrane keratinocytes, fibroblasts and vessels to grow over the scaffold to replace the damaged tissue.[3] Multiple factors determine the post-surgical improvements and overall success of tympanoplasty.

While repairing the tympanic membrane we have to understand that tissue engineering of the tympanic membrane stands on three major pillars: (a) cells for the regeneration of the damaged tissue, (b) a framework to support regrowth, and (c) a bioactive material to stimulate and strengthen the natural healing process of membrane.[4] Several materials like de-cellularized matrices, cross-linked hydrogels, calcium alginate, silk, polyglycerol sebacate, and chitosan have been used as scaffolds to repair TM perforations. [4] In Japan, an artificially designed dermis, majorly containing atelocollagen sponge, is frequently used as a scaffold to close TM perforations because of its easy availability but the results are not very satisfactory. To compensate for this lack of bioactive factor and to improve surgical outcome, various studies have been conducted using platelet rich plasma in tympanoplasty [5] Platelet rich plasma is easy to procure and its addition in the middle ear cavity may have some added improvement in terms of sensorineural hearing loss and conductive hearing loss apart from its benefits in early healing and better success in tympanoplasty. The bioactive molecule secreted by platelet granule are involved in proliferation, differentiation of cells having anabolic and pro inflammatory response optimizing the tissue environment and favoring healing process.[6] The bioactive agents, serotonin, dopamine, adenosine and calcium increase the cell permeability and modulate the repair.

This study has been conducted to evaluate the use of platelet rich plasma placed on both sides of temporal fascia graft in type 1 tympanoplasty, graft take up rate, improvement in air conduction and bone conduction during observation period in tympanoplasty with conventional techniques.

2. Materials and Methods

This hospital based double blind interventional study was conducted in the Department of Otorhinolaryngology at SMS Medical College & Hospital, Jaipur. Sample of 40 cases in each group were taken at 95% confidence and power of 80% to verify expected difference of proportion of 31.7% for hearing improvement in patient undergoing type 1 tympanoplasty in between two groups. Data was collected and stored on the proforma for further analysis using appropriate statistical tools The inclusion criteria was patients diagnosed with dry tympanic membrane perforation for at least 1 month before surgery and patients with healthy middle ear mucosa. The exclusion criteria was revision surgery, patients with cholesteatoma, poor eustachian tube function, attic perforation and suspected ossicular pathology.

The blood was obtained from antecubital vein using 16/18 number scalp vein set in specific tube having 1 ml of anticoagulant, 9.0 ml of blood was collected. Immediate centrifugation of was done at automatic centrifugation machine using 1500 rpm for 15 min. This resulted in two layers, upper one yellowish and lower one dark red. By using a sterile pipette supernatant plasma was transferred to another sterile tube for hard spin. Second centrifugation was done at 3000 rpm for 15 min, upper supernatant platelet poor plasma was sucked gently with pipette after leaving 1 ml of fluid and pallet at the bottom. Now with another sterile pipette the pallet was gently mixed in the fluid, and preserved to be used during surgery. This PRP was added to adjust sufficient quantity of gel foam used in surgery and two drops of PRP was installed on each side of dried temporal fascia graft. The graft was placed medial to handle of malleus after lifting 360° tympano-maetal flap. Repositioning of anterior segment was done meticulously to prevent anterior blunting. PRP wet gel foam placed over the graft pressing the graft will squeeze out any air or blood between the graft and bone. Thin ribbon pack was placed in cartilaginous portion external auditory canal. Pack was observed on alternate days and gentle suction was done over it. Ribbon pack was removed on 7th day and condition of gel foam was observed.

Patient was given 2 days injectable antibiotic and 10 days oral antibiotics along with analgesic antihistamines, vitamin C and Vitamin D supplementation and was followed up at 3 months and 6 months. To avoid personnel bias all surgeries were done by same surgeon and all audiometry evaluations were done by same audiologist who was blinded to the groups.

