

# The effect of topical estrogen on healing of chronic tympanic membrane perforations and hearing threshold

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**Background:** Considering the presence of squamous epithelial cells and fibroblasts in the tympanic membrane (TM), topical estrogen application may influence the repair of TM perforations. Therefore, this study was designed to investigate the healing effect of topical estrogen on chronic TM perforations and improvement in hearing threshold. **Materials and Methods:** Sixty patients were enrolled in a randomized clinical trial. Thirty patients were treated with paper patch and 30 with estrogen-impinged paper patch. Complete closure of the TM perforation and hearing threshold were evaluated in both groups. Chronic unilateral perforations of the TM involving less than 40% of the total area of the TM without active disease in the middle ear were selected. The margin of the perforation was chemically trimmed using 15% trichloroacetic acid and patching performed under an operating microscope. Microscopic examination was performed after 14 days and repeated after 30 days. Complete closure of the TM perforation was considered as successful repair. Hearing threshold was determined before the start of the trial and 30 days after treatment. **Results:** No significant difference was found between the two groups after 14 days in complete closure of the perforation ( $P = 0.310$ ). After 30 days, patients treated with estrogen-impinged paper patch showed a significantly higher rate of closure of the perforation (63.4%) and improvement in hearing threshold ( $P = 0.017$ ). **Conclusion:** Topical estrogen may have a healing effect on chronic TM perforations and improvement in hearing threshold.

**Key words:** Estrogen, paper patch, perforation, tympanic membrane

## INTRODUCTION

Tympanic membrane (TM) perforations can arise from a variety of causes. Major causes include trauma and middle ear disease.<sup>[1]</sup> Most of these perforations especially, those caused by acute trauma heal spontaneously while others remain open and need surgical closure.<sup>[1]</sup>

For surgical treatment of TM perforations use of autologous auto grafts including, muscle fascia or perichondrium is reported in most studies with a success rate between 88% and 97%.<sup>[2-6]</sup> However, surgical treatment requires higher costs, more effort and surgical risks.<sup>[7]</sup> Therefore, many investigators have studied topical use of substances to facilitate TM repair and alternative methods to the surgical repair of TM perforations.<sup>[8-10]</sup>

Estrogen can influence the various phases of wound healing in cutaneous repair described by Clark.<sup>[11]</sup> In the inflammatory phase neutrophils are the first cells to arrive at the wound site in significant numbers.<sup>[12]</sup> Estrogens have been found to enhance the activity of human neutrophils in the wound site.<sup>[13]</sup> In the proliferative phase, estrogens result in increased fibroblast infiltration and consequently collagen

production and angiogenesis<sup>[14,15]</sup> and by alterations of capillary bed promote hyperemia.<sup>[16,17]</sup>

Estrogen regulates the synthesis of Interleukine-1 (IL-1) and platelet-derived growth factor (PDGF) by macrophages and may have an indirect effect on the proliferative phase. IL-1 stimulates hyaluronic acid synthesis and collagen deposition while PDGF stimulates angiogenesis.<sup>[18,19]</sup> It also influences the matrix formation and remodeling phase and increase the tensile strength of the wound.<sup>[20]</sup>

Considering the presence of squamous epithelial cells and fibroblasts in the TM, topical estrogen application may influence the repair of TM perforations. Since there is no report about healing of TM perforations using topical estrogen, this study was performed to investigate the effects of estrogen – impinged paper patch on healing of chronic TM perforations and compares it with simple paper patch in a double-blinded clinical trial.

## MATERIALS AND METHODS

After getting the approval of research and Ethics Committee of Isfahan University of Medical

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Sciences (track number 389141) 60 patients, including 29 men and 31 women, were enrolled in the study between March 2010 and June 2011 at Isfahan Kashani and Azzahra General Hospitals.

