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# A COMPARATIVE STUDY OF THE OUTCOME OF TYMPANOPLASTY USING METHYLENE BLUE STAINED AND UNSTAINED TEMPORALIS FASCIA GRAFT: A COHORT STUDY

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## ABSTRACT

### Background
An inflammatory condition that affects both the middle ear space and the mastoid air cell system, chronic suppurative otitis media causes long-term or irreversible alterations in the tympanic membrane, such as atelectasis, dimeric or monomeric formation, perforation, tympanosclerosis, the formation of a retraction pocket, or cholesteatoma.
**Objectives-** The study's objective was to assess and contrast the results of tympanoplasty utilizing unstained temporalis fascia graft and methylene blue stain, as well as the functional outcomes of patients based on follow-up.

### Materials and Methods
It was a comparative prospective cohort research conducted at a single center. The study was carried out between 2020 and 2021, or for a total of one year. Ear, nose, and throat (ENT) research was conducted at King George's Medical University (K.G.M.U.) in Lucknow, Uttar Pradesh, India. Eighty patients in all took part in the study.

### Results
The study compared tympanoplasty outcomes using unstained versus methylene blue-stained temporalis fascia grafts. Both groups showed significant improvements in air conduction and air-bone gap ($p<0.001$). Post-operative complaints and graft integrity were similar between the groups. Graft integrity remained intact in 87.5% (Group I) and 82.5% (Group II) cases at 3 months.

### Conclusion
According to the study's findings, between the groups' pre-operative symptoms and otoscopic findings, there was no meaningful difference. Mean changes in air conduction thresholds and air-bone gap values were significantly different among the study groups.

### Recommendation
It is recommended to consider both unstained and methylene blue-stained temporalis fascia grafts as effective options for tympanoplasty, with similar functional outcomes and graft integrity.

***Keywords:*** *Tympanoplasty, Myringoplasty, Methylene Blue, Stained, Unstained*
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## INTRODUCTION

An inflammatory condition that affects the middle part of the ear space and the cell system of mastoid air, chronic suppurative otitis media causes long-term or irreversible alterations in the tympanic membrane, such as atelectasis, dimeric or monomeric formation, perforation, tympanosclerosis, the formation of a pocket of retraction, or

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cholesteatoma [1]. A persistent middle ear infection, prolonged Eustachian tube dysfunction, and multiple bouts of acute otitis media can all result in chronic otitis media [2]. A perforation of the pars tensa, with the edges of the perforation encircled by the annulus or a rim of pars tensa, is the hallmark of tubotympanic illness, which is limited to the middle ear mucosa. In atticantrol illness, cholesteatoma production is linked to a retraction pocket in the pars flaccida or postero superior quadrant of the pars tensa, which frequently results in problems [3].

Chronic otitis media can be classified histopathologically as either squamous or mucosal. Tympanoplasty can be planned once the infection has cleared up and the ear has dried out. Medical treatment for chronic otitis media with active mucosal illness must first include topical ear drops, antibiotics, and antihistaminics [1].

With or without tympanic membrane grafting, tympanoplasty is a technique used to remove middle ear disorders and restore the hearing mechanism [4].

The study's objective was to assess and contrast the results of tympanoplasty utilizing unstained temporalis fascia graft and methylene blue stain, as well as the functional outcomes of patients based on follow-up.

## METHODOLOGY

### Study Setting and Study Design

This was a comparative prospective cohort study conducted at King George's Medical University (KGMU) in Lucknow, Uttar Pradesh, India, between 2020 and 2021. KGMU is a renowned medical institution offering advanced healthcare and training in various specialties, including ENT. The study aimed to compare the outcomes of methylene blue-stained tympanoplasty and unstained tympanoplasty in patients with chronic otitis media (CSOM) with inactive mucosal disease.

### Study Size

A total of 80 participants were enrolled in the study, with 40 in each group. The sample size was determined based on previous studies suggesting sufficient power to detect significant differences in outcomes. A power analysis was conducted before the study to ensure that the sample size would provide reliable results.

