Graft Uptake and Hearing Outcomes in Myringoplasty by Conventional Temporalis Fascia Underlay Technique with and without Addition of Platelet Rich Fibrin (Prf): A Prospective, Randomised Double Blind Study

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Myringoplasty, Platelet-rich fibrin (PRF), Hearing improvement, Cellular proliferation.

ABSTRACT
Myringoplasty is a crucial surgical technique employed to repair tympanic membrane perforations, safeguarding the middle ear cavity from external exposure and associated risks. While traditional graft materials such as temporalis fascia, cartilage, and vein grafts have shown graft uptake success rates ranging from 64% to 96%, challenges like graft failure persist. To address this, innovative methods such as the application of platelet-rich fibrin (PRF) have gained attention. PRF, a second-generation platelet concentrate, is rich in growth factors, cytokines, and glycoproteins, promoting accelerated wound healing, cellular proliferation, and matrix remodelling. As an autologous material, PRF offers significant advantages, including safety, cost-effectiveness, and ease of preparation. This study aims to evaluate the efficacy of PRF in enhancing graft uptake and hearing outcomes among patients undergoing myringoplasty. By exploring its potential, this research seeks to establish PRF as an ideal adjunct in surgical management, addressing current limitations and optimizing patient care outcomes.

INTRODUCTION

Chronic otitis media (COM)-mucosal type is the most common ear disease, which leads to the development of hearing loss. It is defined as the persistent inflammation of the middle ear cleft associated with recurrent discharge from the ear for at least six weeks through a non-intact tympanic membrane [1]. Globally, the prevalence of chronic otitis media was estimated to be around 65 to 300 million. Among the affected patients, about 60% of the patients experience hearing loss in their life. COM was more prevalent in developing countries than in developed countries [2]. It is estimated that 22% of the children affected globally are below five years of age. Global COM incidence rates were highest during the first year of life, and it is estimated that 15.4 new cases per one thousand children were affected per year [3]. The reason may be due to the poor environmental status, which leads to recurrent upper respiratory tract infection, which causes eustachian tube dysfunction. It is estimated that around 6% of the Indian population is suffering from chronic ear disease [4].

The COM leads to the development of conductive hearing loss, which occurs due to the non-intact tympanic membrane and damage of the ossicles. It also leads to the development of permanent sensorineural hearing loss because of the damage to the inner ear and also leads to the development of intracranial complications. Due to the defect hearing, most of the patients suffering from the disease had a social and physical impact. It also indirectly affects the socio-economic status of the patients and the quality of life of the patients. So, the disease should be managed appropriately to prevent the development of complications and to improve the quality of life of the patients [3].

Although many treatment modalities are available for the treatment of the disease, the surgical modality is considered the best treatment of the choice to cure the disease completely. And myringoplasty is considered the gold-standard treatment for the management of the disease. Myringoplasty is a surgical technique done for the repair of the non-intact tympanic membrane (tympanic membrane perforation). The tympanic membrane can be repaired by underlay and overlay techniques using various graft materials to seal the perforation in the tympanic membrane. The various graft materials used for sealing the tympanic membrane are temporalis fascia, cartilage, perichondrium, vein graft, periosteum, etc [5].

With the help of the myringoplasty technique, the middle ear cavity can be safely guarded from exposure to the external environment and various pathogens and allergens. However, the outcome of the surgical management depends upon multiple factors such as the size of the perforation, location of the perforation in the pars tensa, status of the ossicular chain, Eustachian tube function, type of the graft material used and type of the surgical procedure employed for the management of the condition [6]. The graft uptake success rate was around 64% to 96% [7]. Based on the reports from the Royal College of Surgeons of England, the success rate of graft uptake was 80% [8].

Thus, some alternate methods have been devised to overcome the problem associated with graft failure during the myringoplasty technique. Some of the technique used to improve the graft uptake rate was plugging the eardrum with gel foam after the removal of the ventilation tubes, the fat plug technique, where the fat taken from the ear lobule or abdomen were used to plug the perforation, use of paper patches, cigarette papers used to close the perforation, usage of stereo strip and the use of platelet-rich fibrin [6]. Platelet concentrates are already in many surgeries by Oro maxilla facial surgeons, plastic surgeons, and orthopaedic surgeons for faster healing purposes. Different platelet concentrates have been studied so far, such as platelet-rich plasma and Platelet-rich fibrin [9]. The use of platelet-rich fibrin was first described by Choukroun [10]. The Platelet-rich fibrin is a second-generation platelet concentrate. It has a rich content of platelets, cytokines, growth factors, glycoproteins, and leukocytes, which were trapped and released over a period of time. This platelet-rich fibrin provides mechanical and inflammatory protection to the tympanic membrane. It accelerates the process of cellular proliferation and the matrix remodeling. It is an autologous material, so it is safe to use with no chance of disease transmission or undesirable tissue reaction. Since it augments in wound healing, it is widely used in various surgical and non-surgical procedures in many departments. The preparation and extraction of the platelet-rich fibrin was easy and quick, which is cheaper to produce and can be easily manipulated during surgical procedures [11].

Although its benefits have already been studied and established in various other surgical procedures, its effect on graft uptake and hearing improvement has yet to be explored. Since it has a strong elastic fibre rich in growth factors, it will act as an ideal agent for the treatment of the tympanic membrane perforation. So, this study has been planned to evaluate the effect of the platelet-rich fibrin application among patients undergoing myringoplasty procedures.

Study Design

Prospective Randomized controlled trial

Study Setting

Department of ENT, Sri Venkateshwaraa Medical College Hospital and Research Centre, Ariyur, Puducherry.

Study Population

All patients who meet the eligibility criteria for the ENT Outpatient Department of Sri Venkateshwaraa Medical College Hospital and Research Centre, Ariyur, Puducherry, were included in the study.