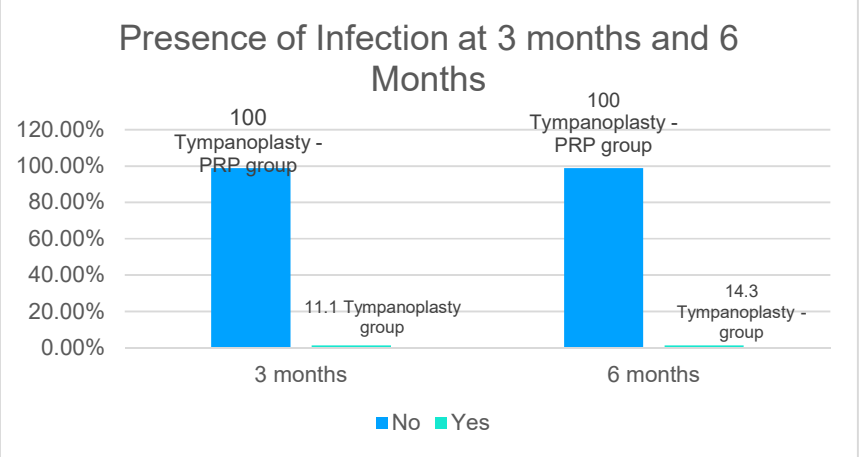
3. Results

Statistical analysis was done with Statistical Package for Social Sciences (IBM SPSS Statistic for window, version 26.0. Armonk, NY: IBM Corp.) at 95% CI and 80% power to the study. Descriptive statistics was performed in terms of, mean and standard deviation, Frequency and Percentage. Repeated Measures ANOVA followed by Post hoc Tukey's test was applied to compare the Audiometric Evaluation between Pre-operative, 3 months and 6 months follow up respectively. Fisher's Exact test and Chi Square test was applied to compare the nominal data between Pre-operative, 3 months and 6 months. Statistical significance was calculated at $p<0.05$ and $p<0.001$ was considered highly significant.

A total of 80 patients were included in the analysis with mean age of 31.29 ± 12.22 years, with the youngest being 12 years and the oldest 58 years. The male population slightly outnumbered the female population in this study as 56.3% were male and 43.8% were female. Based on Otoscopic examination, out of the total sample 40.0% showed left ear central perforation involving more than two quadrants, while 36.3% had similar findings in the right ear; central perforation involving more than one quadrant was observed in 13.8% in the left ear and 10.0% in the right ear in pre-operative assessment.

The infection rate in Tympanoplasty + PRP group at both 3 months and 6 months was that 100% patients had no infection. Whereas, infection rate in Tympanoplasty group at both 3 months was 11.1% and at 6 months was 14.3% (Graph1).

Graph 1: Presence of Infection at 3 months and 6 months



Graft uptake was successful in 100% patients at both 3 and 6 months in Tympanoplasty + PRP group. The p-value was 0.752, again indicating no significant difference between follow-up periods. The graft uptake in Tympanoplasty group at both 3 months was that 85.7% and at 6 months was 82.5% (Table 1).

Table 1: Comparison of Patients at 3 months and 6 months for graft uptake

Tympanoplasty-PRP group						
			Follow up		Total	P value
			3 Months	6 Months		
Graft Uptake	YES	Count	40	40	80	0.752
		% within Follow up	100%	100%	100%	
Tympanoplasty group						
			Follow up		Total	P value
			3 Months	6 Months		
Graft Uptake	YES	Count	35	33	68	0.5
		% within Follow up	85.7%	82.5%	85%	
	NO	Count	5	7	12	
		% within Follow up	14.3%	17.5%	15%	

Table 2: Comparison of Audiometric Evaluation between Preoperative, 3 months and 6 months

