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Main Article

Evaluation of plasma fibronectine level as a probable indicator for tympanosclerosis

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Abstract

Objectives: Wound healing, epithelial regrowth and collagen synthesis are very important factors in the repair of the traumatised tympanic membrane. The aim of the present study was to determine the role of plasma fibronectine in the aetiopathogenesis of tympanosclerosis.

Methods: This prospective study included 58 patients with and 49 without tympanosclerosis. No inflammation or trauma was noted in either patient group. All patients underwent otoscopic and otomicroscopic examination, and the degree of tympanosclerosis was graded from mild (stage I) to severe (stage III). Following otological examination, blood samples were taken for plasma fibronectine measurement.

Results: Following otoscopic and otomicroscopic examinations, patients' tympanosclerosis was graded as follows: 18 patients were stage I; 29 were stage II; and 11 were stage III. Statistical analyses revealed that the plasma fibronectine concentrations were significantly lower in the study group compared with the control group ($p = 0.031$). In addition, fibronectine levels were lowest in the patients with severest tympanosclerosis ($p = 0.0001$ in each comparison).

Conclusion: The results of the present study show that serum fibronectine is important in the development and severity of tympanosclerosis.

Key words: Tympanosclerosis; Fibronectine; Tympanic Membrane; Wound Healing; Extracellular Matrix

Introduction

Tympanosclerosis is a degenerative healing process. It presents as a hyaline degeneration of the fibrous and elastic layers in the lamina propria of the tympanic membrane and the middle-ear mucosa. It is the end stage of otitis media, and develops as a sequel. While Kay *et al.*[1] have reported that tympanosclerosis occurs in 32 per cent of ears after tube insertion, other reports give rates between 7 and 64 per cent. Most cases of tympanosclerosis are asymptomatic or cause only mild hearing loss. However, if the plaques involve relatively larger areas of tympanosclerosis or invade down the bony annulus, ossicles and promontorium, there is a higher risk of marked hearing loss.[2]

Tympanosclerosis develops when irreversible tissue injury occurs in the tympanic membrane. When tissue defects occur, a healing process immediately begins. In this complex chain of events, proliferation and migration of extracellular matrix cells play a key role.

Fibroblasts are the key elements of wound healing, and are known to be directly influenced by the protein fibronectine. Fibronectine is a non-collagen, adhesive protein found in the extracellular matrix. It plays a central role in various processes, including cell-to-cell adhesion, cell–basement membrane adhesion, clot stabilisation, fibroblast migration and regulation of macrophage functions. Functioning fibronectine has two basic forms: soluble and insoluble. The most definitive characteristic of soluble fibronectine is that it circulates in bodily fluids. Insoluble fibronectine, however, is located in the matrix and cell surfaces. Whereas insoluble fibronectine is produced locally in tissues by fibroblasts, chondrocytes, endothelial cells, macrophages and certain epithelial cells, plasma fibronectine is expressed only by hepatocytes.[3,4] Plasma fibronectine appears to be utilised by healing wounds; thus, its concentration decreases in the early post-operative period.[5] Both forms of fibronectine are generated from a single gene, with individual differences.[6]

In the present study, we aimed to assess the relationship between clinical tympanosclerosis and the plasma fibronectine concentration. To our knowledge, this is the first study to investigate whether fibronectine plays a role in the development and severity of tympanosclerosis.

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Materials and methods

Patients

This was a prospective study performed at the Mersin University Hospital. From January to May 2006, a total of 107 cases was enrolled. The study group consisted of 58 patients with tympanosclerosis within an intact tympanic membrane (37 men). The mean age of study group patients was 42 years (range, 18–78 years). The control group consisted of 49 patients without tympanosclerosis (23 men), with a mean age of 43 years (range, 21–76 years). No inflammation or trauma was noted in patients of either group. The study and control subjects were from southern Turkey and were of the white Turkish racial group.