Sampling Method

Double-blinded method

Sampling Technique

Sealed Envelope Technique.

Sample Size

With a 95% confidence interval, 80% Power of the study with an average success rate of 89% and Precision of 7% [4], the expected sample size is 76.

The sample size was calculated using Open Epi software version 3.01.

Duration of the Study

18 Months from October 2022 to March 2024.

Participant timeline

Time for enrolment	5 mins
History & Examination	5 mins
Otoendoscopy	5 mins
Tympanometry	10 mins
Impedance	10 mins
Diagnostic Nasal Endoscopy	10 mins
Surgery duration	2 hour
Total	**2 hours 45 minutes**

Table 1: Details of the participant timeline

Eligibility Criteria Inclusion Criteria
All the patients with the following criteria were included in this study.
1. Patients of both sexes of age more than 18 years and less than 50 years.
2. Patients diagnosed with COM- safe type, inactive stage for at least three months.
3. No active foci of infections in the nose and Paranasal sinuses.
4. The patient should have a normal eustachian tube function.
5. Adequate cochlear reserve.
6. Patients with traumatic tympanic membrane perforation.

Exclusion Criteria
The patients with the following conditions or situations were excluded from this study.
1. Patients with active ear infections,
2. Patients who are diagnosed with COM – unsafe type disease (Attico-Antral Disease),
3. Patients diagnosed with chronic granulomatous diseases of the ear
4. Patients with tumours of the ear.
5. Pregnant women.
6. Patients with bleeding disorders.
7. Unstable hemodynamic disorders.
8. Co-morbidities like uncontrolled Diabetes mellitus with random blood sugar values of >200mg/dl with oral or injectable antidiabetic drugs.
9. Patient with uncontrolled hypertension
10. Any history of ear surgery.
11. When the other ear is dead or unsuitable for hearing aid rehabilitation.
12. Patients who are not willing to have surgery
13. Patients who are not fitted during the anaesthesia check-up.

Interventions
After the initial assessment, the patients were diagnosed and categorised into one of the two groups. The patients in both groups underwent myringoplasty by a post-aural approach using the temporalis fascia graft. The grafts in both groups were placed via the underlay technique. In Group A, or the study group, the fibrin-rich fibrin-soaked gel foam was kept in the external auditory canal after placing the graft by underlay technique. In Group B or the control group, plain gel foam soaked in saline was kept in the external auditory canal after the graft placement.
Following the surgery, the patient was followed up for six months and evaluated at the first, third and sixth months during the follow-up visit. During the follow-up visits, the patient was evaluated for graft uptake by otological examination, improvement in hearing by doing a pure tone audiogram, amount of hearing gain in the pure tone audiogram, and evaluation of graft uptake. The patient has also been evaluated for postoperative infection and ear pain.

Outcomes
Pre- and postoperative improvement of hearing between the two intervention groups has been evaluated between the two intervention groups. The two intervention groups also compared various parameters such as graft uptake, development of postoperative infection, and development of ear pain and any residual perforation.
Data Collection Methods Clinical Proforma (Annexure – A)
Confidentiality of Data
1. Details of the study protocol will be explained to the subjects.
2. Informed consent (Oral and written consent) will be obtained
3. Confidentiality will be ensured.

Statistical analysis:
Data will be entered in a Microsoft Excel sheet. The SPSS app version did analysis; descriptive statistics were obtained for quantitative variables like age and duration of surgery.
Continuous variables like intraoperative blood loss, pain scale, and No. The number of days of hospital stay

was expressed in mean and standard deviation.
Categorical variables like sex and chief complaints were expressed in frequency and percentage. To compare the significant mean difference between the group's student-t tests were used. The chi-square test will be used to test the association between categorical groups. A p-value <0.05 will be considered as statistically significant.

Statistical methods:
All the data will be entered in Excel and analysed using IBM SPSS version 23. The various tests used in the study are in Table 2.

Objectives	Statistical Test
Objective 1: To compare the graft uptake in patients undergoing myringoplasty with and without Platelet-rich fibrin.	Chi-Square test
Objective 2: To compare the hearing outcomes in patients who underwent myringoplasty with and without Platelet-rich fibrin.	unpaired t-test

Table 2: Various objectives and statistics involved in the study.

Operational Definition:
Chronic Otitis media
It is defined as chronic inflammation of the middle ear and mastoid mucosa, with recurrent discharge (at least two weeks) through a chronic tympanic membrane perforation.

Myringoplasty
Myringoplasty can be defined as the surgical repair of the tympanic membrane. In most cases, the damage will likely be a persisting tympanic membrane perforation.

Graft Uptake
Graft uptake may be defined as healing the perforated eardrum to form the healed eardrum called the neotympanum without any residual perforation.

METHODOLOGY
The study was planned to assess the Graft uptake and Hearing outcomes in myringoplasty by conventional temporalis fascia underlay technique with and without the addition of platelet – Rich Fibrin. The study was conducted over 18 months from the approval date of the Scientific Research Committee and the Institute Ethics Committee of the Sri Venkateshwaraa Medical College and Research Institute in the Department of ENT of SVMCH & RC.
The patients attending the ENT Outpatient Department of Sri Venkateshwaraa Medical College Hospital and Research Centre, Ariyur, with signs and symptoms of Chronis Suppurative Otitis Media, safe type with inactive disease, as per inclusion criteria, were included in the study. After the informed written consent, the participants were subjected to proper history taking, clinical examination, and otoscopic examination, followed by Examination Under Microscope. Then, the patient was subjected to the X-ray mastoid law's view for both years.
The case proforma consists of 6 parts. The first part has basic demographic details of the patients like Name, age, sex, education, occupation, socio-economic status (modified BG Prasad Scale), and addresses with contact numbers. The second part consisted of the history of presenting complaints such as ear pain, ear discharge, hard of hearing, tinnitus and vertigo. They were followed by other relevant histories pertaining to the nose and throat, such as nasal block, nasal discharge, snoring, mouth breathing, allergy, sneezing, running nose, and headache. Then, the patient was evaluated for other co-morbidities such as diabetes, hypertension, tuberculosis, asthma and any other chronic illness. Then, the patient was enquired about the treatment history and family history for any allergy in the family. The third part of the proforma consisted of the general physical examination; the patient was evaluated for consciousness, orientation, pulse rate and blood pressure monitoring.
The fourth part of the proforma consisted of the ENT examination; in this part, the patient was evaluated for the external auditory canal, the status of the tympanic membrane regarding the number of perforations, type of perforation, size of the perforation, margins and site of perforation and the status of the middle ear mucosa.

