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ARAŞTIRMALAR / RESEARCH ARTICLES

Ultrastructural Comparison Between Tympanosclerosis and Atherosclerosis

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Timpanoskleroz ve ateroskleroz- ultrastrüktürel karşılaştırma

Amaç: Çalışmanın amacı, timpanoskleroz ile ateroskleroz arasındaki ultrastrüktürel benzerlikleri, ışık mikroskobu ve elektron mikroskobu incelemeleri ile ortaya koyarak, sklerotik dejenerasyona karşı olası bir genetik predispozisyonu araştırmaktır.

Yöntem: Kronik otitis media nedeniyle opere edilen 7 hastadan timpanosklerotik materyal ve koroner arteriyel by-pass greftleme operasyonu uygulanan 4 hastadan aterosklerotik materyal alınarak ışık ve elektron mikroskobu ile incelenmiştir.

Bulgular: Spesmenlerin incelenmesinde; her iki spesmen grubunda da görülen önemli ortak özellikler, kollajen fibrillerde artış, kalsifiye alanların varlığı, infeksiyon hücrelerinin ve fibroblastların varlığı idi.

Sonuç: Timpanoskleroz ve aterosklerozda, reaksiyon temel olarak aynı yolu izler. Her iki hastalıkta gözlenen ultrastrüktürel değişiklikler benzerdir ve her iki hastalıkta da cinsiyet farkı mevcuttur. Bu bulgular ile birlikte, aterosklerotik bireylerde timpanoskleroz görülme insidansının normal popülasyona göre fazla olması; tüm vücut dokularında sklerotik dejenerasyona karşı bir genetik-ailesel yatkınlığın varolabileceğini düşündürmektedir. Timpanoskleroz, bu genetik karakteristiğin orta kulaktaki görüntüsü olabilir.

Anahtar Sözcükler: Timpanoskleroz, ateroskleroz, kalsifikasyon, genetik yatkınlık.

Absctract

Objectives: The aim of this study is to identify the shared similar characteristics of tympanosclerosis and atherosclerosis on light and electron microscopy and to find out if there is a genetic predisposition between these two conditions.

Methods: Tympanosclerotic materials were taken from 7 patients who were operated on due to chronic otitis media and atherosclerotic materials were taken from 4 patients on whom coronary arterial bypass grafting operations had been performed. Tympanosclerotic and atherosclerotic specimens were examined under light and electron microscopy.

Results: In the examination of the specimens, the important common characteristics in both specimen groups were increase in collagen fibers, presence of calcified areas, increase of the infectious cells and especially the fibroblasts.

Conclusion: In tympanosclerosis and atherosclerosis, the basic mechanism of the reaction goes through the same routes. Both the ultrastructural similarities between atherosclerosis and tympanosclerosis as well as the sexual difference seen in each of the two diseases, in addition to the incidence of tympanosclerosis observed on atherosclerotic patients in comparison to the normal population, is indicating that the genetic-familial predisposition of sclerotic changes is shared by all tissues, and that tympanosclerosis must be the appearance of the same genetic characteristic in the middle ear.

Key Words: Tympanosclerosis, atherosclerosis, calcification, genetic predisposition.

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Introduction

Tympanosclerosis is a clinical entity appearing with the increase of collagen tissue, hyaline degeneration

and calcification in the tympanic membrane and mucosa of the middle ear.[1-4] Myringotomy incisions, application of ventilation tubes, infections, physical trauma, various chemical agents, immunologic reactions and local metabolic changes are blamed as the reason for this mucosal reaction to appear.[3-10]

Atherosclerosis is a disorder wherein the arterial walls become thicker and loose their elasticity, starting with localized lesions in the vascular endothelium. It is a slow advancing, progressive disease starting at childhood.[11-12] For the appearance of atherosclerosis, hyperlipidemia, hypertension, smoking, diabetes mellitus, obesity and familial predisposition and other genetic and habitual factors are being cited.[13-16]