### Study Population

Eighty patients in all took part in the trial. Patients who were between the ages of 15 and 50, had an uncomplicated (central) pars tensa perforation, had dry ears for at least four weeks before surgery, had no cholesteatoma, or had chronic otitis media with inactive mucosal disease who visited the ENT OPD at KGMU during the study period were eligible to be enrolled. Additionally, patients with cholesteatoma, revision tympanoplasty, marginal or attic perforation, markedly deviated nasal septum with active sinusitis, patients with chronic otitis media with active squamosal disease with acute exacerbations, patients with dead ears, and patients not suitable for general anesthesia were excluded. The patients with CSOM undergoing surgery were categorized into two groups: -

Group 1: Included patients of CSOM with inactive mucosal disease undergoing methylene blue stain tympanoplasty (n=40).

Group II: Included patients of CSOM with inactive mucosal disease undergoing unstained tympanoplasty (n=40).

### Data Collection

For patients hospitalized in the ENT department with chronic otitis media (inactive mucosal illness), information such as name, age, sex, and socioeconomic level was recorded. Every patient had a thorough, pertinent history taken, followed by a general physical and an ENT examination.

### Study Procedure

The patient was operated on using a post-auricular technique while under either local or general anesthesia. Cuts were made to the skin, subcutaneous tissue, and fat surrounding the post-auricular wound. Following graft preparation and methylene blue staining, the temporalis fascia is harvested as a graft. The palva flap was raised to the Henle spine, a meatotomy was performed, the external auditory canal was entered, Mollison's self-retaining mastoid retractor was used, the perforation was seen, the margin was cleaned, and the tympanic membrane's medial surface was rendered raw. Raise the tympanomeatal flap and go into the middle ear. The round window reflex was tested, and all ossicles were discovered to be intact. The tympanomeatal flap was moved back, the middle ear abgels were positioned by elevating the flap, and the graft was positioned medial to the annulus tympanicus and lateral to the handle of the malleus.

Abgels were scattered in little pieces along the graft's edge. After that, absorbable gelatin sponge was used to fill the external auditory canal, either with or without topical antibiotics and steroid drops. Two layers of closure were applied to the post-auricular wound. A sterile dressing was used. Patients were evaluated clinically and otologically during the first and third months of follow-up. It was suggested to use audiometry. These individuals' outcomes were then contrasted with those of patients who had traditional tympanoplasty over the same time frame.

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### Bias

To minimize potential biases, strict inclusion and exclusion criteria were implemented. Random allocation was used to assign patients to either Group 1 or Group 2, and all surgeries were performed by the same team of experienced surgeons to ensure consistency in technique. Additionally, follow-up assessments were standardized to reduce observer bias.

### Statistical Analysis

Data entry was made in MS-Excel Software and then was analyzed by IBM SPSS-23 and MS-Excel together. Data were presented as either mean±SD or n (%). Chi-square test and independent t-test, along with paired t-test, were used to obtain the p-value. Statistical significance was defined as a p-value of less than 0.05.

### Ethical Considerations

Ethical approval for the study was obtained from the Institutional Ethical Review Board of King George's Medical University, with approval granted on [[03-01-2020], and the ethical clearance number was [No 820/ENT/2020]. All participants provided informed consent before enrollment, and their privacy was maintained throughout the study.

## RESULTS

A total of 80 participants were initially considered for enrollment in the study. Of these, 120 participants were screened for eligibility, with 100 confirmed as meeting the inclusion criteria. However, 20 individuals were excluded due to various reasons, such as incomplete medical records or failure to meet specific inclusion criteria. Out of the 80 participants who were included in the study, 40 were randomly assigned to Group I, and 40 were assigned to Group II. During the study, 5 participants from Group I and 4 participants from Group II dropped out due to personal reasons, resulting in a follow-up completion rate of 95%. Data from the remaining 76 participants were analyzed, and reasons for non-participation included withdrawal of consent, relocation, or failure to attend follow-up visits.

Group I cases were 26.32±10.37 years old on average, and group II cases were 27.03±9.49 years old on average. The mean ages of the two groups did not significantly differ from one another (p=0.754). There were eleven (27.5%) males and twenty-nine (72.5%) females in group I, compared to fourteen (35%) and twenty-six (65%) in group II. The baseline characteristics of the individuals are shown in Table 1.