Then, the patient was subjected to various tuning fork tests such as Rinne's, Weber's, and absolute bone conduction tests.
The fifth part of the proforma consisted of pure tone audiometry findings; the patient was evaluated for the presence of hearing loss, degree of hearing loss, type of hearing loss whether it is a conductive, sensorineural or mixed hearing loss and the presence of the air-bone gap. Then, the sixth part consisted of a diagnostic nasal endoscopy, and the patient was evaluated based on the three-point scoring test as in Table 3.

Nasal endoscopy (3ET) 3-POINT SCORING TEST	none	mild	severe
1)Edema	0	1	2
2)Erythema	0	1	2
3)Exudate	0	1	2
4)Tubal tonsil	0	1(present, not involving lumen)	2(present, involving lumen)
Total			

Table 3: Three-point scoring test for the evaluation of the nasal cavity by DNE

Then, an x-ray of the mastoid for both sides, the law's view to assess the cellularity of the mastoid, was recorded on the proforma. Then, the seventh part of the proforma consisted of the postoperative complaints such as ear pain, ear discharge, hard of hearing, and otoendoscopy findings to evaluate the graft uptake and assessment of the pure tone audiometry. All these findings were recorded three times during the follow-up visits during the first, third and sixth months.

After getting informed and written consent, the patients were selected based on the inclusion and exclusion criteria and were subjected to an initial assessment regarding the demographic details. After that, the patients were enquired about were subjected to complete history taking regarding the ear complaints. The patient was enquired about the presence and duration of ear pain, ear discharge, hard of hearing, aural block, tinnitus and vertigo. They were enquired about nasal complaints such as nasal block, nasal discharge, sneezing, mouth breathing, snoring, and headache per the case proforma.

Then, the patients were subject to the proper ENT examination; in the examination of the external auditory canal, any discharge, swelling or was had been noted. Then, the status of the tympanic membrane has been evaluated. In the tympanic membrane, presence of perforation, number, type, size, site, and margins of the perforation were noted. The status of the middle ear mucosa has also been evaluated. The three-finger test has been assessed to see the presence of the mastoid infection.

Then, all the patients were subjected to a tuning fork examination. In the tuning fork test, they were subjected to Rinne's, Weber's, and absolute bone conduction tests. The tuning fork test was done to evaluate the presence of hearing loss and the degree and type of hearing loss. Then, the patient was subjected to a pure tone audiogram. There, the finding of the tuning fork was confirmed, and the degree and type of hearing were evaluated. The air-bone gap has been found—the assessed existing eustachian tube pathology with the help of the diagnostic nasal endoscopy. The nose was packed with adrenaline lignocaine cotton pledgets, and the nasal cavities were evaluated systematically. The Eustachian tube pathology was assessed with the help of three-way three-point scoring systems.

After the history, examination and investigation, all the patients were divided into Groups A and B, based on the simple random sampling technique through the computer- generated random numbers. The allocation was concealed with the help of the sequentially numbered sealed opaque envelopes. The envelope was handed over to the staff nurse in the operation theatre and sealed co. Sealed opened only at the time of the entry of the eligible participants in the operation theatre. The patient in Group A underwent myringoplasty with the platelet-rich fibrin-soaked gel foam. In contrast, in Group B, the patients underwent myringoplasty with the help of saline-soaked gel foam.

Since it is a double-blinded study, all the patients selected collected 10 ml of their own before the surgical

procedure to prevent the surgeon from getting biased. After collecting the sterile container just before the surgery, the blood sample was taken to the blood bank and centrifuged using a table model centrifuge machine at 2,700 rpm for 12 minutes, as in Figure 1.

Figure 1: Tabletop centrifuge machine

This centrifugation results in the formation of the three separate layers. The bottom layer with red blood cells, the middle layer with platelet-rich fibrin, and the above or the third layer with plasma, as shown in Figure 2.

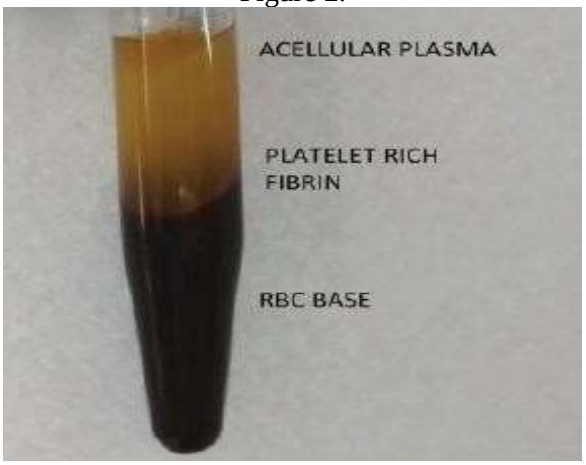

Figure 2: Post-centrifugated blood showing different layers of blood

The topmost plasma layer is deficient in platelets. The middle layer was separated with the help of the laminar flow hood under the aseptic precautions and was sent to the operation theatre under sterile conditions in a sealed container.