Although they appear in two different tissues in the body, we can consider that there are many similarities between atherosclerosis and tympanosclerosis either from a physiopathological or from a histopathological point of view. In both conditions, it is not known exactly what starts the lesion principally. Although the progression of atherosclerosis speeds up with some risk factors, mainly the characteristics of being a chronic inflammation developing against irritation also appears similarity in tympanosclerosis. The fact that neither situation, seen as the last reaction of inflammation in connective tissue, appears in every individual subjected to the same risk, is making it considerably possible that genetic factors play a role in either atherosclerosis or tympanosclerosis.[9,11,17]

The fact that atherosclerotic disease is seen in persons not carrying the known risk factors, as well as the presence of persons who do not display atherosclerotic lesions clinically, although they possess more than one of the risk factors, is a well known situation. Also due to the fact that family predisposition is seen as the most important risk factor, we may think that atherosclerotic lesions basically have a genetic base.[13-14] This situation is an event which must be discussed from the point of importance of the risk factors. Sclerotic process is not seen in all cases with otitis media with effusion, acute or chronic otitis media and cholesteatoma.[2-3,10] It is speculated that chronic hypercholesterolemia, hypertension and smoking start the procedure of atherosclerosis by the way of the endothelial injury and increase in the permeability of endothelium. Nevertheless, the presence of healthy people subjected to major risk factors show that the reaction to risk factors of the different tissues may be different.[11-12]

In our previous studies, we observed that the incidence of healing with tympanosclerosis in children treated with tympanostomy tube insertion is higher in boys than girls.[18] And we also found the incidence of tympanosclerosis in the atherosclerotic patients higher than the normal population. By the way, another finding of the study is the higher incidence of tympanosclerosis in atherosclerotic patients who had ear infections in previous medical history than the non-atherosclerotic patients with previous ear infections.[19]

In this study, materials from atherosclerotic and tympanosclerotic tissues were studied by light and electron microscopes to find out the ultrastructural correlations of both conditions.

Materials and Methods

The materials for the ultrastructural study were taken from 7 patients who were operated on due to chronic otitis media, and 4 patients on whom coronary arterial bypass grafting operations had been performed. Of the tympanosclerotic patients whose ages were between 27 and 49, 4 were men and 3 were women. The length of the period of the disease was between 8 and 24 years. The sclerotic tissues were taken from the promontorial mucosa, from the oval window niche region and from the epitympanum. The tympanosclerotic tissues, were spotted under the microscope with their typical appearance, and were obtained totally or partially from the underlying mucosa or osseous tissue. The ages of the 4 male patients in whom the coronary obstructions were diagnosed through coronary angiography, were between 56 and 68 years. During the operation, the artery segment wherein the obstruction existed was removed together with the atheroma plaques. Immediately after all the materials were removed, they were divided in order to be examined under the light microscope and the electron microscope and were fixed. For the light microscope study, after the specimens were fixed for 24 hours in neutral buffered formaldehyde, they were taken into routine tissue observation. They were embedded into paraffin blocks, and dyed with Hematoxylin-Eosin and Van Gieson. The sclerotic specimens taken for the electron microscope, were split into 1 mm^3 pieces, and were fixed for a peri-

od of 2 hours at +4°C inside a 2% glutaraldehyde (pH=7.4; 0.1 M phosphate buffer). The secondary fixation was provided for a 1 hour period in 1% osmium tetroxide (OsO_4). The specimens were passed through a gradual ethyl alcohol series and after the process of being rendered transparent with toluene, were blocked with Epon. Sections of 1 micrometer thickness were taken from the Epon blocks with the ultramicrotome for light microscope examinations, were dyed with 1% toluidine blue and the required areas were investigated. Subsequently, thin sections were taken and examined under the Geol Transmission Electron Microscope.