Tympanoplasty + PRP group					
		N	Mean	Std. Deviation	P value
Air Conduction	Preoperative	40	40.7588	8.63179	<0.001**
	3 months	40	21.3038	5.09394	
	6 months	40	18.7875	4.07740	
Bone Conduction	Preoperative	40	15.1975	4.88773	<0.001**
	3 months	40	10.3538	3.20971	
	6 months	40	10.1625	3.53444	
Air Bone Gap	Preoperative	40	25.5862	5.70686	**<0.001****
	3 months	40	10.8875	4.48060	
	6 months	40	8.6250	3.43981	
Tympanoplasty group					
		N	Mean	Std. Deviation	P value
Air Conduction	Preoperative	40	41.6754	7.76456	<0.05
	3 months	40	39.3038	5.09394	
	6 months	40	35.7875	4.07740	
Bone Conduction	Preoperative	40	15.1235	5.67773	<0.05
	3 months	40	14.3538	4.78671	
	6 months	40	14.7865	4.98676	
Air Bone Gap	Preoperative	40	24.7862	4.98686	<0.05
	3 months	40	20.6545	5.56460	
	6 months	40	18.1344	4.98761	

Air Conduction in Tympanoplasty + PRP group significantly improved from 40.76 ± 8.63 dB (Preoperative) to 21.30 ± 5.09 dB (3 months) and 18.79 ± 4.08 dB (6 months) with the $p<0.001$ as statistically significant. Whereas in Tympanoplasty group there was minimal improvement from 41.67 ± 7.78 dB (Preoperative) to 39.30 ± 5.09 dB (3 months) and 35.78 ± 4.08 dB (6 months) with the

p<0.05 as statistically non-significant. Bone Conduction in Tympanoplasty + PRP group significantly improved from 15.20 ± 4.89 dB (Preoperative) to 10.35 ± 3.21 dB (3 months) and 10.16 ± 3.53 dB (6 months) with the p<0.001 as statistically significant. Whereas in Tympanoplasty group there was no improvement from 15.12 ± 5.67 dB (Preoperative) to 14.35 ± 4.78 dB (3 months) and 14.78 ± 4.98 dB (6 months) with the p<0.05. Air-Bone Gap reduced significantly in Tympanoplasty + PRP group from 25.59 ± 5.71 dB (Preoperative) to 10.89 ± 4.48 dB (3 months) and 8.63 ± 3.44 dB (6 months) with the p<0.001 as statistically significant. Whereas in Tympanoplasty group there was no improvement 24.78 ± 4.98 dB (Preoperative) to 20.65 ± 5.56 dB (3 months) and 18.13 ± 4.98 dB (6 months) with the p<0.05 (Table 2).

The pairwise comparison of air conduction thresholds revealed a highly statistically significant improvement from preoperative to 3 months postoperatively (p<0.001), and from preoperative to 6 months postoperatively (p<0.001), indicating a substantial gain in hearing thresholds following surgical intervention in Tympanoplasty + PRP group. However, the difference between 3-month and 6-month air conduction values was statistically significant (p<0.05). In terms of bone conduction thresholds, there was a highly statistically significant improvement from preoperative to 3 months (p<0.001) and from preoperative to 6 months (p<0.001) in Tympanoplasty + PRP group, reflecting enhancement in inner ear function or middle ear mechanics postoperatively. However, no statistically significant difference was observed between 3 months and 6 months (p=0.950), indicating that most improvements in bone conduction occurred within the initial 3-month period with stabilization thereafter. The air-bone gap significantly reduced over time in Tympanoplasty + PRP group, with highly statistically significant differences between preoperative and 3 months (p<0.001) and between preoperative and 6 months (p<0.001), suggesting effective closure of the conductive component of hearing loss following surgery. The reduction in the air-bone gap from 3 months to 6 months was also statistically significant (p=0.006).

Preoperatively, 100.0% patients had BC>AC, indicating conductive hearing loss. At 3 and 6 months, all 100.0% in Tympanoplasty + PRP group showed AC>BC, indicating resolution. The change was highly statistically significant (p<0.001). In Tympanoplasty group at 3 months 88.9% and at 6 months 85.7% showed resolution with AC>BC whereas 11.1% and 14.3% at 3 and 6 months respectively showed BC>AC indicating no hearing repair.