This sterile container was handed over to the operation theatre. During the procedure, based on the group on the sealed envelope, the scrub nurse will provide the platelet-rich fibrin-soaked gel foam or the saline-soaked gel foam to the surgeon operating on the table.

General Surgical Practices

Post-aural myringoplasty was performed on every patient in both groups. Under intravenous sedation; all the patients had surgery and underwent the underlay graft placing technique, applied mastoid bandages, and followed up for six months.

Myringoplasty Technique

Under intravenous sedation, after microscopic examination, four quadrant and postauricular infiltration were done with 2% lignocaine with adrenaline solution. Margins of the perforation in the tympanic membrane were freshened first. William Wilde's postauricular incision was made about 10 mm behind the postauricular grove and tissues in postauricular region were dissected. Temporalis fascia graft was identified and harvested. The external auditory canal's cartilaginous portion was then located. Tissues were pulled back after an incision was made on the external auditory canal at the bone cartilaginous junction. The tympanic membrane was then raised, the tympanic annulus was raised, and the skin from the bone canal was removed.

The middle ear was next examined to determine the ossicular state in terms of the middle ear mucosa's mobility, fixation, and inflammation. After maintaining gel foam in the middle ear, the underlay approach was used to preserve the dried temporalis facia graft behind the annulus. The skin flaps were repositioned.

At this stage, platelet-rich fibrin-soaked gel foam was kept over the graft and in the external auditory canal for group A patients. For Group B patients, saline-soaked gel foam was kept over the graft and in the external auditory canal. Then, the cotton ball was placed over the end of the external auditory canal, and a mastoid bandage was applied. The patient was shifted to the postoperative room. All the patients were discharged during the second postoperative day. All the patients were given oral antibiotics and topical antibiotic ear drops during the follow-up postoperative visits.

The patients were followed for six months. They were evaluated at the end of the first, third and sixth months for graft uptake, development of postoperative ear infection, and improvement in the threshold of hearing.

Rational for the follow-up visits

In the present study, the graft uptake begins only four weeks after the surgery and the hearing outcomes were assessed with the help of the pure tone audiometer after three months. By the end of the 6^{th} month, a complete graft uptake will be seen. The study protocol is given below figure 3.

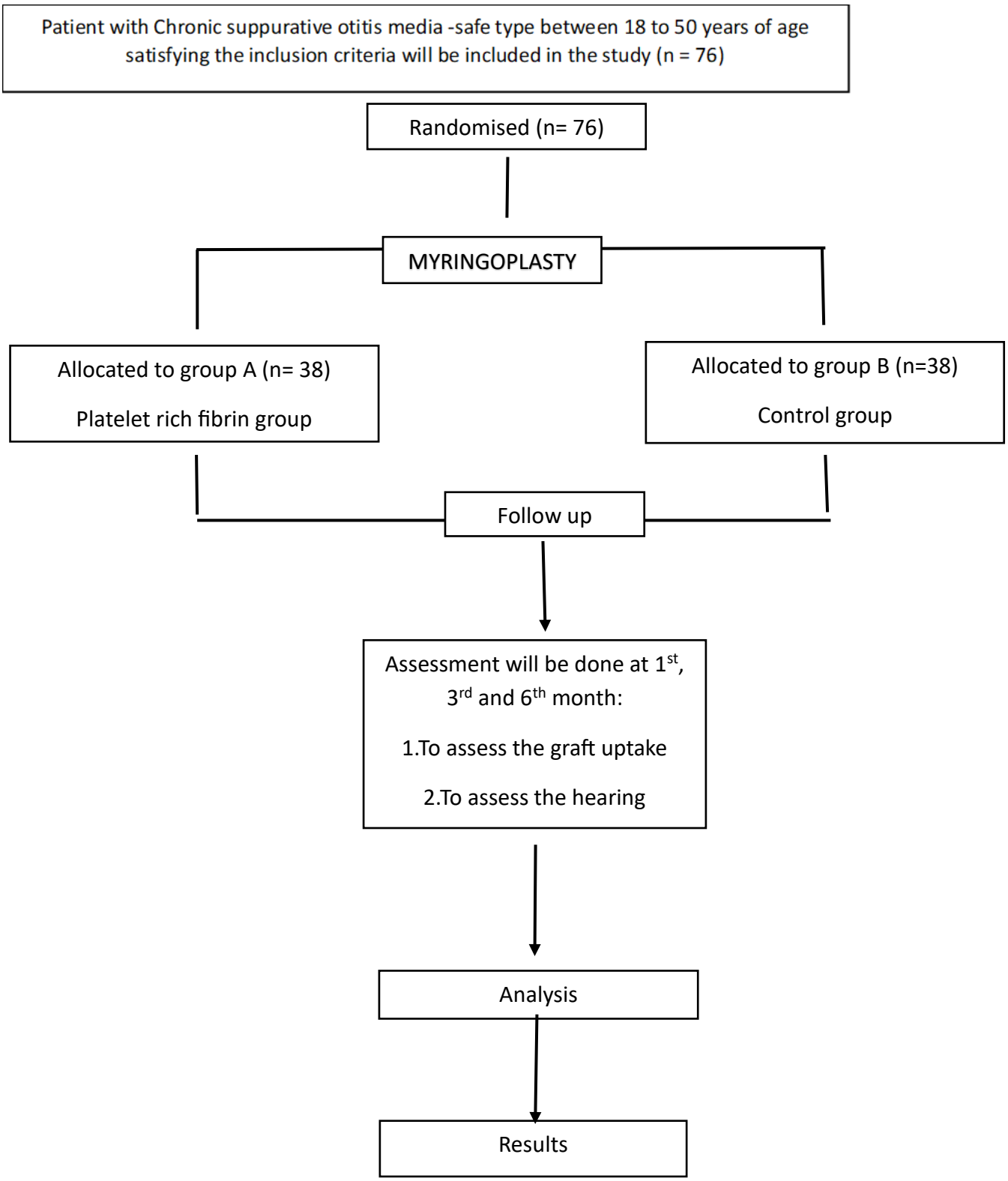

Figure 3 – Overall study protocol

RESULT

The study included 78 patients who were diagnosed with chronic otitis media mucosal type. The double-blind method's randomization methodology was used to split the entire patient population into two groups. Table 4 shows the study participants' age distribution. Of the patients, 19.8% were between the ages of 36 and 40, while nearly 25% were between the ages of 31 and 35. Approximately 18% of the patients were between the

