Endoscopic vs Microscopic Overlay Tympanoplasty for Correcting Large Tympanic Membrane Perforations: A Randomized Clinical Trial

Yuvatiya Plodpai, MD

Abstract

Objective. Although overlay grafting for complicated tympanic perforations offers a high success rate, potential complications may outweigh its advantages. This study aimed to assess endoscopic overlay tympanoplasty (EOT), compared with microscopic overlay tympanoplasty (MOT), to optimize outcomes while minimizing complications associated with large tympanic perforations.

Study Design. Nonmasked, randomized.

Setting. Tertiary care university hospital.

Subjects and Methods. Altogether, 70 patients with large tympanic perforations were randomized to undergo overlay tympanoplasty between June 2014 and July 2016. Primary outcome was the visual analog scale (VAS) of pain. Secondary outcomes were anatomic closure, hearing results, middle ear findings, and postoperative complications.

Results. Overall, 34 EOT patients and 30 MOT patients completed the follow-up. VAS scores at 4, 24, and 48 hours in EOT and MOT groups were, respectively, 3 and 8, 1.7 and 6.0, and 0.6 and 4.1. Postoperative pain was less in the EOT group \((P < .001)\), and canalplasty was not required \((P = .003)\). Graft “take” rates for EOT and MOT were 97.1% and 93.3%, respectively \((P = .60)\). Postoperative air-bone gap was lower with EOT \((5.0 \text{ vs } 10.3 \text{ dB})\) \((P = .01)\). Various middle ear structures were more visible after EOT than after MOT \((P < .001)\). Ear protrusion \((P = .008)\) and postauricular numbness \((P < .001)\) occurred after 50 MOTs.

Conclusion. EOT for repairing large tympanic perforations provides more favorable anatomical and audiometric outcomes. It also offers superior visibility of middle ear structures without lifting the annulus, with fewer complications and less invasiveness than MOT.

Keywords

dendoscopy, overlay, tympanoplasty, lateral placing, tympanic membrane perforation

Received March 17, 2018; revised May 9, 2018; accepted June 14, 2018.

Tympanoplasty is a surgical procedure used to repair tympanic membrane (TM) perforation and possible impairment of the ossicles. Among the various contemporary grafting techniques, the most common are the overlay and underlay methods, wherein the graft is placed laterally and medially, respectively, on the fibrous annulus and tympanic remnant. The overall success rates for these 2 techniques are not significantly different,\(^1\)\(^2\) with each having its advantages and limitations.

Rizer\(^3\) and Angeli et al\(^4\) recalled that, during the 1960s, House and Sheehy reported that “the onlay or overlay technique” provided satisfactory results and was still widely used. The overlay technique is the ideal technique for all types of TM perforations because the middle ear space is not reduced, there is a 2-fold blood supply, and it provides support for the graft.\(^2\) Many studies also reported successful outcomes of the overlay technique, especially in more challenging cases, such as revision tympanoplasty, mesotympanic cholesteatoma, extensive tympanosclerosis, and anterior and large perforations.\(^4\)\(^6\) Its several disadvantages include the fact that it is more technically demanding, the healing process takes longer, and the potential for postoperative inflammatory ear canal stenosis exists.\(^2\) In addition, anterior blunting and lateralization of the graft can occur if the surgical technique and graft placement are not appropriately applied. In general, these problems arise if the exposure is inadequate.

Excellent visualization of the entire TM is an essential requirement for a successful overlay technique. However, transcanal visibility is limited when operating via a microscope. Traditionally, the postauricular surgical approach of the overlay technique is used most commonly because it

\(^1\)Department of Otolaryngology, Faculty of Medicine, Prince of Songkla University, Hatyai, Songkhla Province, Thailand

Corresponding Author: Yuvatiya Plodpai, MD, Department of Otolaryngology, Faculty of Medicine, Prince of Songkla University, Hatyai, Songkhla Province 90110, Thailand. Email: yuvatiya.plodpai@gmail.com, yuvatiya.p@psu.ac.th
provides excellent exposure of the operative site. Nevertheless, it is more invasive than other procedures and may still provide inadequate exposure of the entire TM. Moreover, canalplasty must be performed to enhance the visibility of the whole TM, thereby ensuring complete deepithelialization and satisfactory graft placement.