Otoscopic and otomicroscopic examination of all patients was performed. Patients' degree of tympanosclerosis was graded (as described by Bluestone in 2003)[7] as follow: stage I, limited to one quadrant of the pars tensa; stage II, limited to two or more quadrants; stage III, total involvement of the tympanic membrane. Control group patients were randomly chosen from ENT clinic attendees without tympanosclerosis. After written informed consent was obtained, 5 ml of blood was taken from both groups for serum fibronectine assay.

Fibronectine assay

The fibronectine assay was used to determine the fibronectine concentration of subjects' serum specimens. Blood samples were taken using standard sampling tubes. The serum contents were separated by centrifugation at 4000 rpm for 10 minutes. Serum samples were stored at −20°C until analysis. All samples were transferred to the laboratory for analysis of fibronectine under appropriate conditions. Fibronectine concentration was measured via nephelometry (BN 100, Dade Behring, Marburg, Germany), using fibronectine antiserum (human fibronectine antiserum, OUND 09, Dade Behring). Fibronectine levels were expressed as g/l of serum.[4]

Statistical analysis

The serum fibronectine concentrations of the tympanosclerosis and control groups were statistically compared, using the Mann–Whitney U test as the normality assumption was not satisfied. Differences between the three tympanosclerosis groups were analysed by one-way variance analysis after using Student Neyman-Keuls Test. A p value of less than 0.05 was considered significant.

Results

The serum fibronectine concentrations in the tympanosclerosis group were statistically significantly less than those in the control group ($p = 0.031$). Results for the two groups are shown in Table I. In addition, serum fibronectine levels were statistically significant less in patients with stage III tympanosclerosis than in those with stages I and II (Table II).

TABLE I

PLASMA FIBRONECTINE LEVELS IN TYMPANOSCLEROSIS AND CONTROL GROUPS

Group	*n*	Mean (g/L)	SD (g/L)	SEM (g/L)
Tympanosclerosis	58	0.260	0.077	0.010
Control	49	0.370	0.146	0.020

SD = standard deviation; SEM = standard error of the mean

TABLE II

PLASMA FIBRONECTINE LEVELS FOR EACH TYMPANOSCLEROSIS STAGE

Stage	*n*	Mean (g/L)	SD (g/L)	SEM (g/L)	Min (g/L)	Max (g/L)
I	18	0.3755	0.0053	0.0125	0.3670	0.3840
II	29	0.2260	0.0085	0.0015	0.2120	0.2400
III	11	0.1800	0.0033	0.0010	0.1750	0.1850
Total	58	0.2606	0.0778	0.0102	0.1750	0.3840

SD = standard deviation; SEM = standard error of the mean; min = minimum; max = maximum

Definitive statistical results for each tympanosclerosis stage are shown in Table II. There were statistically significant differences between the serum fibronectine levels for tympanosclerosis stages I and II ($p = 0.0001$), stages I and III ($p = 0.0001$), and stages II and III ($p = 0.0001$). The highest fibronectine levels were found in stage I tympanosclerosis and the lowest in stage III. Figure 1 shows the means and standard deviations for the fibronectine levels of the three tympanosclerosis groups and the controls.

Discussion

In the present study, a statistically significant difference was noted between the plasma fibronectine levels of the tympanosclerosis and control groups. Furthermore, statistically significant differences were also found amongst the three tympanosclerosis stages, with the severest form having the lowest plasma fibronectine level.