ages of 26 and 30 and 41 and 45, respectively. Just 7% of the patients were between the ages of 46 and 50, while 10% of the patients were between the ages of 18 and 25.

Age	Number	Percentage (%)
18-25	8	10.5
26-30	14	18.4
31-35	19	25.0
36-40	15	19.8
41-45	14	18.4
46-50	6	7.9

Table 4: Age Wise distribution of the study population (n=76)

The patient's age distribution by group is displayed in Table 5. About 13% of the patients in Group A were between the ages of 31 and 35, whereas 9% were between the ages of 26 and 30; 36 and 40; and 41 and 45. Just 3% of the patients were in the 46–50 age range, while 5% of the patients were in the 18–25 age range. In group B, most of the patients in the age group 31 to 35 were 11% and 10% of them were 36 to 40, followed by 9% in 26 to 30 years and 41 to 45. In group A 5% were in the younger age group and 3% in the older age group.

	Group A	Group B
Age	N(%)	N(%)
18-25	4 (5.3)	4 (5.3)
26-30	7 (9.2)	7 (9.2)
31-35	10 (13.2)	9 (11.8)
36-40	7 (9.2)	8 (10.6)
41-45	7 (9.2)	7 (9.2)
46-50	3 (3.9)	3 (3.9)

Table 5: Age-wise distribution of the study population based on the intervention group (n=76)

Regarding the distribution of the gender between the two groups; total of 42 males and 34 females participated in the study. Among the distribution of the participants between the interventional groups, in group A, about 52.6% of the males and 47.4% of the females participated in the study, as in Table 6. In group B, about 57.9% of the males and 42.1% of the females participated with the diagnosis of Chronic Otitis Media Mucosal type. The mean age of the participants in Group A was found to be 33.8$\pm$8.6 years, and in Group B, it was found to be 30.12$\pm$95 years.

	Total Number	Group A	Group B
Age		N(%)	N(%)
Male	42	20 (52.6)	22 (57.9)
Female	34	18 (47.4)	16 (42.1)
Total	76	38(100)	38(100)

Table 6: Gender distribution of the study participants

Regarding the distribution of the nature of the disease, whether the COM is present in one ear or both ears. In Group A, about 92.1% of the patients only had a perforation in one ear. That is, the patients had unilateral COM, and 7.9% of the patients had bilateral COM. In the Group B patients, about 78.9% had unilateral COM, and about 21.1% had perforation in both ears, as in Table 7.

	Total Number	Unilateral COM	Bilateral COM
Group		N(%)	N(%)
Group A	38	35 (92.1)	3 (7.9)
Group B	38	30 (78.9)	8 (21.1)

Table 7: Distribution of the participants based on the nature of the disease

Based on the size of the tympanic membrane perforation between the two groups, in group A about

50% had large central perforation, followed by 28.9% of the participants had medium size perforation, about 13.2% had small perforation, and only 7.9% of the participants had subtotal perforation. In group B about one third of the participants, 36.8%, have large perforation, 26.3% had medium perforation, 21.1% had a subtotal perforation, and 15.8% of the study participants had small perforations, as in Table 8.

Size of Perforation	Group A	Group B
	N(%)	N(%)
Small	5 (13.2)	6 (15.8)
Medium	11 (28.9)	10 (26.3)
Large	19 (50.0)	14 (36.8)
Subtotal	3 (7.9)	8 (21.1)

Table 8: Distribution of the Study participants based on the size of TM perforation

In evaluating the degree of hearing loss among the two groups, the degree of hearing loss was classified based on the American Speech-Language-Hearing Association (ASHA). In Group A, , 71.1% majority of the patients had moderate degree of hearing loss, followed by 13.2% of the participants, who had mild and moderate to severe degree of hearing loss, and only 2.6% of the participants had severe degree of hearing loss. In group B, similar to group A, 73.7% majority of the study participants had a moderate degree of hearing loss, followed by 15.8%, who had a mild degree of hearing loss, moderate to severe degree of hearing loss were found in 7.9% and only 2.6%, only one patient had severe degree of hearing loss as per ASHA classification as in Table 9. It is noted that none of the patients who participated in this study had profound hearing loss.

Degree of Hearing Loss	Group A	Group B
	N(%)	N(%)
Mild	5 (13.2)	6 (15.8)
Moderate	27 (71.1)	28 (73.7)
Moderate to Severe	5 (13.2)	3 (7.9)
Severe	1 (2.6)	1 (2.6)

Table 9: Distribution of the Study participants based on Degree of Hearing Loss

The distribution of patients was based on the presentation of the symptoms. 52.6% of the patients in Group A experienced ear discharge, while the majority of patients (84.2%) had a history of hard hearing. According to Table 10, only 21.1% of the patients had a history of tinnitus, while 44.7% of the patients had a history of ear pain. Additionally, 78.9% of Group B's members were hard of hearing, and 63.2% of them experienced ear discharge. 13% of the patients had tinnitus, and 52% had a history of ear pain. None of the patients experienced ear discharge at the time of surgery, despite the fact that half of the patients in each group had ear discharge in the past. The ear discharge's most recent occurrence occurred at least six weeks ago.

