*Int. Adv. Otol. 2009; 5:(1) 17-23*

ORIGINAL ARTICLE

# Effects of Vitamin E on the Amount of Free Radicals in Traumatized Middle Ear Tissue

**Murat Sari, Serdar Baylancicek, Tekin Baglam, Nevruz Ozdemir, Cuneyd Uneri**

*Marmara University School of Medicine, Department of Otorhinolaryngology, Head and Neck Surgery, Istanbul-Turkey (MS, NO, CU)*
*Şişli Etfal Training and Research Hospital, Ear Nose and Throat Clinic, Istanbul-Turkey (SB)*
*Gaziantep University School of Medicine, Department of Otorhinolaryngology, Head and Neck Surgery, Gaziantep-Turkey (TB)*

**Objective;** Tympanosclerosis is a common sequela of tympanoplasty operations causing hearing disability. It is associated with an increased production of free radicals (also known as reactive oxygen species) after trauma. Vitamin E is a scavenger of different free radicals by working as an antioxidant. The aim of the present study was to evaluate the effect of vitamin E-soaked absorbable gelatin sponge placement to the middle ears at quantity of free radicals in rat middle ear.

**Materials & Methods;** This prospective, controlled animal study consisted of Sprague-Dawley rats divided into two groups of 8 animals each. Middle ear trauma was applied following myringotomy to the right ears of the first group and vitamin E-soaked absorbable gelatin sponge were placed to the middle ear of the right ears of the second group. The left ears were used as controls. Then, the animals were killed and chemiluminescence measurements were made for middle ear tissues.

**Results;** Reactive oxygen species levels were significantly increased in right ears of the first group when compared with the control ears ($p < .0001$), and the levels were significantly decreased in right ears of the second group as compared with the operated ears of the first group ($p < .005$). The free radical levels of right and left ears in the second group were similar.

**Conclusion;** Our results indicate that vitamin E- soaked absorbable gelatin sponge placement to the middle ear decreases the quantity of reactive oxygen species after trauma.

*Submitted : 03 September 2008* *Revised : 19 December 2008* *Accepted : 07 January 2009*

Tympanosclerosis (TS) is a pathologic degenerative condition that is characterized by calcification of connective tissue in the middle ear and tympanic membrane (TM). It is seen as white plaques of sclerotic material in the initial stages and becomes a harder, bony material by accumulation of calcium and phosphorus [1, 2] at the later stages. The most common localization of the process in TM is called myringosclerosis and typically involves lamina propria [3]. Histologically there is an increase in collagen fibers as well as hyaline degeneration within the lamina propria [4]. When the process involves ossicles, patients develop hearing loss. Since the surgery has low success rates and high recurrence rates [5, 6], the preventive modalities seem to be more logical.

Tympanosclerosis often occurs in patients who underwent tympanoplasty and had ventilation tubes (VT) inserted [7]. The etiology of this condition is unknown, but it has been suggested that tympanosclerosis is caused by an immunological hypersensitivity reaction, infection, inflammation, genetic tendency, or trauma [7-10]. Recent studies have established the relationship between the reactive oxygen species (ROS) and the development of

**Corresponding address:**
Murat Sari, MD
Marmara Universitesi Hastanesi Kulak Burun Bogaz ABD
Tophanelioglu Cad. No: 13-15 Altunizade 34662, Istanbul-Turkey
Phone: + 90 216 5450047; Fax: + 90 216 3269637; E-mail: msari@marmara.edu.tr

myringosclerosis after tympanic membrane trauma and/or VT insertion [11-14]. Additionally, some studies have reported the preventive effect of different antioxidants both in animals and in humans.[15-17]

Free radicals are highly reactive molecular species with one or more unpaired outer orbital electrons that cause tissue damage by chemically modifying cellular lipids, proteins, carbohydrates and DNA. ROS are produced by normal oxidative metabolism and also may result from inflammation, trauma, oxygen toxicity, perfusion injury, and radiation. Under normal conditions, the enzyme systems present in the living organism protect it against the damage, but in disease states, the antioxidant defense system is weakened and increased ROS levels may contribute to the tissue injury [15]. Strong evidence is present in the literature to support the relationship between ROS and TS.[11,12,13]

Chemiluminescence is the production of light generated from chemical sources. It may be used as a direct method for measuring reactive oxygen species [18]. Chemiluminescence is a universal property of organic substances that are able to undergo an oxidation reaction sufficiently exothermic to produce an emitting state [19]. Investigators have introduced the use of enhancer compounds, such as luminol, because of limitations with potential variability and the low intensity of native chemiluminescence [20]. These compounds were selected primarily because of their high quantum efficiency after oxidation. These compounds function as bystander substrates for oxygenation and form high levels of excited state products and chemiluminescence when added to an in vitro biologic system.