The endoscope has played an increasing role in otology over the past few years.7-13 Many studies revealed that the success of the endoscopic approach for underlay tympanoplasty was comparable to that of the microscopic approach.14-19 There is still insufficient evidence, however, to demonstrate the benefits of the endoscopic approach for overlay tympanoplasty. Therefore, we conducted a randomized study to determine the outcomes of overlay tympanoplasty for correcting large TM perforations according to the approach used: endoscopic or microscopic.

Materials and Methods
Study Design
A single-center, 12-month, nonmasked, randomized study was carried out at the Department of Otolaryngology, Faculty of Medicine, Prince of Songkla University, June 2014 to July 2016. This study was approved by the institutional review board of Prince of Songkla University, and written informed consent was obtained from all patients. The study adhered to the tenets of the Declaration of Helsinki and was performed according to the principles of Good Clinical Practice and the Consolidated Standards of Reporting Trials (CONSORT) statement. The trial was registered at the Clinical Trials Registry (NCT02331797).

Study Population
In all, 73 patients were assessed for eligibility, among whom 70 patients satisfied the inclusion and exclusion criteria.

Figure 1. Flow diagram according to the Consolidated Standards of Reporting Trials (CONSORT) statement shows patient flow in this study. EOT, endoscopic overlay tympanoplasty; MOT, microscopic overlay tympanoplasty.

Randomization and Masking
Randomization was performed using computer-generated block randomization. Patients were randomized 1:1 into 2 treatment groups (n = 35/group): endoscopic group and microscopic group. The endoscopic overlay tympanoplasty (EOT) group was defined as patients in whom the procedure was performed using an endoscope with the transcanal approach. The microscopic overlay tympanoplasty (MOT) group was defined as patients in whom the procedure was performed using an operating microscope via a postauricular incision. The allocation was performed using a sealed envelope system. The surgeon (Y.P.) and participants were unmasked to the treatment allocation.

Surgical Instruments and Technique
For the endoscopic group, 3 rigid endoscopes—(1) 3 mm diameter/14 cm long/0° angle of view, (2) 3 mm diameter/14 cm long/45° angle of view, and (3) 2.7 mm diameter/18 cm long/30° angle of view—were used together with a high-definition camera head and monitor (Karl Storz, Tuttingen, Germany). For the microscopic group, a Zeiss OPMI 111 (Carl Zeiss,
A Jena, Germany) operating microscope was used. Similar standard ear surgery instruments were used in both groups. All patients underwent tympanoplasty with the overlay grafting technique coupled with a temporalis fascia graft under local anesthesia by the same surgeon (Y.P.) in an outpatient setting. The external ear canal and postauricular skin were injected with 2% xylocaine with a 1:80,000 dilution of epinephrine (M&H Manufacturing, Samutprakarn, Thailand).

For the endoscopic group, the graft material was temporalis fascia that was harvested from a small postauricular incision. The transcanal approach was performed in all patients (Figure 2). The margin of the perforation was circumferentially trimmed using a Rosen needle. An incision was made laterally about 3 to 5 mm from the annulus, and the tympanomeatal flap was elevated. When the level of the fibrous annulus was reached, the squamous epithelium of the TM was raised from the tympanic remnant. The lateral process and handle of the malleus were denuded. The canal skin was not removed as a free graft, but the tympanomeatal flap was pedicled posterosuperiorly to attach to the vascular strip. The mobility of the ossicular chain and middle ear status were evaluated by passing the endoscope through the perforation site, after which the middle ear cavity was packed with the absorbable hemostatic gelatin sponge. The temporalis fascia was placed over the fibrous annulus, and the tympanic remnant and the tympanomeatal flap were repositioned. The absorbable hemostatic gelatin sponge was packed over the tympanomeatal flap and the graft to prevent anterior blunting and lateralization.

In the microscopic group, the surgical steps and sequence were similarly executed except all procedures were performed under the microscope, and the postauricular approach was employed.