Results

When the tympanosclerotic specimens here examined under the light and electron microscope, first finding was in all the areas, irregular but intense fibrotic tissue increase in thickened submucosa. The collagen fibril bundles which constitute this typical appearance were extending into different directions irregularly (Figure 1). Degenerative alterations and hyaline degeneration were spotted in the shape of calcium deposits in localities. Hyaline regions were metachromatically dyed with toluidine blue and were observed in almost all of the specimens (Figure 2). In affected areas, calcium deposits were seen as basophilic colorations. In some of the specimens, the upper epithelium was intact. Simple columnar and cuboidal epithelium was encountered. Epithelium was not present in the other specimens.

Vascularization was quite sparse in tympanosclerotic tissues. In one single specimen, bone tissue which was in contact with the collagen tissue, considered to belong to one of the middle ear ossicles was determined. Glands were scarcely observed in the samples (Figure 3). In some of the specimens glands were not observed at all. When the few cellular elements were examined in between the fibrotic tissue and around its vicinity, it was observed that the dominant cells were fibroblasts (Figure 4). The number of cells other than fibroblasts was few. It was seen that this few number of cells were polymorphonuclear leucocytes and macrophages. These cells had been located basically as heaps in between the fibrotic tissue bundles (Figure 5). Contact of these cell groups with the calcified and hyaline regions were not determined. It was seen that the fibroblasts were distributed widely both in between the connective tissue bundles and in all the other areas. Another important finding in the submucosal layer, was the presence of calcified regions. The calcium deposits, both in between and around the collagen fibers, as well as in hyaline regions, were widely distributed as basophilic colored areas and creating the basic degenerative modification of the tympanosclerotic specimens together with the fibrotic tissue increase (Figure 4).

Distinctive findings in tympanosclerotic specimens were determined to be thickening in the lamina propria layer, the presence of a small number of cellular elements, the increase of collagen fibrils and hyaline degeneration.

When the tympanosclerotic specimens are examined with a transmission electron microscope, a distinct increase became apparent in collagen fibrils. The collagen fibrils were randomly extended and superimposed in localities. They were joined in some areas in a loose manner and tightly in other areas. There were a large number of calcium deposits in between the collagen fibrils. The calcified material took the place of the collagen fibrils in various locations. Infectious cells were observed with the electron microscope and it was found that these cells did not carry a structural abnormality. The fibroblasts made up the actual cell group. It was seen that these cells were irregularly distributed (Figures 6 and 7).

Sections taken from the atheromatous plaque and the sclerotic artery wall were examined under the light and electron microscopes. When the atherosclerotic specimens were examined with the Hematoxylin-Eosin and Van Gieson first with the light microscope and subsequently with the electron microscope, similar to the tympanosclerotic specimens, it was seen that there was an advanced and irregular increase in the collagen tissue (Figures 8 and 9). Especially prominently in the section of the atheroma plate close to the arterial lumen, heaps of cell groups were observed between and around the collagen fibers (Figure 9). These cell groups made up of the macrophages, leucocytes and platelets, were observed in less intensity in the arterial walls. It was determined with the electron microscope that these cells were structurally normal. It was also

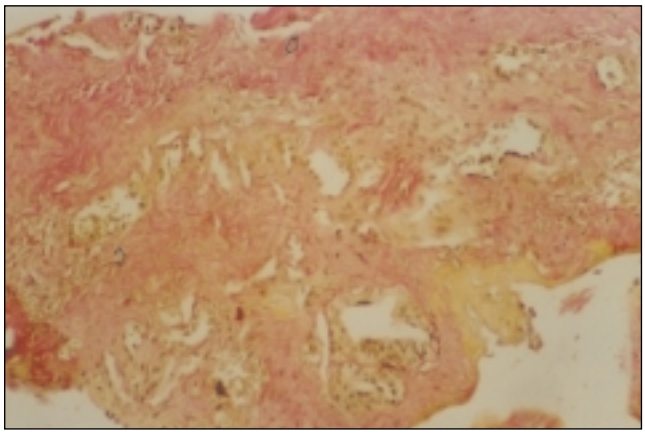

Figure 1. Tympanosclerotic tissue. Light microscopy (**Van Gieson x100**). **Arrows:** collagen fibrils.