In Tympanoplasty – PRP group, pre-operatively 55% had lateralization towards the left and 45% towards the right. By 3 and 6 months, all 100.0% patients showed centralized results. The difference was highly statistically significant (p<0.001). In Tympanoplasty group, pre-operatively 52.5% had lateralization towards the left and 47.5% towards the right. By 3 and 6 months, 90.0% and 87.5% patients showed centralized results whereas in the infected cases 3 months – 5% and 6 months – 7.5% showed lateralization towards the infected side (Table 8). The difference was highly statistically significant (p<0.001).

4. Discussion

The tympanic membrane is an integral part of the ear, contributes majorly to the activity of hearing. Functionally, it collects and transmits sound vibrations covering the middle ear. The tympanic membrane (eardrum, myringa) is a thin, semi-transparent, oval cone shaped membrane, approximately 1 cm in diameter, which separates the external acoustic meatus from the tympanic cavity or outer ear from the middle ear. The tympanic membrane can be visualized during an otoscopic examination, when light is directed at the tympanic membrane, a characteristic feature to be observed is the anterior cone of light, which radiates antero-inferior from the umbo. The tympanic membrane should be pearly-grey in color, translucent, shiny, and mobile on insufflation. If any of the aforementioned features of the tympanic membrane is altered, a pathological process, usually in the tympanic cavity, should be considered. [7] In our study, otoscopic examination revealed that 40.0% showed left ear central

perforation involving more than two quadrants, while 36.3% had similar findings in the right ear whereas central perforation involving one quadrant was observed in only 13.8% cases in the left ear and 10.0% in the right ear.

The major etiological factors are acoustic trauma (loud noise), otitis media, ear infections, insertion of some foreign materials in the ear, physical trauma, and so on. Untreated perforations may progress to hearing loss, tinnitus (ringing of ears), Cholesteatoma (noncancerous skin growth often developed as a cyst), otalgia, otorrhea, and severe calcifications. The pathophysiology behind tympanic membrane rupture depends on the etiology of the rupture itself. In our study patients experiencing tympanic membrane perforation reported with complain of sudden onset of pain accompanied by hearing loss, bloody otorrhea, hearing loss, vertigo or tinnitus which was similar to the study by Yavuz R et al[8] who evaluated that most common presenting symptom was otorrhea (81.5%), followed by otalgia (72.8%) and tinnitus (55.7%).

Tympanic membrane rupture can occur at any age, although it is mainly seen in the younger population, associated with acute otitis media and in older age trauma becomes a more likely cause. In our study the mean age of the patients was 31.29 years, with the youngest being 12 years and the oldest 58 years so our study included a mixed age sample but with similar causative factors. In a study by Sagiv et al[9] the average age of patients was 26.7, with children making up 25% of the sample size whereas, Naseema et al[10] took the sample of age distribution from 14 years to 72 years with mean age of 41.78 years. Pannu et al[11] in their study of 100 patients noted the mean age of patients as 28.27. Taneja[8] who compared the efficacy of platelet rich plasma (PRP) in tympanoplasty concluded that elderly age of presentation may have a higher risk of recurrence or complications when compared to the younger age of presentation. Men are more likely to experience tympanic membrane perforation compared to women. In our male population slightly outnumbered the female population in this study with a ratio of 1.31:1. This was similar to other studies by Dohli N et al who showed that men more commonly had traumatic rupture compared to women in a ratio of 1.49 in a study of 1000 patients and similar findings were also observed in a study of 529 patients by Yavuz R[8] et al who found out the ratio to be 2:1 and Naseema et al[10] who had the Male: female ratio of 1.59:1. There are also studies in literature that differed from the above statistics like Pannu et al[11] who showed the male to female ratio as 1:1 and Maharajan et al[12] who noted a female preponderance in their study. However, in general no specific trends were noted with regard to gender preponderance.[13]

