■ BASIC INVESTIGATION

Connections Between Bone Marrow and Mesenchyme of the Middle Ear

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Objective: To investigate the presence of connections between the bone marrow of the ossicles and the mesenchyme that fills the future tympanic cavity.
Material and methods: Ninety temporal bones from embryos and foetuses were examined, selecting 15 aged between 20th to 30th weeks of development, to show connections between ossicle marrow and mesenchyme.
Results: The connections are transitory and appear in the malleus and the incus between 20th to 24th weeks of development, while in the stapes appear later, being between 24th to 28th weeks.
Conclusions: These connections may have an important role in the phagocytosis of the mesenchymal remains and join in the detritus elimination mechanisms produced during the regression.

Key words: Tympanic ossicles. Connections. Marrow-mesenchye.

Conexiones entre la médula ósea de los osículos y el mesénquima del oído medio
Objetivo: Determinar si existen conexiones entre la médula ósea de los osículos timpánicos y el mesénquima que rellena la futura cavidad timpánica.
Material y métodos: Se han examinado 90 huesos temporales pertenecientes a embriones y fetos, y se han seleccionado 15 de edades comprendidas entre las semanas 20 y 30 del desarrollo al presentar conexiones entre médula del osículo y el mesénquima.
Resultados: Las conexiones son de tipo transitorio y aparecen en el martillo y el yunque entre las semanas 20 y 24 de desarrollo, mientras que en el estribo se manifiestan posteriormente, entre las semanas 24 y 28.
Conclusiones: Estas conexiones pueden tener un papel importante en la fagocitosis de los restos mesenquimales y sumarse a los mecanismos de eliminación de los detritus producidos durante la regresión.

Palabras clave: Osículos timpánicos. Conexiones. Médula-mesénquima.

INTRODUCTION

In 1997 Linthicum[1] described the connections between the haematopoietic bone marrow of the temporal bone making up the wall of the tympanum and the remains of the mesenchyme that persist once pneumatization has taken place. These connections may contribute to protecting the middle ear from otitis media in the newborn.

The tympanic ossicles present a cavity-like structure during the foetal stage of development and the first years of postnatal life, with a large medullar sinus inside, with abundant haematopoietic cells[25].

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With this background information in mind, we have analyzed whether the tympanic ossicles, given their peculiar morphology might present marrow-mesenchyme connections during development.

MATERIAL AND METHOD

Ninety temporal bones belonging to embryos and foetuses have been reviewed and 15 have been selected with ages ranging from 20 to 30 weeks of development.

The specimens come from medical-legal autopsies of deceased foetuses from miscarriage, intrauterine death or that died at the time of birth.

In order to date the foetuses we have used the tables of O'Rahilly and Muller (1996) based on the relationships between different measurements (maximum length, head-to-heel length, biparietal diameter, abdominal circumference and head circumference) and body weight. These measurements were compared with the data

provided in the clinical history and the sonogram when available.

In the case of embryos and foetuses of less than 12 weeks of development, the entire head was fixed, whereas in the case of the older specimens, a meticulous dissection of the temporal bones *en bloc* was performed. All samples were fixed in 10% formaldehyde, decalcified using 2% nitric acid at a temperature of 25°C. The mean time of decalcification varied between 1 and 15 days, depending on the size and thickness of the specimen. After the decalcification process, the acid was eliminated by flushing with running water.

After dehydrating the samples in progressively more concentrated alcohols, they were embedded in paraffin, sliced with a Leitz microtome into 7 mm sections and serially stained following Martin's trichrome technique.

RESULTS

During ossification, the tympanic ossicles present connections between the mesenchyme that fills the cavum timpani and the haematopoietic tissue inside the cavity of the middle ear. We have observed these connections during the times at which the ossicles of the perichondral bone appear, during formation of the cavity of the middle ear, the endochondral bone and at the beginning of the formation of the trabecullar systems.

In the malleus, these connections have been observed in the head, at the level of its external cortical portion when it is being formed by means of perichondral ossification, in foetuses at 20 to 23 weeks of development, and disappear at 24 weeks when the perichondral bone trabeculae have consolidated by fusion (Figure 1).

Insofar as the body of the incus is considered, in its internal cortical fascicle, we have observed these connections in foetuses of the same developmental age as the ossicle previously described (Figure 2), that then also disappear at week 24. At the level of the stapes, we have been able to observe these connections in two areas, in the anterior and posterior crura and in the platina. They appear later in both locations, between weeks 24 and 28 of development, when signs of remodelling are observed, with the subsequent disappearance of the internal cortex in both branches (Figure 3A) and part of the platina, leaving only the tympanic bone (Figure 3B).