	Group A		Group B	
Symptoms	n	%	n	%
Hard of Hearing	32	84.2	30	78.9
Ear Discharge	20	52.6	24	63.2
Ear Pain	17	44.7	20	52.6
Tinnitus	8	21.1	5	13.2
Tinnitus	8	21.1	5	13.2

Table 10: Distribution of patients based on Presentation of Symptoms

The study also evaluates the presence of the intra-operative middle ear inflammation between the study groups; in Group A, 7.9% of the patient had middle ear inflammation on raising the tympanomeatal flap and 92.1% of the patient didn't have any middle ear inflammation during the surgery. In group B, about 5.3% of the patients had middle ear inflammation during the surgery and 94.7% didn't have any middle ear inflammation, as in Table 11.

Intra operative middleear inflammation	Group A		Group B	
	n	%	n	%
Present	3	7.9	2	5.3
Absent	35	92.1	36	94.7
Total	38	100	38	100

Table 11: Distribution of the patients based on the presence of intraoperative middle ear inflammation

Graft uptake was evaluated thrice in the post-operative period, during the first, third and sixth months of post-operative days. On the first post-operative month, in Group A, the graft uptake was seen among 94.7% and graft failure was noted in two patients, 5.3% of the patients. In group B, graft uptake was noted in 89.5% and failure was seen among 10.5% as in Table 12. This difference does not show any statistically significant difference with the p- value of 0.67, analysis was done by the Fisher exact test.

Graft Uptake	Group A		Group B		P value
	n	%	n	%	
Success	36	94.7	34	89.5	
Failure	2	5.3	4	10.5	
Total	38	100	38	100	0.6745

Table 12: Distribution of patients based on the Uptake of Graft at one month

The evaluation of the graft uptake at three months in group A was in 94.7% of the patients and 5.3% showed failure as in the one-month evaluation. However, in group B, graft uptake was noted among 84.2% of the patients, and failure was seen among 15.8% of the patients, which is more than one-month follow-up, as in Table 13.

Graft Uptake	Group A		Group B		P value
	n	%	n	%	
Success	36	94.7	32	84.2	
Failure	2	5.3	6	15.8	
Total	38	100	38	100	
Total	38	100	38	100	0.2619

Table 13: Distribution of patients based on the Uptake of Graft at three-month

The evaluation of the graft uptake was 94.7% on the sixth post-operative month in Group A and failure was 5.3% of the patients. In Group B, graft uptake was seen among 84.2% and failure was noted among 15.8% of patients, as in Table 14. This was consistent with the third month result.

Graft Uptake	Group A		Group B		P value
	n	%	n	%	
Success	36	94.7	0.2619	84.2	
Failure	2	5.3	6	15.8	
Total	38	100	38	100	0.2619

Table 14: Distribution of patients based on the Uptake of Graft at six-month

The development of the complications between the two groups in the post- operative period, in Group A, about 7.9% of the patients developed ear discharge during the post-operative period and 92.1% had no discharge during the post-operative period. In group B, only 78.9% of the patients had no complications during the post-operative period, about 15.8% developed ear discharge, and 5.3% developed otomycosis in the operated ear during the post-operative period, as in Table 15.

Complications	Group A		Group B	
	n	%	n	%
None	35	92.1	30	78.9
Ear Discharge	3	7.9	6	15.8
Otomycosis	0	0	2	5.3

Table 15: Development of Complications in the post-operative period between the two groups

On evaluating the pre-operative and post-operative hearing threshold between the groups, it was found that in Group A, the pre-operative pure tone audiometry was found to be 44.56 dB and the post-operative pure tone audiometric hearing threshold was found to be 23.44 dB. This value shows a significant difference between the two threshold values. In Group B, the pre-operative pure tone audiometry was found to be 45.88 dB, and the post-operative pure tone audiometric hearing threshold was found to be 26.45 dB with a statistically significant difference between the two with the p-value of less than 0.01 as in Table 16

Pure tone Audiometry	Preoperative PTA		Postoperative PTA		p-value
	Mean	Standard Deviation	Mean	Standard Deviation	
Group A	44.56	5.32	23.44	4.12	<0.01
Group B	45.88	4.61	26.45	3.43	<0.01

Table 16: Comparison of hearing improvement in patients between two groups.

On comparing the hearing gain between the two groups, it was noted that the mean hearing gain in Group A was found to be 21.12 decibels and in Group B it was found to be 19.43 decibels. There is a statistically significant difference between the two mean values less than 0.01, as in Table 17.

Hearing Gain	Mean	Standard Deviation	p-value
Group A	21.12	1.2	<0.01
Group B	19.43	1.18	

Table 17: Comparison of hearing gain between the two groups

DISCUSSION:

The distribution of the study population based on the size of the tympanic membrane perforation in Group A, about 50 percentage of the patients had large central perforation, followed by 28.9% of the patients had medium-sized perforation and 13.2% had small perforation. And only 7.9% of the patients had subtotal perforation. In Group B similar to Group A, 36.8% of the patients had large central perforation, followed by medium-sized perforation among 26.3% of the study population. 21.1% had subtotal perforation, and 15.8% of the patients had small perforation. The Shetty *et al.*, [12] study shows that in the study group, most % of the patients 45.16% had medium-sized perforations, followed by 29.03% of the patients had large central perforations, and 25.81% had small perforations. In the control group, 61.29% of the patients had medium perforation, 81% of the patients had small perforation, and large perforation was seen among 12.9% of the patients in group B. The study by Nair *et al.*,., showed that about 18 patients had subtotal perforation followed by 14 patients who had medium perforation. Large central perforation was noted among ten patients, and small central perforation in one patient. In Group B, about 28 patients had subtotal central perforation, while six had medium and large central perforation and two patients had small perforations. Awady *et al.*, [13] compared the perforation size between the study and control groups. In the study group, 40% of the patients had small perforation, followed by 35% with medium perforation, and large perforation was seen among 25% of the patients. In the control group, 40% of the patients had medium perforation, followed by large perforation seen among 35%. 25% of the patients had small perforations.