The intraoperative data collected were the need for canaloplasty, the state of the middle ear structures, and the pathology found (ie, middle ear mucosa, presence of tympanosclerosis, round window, promontory, eustachian tube patency, status of the incudostapedial joint, staples, tympanic segment of the facial nerve, malleus adhesion). Operative time, defined as the duration from the beginning of local anesthetic injection to the completion of the wound dressing, was also recorded. Patients were discharged 2 hours after surgery. Each patient was prescribed oral ciprofloxacin for 1 week. All patients were prescribed oral acetaminophen every 6 hours for 2 days and nonsteroidal anti-inflammatory drugs as part of multimodal analgesia for management of postoperative pain in patients without contraindications. We also considered giving a weak opioid in patients who had a pain score >4 postoperatively.

Follow-up Evaluations and Outcome Measures

The outcome measurements were evaluated postoperatively at 3 weeks and 2, 6, and 12 months. The outcome measures were postoperative pain scores, graft “take” rate, hearing improvement, and postoperative complications. Postoperative complications (ie, myringitis, retraction, ear protrusion, postauricular numbness, anterior blunting, lateralization, epithelial cysts) were recorded. Postoperative pain was also measured and recorded. All patients were asked to score their pain on a visual analog scale (VAS), comprising a 10-cm line, at 4, 24, and 48 hours postoperatively. We instructed the patients to place a mark on the line to indicate how much pain they were feeling. The left end indicated no pain and the right end the worst possible pain.

All patients underwent preoperative and postoperative audiometric evaluations that included pure-tone threshold averages of air conduction and bone conduction at frequencies of 0.5, 1, 2, and 4 kHz. The air-bone gap (ABG)—the difference between air conduction and bone conduction averages—was recorded. The audiometric evaluation was performed within 3 months before the operation and at 2, 6, and 12 months postoperatively.

Statistical Analysis

The statistical analysis was performed using R Statistical Software (version 3.3.0; Foundation for Statistical Computing, Vienna, Austria). A value of \( P < .05 \) was considered to indicate statistical significance. The continuous data were presented as means with standard deviations. Categorical variables were presented as counts and percentages. The Shapiro-Wilk test was used to assess the normality of the findings. Patient characteristics were analyzed by Student t test or Wilcoxon’s rank-sum test for continuous variables. Categorical variables were analyzed by Pearson’s \( \chi^2 \) test or Fisher’s exact test.

Results

A total of 64 patients (34 in the endoscopic group, 30 in the microscopic group) with large TM perforations underwent...
Anatomical Closure of Tympanic Membrane

The graft take rates at 3 weeks and 2, 6, and 12 months were comparable in the EOT and MOT groups (Table 1).

Intraoperative Variable Results

Canalplasty was required in 23.3% (7 patients) of the MOT group. No canalplasty was needed in the EOT group (P = .003). The mean operating times of the EOT and MOT groups were 101.6 and 131.6 minutes, respectively, with the difference reaching significance (P < .001). There were no significant differences in visibility between the 2 groups regarding the middle ear mucosa, promontory, presence of tympanosclerosis, or adhesions binding the handle of the malleus to the promontory (Table 2). Endoscopically, we could visualize the middle ear status—including such items as the round window, eustachian tube patency, the incudostapedial joint, stapes, and tympanic segment of the facial nerve—without having to lift the annulus (Table 2). Four patients in the EOT group had adhesions surrounding the ossicular chain that were lysed using a fine micropick.

Postoperative Hearing Results

The audiometric data of the 2 groups are shown in Table 1. There were no significant differences between the groups regarding the preoperative air conduction threshold, preoperative ABG, or preoperative and postoperative bone conduction thresholds. Remarkably, the mean postoperative ABG and mean postoperative air conduction threshold were satisfactory in the EOT group.

Postoperative Complications

At the end of 12 months, there was no ear protrusion in the EOT group, whereas 4 (13.3%) patients in the MOT group complained of misalignment of the pinna (P = .008). One EOT patient had postauricular numbness, whereas 9 (30%) MOT patients had the same complaint (P < .001). Postoperative myringitis was found in 4 (11.8%) EOT patients and 4 (13.3%) MOT patients (P = 1.00). Epithelial cysts were found in 3 (8.8%) EOT patients and 5 (16.7%) MOT patients (P = .46). There were no cases of TM retraction. One case of anterior blunting (P = .22) and 2 cases of lateralization (P = .10) occurred in the MOT group, whereas none of these complications occurred in the EOT group. There were no statistical differences between the 2 groups regarding anterior blunting, epithelial cyst, lateralization, or myringitis. It is notable that postoperative ear protrusion and postauricular numbness were found more often in MOT patients, with the difference reaching statistical significance.