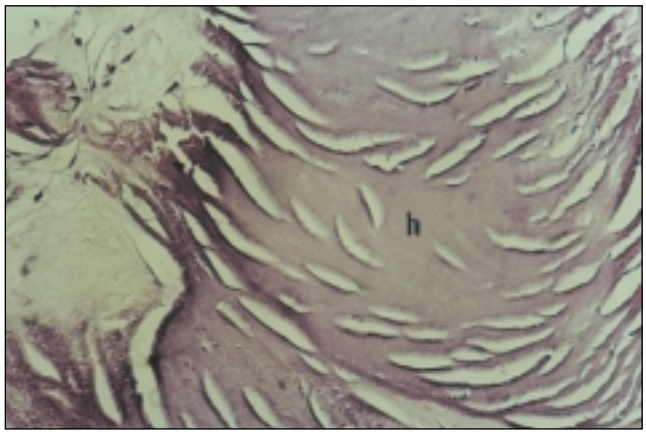

Figure 2. Tympanosclerotic tissue. Light microscopy (**HE x250**). **h:** hyaline regions.

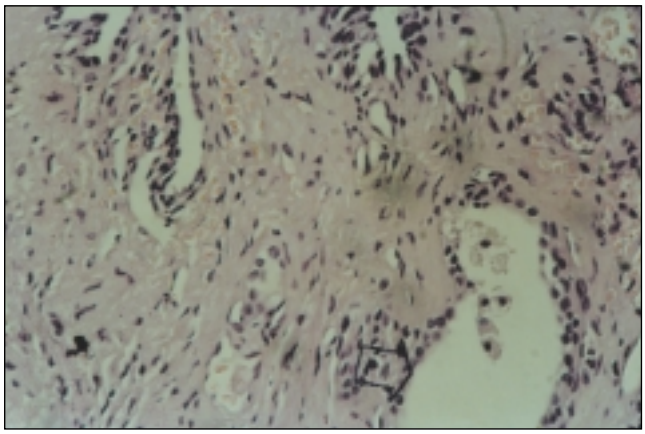

Figure 3. Tympanosclerotic tissue. Light microscopy (**HE x250**). **Arrow:** gland.

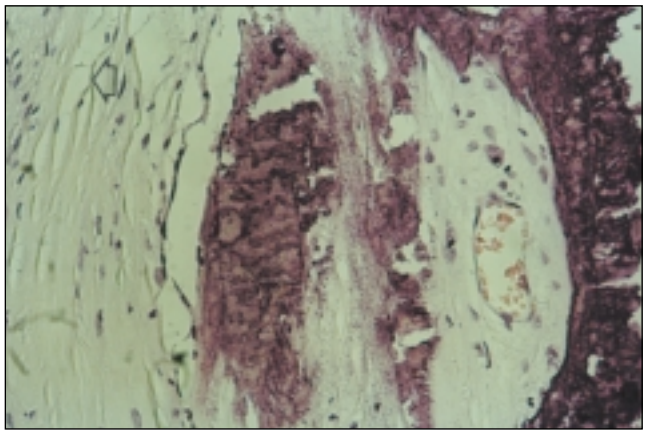

Figure 4. Tympanosclerotic tissue. Light microscopy (**HE x250**). **Arrow:** fibroblast, **c:** calcification.