It can be speculated that plasma fibronectine has a possible role in preventing tympanosclerosis

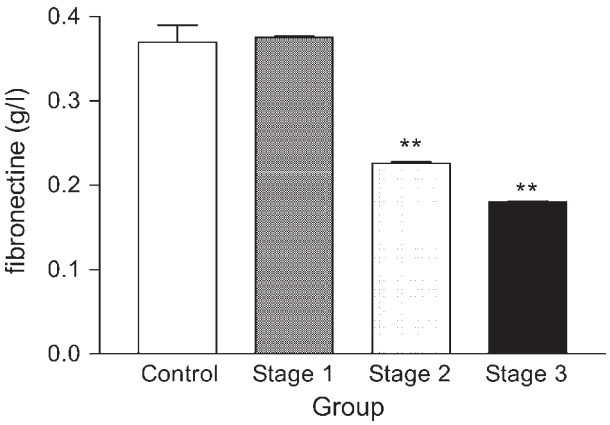

FIG. 1

Box plots of plasma fibronectine levels for each tympanosclerosis stage and for controls. **p < 0.05.

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development. Fibronectin and fibrin are known to concentrate at damaged sites immediately after injury, and simultaneously act together to form a provisional matrix that favours the development of granulation tissue.[6] During normal wound healing, keratinocytes migrate over this provisional matrix to close the wound, and plasma fibronectin is extravasated.[8] Plasma fibronectin appears to be utilised at healing wounds; thus, its concentration decreases during the early post-operative period. However, in our study groups, the plasma fibronectin level changed as the inflammation and tissue injury process progressed. This alteration may be due to a genetic control mechanism which is entirely independent of the pathology. In other words, the risk of tympanosclerosis may be higher in patients with lower plasma fibronectin levels.

A genetic influence[9,10] has been shown in the aetiopathogenesis of tympanosclerosis, along with possible roles for infection, inflammation,[11] immunological sensitivity,[12,13] oxidative stress,[14–16] mechanical injury, metabolic disturbance[17] and reduction of tympanic membrane mobility.[18] On the other hand, all these factors are related to tissue injury and inflammatory processes. However, to date, the most popular theory of tympanosclerosis pathogenesis is that tissue injury is followed by the release of abundant oxygen free radicals, leading to increased inflammation.[14–16]

Theoretically, in order to prevent tympanosclerosis, tissue injury must be diminished or self-healing events must be induced. Our previous studies focussed on diminishing tissue injury; we found increased fibroblast activity in rats in which tympanosclerosis had been prevented by using selenium, L-carnitine and N-acetylcysteine as free oxygen radical scavengers.[19–21] In the present study, however, we aimed to assess the second preventive mechanism, i.e. inducing self-healing events related to fibronectine.

- **Wound healing, epithelial regrowth and collagen synthesis are very important factors in the repair of the traumatised tympanic membrane**
- **The aim of this study was to determine the role of plasma fibronectine in influencing the severity of tympanosclerosis, as a step towards further understanding of the aetiopathogenesis of this condition**
- **Serum fibronectine levels were found to be statistically significantly lower in the tympanosclerosis group compared with the control group**
- **The results of this study suggest that fibronectine is an important substance in determining the development and severity of tympanosclerosis**

The most important process of tissue repair is synthesis of extracellular matrix proteins and collagen deposition.[22] Collagen and glycosaminoglycan are two of the most important ingredients in connective tissue healing. However, fibronectine is the key factor leading to the production and release of these two substances and subsequent fibroblast activation. Hence, plasma fibronectine concentration may be much more indicative of the healing response. For tissues that are incapable of repair, wound healing is accomplished by connective tissue formation, producing a scar and calcium deposition. Thus, parenchymal cells are replaced by scar tissue.[23] It has been reported that the integrity of the collagenous layer of the pars tensa is essential for the proper mechanical functioning of the tympanic membrane.[24,25] If the connective tissue bridge is not assembled properly, epithelial and mucosal growth may not occur. In the perforated tympanic membrane, fibronectine provides the necessary environment for cell migration and proliferation and acts as a stimulus for the re-epithelialisation process.[26] Interestingly, while topical fibronectine has been shown in various studies to enhance skin wound healing,[22,27] Hellström *et al.*[28] stated that exogenous fibronectine does not appear to enhance the healing rate in a tympanic membrane perforation model.

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