Trauma occurs in the middle ear tissue during tympanoplasties, and this is a possible etiologic factor for TS after surgery. An antioxidant material soaked in an absorbent gelatin sponge and placed in the middle ear cavity could prevent TS. So the aim of the study was to quantify ROS levels in middle ear tissue of rats by measuring luminol amplified chemiluminescence and evaluating changes in ROS levels following middle ear trauma, with and without concurrent vitamin E treatment.

The aim of the present study was to evaluate the effect of vitamin E-soaked absorbable gelatin sponge placement to the middle ears at quantity of free radicals in rat middle ear.

## Materials & Methods

The present study was approved by the Animal Ethics Committee of the Marmara University School of Medicine (Istanbul, Turkey). Sixteen healthy Sprague-Dawley rats (weight, 280-400 g) were used. All animals had been kept in ordinary cages with free access to food and water. The animals were anesthetized with 50 mg/kg ketamine hydrochloride (Ketalar, Eczacibasi, Warner Lambert, Istanbul, Turkey) and 10 mg/kg xylazine (Rompun, Bayer, Istanbul, Turkey) by intraperitoneal injection. Animals were examined and assessed otoscopically for evidence of an ear disease, and any animal that showed signs of any ear disease before or after surgery was excluded from the study. The animals were randomly assigned into two groups of eight each. In both groups, middle ear mucosal trauma was performed with a lancet following myringotomy to the right ears (8 ears), through an aural speculum under otomicroscopy (S1; Zeiss, Jena, Germany, with a 300-mm objective lens) with a sterile technique. The left ears in both groups constituted the control group and an intervention was not applied. Vitamin E soaked absorbable gelatin sponges (Spongostan, Johnson & Johnson Medical Limited, Gargrave, Skipton, UK), were put in the middle ear of the right ears in group 2 (8 ears) after the surgical procedure. Saline soaked Spongostans were put in the middle ear of the right ears in group 1 (8 ears) after the surgical procedure.

Twenty-four hours after myringotomy, the rats were killed with an overdose of ketamine injected intraperitoneally. For chemiluminescence measurements, tympanic bullae were cracked with scissors. Under a dissecting microscope, the middle ear mucosa was peeled off. The tissues were washed in an ice-cold saline solution and analyzed as described later in this section. After ten minutes, the specimens were studied to detect ROS levels with chemiluminescence.

Chemiluminescence measurements were made at room temperature using a Mini Lumat LB 9506 luminometer (EG&G Berthold) in the presence of 0.2 mmol/L luminol. Counts were obtained at 5-second intervals and the results were given as the area under curve (AUC) for a luminol chemiluminescence counting period of 5 minutes. Counts were corrected for wet tissue weight (rlu, or relative light unit, per milligram of tissue).

SPSS 16 for Windows software package was used to perform the statistical analysis. Bonferroni Multiple Comparisons Test was used to compare the means of groups. The differences were considered statistically significant when the probability was $P < 0.05$.

## Results

Luminol-amplified chemiluminescence levels in rat middle ear tissues of all groups are shown in Table 1 and the statistical analysis is summarized in Table 2 and 3. Mean luminol chemiluminescence level was found as 5667±837.81 RLU/mg tissue in the left middle ear tissue and 7881.5±1172 RLU/mg tissue in the right middle ear tissue in the first group. In this group, trauma to the right middle ear caused a statistically significant increase in ROS as measured by luminol chemiluminescence ($P< 0.0001$) (Fig. 1).

Insertion of Vitamin E soaked Spongostan into the right middle ears of the second group caused a significant decrease in ROS levels (mean, 5982.1±690.5) when compared with saline soaked Spongostan within the middle ear ($P< 0.005$, Fig. 1). Nonetheless, statistically significant differences were not found in terms of ROS levels between the right and left ears of the second group and the un-operated ears of the first group (Table 3).