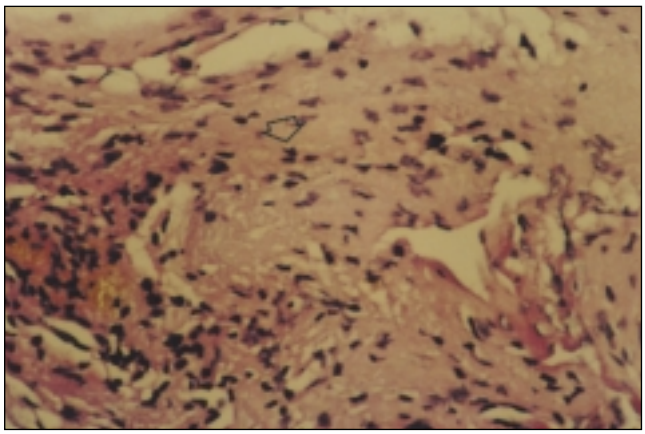

Figure 5. Tympanosclerotic tissue. Light microscopy (**HE x200**). **Arrow:** infectious cells.

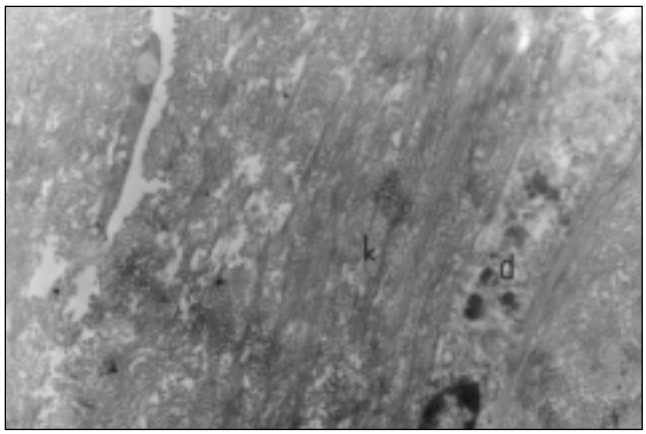

Figure 6. Tympanosclerotic tissue. Electron microscopy (**x8500**). **k:** collagenous fibrils, **d:** calcification.

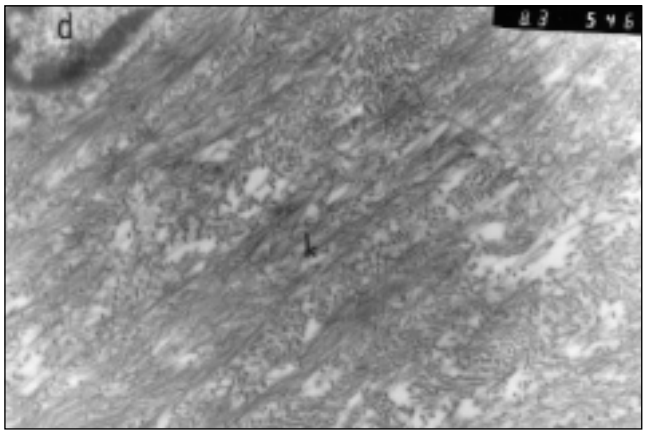

Figure 7. Tympanosclerotic tissue. Electron microscopy (**x14000**). **k:** collagenous fibrils, **d:** calcification.

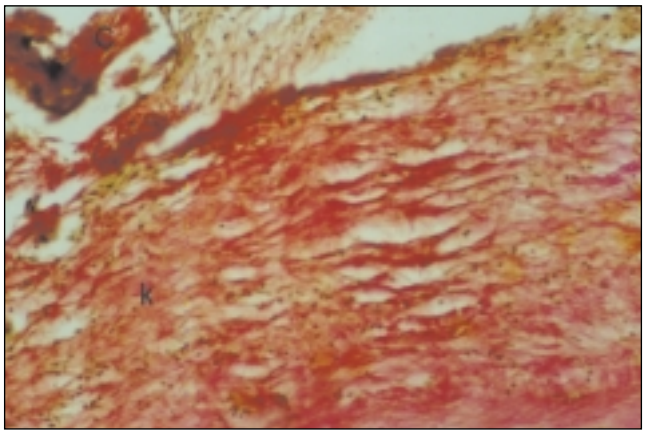

Figure 8. Atherosclerotic tissue. Light microscopy (**Van Gieson x100**). **c:** calcification, **k:** collagenous fibrils.