Discussion

The choice of the optimal surgical technique for tympanoplasty is an ongoing debate. The tympanoplasty technique often depends on the surgeon’s skills and familiarity with a particular technique. There are advantages to applying overlay tympanoplasty for large perforations, including that the middle ear space is not reduced, thereby avoiding atelectasis and medialization. In addition, overlay tympanoplasty is suitable for perforations of all locations and sizes, the chorda tympani are not injured, and the graft take rate is high. This technique is typically reserved for complicated cases and perforations located in the anterior TM.6,20 This finding is consistent with our study in which anatomic closure was achieved in 95.3% of all patients. The anatomic closure rates of EOT and MOT were comparable.

The overlay technique has been abandoned over the years because great surgical skill is required, and it is laborious. It is therefore used mainly by senior surgeons.6 A significant problem with the overlay technique is the need for an adequate surgical approach. The overlay technique also requires more work on the bony canal for broader exposure and is technically more difficult. Sheehy and Anderson21
reported satisfactory outcomes of overlay tympanoplasty when the surgeon used the postauricular approach. There are several drawbacks with this approach, however, including the following: it has a longer operative time and more demanding postoperative care, it is more invasive than other approaches, and visualization is limited in the

### Table 1. Audiometric and Surgical Outcomes between the Endoscopic Group and Microscopic Group.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Endoscopic Group (n = 34)</th>
<th>Microscopic Group (n = 30)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air conduction, mean (SD), dB</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preoperative</td>
<td>46.4 (11.8)</td>
<td>48.1 (14.5)</td>
<td>.62a</td>
</tr>
<tr>
<td>Second month postoperation</td>
<td>30 (11.4)</td>
<td>36.8 (12.4)</td>
<td>.04a</td>
</tr>
<tr>
<td>Sixth month postoperation</td>
<td>29.2 (10.7)</td>
<td>35.2 (11.4)</td>
<td>.03a</td>
</tr>
<tr>
<td>Twelfth month postoperation</td>
<td>28.1 (14)</td>
<td>35.4 (14.4)</td>
<td>.02a</td>
</tr>
<tr>
<td>Bone conduction, mean (SD), dB</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preoperative</td>
<td>21 (11.2)</td>
<td>25.3 (9.7)</td>
<td>.11a</td>
</tr>
<tr>
<td>Second month postoperation</td>
<td>22 (11.4)</td>
<td>23.4 (8.7)</td>
<td>.34a</td>
</tr>
<tr>
<td>Sixth month postoperation</td>
<td>21.6 (9)</td>
<td>24.4 (8.6)</td>
<td>.20a</td>
</tr>
<tr>
<td>Twelfth month postoperation</td>
<td>23.1 (10)</td>
<td>25 (8.6)</td>
<td>.41a</td>
</tr>
<tr>
<td>Average ABG at month 12, mean (SD), dB</td>
<td>5 (5.4)</td>
<td>10.3 (9.6)</td>
<td>.008b</td>
</tr>
</tbody>
</table>

No. (%) of patients at each range of ABG at 12 months postoperation

<table>
<thead>
<tr>
<th>Range</th>
<th>Endoscopic Group (n = 34)</th>
<th>Microscopic Group (n = 30)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-10 dB</td>
<td>30 (90.9)</td>
<td>18 (64.3)</td>
<td>.02b</td>
</tr>
<tr>
<td>11-20 dB</td>
<td>2 (6.1)</td>
<td>7 (25)</td>
<td>.02b</td>
</tr>
<tr>
<td>21-30 dB</td>
<td>1 (3)</td>
<td>2 (7.1)</td>
<td>.02b</td>
</tr>
<tr>
<td>31-40 dB</td>
<td>0 (0)</td>
<td>1 (3.6)</td>
<td>.02b</td>
</tr>
</tbody>
</table>

Graft take rate, No. (%)