## Discussion

In the present study, specimens from traumatized middle ear tissues have been investigated in respect to ROS levels. ROS levels measured in the specimens from the middle ear were higher in the saline treated group when compared with the Vitamin E treated group. Vitamin E caused a significant decrease of free oxygen radicals which was responsible for tissue damage.

The term TS is used to describe a sclerotic or a hyaline degeneration of the fibrous and elastic fibers in the lamina propria of the TM and middle ear mucosa. It appears to be a sequela of acute or chronic ear infection and it appears to occur after ventilation tube insertion or tympanoplasties [21, 22].

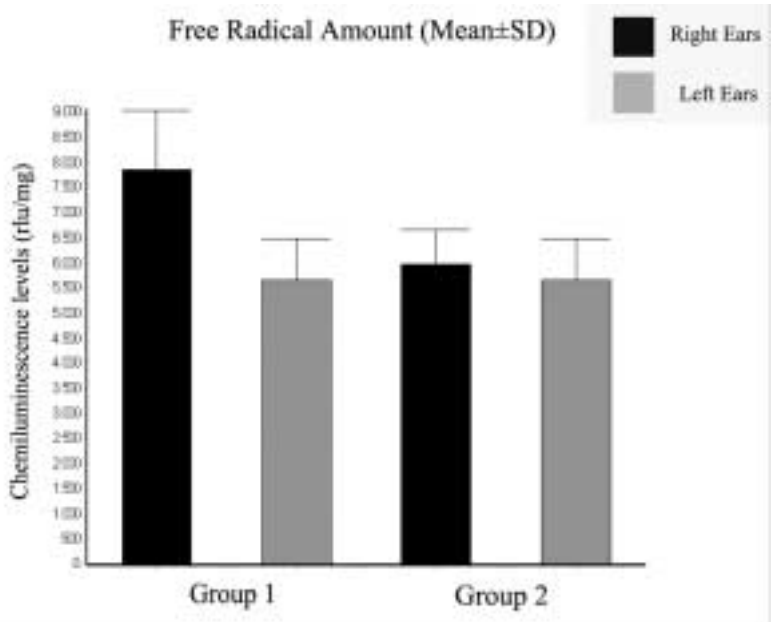


**Figure 1.** Comparison of mean±SD values for free radical amount. Chemiluminescence levels in middle ear tissues are shown to be significantly higher in right ears of the first group compared with the control ears. Placement of vitamin E-soaked gelatin sponges (group 2-right ears) statistically significantly decreases the reaction oxygen species levels compared with the right ears of the first group.

**Table 1.** Luminol-amplified chemiluminescence values in rats middle ear mucosa (rlu [relative light unit] per Milligram Tissue).

| | Group I | | Group II | |
|---|---|---|---|---|
| **Animal Number** | **Right ear** | **Left ear** | **Right ear** | **Left ear** |
| **1** | 9554 | 6887 | 6402 | 7012 |
| **2** | 7943 | 5416 | 4997 | 6281 |
| **3** | 7103 | 5213 | 5128 | 4985 |
| **4** | 8221 | 6675 | 5912 | 4639 |
| **5** | 8554 | 5102 | 7032 | 5543 |
| **6** | 6753 | 4378 | 6514 | 4761 |
| **7** | 8879 | 6061 | 6119 | 5998 |
| **8** | 6045 | 5604 | 5753 | 6107 |

*Group 1 right ear, Vitamin-E treated group; Group 2 right ear, saline treated group; Left ears, control groups.*

**Table 2.** Descriptive Statistical Summary of Middle Ear ROS Levels.

| | Group I | | Group II | |
|---|---|---|---|---|
| | **Right ear** | **Left ear** | **Right ear** | **Left ear** |
| **Median (Min-Max)** | 8082 (6045.0-9554.0) | 5510 (4378-6887) | 6015.5 (4997-7032) | 5770.5 (4639-7012) |
| **Mean± Standard Deviation** | 7881.5±1172 | 5667±837.81 | 5982.1±690.5 | 5665.8±832.15 |
| **Standard Error Mean (SEM)** | 414.38 | 296.21 | 244.13 | 294.21 |
| **95% Confidence Interval** | 6901.5-8861.5 | 4966.5-6367.5 | 5404.8-6559.5 | 4969.9-6361.6 |
| **Size** | 8 | 8 | 8 | 8 |

*Group 1 right ear, Vitamin-E treated group; Group 2 right ear, saline treated group; Left ears, control groups*