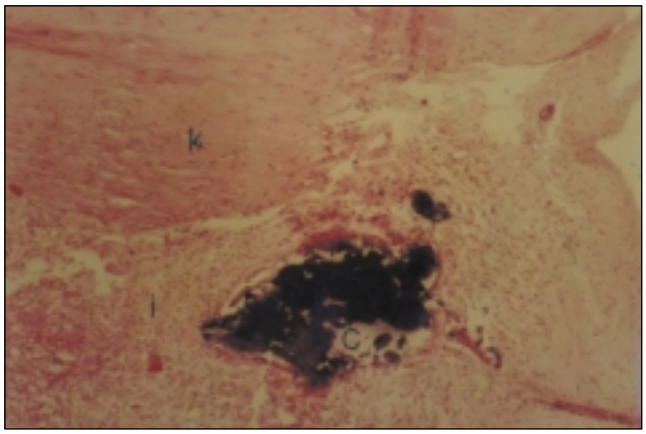

Figure 9. Atherosclerotic tissue. Light microscopy (**HE x40**). **c:** calcification, **k:** collagenous fibrils, **i:** infectious cells.

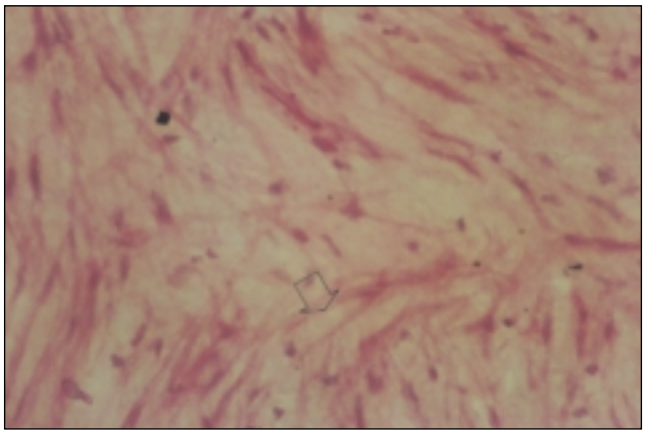

Figure 10. Atherosclerotic tissue. Light microscopy (**HE x100**). **Arrow:** fibroblast.

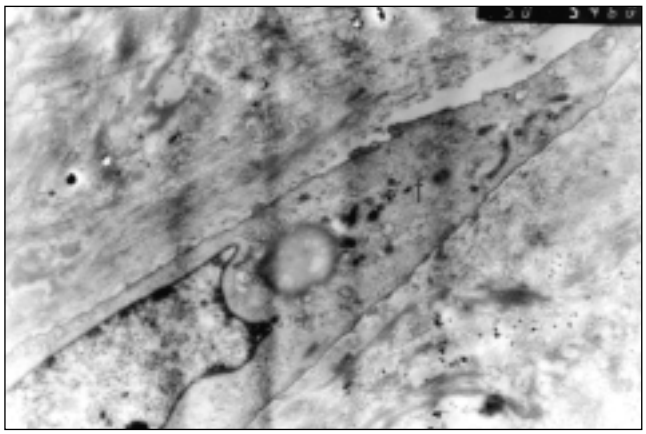

Figure 11. Atherosclerotic tissue. Electron microscopy (**x8500**). **f:** fibroblast.