Numbers of patients was achieved according to this formula, 25 patients for each group were calculated. But we selected 30 patients for each group.

$$n = \frac{Z(1-\alpha/2) + Z(1-\beta/2)}{d^2} \times \{P1(1-P1) + P2(1-P2)\}$$

$\alpha$  = significant level = 95%

$Z(1-\alpha/2) = 1.96$

$(1-\beta)$  = power of study

$Z(1-\beta) = 80\%$

$P1 = P2$ : With the conception of equal repair ratio in two groups

$d$  = minimum of significant difference of repair ratio in two groups = 40%

$n = 25$ .

Inclusion criteria included: (1) Presence of perforation for more than 3 months, (2) unilateral perforation, (3) perforation size less than 40% of the total area of the TM. Exclusion criteria included: (1) Perforation size greater than 40% of the total area of the TM, (2) presence of cholesteatoma or granulation tissue or polyp in the middle ear, (3) presence of otorrhea in the past 3 months, (4) presence of anterior perforation, marginal perforation or acute perforation of the TM.

After getting informed consent, patients were randomly allocated to two groups. One group was treated with paper patch impinged with 1% tetracycline ointment (Hakim factory, Iran) to act as the control group. The second group was treated with 1% estrogen ointment dissolved in 1% tetracycline ointment obtained from Pharmacology Department of Isfahan University of Medical Sciences. Paper patches were prepared from cigarette paper sterilized with ethylene oxide gas.<sup>[21]</sup> Using an operating microscope, local anesthesia was administered using cotton soaked with 4% lidocaine and 1/100,000 epinephrine solution around the perforation.<sup>[21]</sup> The margin of the perforation was chemically trimmed to create a fresh wound. This was achieved using a tiny drop of 15% trichloroacetic acid on a 1 mm hook. Patches were placed and stabilized with gelfoam filling the external ear canal.<sup>[21]</sup> Ciprofloxacin drop (Sina Daru, Iran) was administered three drops every 8 h for 1 week for post-operative care. Gelfoam was suctioned out after 2 weeks using an operating microscope and the TM was examined for complete closure of perforation. A second

similar examination was performed 30 days after the procedure. Only complete closure of TM perforation was considered as successful repair. Pure tone audiometry (PTA) was obtained before the procedure and 30 days after the procedure and hearing threshold was calculated as the mean value of threshold at 500 Hz, 1000 Hz and 2000 Hz.

Statistical analysis was performed using SPSS 17 software. Mean age and frequency of hearing threshold more than 10 db were evaluated and compared using the independent *t*-test and Chi-square. Distribution of sex and side of perforation were analyzed using the Fischer's exact Chi-square test. Logistic regression was used to evaluate and compare hearing threshold improvement and complete closure of the TM perforation.

## RESULTS

The control group included 16 men and 14 women and the estrogen group included 13 men and 17 women. No statistically significant difference was found between the two groups regarding sex distribution ( $P = 0.438$ ). The mean age was 39.5 (SD = 15.62) years old in the control group and 40.4 (SD = 16.89) years old in the estrogen group with no statistically significant difference between two groups ( $P = 0.83$ ).

The perforation was right sided in 29 patients (48.3%) and left sided in 31 patients (51.7%), revealing no significant difference ( $P = 0.438$ ).

After 2 weeks, complete closure of the TM perforation was achieved in four patients (13.3%), including three men and one woman, in the control group. In the estrogen group nine patients (30%) achieved complete closure after 2 weeks, including four men and five women. No statistically significant difference was found in complete closure rate after 2 weeks between the two groups ( $P = 0.31$ ).

After 30 days complete closure of the TM perforation was achieved in eight patients (26.7%) in the control group, including four men and four women. In the estrogen group 19 patients (63.3%), including 8 men and 11 women, achieved complete closure. Statistical analysis revealed a significant difference between the two groups in complete closure of the TM perforation after 30 days ( $P = 0.017$ ). Female sex had no impact on achieving a complete closure ( $P = 0.586$ ) [Table 1].