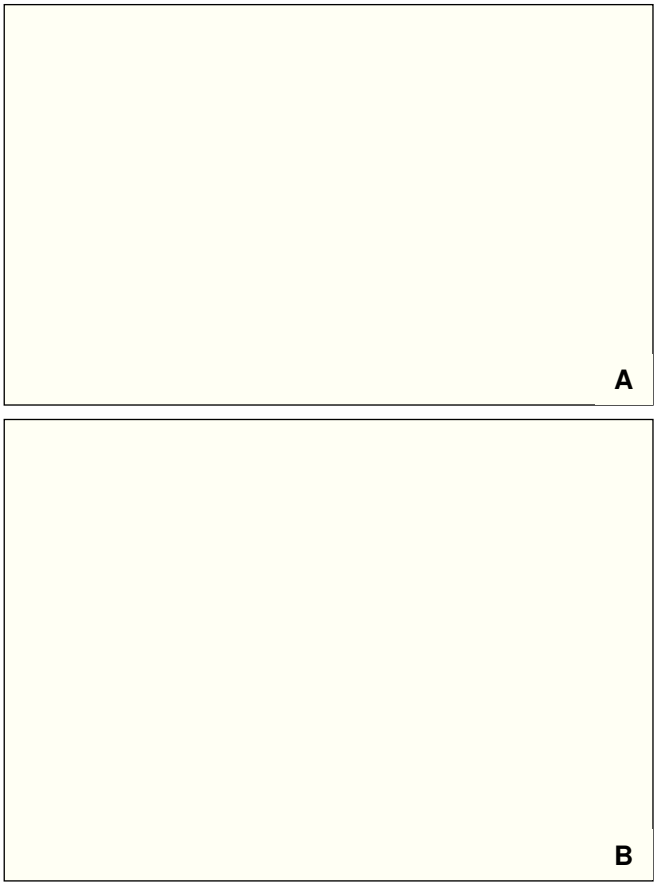

Figure 1. Twenty-three week human foetus (220 mm). Panoramic view and close-up. Note the presence of connections (arrow) between the cavity of the middle ear of the head of the malleus and mesenchyme of the cavum timpani at the level of the attic. (Martin's trichromic technique, □4 and □20.)

DISCUSSION

In 1997, Linthicum et al described the presence of connections between the bone marrow of the walls of the cavum timpani and the mesenchyme that fill in during the middle stages of development.

Said connections appear in foetuses at 21 weeks of development when the intrachondral bone and the primitive marrow are replaced by endochondral bone and mature haematopoietic marrow, connections that will be more and more frequent until birth. These connections will serve a protective role against acute ear infections in newborns, by invading the cavum timpani with white blood cells that come from the bone marrow, revealed by the use of anti-CD15 antibodies (marker for granulocytic leukocytes).

We have observed these connections temporarily in the tympanic ossicles. They are visible in the malleus and the incus starting between week 21 and 24 of development, whereas in the stapes, they appear later, between weeks 24 and 28. We point out that in the malleus and the incus they appear during the formation of perichondral bone and disappear once the cortical bones have formed; in the stapes, these connections appear in both branches as a result of the erosion and dissolving of the internal cortical bone, whereas in the platina, it is due to the disappearance of part of it.

We have seen connections coinciding with the disappearance of the mesenchyme, the phenomena of reabsorption and pneumatization of the cavum timpani. We have observed the disappearance of the mesenchyme as of 21 weeks of development, finalizing the pneumatization of the cavum timpani towards week 33, except for the epitympanum where it will take place 4 weeks later, as described by Buch et al[6] (1963), Rauchfuss[7] (1989) and Piza et al[8] (1998).

Reabsorption continues throughout childhood, since, as Takahara et al[9] (1986), Kasemsuwan et al[10] (1996) and Linthicum et al[1] (1997) observed, the amount of mesenchymal tissue that remains in the temporal bone forms a layer