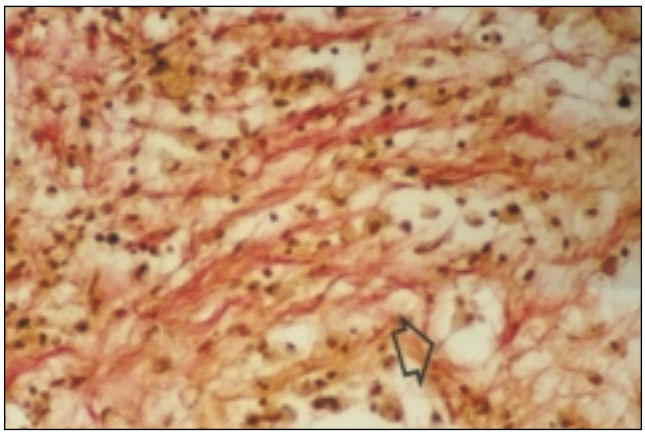

Figure 12. Atherosclerotic tissue. Light microscopy (**Van Gieson x40**). **Arrow:** histiosites.

determined that the cells in dominant numbers amongst the cells seen in atherosclerotic specimens were fibroblasts. Fibroblasts were distributed in all specimens and almost in all the areas among the collagen fibers widely (Figures 10 and 11). Another cell group was the foam-like histiocytes seen in focal regions (Figure 12). It was observed in the specimens that the smooth muscle tissues were excessively proliferated and were advancing towards the intima and the arterial lumen. The collagen fibrils, together with the proliferation of the smooth muscle cells, displayed another characteristic of the samples, the calcium deposits. The calcium deposits were widely distributed in between the collagen structures (Figure 13).

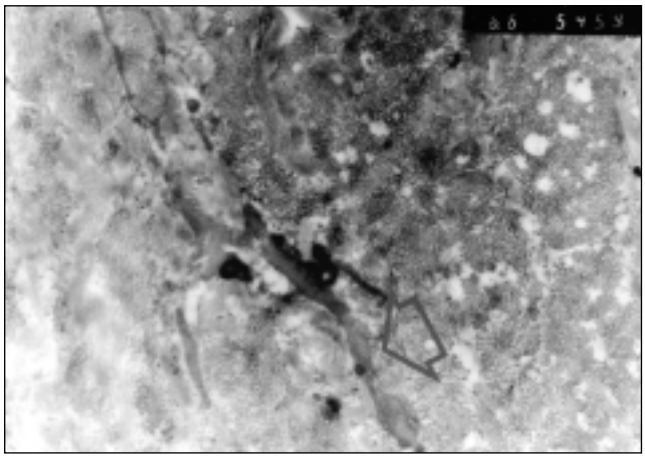

Figure 13. Atherosclerotic tissue. Electron microscopy (**x11000**). **Arrow:** calcification.

When the light and electron microscope examinations of the tympanosclerotic and atherosclerotic tissue samples were compared, the most important common characteristic in both specimen groups was a distinct increase in collagen fibers and the presence of calcified areas. The collagen fibers were irregularly distributed in both groups, and calcified deposits were observed in between and in the vicinity of the fibers. Again in both of the specimen groups, the increase of the infectious cells and especially of the fibroblasts was another common characteristic. The foam-like histiocytes and flat muscle cell groups observed in atherosclerotic specimens were not seen in the tympanosclerotic specimens.

Discussion

It is noteworthy that two different tissues gave a reaction over the same pathological route against different irritants and that this reaction mechanism is similar in both cases. The arterial wall and the tissue covering the middle ear cavity give the same connective tissue response against irritation. This response is characterized through an abnormal increase in the connective tissue, demonstrated through both the light microscope, as well as electron microscopy, in our tympanosclerotic and atherosclerotic specimens. Calcium ions are deposited over the mass of connective tissue created in both of the above tissues, thus completing the degeneration, and the tissue which is subjected -depending on the organ- creates different clinical findings. Patients whose movements of the middle ear ossicles are prevented due to sclerotic tissue are encountering hearing loss, whereas patients whose arterial flow is disrupted are encountering complaints related to acute or chronic circulation deficiencies. Even though the reasons bringing the patient to search for medical attention may be different, the pathological structure causing these symptoms in all of the patients is the same.[2-3,11-12]