<table>
<thead>
<tr>
<th>Time Period</th>
<th>Endoscopic Group (n = 34)</th>
<th>Microscopic Group (n = 30)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Third week</td>
<td>30 (94.1)</td>
<td>28 (93.3)</td>
<td>.01b</td>
</tr>
<tr>
<td>Second month</td>
<td>33 (97.1)</td>
<td>28 (93.3)</td>
<td>.60b</td>
</tr>
<tr>
<td>Sixth month</td>
<td>33 (97.1)</td>
<td>28 (93.3)</td>
<td>.60b</td>
</tr>
<tr>
<td>Twelfth month</td>
<td>33 (97.1)</td>
<td>28 (93.3)</td>
<td>.60b</td>
</tr>
</tbody>
</table>

Operative time, mean (SD), min

<table>
<thead>
<tr>
<th>Time Period</th>
<th>Endoscopic Group (n = 34)</th>
<th>Microscopic Group (n = 30)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>101.6 (26)</td>
<td>131.6 (31.3)</td>
<td>.001a</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: ABG, air-bone gap; dB, decibel; SD, standard deviation.

aIndependent t test.

bFisher exact test.

### Table 2. Visibility of Middle Ear Structures Based on Endoscopic and Microscopic Views.

<table>
<thead>
<tr>
<th>Middle Ear Status</th>
<th>Endoscopic Group (n = 34), No. (%)</th>
<th>Microscopic Group (n = 30), No. (%)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Location of perforation</td>
<td></td>
<td></td>
<td>.32b</td>
</tr>
<tr>
<td>Anterior perforation</td>
<td>8 (23.5)</td>
<td>8 (30.0)</td>
<td></td>
</tr>
<tr>
<td>Posterior perforation</td>
<td>1 (2.9)</td>
<td>1 (3.3)</td>
<td></td>
</tr>
<tr>
<td>Central perforation</td>
<td>25 (73.6)</td>
<td>20 (66.7)</td>
<td></td>
</tr>
<tr>
<td>Middle ear mucosa</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>29 (85.3)</td>
<td>26 (86.7)</td>
<td></td>
</tr>
<tr>
<td>Swelling</td>
<td>5 (14.7)</td>
<td>4 (13.3)</td>
<td></td>
</tr>
<tr>
<td>Promontory</td>
<td>34 (100)</td>
<td>30 (100)</td>
<td>.62a</td>
</tr>
<tr>
<td>Round window</td>
<td>33 (97.1)</td>
<td>14 (46.7)</td>
<td>&lt;.001a</td>
</tr>
<tr>
<td>Incudostapedial joint</td>
<td>34 (100)</td>
<td>13 (43.3)</td>
<td>&lt;.001a</td>
</tr>
<tr>
<td>Stapes</td>
<td>28 (82.4)</td>
<td>10 (33.3)</td>
<td>&lt;.001a</td>
</tr>
<tr>
<td>Tympanic segment of facial nerve</td>
<td>28 (82.4)</td>
<td>4 (13.3)</td>
<td>&lt;.001a</td>
</tr>
<tr>
<td>Eustachian tube opening</td>
<td>32 (94.1)</td>
<td>10 (33.3)</td>
<td>&lt;.001a</td>
</tr>
<tr>
<td>Malleus adhere to promontory</td>
<td>4 (11.8)</td>
<td>3 (10.0)</td>
<td>1a</td>
</tr>
<tr>
<td>Tympanosclerosis</td>
<td>5 (14.7)</td>
<td>6 (20.0)</td>
<td>.82a</td>
</tr>
</tbody>
</table>

aPearson’s χ².

bFisher exact test.
presence of bony canal wall overhangs. In the current study, the postoperative pain score and operative time were significantly higher in the MOT group, in which the postauricular approach was used. These findings may be attributed to the need for a larger postauricular incision, which may result in greater postoperative pain and ear numbness. Postauricular numbness was found in 30% of the MOT patients. A similar finding was reported in 26% of patients who underwent the postauricular approach in otologic surgery, sometimes with ongoing numbness lasting >8 months. In addition, 13.3% of the patients in the MOT group experienced some change in auricular positioning, a finding in accordance with previous reports.