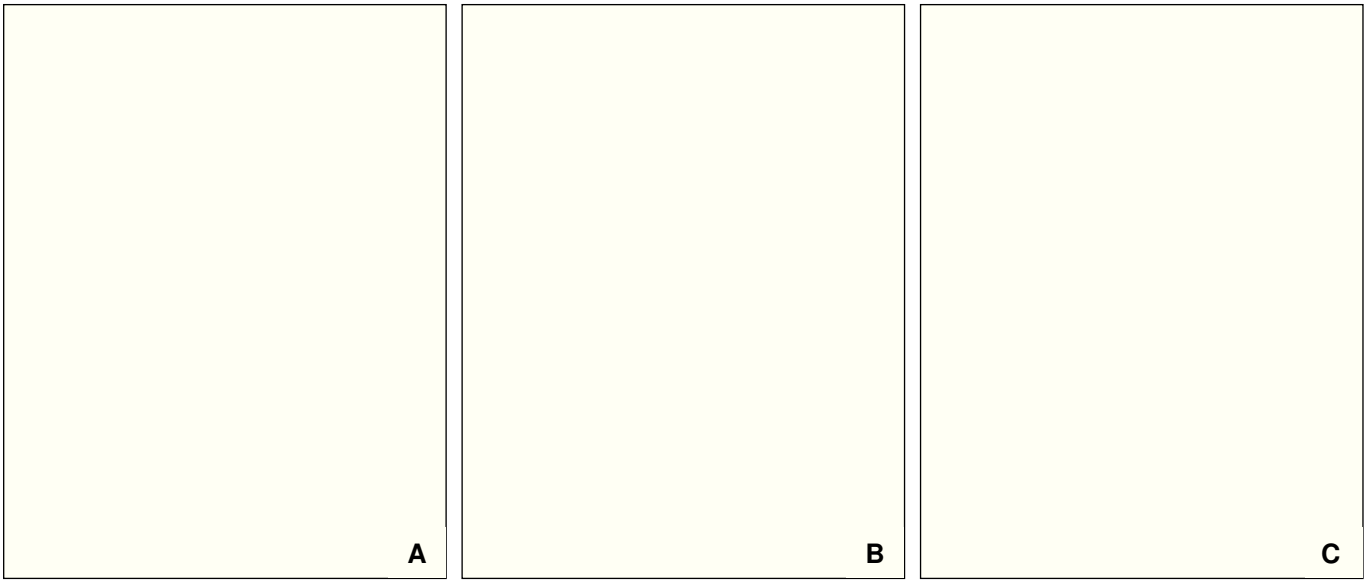

Figure 2. 22-week human foetus (212 mm); B: 23-week human foetus (220 mm), and C: 24-week human foetus (228 mm). In A and B, the medulla-mesenchyme connections can be seen (arrows) in the body of the incus and they disappear (C) when the cortices becomes consolidated. (Martin's trichromic technique, ×4.)

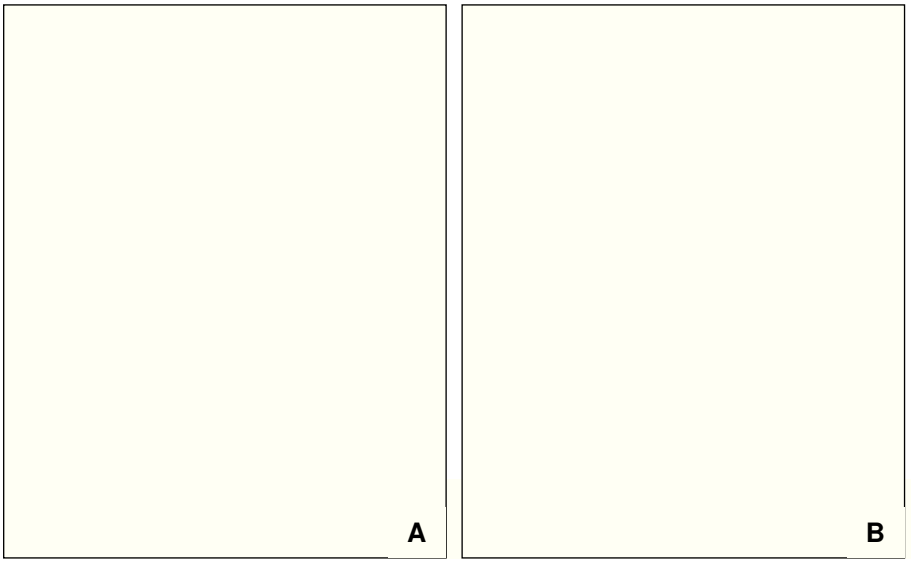

Figure 3. A: 25-week human foetus (242 mm). B: 24-week human foetus (228 mm). Note in A) that the branch of the stapes at the level of the internal cortex presents connections between the medulla and the mesenchyme (arrow) where haematopoietic tissue emerges towards the cavum timpani. There are signs of erosion in B) the platina where the medulla connects with the mesenchyme. (Martin's trichromic technique, ×4.)

persisting in the walls of the middle ear, thicker at the epitympanum, in the tympani sinus and in the remains of the antrum, and decreasing as the child grows older. We have observed the connections at the time the mesenchyme begins to disappear and therefore thought that they may play an important role in the phagocytosis of the mesenchymal remains and combine with the mechanisms for elimination of the dendrites produced during regression. This role has been corroborated by Palva and Ramsay[11] (2002), who state that the bone marrow cells contribute to destroying these remains.

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