Tympanic membrane perforation can be acute or chronic depending on the duration. Acute and traumatic perforations usually heal spontaneously but if the perforation is present for a duration of more than 3 months, it is chronic. Various techniques have been used in the attempt of repairing perforations. Myringoplasty is the conventional method of treatment (procedure of closure of the perforation present in pars tensa of the tympanic membrane) and Tympanoplasty (myringoplasty along with ossicular reconstruction). But the terms myringoplasty and tympanoplasty type 1 have been used interchangeably.[14] The term myringoplasty was coined by Berthold but the first successful myringoplasty was performed by Marcus Bancer in 1640. The aim of the operation includes closure of perforation with a dry stable grafted membrane and improvement in hearing levels. Grafting the tympanic membrane with autologous temporal fascia helps in restoration of its integrity, as temporalis fascia is a thin, non-shrinking tissue with low metabolic rate resembling tympanic membrane in texture, thickness and structure. Various materials like vein grafts, fat grafts, split and full thickness skin grafts, perichondrium, periosteum, fascia, cartilage graft, and others, have been used in the past to repair tympanic membrane perforations. Out of these, fascial graft has been used widely because of its internal structure, and it is also easily available for harvesting.[15] Tympanoplasty with autografts (e.g., temporalis fascia) is the gold standard surgical treatment for tympanic membrane perforation and achieves high success rates. However, an ideal repair material has yet to be identified because of various limitations like re-perforation, middle ear infections or other complications after surgeries.

Thus, it is essential to explore options to improve the efficacy of repair and over the past decades, alternatives to resolve the limitations of conventional tympanoplasty have been widely explored, including the use of scaffold materials, bioactive molecules and cells. These new technologies should ideally yield more advantages than conventional tympanoplasty. Among them, PRP is reportedly effective in wound healing and present great potentiality for tympanic membrane repair.[6, 16] Hence the main aim of this study was to evaluate the potential of Platelet Rich Plasma (PRP) in Type 1 tympanoplasty.

Platelet-rich fibrin (PRF) was first described in 2001 and PRF is often referred as second-generation platelet-rich plasma (PRP). We can define platelet rich plasma as a volume of autologus plasma of blood which has been enriched with platelets and growth factors. In 2006, Dohan *et al*. made a detailed biochemical analysis of PRF and reported that PRF is surrounded by a polymerized fibrin network, containing cytokines, glycolic chains, and structural glycoproteins. In addition to a high platelet concentration, PRF contains various growth factors. PRF also contains white blood cells, phagocytic cells, vasoactive amines, and chemotactic agents with high fibrinogen concentration. These biochemical components have a well-known synergistic effect on the healing process.[17] The reason for using this in our study was that, Platelet rich plasma is easiest to procure and to use it in human body and it is autologous so does not have any significant side effect. Platelet rich plasma is already being used as a treatment modality in various other fields of medicine, such as cardiothoracic surgery, orthopedics, neurosurgery, and plastic surgery, among others but its use in ENT is not yet well studied and hence the objective of our study was to add to this missing literature. In our study the PRP preparation was done according to the method explained by Dhurath et al and a literature based double spin method as explained by Mazzacco et al. PRP has several advantages like safety and low toxicity; does not induce any undesirable tissue reactions; easy, quick and inexpensive to produce and can easily be manipulated during surgical procedure and the healing time of tympanic membrane regeneration and hospitalization duration were shortened.[18]