PTA obtained before the start of the trial showed a 15.7 dB air-bone gap in the control group and a 16.2 dB air-bone gap in the estrogen group ( $P = 0.781$ ). After 30 days the hearing threshold improved by 10 dB or more in 12 patients (40%) in the control group and in 18 patients (60%) in the estrogen group ( $P < 0.001$ ) [Table 2]. In the follow-up period no

complications including mucopurulent otorrhea or pain were observed. Results regarding prognostic factor in outcome (repair of perforation and improvement in hearing threshold has been shown in Table 3).

## DISCUSSION

Our results showed that estrogen paper patch improved hearing threshold and repaired of TM perforation after 30 days. Early closure with paper tape patching for traumatic perforations of the TM has been evaluated in previous studies.<sup>[22,23]</sup>

In chronic perforations the squamous epithelium grows over the edge of the perforation preventing spontaneous healing by blocking vascular proliferation.<sup>[24]</sup> Creation of a fresh wound margin is the initial step in treating chronic TM perforations. A guide surface in contact with the fresh wound and induction of cellular proliferation and migration are the next steps in healing of the TM perforation.

Numerous studies have used experimental materials as guide surface to promote the healing of the TM perforation.<sup>[25-27]</sup> Kim *et al.* compared chitosan patch with no

patching in the treatment of acute TM perforation in adult rats and found a beneficial effect with the use of chitosan patch.<sup>[28]</sup> In a recent animal study silk patch closed acute TM perforations in 92% of cases compared with 67.5% closure rate by using paper patch.<sup>[29]</sup>

Numerous growth factors have been investigated to stimulate healing of TM perforations including, epidermal growth factor (EGF),<sup>[30,31]</sup> fibroblast growth factor,<sup>[32,33]</sup> and platelet-derived growth factor<sup>[34]</sup> with mixed results. Topical insulin application in acute TM perforations in guinea pigs showed no beneficial effect on closure rate, epithelial thickness and TM thickness.<sup>[35]</sup>

Chronic perforation is deficient in growth factors. Very few studies have investigated the use of growth factors in chronic perforations in humans. Ramsay *et al.* investigated topical EGF in promoting complete closure of chronic TM perforations in humans and found no beneficial effect.<sup>[36]</sup> Hakuba *et al.* used basic fibroblast growth factor (bFGF) with atelocollagen for closing chronic TM perforations in humans and achieved a 92% closure rate after 4.8 weeks and 1.8 attempts on average.<sup>[27]</sup> In our study, simple paper patching achieved a 26.7% closure rate while estrogen-impinged paper patching achieved a 63.3% closure rate in chronic perforations of the TM after 30 days. The higher closure rate attained by Hakuba is probably due to the combined use of bFGF as growth factor and atelocollagen as guide surface in addition to repeat attempts to treat incomplete closures.

Hearing threshold improved by 10 dB or more in 70.1% of patients in Hakuba's study. In our study, this was achieved in 60% of patients treated with the estrogen-impinged paper patching technique.

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**Table 1: Outcome of different treatment of tympanic membrane perforations with respect to sex**

Outcome	Sex	Group		P value
		Paper patch (%)	Estrogen-impinged paper patch (%)	
Repair	Male	4 (13.33)	8 (26.67)	0.586
	Female	4 (13.33)	11 (36.67)	
Failure	Male	12 (40)	5 (16.67)	0.586
	Female	10 (33.33)	6 (20)	
Total		30 (100)	30 (100)	0.017

**Table 2: Hearing threshold improvement in patients**

Groups	Mean (SD) hearing threshold gap		P value
	Before intervention	After intervention	
Estrogen patch	15.76 (5.41)	6.23 (1.23)	0.015
Paper patch	16.23 (7.39)	12.45 (2.34)	

**Table 3: Prognostic factors in patient's outcome (healing effect and hearing threshold)**

Outcome	Variable	P value	Odds ratio	95% confidence interval
Healing of TMP	Estrogen patch	0.016	26.98	1.826-397.013
	Age	0.085	1.068	0.991-1.150
	Sex	0.586	1.66	0.268-10.293
	Perforation site	0.064	0.122	0.013-1.135
Hearing improvement threshold	Gap improvement	0.000.000	0.005	0.000-0.090

TMP=Tympanic membrane perforation

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