**Table 3.** The Comparison of Reactive Oxygen Species Levels in Middle Ear Tissues of Rats (Mean±Standard Deviation [SD]).

| | **Right Ears (Mean±SD)** | **Left Ears (Mean±SD)** | ***p Value*** |
|---|---|---|---|
| **Group 1** | 7881.5±1172 | 5667±837.81 | 0.0007* |
| **Group 2** | 5982.1±690.5 | 5665.8±832.15 | NS |
| ***P Value*** | 0.0015* | NS | |

**Bonferroni Multiple Comparisons Test (p< .005 was significant).*
*NS: not significant (p>0.05).*

The irreversible calcification involving the ossicular chain results in conductive or mixed hearing loss. Surgical removal of the calcified lesion, followed by reconstruction of the sound conduction mechanism, is the only way to restore hearing. However, postoperative hearing results are not always satisfactory, particularly when the stapes is involved and fixed in the sclerotic lesion [21, 23, 24].

One of the possible etiologic factors for TS is tympanoplasty operations. Middle ear trauma can happen during these surgeries. Some absorbable gelatin sponges are placed in the middle ear to support the graft material. Theoretically, soaking sponges with an antioxidant material would prevent the development of TS.

In an animal study, Mattsson et al. [11] determined that an increased oxygen concentration in the atmosphere of the ear is associated with an increased myringosclerosis development of traumatized tympanic membranes. Similarly Dawes et al. [25] hypothesized that the risk of development of MS is increased where there is traumatic tube insertion, hemorrhage, or excessive aspiration of middle ear fluid.

In recent studies, some antioxidant enzymes and elements were used to reduce oxidative damage in myringotomized tympanic membranes. Polat et al. [13] directly measured the levels of ROS in the tympanic membrane and middle ear mucosa in myringotomized rats and also showed that Vitamin E is effective in decreasing the levels of ROS. Üneri et al. [15] reported that Vitamin E-coated tube insertion both experimentally and clinically decreases the quantity of reactive oxygen species in tympanic membrane after myringotomy and tympanostomy tube insertion. The topical treatment with copper zinc-superoxide dismutase plus catalase and deferoxamine was found to decrease the development of myringosclerosis [12]. Spratley et al. [14] used topical ascorbic acid as a free radical scavenger in myringotomized rat ears and demonstrated that ascorbic acid application reduces the occurrence of myringosclerosis. Özcan et al. [26] applied N-acetylcysteine topically to myringotomized rat tympanic membranes and found that N-acetylcysteine is effective in the prevention of sclerotic lesions. Karlıdağ et al.[17] observed that in chronic otitis media patients, nitric oxide and malondialdehyde levels were higher and catalase activity levels were lower in patients with tympanosclerosis compared to patients without tympanosclerosis.

ROS have extremely brief half-lives; therefore, free radical production is difficult to identify and quantify. A study by Mattson et al. [27] revealed that myringosclerosis develops within nine hours of myringotomy, and inflammatory reaction is most intense in the first twenty-four hours after myringotomy. This is why we measured ROS levels twenty-four hours after myringotomy.

## Conclusion

Our results showed that middle ear trauma increases ROS levels, and placement of Vitamin E soaked Spongostan can decrease ROS levels in the middle ear. This can help to prevent the development of TS, which results from tympanoplasty operations. We believe that further clinical studies should concentrate on the effects of Vitamin E or other antioxidants on TS development after tympanoplasty operations.

## References

1. Tos M, Bak-Pedersen K. Middle-ear mucosa in tympanosclerosis. J Laryngol Otol 1974; 88:119-126.

2. Bhaya MH, Schachern PA, Morizono T, Paparella MM. Pathogenesis of tympanosclerosis. Otolaryngol Head Neck Surg 1993; 109:413-420.

3. Costa SS, Paparella MM, Scachern PA, Yoon TH, Kimberley BP. Temporal bone histopathology in chronically infected ears with intact and perforated tympanic membranes. Laryngoscope 1992; 102:1229-1236.

4. Russell JD, Giles JJ. Tympanosclerosis in the rat tympanic membrane: an experimental study. Laryngoscope 2002; 112:1663-6.

5. Flodin MF, Hultcrantz. Possible inflammatory mediators in tympanosclerosis development. Int J Pediatr Otorhinolaryngol 2002; 63:149-154.