Surgical success is dependent on the optimal contact between the newly placed graft and the remaining tympanic membrane, and failure can occur due to medialization or lateralization of graft. Any substance that helps in rapid healing will, in turn, help in reducing the chances of graft migration leading to increase in the graft uptake rate. In our study, in Tympanoplasty + PRP group 100% patients had no infection at 3-month and 6-month interval and showed a consistent postoperative outcome whereas 11-17 % patients had infection in only Tympanoplasty group indicating superior healing in PRP group. Graft uptake was successful in 100% patients at both 3 and 6-months indicting that use of PRP in Type 1 Tympanoplasty was successful as compared to regular treatment where the graft non-uptake was 15-18%. Similarly, Ma Y et al also concluded that average closure rate in the PRP treatment groups was 93.4%, compared to 78.6% in the control groups with conventional surgery alone and application of PRP along with autografts achieve a higher success rate in repairing perforations. El-Anwar et al[5] also showed success rate for patients who underwent tympanoplasty with PRP was 100%, compared to patients who underwent only tympanoplasty, had a success rate of 81.25%. Gokce Kutuk et al[19] and Yadav et al[20] also had similar results to our study concluding that graft survival rates were significantly higher in those platelet rich fibrin was applied.

In addition to the healing results, hearing improvements also play an important role in the evaluation of alternative strategies. In our study Air Conduction significantly improved from 40.76 ± 8.63 dB (Preoperative) to 21.30 ± 5.09 dB (3 months) and 18.79 ± 4.08 dB (6 months) in Tympanoplasty + PRP group. Whereas in Tympanoplasty group there was minimal improvement from 41.67 ± 7.78 dB (Preoperative) to 39.30 ± 5.09 dB (3 months) and 35.78 ± 4.08 dB (6 months). Bone Conduction also showed improvement from 15.20 ± 4.89 dB (Preoperative) to 10.35 ± 3.21 dB (3 months) and 10.16 ± 3.53 dB (6 months) in Tympanoplasty + PRP group. Whereas in Tympanoplasty group there was no improvement from 15.12 ± 5.67 dB (Preoperative) to 14.35 ± 4.78 dB (3 months) and 14.78 ± 4.98 dB

(6 months). Air-Bone Gap reduced significantly from 25.59 ± 5.71 dB (Preoperative) to 10.89 ± 4.48 dB (3 months) and 8.63 ± 3.44 dB (6 months) Tympanoplasty + PRP group. Whereas in Tympanoplasty group there was no improvement 24.78 ± 4.98 dB (Preoperative) to 20.65 ± 5.56 dB (3 months) and 18.13 ± 4.98 dB (6 months). This implies that there was a significant improvement from preoperative to 3 months postoperatively and from preoperative to 6 months postoperatively, indicating a substantial gain in hearing thresholds following surgical intervention. In terms of bone conduction thresholds, there was a significant improvement from preoperative to 3 months and from preoperative to 6 months, reflecting enhancement in inner ear function or middle ear mechanics postoperatively. However, no significant difference was observed between 3 months and 6 months, indicating that most improvements in bone conduction occurred within the initial 3-month period with stabilization thereafter. The air-bone gap significantly reduced over time, between preoperative and 3 months and between preoperative and 6 months suggesting effective closure of the conductive component of hearing loss following surgery. The results of our study were similar to Yadav et al[20] who indicated that TFT with the addition of PRP can provide greater hearing improvement. El-Anwar et al[5] obtained better postoperative ABG values with the use of PRF in their study with change of 10 dB and above in ABG as hearing gain.

Overall, tympanic membrane perforation has a favourable prognosis with a small risk of complications but it is important to know when intervention and early referral is required, based on size, location, and symptoms associated with the perforation. In general, it can be recommended that PRP has definite qualities which can help achieve better success in conjunction with Type 1 Tympanoplasty with respect to healing, graft uptake, infection rate and hearing improvement.[21]

5. Conclusion

Our study showed that using PRP during tympanoplasty increased the complete healing rate, hearing improvement thus emphasizing the benefits of using PRP in the tympanic membrane perforation repair. Hence, Platelets rich plasma (PRP) can be considered as an effective material in tympanic membrane regeneration along with Type 1 Tympanoplasty as it enhanced healing rate and provided potentially better hearing outcome with no risk of infection. This is an innovative application and it can be used a jumpstart for more widespread usage in the future in multiple fields of medicine.

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