In the present study, regarding the distribution of the patients based on the presentation of the symptoms, in Group A, 84.2% of the patients presented with hard of hearing, followed by 52.6% of the patients had a history of ear discharge. And ear pain was present in 44.7% of the patients. Tinnitus was present in only 21.1% of the patients in Group A. In Group B, like in other groups, 78.9% of the patients had a history of hard of hearing at the time of presentation to the hospital, 63.2% had a history of ear discharge, and 52.6% had an ear pain history.

And tinnitus was present only in 13.2% of the patients. The ear discharge was present at least six weeks before the presentation to the hospital, and no patient had an ear discharge. Similar results were also seen in the study by Vignesh *et al.,*. [14] In the study group, nearly half of the patients, 55.3%, present with hard of hearing. The ear discharge was present in 26.3% in the study group and 18.4% in the control group. It was noted that 47.4% of the patients in the study group had a history of earache, and 28.9% had an earache in the control group. And it is surprising that 55.3% of tinnitus in the study group and 47.5% in the control group.

The present study also evaluates the presence of middle ear inflammation during the intraoperative period after the elevation of the tympanometry flap. In Group A, about 7.9% of the patients had middle ear inflammation and was absent in 92.1% of the patients. In Group B, about 5.3% of the patients had middle ear inflammation, and 94.7% didn't have any middle ear inflammation during the intraoperative time. The study by Nair *et al.,* [4] also evaluates the presence of intraoperative middle ear inflammation. Their study found middle ear inflammation in the study group was found in 5 patients and absent in 38 patients. In the control group, middle ear inflammation was found in 4 patients, and 38 patients didn't have middle ear inflammation during the surgery. The study by Riaz *et al.,* [15] also evaluates the presence of middle ear mucosa congestion during the surgery. The study found that in the case group, all patients had normal, healthy middle ear mucosa. About 12% of patients in the control group had congested middle ear mucosa, and 88% had normal, healthy middle ear mucosa.

Regarding the graft uptake at the 1st follow-up post-operative month, this study showed that in Group A, there is a graft uptake of 94.7% and a failure rate of 5.3%. In group B, the graft uptake rate was found to be 89.5%, and the failure rate was found to be 10.5%. There was no statistically significant difference with the p-value of 0.6745, similar study was noted in the study by Shetty *et al.,* [12], wherein the follow-up results on the first post-operative month, in group A, the graft uptake was seen among 96.6% of the patients, and failure was seen among 3.3% (1 patient). And in Group B (Control Group), graft uptake was noted in 96.6% of the patients. Failure was seen in 3.3% of the patients in Group A. Similar results were also noted in the study by Nair *et al.,* [4], where the graft uptake in the study group was 97.7%, and failure was noted in 2.3% (one patient). In control, the graft uptake was found to be only 81%, and the failure rate was found to be 19%. The study by Vignesh *et al.,* [14] showed that graft uptake in the study group was higher at 97.4% than in the control group, with a graft uptake rate of 89.5% of the patients. The study by Awady *et al.,* [13] also showed similar results to ours, with graft uptake higher in the PRF-used group at 95% than in the non-PRF-used group with an uptake rate of 70% only.

Regarding graft uptake in the third post-operative month, in Group A, the graft uptake was found to be 94.7%, and the failure rate was found to be 5.3%. However, in Group B, the graft uptake rate was found to be 84.2%, and the failure rate was found to be 15.8%. The failure rate in Group B is increased from the first post-operative month to the third post-operative month. The difference shows no statistically significant difference with the p-value of 0.2619. but the graft uptake was superior with the Platelet-rich fibrin group compared with the other group in this study. The study by Shetty *et al.,* [12] also showed similar results to our study, where the graft uptake rate in the study group was found to be 96.6%, which is consistent with the first-month follow-up rate. The failure rate was found to be 3.3%. In Group B, the graft uptake was seen among 93.3%, which was decreased from the previous first-month graft uptake, which was found to be 96.6%. The failure rate in the control group was found to be 6.6%. And the results also didn't show any statistically significant difference with the p-value of 1. But like in our study, the graft uptake was superior with the PRF group than with the control group.

The Vignesh *et al.,* [14] Study also showed similar results to our study. The graft uptake was 97.4% in the study group and 84.2% in the control group. It was noted that the graft uptake remained static in the study group using platelet-rich fibrin, but it was decreased in the control group compared with the 1st-month follow-up results. The graft uptake decreased from 89.5% to 84.2% in the third follow-up month. The results of this study are also consistent with the earlier study which showed the three-month graft uptake was 95% in the study group using PRF and 85% in the control group. However, the studies by El-Anwar *et al*., and Fawzy *et al.,* showed a lower success rate of 84% regarding graft uptake than our present study [16,17]. The increased graft failure rate in their studies was due to the recurrent upper respiratory tract infection, development of eustachian tube dysfunction, poor patient compliance regarding the post-operative advice and follow-up, as well as the general hygiene of the patient during the post-operative period.