The collagen fibrils which we observed in light microscope studies extending in all directions in both atherosclerosis as well as tympanosclerosis and the calcified regions between them, were also supported with electron microscope work. The lipids and the smooth muscle cells are the elements which participate secondary to the degeneration created in the tissue. The inflammation which starts when there is damage in the artery endothelium, from one side causes an increase in collagen by increasing the number of fibroblasts, from the other side stimulates the smooth muscle cells. The number of smooth muscle cells increase and these cells participate in the lesion, however this stimulation and participation takes place completely due to presence of these cells in the medium and from the primary pathological process. The smooth muscle cells and the lipid elements are not effective in the start of the lesion, they only play a role in the progression of the lesion. Since the creation of atherosclerotic tissues is a process which continues for long years, it is a rule for these cells to participate in the reaction. The fact that smooth muscle and lipid elements are not observed in our middle ear

specimens, supports our view that sclerotic tissue can be created without need for these structures and that these structures have secondary roles.

In both of our tissue examinations, the presence of infectious tissues is noteworthy even if they may be in smaller numbers. The proliferation of infectious tissues in the intimal layer of the artery, can be explained as "a reaction against damage". It is considered that the small thrombus constituted of thrombosis, macrophage and leukocytes, gather over the endothelial damage, become organized, and participate in the degenerative process. These cells which are the indication of small level inflammations in the arterial wall, also take place in the sclerotic structure which we meet similarly in the middle ear. The presence of these cells, may be indicating the contribution of the effusion in the middle ear or the infectious exudates in the creation of the tympanosclerosis. These cells which are distributed in both tissues among the increased connective tissue, are indicating that infectious response is taking place in the pathogenesis.[1,8,20-28]

Atherosclerosis and tympanosclerosis are the final appearances of a process which continues for long years and starts due to a reason such as trauma, endothelium damage and infection. This process continues with thickening, hyalinization and calcification as a result of the reaction given by the connective tissue to the damage.[1,3,26] This situation which we observed in both the arterial wall, the atheroma plaque and the middle ear with our light and electron microscope studies even if the factor causing the damage and the characteristics of the individuals subjected to it may be different, demonstrate that the reaction given by the tissues to any kind of reaction and the histopathological appearance of this reaction is fundamentally the same. Some specialized cell groups added to the structure of the tissues depending on the functions of the organs, are also affected from this process and participate in the degeneration. This participation, as it is completely a secondary effect due to their presence in the medium, it would be wrong to think that the pathological appearances in different tissues would be different.

The fact that the basic mechanism of the reaction goes through the same routes and the similar appearance of the final response, is indicating the effect of the risk factors to the tissue alterations. The risk factors of atherosclerosis have been described since many years with detailed clinical and experimental studies and took their place in the therapeutic protocols. Still carrying all the known risk factors, is not sufficient for the development of atherosclerosis. The indication of the presence of atherosclerotic disease in the family as the most important risk factor, is demonstrating that the disease has a genetic foundation. Diabetes mellitus, hypertension and hyperlipidemia are the other risks which are less important factors added on top of this genetic foundation.[11-16]

Both the ultrastructural similarities between atherosclerosis and tympanosclerosis, as well as the gender difference seen in each of the two diseases, in addition to the incidence of tympanosclerosis observed on atherosclerotic patients in comparison to the normal population, may indicate that the basis of these two entities, the genetic-familial predisposition is shared by all tissues, and that tympanosclerosis is the appearance of the same genetic characteristic in the middle ear. This subject must be supported with further studies, and it must be demonstrated whether tympanosclerosis could be accepted as the early indication of an atherosclerotic lesion which could develop in the advanced periods of human life.

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