Many authors have recommended routine drilling of the bony ear canal in all cases of overlay tympanoplasty to facilitate good exposure, which allows deepithelialization and optimal graft placement. Technical skill is essential for canalplasty to avoid exposure of the temporomandibular joint while removing anterior canal bulges.

The use of minimally invasive surgery is trending upward in the field of otologic surgery, with endoscopy currently having a wide range of applications. Endoscopic views from the natural ear opening allow inspection of angles in hidden areas of the middle ear and clear depiction of the whole TM without need for an extensive postauricular approach. Other studies also demonstrated the superiority of the endoscopic views over microscopic views via a transcanal approach regarding visualization, operability, and no need for canalplasty. With endoscopic visualization, surgeons are better able to see the anterior meatal angle, which is vital in cases of large or anterior perforations.

Middle ear structures (ie, incudostapedial joint, stapes, tympanic segment of the facial nerve, orifice of the eustachian tube, round window) were better visualized in EOT patients than MOT patients (Table 2). Under 30° and 45° endoscopic views, the ossicular chain could be assessed without canal wall curettage or lifting the annulus, which is usually challenging but needed with the microscopic approach. A study by Farahani et al showed that the diagnostic performances for viewing the mobility of the ossicular chain, the round window reflex, and ossicular chain erosion with the microscopic and endoscopic approaches were comparable, but the visibility provided by endoscopy for viewing the epitympanum, posterior mesotympanum, and hypotympanum was superior (vs microscopy). Middle ear pathologies, such as adhesions in the ossicular chain, were also manipulated more accurately. In our study, 4 patients who had concomitant middle ear pathology were manageable under endoscopic views. Consequently, without the interference of the middle ear space, the risk of further adhesions decreased.

In a systematic review and meta-analysis of patients who underwent the underlay technique, Tseng et al reported...
improvement in the ABG ranging from 6.6 to 18.1 dB in the endoscopic group, whereas in the microscopic groups, the improvement ranged from 6.2 to 16.9 dB. In our study, the decreases in the ABG were 5 dB for EOT patients and 10.3 dB for MOT patients. These improvements in the ABG were comparable to, and consistent with, previously published studies that used the overlay technique for large TM perforations.4,5 Nardone et al6 reported a significantly higher long-term success rate with the overlay technique than with the underlay technique, which could be explained by the better 2-fold vascularization, preservation of the annulus, and better exposure.

The strengths of the present study are its prospective randomized design, all procedures being performed by a single surgeon, and the large sample size. The study has reported on the unique results regarding the postoperative pain score comparing both techniques. Nonetheless, there are some limitations of the study. This is the nonmasked randomized study. The surgical procedures were performed under local anesthesia, which might affect the postoperative pain score. The criteria for inclusion and exclusion may not be applicable for the treatment of the other size of the perforated TM. There are also some limitations of the endoscopic approach. The endoscopy is a one-handed technique. It is difficult to control bleeding at the time of elevating the tympanomeatal flap. There is the loss of depth perception via the endoscopic approach, although this problem can be minimized with a high-definition monitor with a charge-coupled device camera.

Conclusion

Overlay tympanoplasty by the endoscopic approach has favorable surgical and audiometric outcomes for correcting large tympanic membrane perforations. The ossicular chain was assessed and manipulated without lifting the fibrous annulus, and canalplasty was not required under the endoscopic view. Endoscopic overlay tympanoplasty seems to be associated with less postoperative pain, fewer complications, and a shorter operative time than microscopic overlay tympanoplasty.

Acknowledgments

The author thanks the Faculty of Medicine, Prince of Songkla University for funded support; Ms Nannapat Pruphetkaew, who had served as a biostatistician of this study; and Nancy Schatken, MT, from Edanz Group (www.edanzediting.com/ac) for editing a draft of this manuscript.

Author Contributions

Yuvatiya Plodpai, conception, design, and supervise the study, corresponding author, data analysis and interpretation, manuscript writing and editing, final approval the study.

Disclosures

Competing interests: None.

Sponsorships: None.

Funding source: Faculty of Medicine, Prince of Songkla University, Thailand (grant REC 57-0123-13-1). The funding organization had no role in the design or conduct of this research.

References