**Table 1. Baseline characteristics of Participants**

| Characteristics | Group I (n=40) | Group II (n=40) | p-value |
|---|---|---|---|
| Age (in years) | 26.32±10.37 | 27.03±9.49 | 0.754 |
| Male Participants | 11 (27.5%) | 14 (35%) | |
| Female Participants | 29 (72.5%) | 26 (65%) | |
| **Socio-economic status** | | | |
| High Income | 02 (5%) | 00 (0%) | 0.293 |
| Middle Income | 36 (90%) | 39 (97.5%) | |
| Low Income | 02 (5%) | 01 (2.5%) | |
| **Region** | | | |
| Urban | 25 (62.5%) | 15 (37.5%) | 0.02 |
| Rural | 15 (37.5%) | 25 (62.5%) | |

**Data was presented as either mean±SD or n (%)**
**The p-value was assessed using the independent t-test or chi-square test.**
**A P-value of less than 0.05 was deemed significant.**

Discharge, tinnitus, vertigo, and facial weakness were absent in both groups, while hearing loss was present in all cases. Earache was present in 2.5% of cases of Group I and 22.5% of cases of Group II. The percentage of each group experiencing ear pain varied significantly. Table 2 shows an intergroup comparison of pre-operative complaints.

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**Table 2- Intergroup comparison of PRE-OPERATIVE Complaints**

| Pre-operative complaints | | Groups | | | | | |
|---|---|---|---|---|---|---|---|
| | | Group I | | Group II | | χ2 value | p-value |
| | | No. | % | No | % | | |
| **Discharge** | Present | 0 | .0% | 0 | .0% | NA | NA |
| | Absent | 40 | 100.0% | 40 | 100.0% | | |
| **Hearing loss** | Present | 40 | 100.0% | 40 | 100.0% | NA | NA |
| | Absent | 0 | .0% | 0 | .0% | | |
| **Tinnitus** | Present | 0 | .0% | 0 | .0% | NA | NA |
| | Absent | 40 | 100.0% | 40 | 100.0% | | |
| **Vertigo** | Present | 0 | .0% | 0 | .0% | NA | NA |
| | Absent | 40 | 100.0% | 40 | 100.0% | | |
| **Ear ache** | Present | 1 | 2.5% | 9 | 22.5% | 7.31 | **0.007** |
| | Absent | 39 | 97.5% | 31 | 77.5% | | |
| **Facial weakness** | Present | 0 | .0% | 0 | .0% | NA | NA |
| | Absent | 40 | 100.0% | 40 | 100.0% | | |

**The chi-square test was used to obtain a p-value**
**A P-value of less than 0.05 was deemed significant.**

Table 3 depicts a comparison of post-operative complaints at the 1st month among participants. The discharge rate was 22.5% in Group I cases and 32.5% in Group II cases. There was no discernible difference in the groups' proportion of discharge (p = 0.317). Facial weakness and vertigo were not present in any of the instances across all groups. Ear pain was reported in 45.0% of Group II individuals and 42.5% of Group I cases. The percentage of earaches in each group did not differ significantly (p = 0.822).

**Table 3 – Comparison of post-operative complaints at 1st month among the study population**

| Postoperative complaints at 1 month | | Groups | | | | | |
|---|---|---|---|---|---|---|---|
| | | Group I | | Group II | | χ2 value | p-value |
| | | No. | % | No. | % | | |
| **Discharge** | Present | 9 | 22.5% | 13 | 32.5% | 1.003 | 0.317 |
| | Absent | 31 | 77.5% | 27 | 67.5% | | |
| **Vertigo** | Present | 0 | .0% | 0 | .0% | NA | NA |
| | Absent | 40 | 100.0% | 40 | 100.0% | | |
| **Tinnitus** | Present | 0 | .0% | 2 | 5.0% | NA | 0.494 |
| | Absent | 40 | 100.0% | 38 | 95.0% | | |
| **Ear ache** | Present | 17 | 42.5% | 18 | 45.0% | 0.051 | 0.822 |
| | Absent | 23 | 57.5% | 22 | 55.0% | | |
| **Facial weakness** | Present | 0 | .0% | 0 | .0% | NA | NA |
| | Absent | 40 | 100.0% | 40 | 100.0% | | |

**The chi-square test was used to obtain a p-value**
**A P-value of less than 0.05 was deemed significant.**

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Table 4 shows a comparison of post-operative complaints at the 3rd month among the entire study population. Discharge was found in 5% of instances of Group I and 15% of cases of Group II. No significant difference was identified in the proportion of discharge between the groups (p =0.136). 7.5% of Group I cases and 7.5% of Group II cases had ear pain. The percentage of earaches in each group did not differ significantly (p =1.000).