In the present study, the graft uptake was evaluated in the sixth post-operative month. In Group A, graft uptake was seen in 94.7%, and failure was seen among 5.3%, consistent with the first and third-month post-operative

follow-up. However, in Group B, the graft uptake was 84.2%, and the failure rate was 15.8%. This graft uptake rate is consistent with the third-month follow-up evaluation but lower than the first-month follow-up evaluation. And this difference doesn't show any statistically significant difference with the p-value of 0.2619. but the p-value seemed to be decreased than the first-month p-value. Similar to this study, Mekhemar *et al.,* [5] regarding the post-operative tympanic membrane status in the 6th post-operative month. The study shows in the study group, the graft uptake was found to be 90%, and failure was found to be only 10%. However, in the control group, it is surprising that the graft uptake was only 40%, and the failure rate was 60%. The increased graft uptake in the patients in the platelet-rich fibrin group was due to the increased amount of platelets and other growth factors in the concentrate.

The platelet which is present in the platelet-rich fibrin is the main factor responsible for the biological activity of the PRF. The platelet-derived protein molecules present in the platelet are involved in the signalling of the wound-healing cascade. Apart from the platelets, other growth factors such as transforming growth factor β (TGF-β), platelet-derived growth factor (PDGF), insulin-like growth factor 1 (IGF1), vascular endothelial growth factor (VEGF), and epidermal growth factor (EGF) and cytokines such as interleukin (IL)-1β, IL-6, IL-4, and tumour necrosis factor (TNF)-α are also present in the PRF which are responsible for the increased graft uptake in the patients than the control group.

Regarding the distribution of the patients about the development of the post-operative complications between the two groups, it was known that the rate of development of post-operative complications was lower in the PRF group (Group A) compared with Group B. In Group A, about 7.9% of the patients developed ear discharge during the post-operative period. About 92.1% of the patients had no complications in the post-operative period. However, in Group B, 15.8% of the patients developed ear discharge during the post-operative period and about 5.3% developed otomycosis. And only 78.9% of the patients didn't develop any complications during the post-operative period. Similar results were also seen in the study by Riaz *et al.,* [15], where only 8% of the patients developed a post-operative infection in the PRF group. At the same time, in the control group, about 32% of the patients developed post-operative complications.

The Study by Fawzy *et al.,* [18] also showed a decreased incidence of post-operative infection in the patients undergoing tympanoplasty using platelet-rich fibrin. This decreased incidence of post-operative infection is due to the fact that in the platelet-rich fibrin, there is an increased concentration of platelets, white blood cells and other cytokines, which have a role against the infection with their bactericidal action. This causes a decreased infection rate in the platelet-rich fibrin group compared to the other groups.

The present study also evaluated the hearing improvement in the patients before and after the procedure. The hearing assessment was done with the help of the hearing threshold, which was measured in pure tone audiometry. The mean pure tone audiometry values were compared in the pre-operative and post-operative periods to look for significant improvement in hearing between the groups. In group A (PRF group), the mean hearing threshold was 44.56 decibels, and the post-operative hearing threshold was 23.44 decibels. And this hearing threshold difference is statistically significant with a p-value of less than 0.01 and in group B, the pre-operative mean hearing threshold was found to be 45.88 decibels and the post-operative hearing threshold was found to be 26.45 decibels. These values also show a statistically significant difference with a p-value of less than 0.01.

The study by Shetty *et al., [*12] also showed similar results to this study. The pre-operative pure tone audiometry in group A was found to be 37.58 decibels; in the post-operative PTA, it was between 21.258 decibels. In Group B, the control group, the pre-operative PTA was found to be 38.803 decibels, and the post-operative PTA hearing threshold was 23.339 decibels.

The study by Turhal *et al., [19*] also compared the pre-operative and post-operative pure tone audiometric threshold between the groups. In the study in the PRF group, the pre-operative air conduction hearing threshold mean was found to be 35.53 decibels, and the post-operative hearing threshold was found to be 26.33 decibels with a statistically significant difference with a p-value of less than 0.01. In the control group, the pre-operative hearing threshold was found to be 39.16 decibels, and the post-operative hearing threshold was found to be 28.41 decibels. And there is a statistically significant difference between the pre-operative and the post-operative PTA hearing threshold. Similar results were also noted in the study by Vignesh *et al., [14].*

Study by Vignesh compares the pre-operative hearing threshold with the post-operative hearing threshold at the one-month and three-month follow-up [14]. In the PRF group, the pre-operative hearing threshold was found to be 33.63 decibels, the post-operative hearing threshold at one month was found to be 27.71 decibels, and the third month was found to be 25.61 decibels. In the control group, the pre-operative hearing threshold

was found to be 33 decibels, the post-operative hearing threshold at one month was found to be 28.7 decibels, and the third month was noted to be 28.47 decibels. The PRF group shows more hearing improvement compared to the control group. A similar study by Sharma *et al.,* [10] among 100 patients also showed significant improvement in the air-bone gap at the speech frequencies. These results show that more hearing gain was seen in the platelet-rich fibrin group than in the other group. These could be due to the increased hearing nature of the tympanic membrane produced by the reaction of the PRF.

This present study also compared the hearing gain in both groups. In Group A, the mean hearing gain between the pre and post-operative pure tone audiometry was 21.12 decibels; in Group B, the mean hearing gain was 19.43 decibels. There is a statistically significant difference between the two values with a p-value of less than 0.01. the study by Shetty *et al.,* [12] also showed similar results to the present study. The Shetty study shows the mean hearing gain in the study group was found to be 16.31 decibels, and in Group B, it was found to be 15.481 decibels. The Shetty's study didn't show any significant difference between the hearing gain in both groups. A similar study by Anwar *et al.,* [20] among seventy patients showed an audiological improvement of more than ten decibels in 88.6% of their cases and only 77% in their control groups.

The present study shows the increased graft uptake rate and improved hearing outcomes in the PRF-aided myringoplasty than the other group, and it has a lesser post-operative infection rate. And the production of platelet-rich fibrin is simple, safe, cost-effective and readily available. The study concluded that myringoplasty with platelet-rich fibrin has a success rate, and it can be used as an alternative to the plain or saline-soaked gel foam sponges during the surgery.

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