6. Wielinga EWJ, Kerr AG. Tympanosclerosis. Clin Otolaryngol 1993; 18:341-349.

7. Tos M, Stangerup SE. Hearing loss in tympanosclerosis caused by grommets. Arch Otolaryngol Head Neck Surg 1989; 115:931-935.

8. Koc A, Uneri C. Sex distribution in children with Tympanosclerosis after insertion of a tympanostomy tube. Eur Arch Otorhinolaryngol 2001; 258:16-19.

9. Schiff M, Poliquin JF, Catanzaro A, Ryan AF. Tympanosclerosis: a theory of pathogenesis. Ann Otol Rhinol Laryngol 1980; 89(Suppl 70):1-15.

10. Koc A, Uneri C. Genetic predisposition for tympanosclerotic degeneration. Eur Arch Otorhinolaryngol 2002; 259:180-183.

11. Mattsson C, Magnuson K, Hellström S. Myringosclerosis caused by increased oxygen concentration in traumatized tympanic membranes. Ann Otol Rhinol Laryngol 1995; 104: 625-632.

12. Mattsson C, Marklund SL, Hellstro¨m S. Application of oxygen free radical scavengers to diminish the occurrence of myringosclerosis. Ann Otol Rhinol Laryngol 1997; 106: 513-518.

13. Polat S, Oztürk O, Uneri C, Yüksel M, Haklar G, Bozkurt S, Küllü S. Determination of reactive oxygen species in myringotomized tympanic membranes: effect of vitamin e treatment. Laryngoscope 2004; 114:720-5.

14. Spratley JE, Hellström S, Mattsson C, Pais-Clemente M. Topical ascorbic acid reduces myringosclerosis in perforated tympanic membranes. Ann Otol Rhinol Laryngol 2001; 110:585-591.

15. Uneri C, Sari M, Akboğa J, Yüksel M. Vitamin e-coated tympanostomy tube insertion decreases the quantity of free radicals in tympanic membrane. Laryngoscope 2006; 116:140-143

16. Uneri C, Bağlam T, Yazici M. The effect of Vitamin E treatment on the development of myringosclerosis after ventilation tube insertion. Int J Pediatr Otorhinolaryngol 2006; 70:1045-8.

17. Karlidağ T, Ilhan N, Kaygusuz I, Keleş E, Yalçin S. Comparison of free radicals and antioxidant enzymes in chronic otitis media with and without tympanosclerosis. Laryngoscope 2004; 114:85-9.

18. Gutteridge JM, Halliwell B. The measurement and mecha nism of lipid peroxidation in biological systems. Trends Biochem Sci 1990; 15:129-135.

19. Van Dyke K, Castranova V, eds. Cellular Chemiluminescence. London: CRC Press 1987; 1-67.

20. Allen RC, Loose LD. Phagocytic activation of a luminoldependent chemiluminescence in rabbit alveolar and peritoneal macrophages. Biochem Biophys Commun 1976; 69:45-53.

21. Teufert KB, De La Cruz A. Tympanosclerosis: long-term hearing results after ossicular reconstruction. Otolaryngol Head Neck Surg 2002; 126:264-72.

22. Maw AR. Development of tympanosclerosis in children with otitis media with effusion and ventilation tubes. J Laryngol Otol 1991; 105:614-7.

23. Vincent R, Oates J, Sperling NM. Stapedotomy for tympanosclerotic stapes fixation: is it safe and efficient? A review of 68 cases. Otol Neurotol 2002; 23:866-72.

24. Tsuzuki K, Yanagihara N, Hinohira Y, Sakagami M. Tympanosclerosis involving the ossicular chain: mobility of the stapes in association with hearing results. Acta Otolaryngol 2006; 126:1046-52

25. P.J. Dawes, B.J. Bingham, R. Ryhs, M.V. Griffiths, Aspirating middle ear effusions when inserting ventilation tubes: does it influence post-operative otorrhoea, tube obstruction or the development of tympanosclerosis? Clin. Otolaryngol 1991; 16:457-461.

26. Ozcan C, Görür K, Cinel L, Talas DU, Unal M, Cinel I. The inhibitory effect of topical N-acetylcysteine application on myringosclerosis in perforated rat tympanic membrane. Int J Pediatr Otorhinolaryngol 2002; 63:179-84.

27. Mattson C, Johansson C, Hellström S. Myringosclerosis develops within 9h of myringotomy. ORL J Otorhinolaryngol Relat Spec 1999; 61:31-36.