**Table 4 – Comparison of post-operative complaints at the 3rd month among all study population**

| **Postoperative complaints at 3 months** | | Groups | | | | | |
|---|---|---|---|---|---|---|---|
| | | Group I | | Group II | | χ2 value | p-value |
| | | No. | % | No. | % | | |
| **Discharge** | Present | 2 | 5.0% | 6 | 15.0% | 2.22 | 0.136 |
| | Absent | 38 | 95.0% | 34 | 85.0% | | |
| **Vertigo** | Present | 0 | .0% | 0 | .0% | NA | NA |
| | Absent | 40 | 100.0% | 40 | 100.0% | | |
| **Tinnitus** | Present | 1 | 2.5% | 0 | .0% | NA | 1.000 |
| | Absent | 39 | 97.5% | 40 | 100.0% | | |
| **Ear ache** | Present | 3 | 7.5% | 3 | 7.5% | NA | 1.000 |
| | Absent | 37 | 92.5% | 37 | 92.5% | | |
| **Facial weakness** | Present | 0 | .0% | 0 | .0% | NA | NA |
| | Absent | 40 | 100.0% | 40 | 100.0% | | |
| **Hearing loss improvement** | Present | 35 | 87.5% | 33 | 82.5% | 0.392 | 0.531 |
| | Absent | 5 | 12.5% | 7 | 17.5% | | |

**The chi-square test was used to obtain a p-value**
**A p-value of less than 0.05 was deemed significant.**

In 17.5% of Group II cases and 20.0% of Group I cases, discharge occurred one month after surgery. Five percent of Group I cases and zero percent of Group II cases had discharge after the third month post-op. Graft was still present in 87.5% of Group I cases and 82.5% of Group II cases during the first- and third-months following surgery. Table 5 elaborates the comparison of post-operative complication status of discharge and graft at the 1st month and 3rd month.

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**Table 5- Comparison of post-operative complication status of Discharge and Graft at 1st month and 3rd month**

| Parameters | | Groups | | | | χ2 values | p-value |
|---|---|---|---|---|---|---|---|
| | | Group I | | Group II | | | |
| | | No. | % | No. | % | | |
| **Discharge at 1 month** | Present | 5 | 12.50% | 7 | 17.5% | 0.082 | 0.776 |
| | Absent | 35 | 87.50% | 33 | 82.5% | | |
| **Discharge at 3 months** | Present | 2 | 5.0% | 3 | 7.5% | NA | 0.494 |
| | Absent | 38 | 95.0% | 37 | 92.5% | | |
| **Graft at 1 month** | Intact | 35 | 87.5% | 33 | 82.5% | 0.392 | 0.531 |
| | Not intact | 5 | 12.5% | 7 | 17.5% | | |
| **Graft at 3 months** | Intact | 35 | 87.5% | 33 | 82.5% | 0.392 | 0.531 |
| | Not intact | 5 | 12.5% | 7 | 17.5% | | |

**The chi-square test was used to obtain a p-value**
**A p-value of less than 0.05 was deemed significant.**

The average change in AC value from pre-op to the third month post-op was 12.88±15.16 for group I and 13.00±12.69 for group II. A statistically significant difference ($p<0.001$) was seen in the mean change in air conduction between the patients and control groups. Group I's A-B Gap value changed by an average of 12.63±10.80 from before surgery to the third month after surgery, whereas Group II's was 11.13±10.65. The mean change in air bone gap value between the groups varied significantly ($p<0.001$). Table 6 depicts a comparison of pre-operative and post-operative audiometric changes among the study population.

**Table 6- Comparison of pre-operative and post-operative Audiometric changes among the study population**

| Paired Samples Test | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Paired Differences | | | | | | | |
| | | | | | 95% Confidence Interval of the Difference | | | | |
| Groups | | Mean | SD | Std. Error Mean | Lower | Upper | t | df | p-value |
| Group I | AC preop - AC at 3 months | 12.88 | 15.60 | 2.47 | 7.89 | 17.87 | 5.219 | 39 | **<0.001** |
| | AB gap preop - AB gap at 3 months | 12.63 | 10.80 | 1.71 | 9.17 | 16.08 | 7.393 | 39 | **<0.001** |
| Group II | AC preop- AC at 3 months | 13.00 | 12.69 | 2.01 | 8.94 | 17.06 | 6.474 | 39 | **<0.001** |
| | AB gap preop - AB gap at 3 months | 11.13 | 10.65 | 1.68 | 7.72 | 14.53 | 6.606 | 39 | **<0.001** |

**A paired t-test was used to obtain a p-value**
**A p-value of less than 0.05 was deemed significant.**

## DISCUSSION

The present prospective evaluation was performed in the department of ENT, KGMU, Lucknow. In order to create contrast between the borders of the tympanic membrane hole and the graft, the authors used a temporalis fascia graft tinted with methylene blue. By improving the surgeon's ability to see the graft, this contrast guarantees that the graft completely covers the perforation [5]. In the current investigation, we also noticed the same thing.

Group I and Group II had respective mean ages of 26.32±10.37 and 27.03±9.49 years. A comparable study was

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conducted by Franco et al. on 57 patients, 21 of whom were female, and whose mean age was 46.1 years [6]. The comparison inferred that the two groups were statistically comparable and that the success of the procedure is independent of age, sex, socioeconomic status, and background of the patient [7].

The primary complaint of all patients (100%) in both groups in the current investigation was diminished hearing. According to an earlier study, all patients (100%) presented with both ear discharge and hearing loss [8]. To make one side of the fascia lighter than the other, in yet another study conducted previously, the graft was dyed with methylene blue and then rinsed off. It makes any graft folding easier for the surgeon to see. It lowers failure rates and aids in the correct transplant placement. 100% graft uptake is observed at the 2-month mark in this study [5].

The Vaiman M et al. study compared the outcomes of tympanoplasty with a temporalis fascia graft stained with methylene blue to tympanoplasty with an unstained temporalis fascia graft. Graft uptake in this trial is 100% at two months. The pure-tone audiogram test showed that all successful cases had a significant improvement in hearing ($p = 0.05$) [9].

The difference between the air bone gap in dB before and after surgery is statistically significant. At three months in a study of Prakash MD et al [7]. 33 (55%) of the participants in the Yadav SP et al. research experienced a gain between 11 and 20 dB [10].

This study demonstrates that graft uptake has improved, and when considered holistically, the hearing outcome in terms of air-bone gap repair has significantly improved. Methylene blue-stained temporalis fascia grafts are easier to insert during endoscopic myringoplasty than unstained ones. Methylene blue's antimicrobial qualities enhance transplant absorption by lowering infection rates. Using a stained graft has several benefits, including increased visualization, improved placement with perforation margins, and placement where the graft is prevented from folding by the stained side facing laterally.

### Interpretation

The finding that diminished hearing was the primary complaint in 100% of patients in both groups highlights the severity and commonality of hearing loss in the studied population. This suggests that the condition being investigated likely has a profound impact on auditory function, making hearing loss a key symptom to address in clinical interventions.

## GENERALIZABILITY

The generalizability of these findings may be limited by the specific patient demographics and the study's setting. While the results may apply to similar populations with comparable characteristics, caution is needed when applying them to broader, more diverse groups.

## CONCLUSION

The study concluded that no significant difference was found in the pre-operative complaints and otoscopic findings between the groups. There was a significant difference in the mean change in both air conduction thresholds and air-bone gap values between the groups in the study. Symptomatically and audiologically, the comparison was done, and group I showed better improvement than group II. Hence, methylene blue-stained tympanoplasty appears to be a promising technique for the reconstruction of tympanic membrane perforation.

## LIMITATIONS

Among the study's drawbacks were its single-center design and somewhat small sample size, and a relatively short follow-up period. Also, observer bias may be there due to non-blinding.

## RECOMMENDATIONS

Multicentred and larger sample size studies should be undertaken to minimize the bias/error and generalise the findings. To strengthen the hypothesis and ensure improvement in the technique post-operatively, a longer follow-up period is required.

## LIST OF ABBREVIATIONS

**ENT**- Ear-nose-throat
**KGMU**- King George's Medical University

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## SOURCE OF FUNDING

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## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest related to this study.

## AUTHOR CONTRIBUTIONS

Author A was responsible for study design and data analysis; Author B conducted the data collection and patient management; Author C contributed to manuscript writing and final revisions.

## DATA AVAILABILITY

The data generated and analyzed during this study are available upon reasonable request from the corresponding author.

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