## Cholesteatoma affected incus bone surface shows unusual iron-rich crystals, microvesicles and altered bone turnover

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Cholesteatoma is a noncancerous cystic lesion consisting in an abnormal growth of keratinizing squamous epithelium that invades the middle ear cavity. Due to its capacity of intracranial complications, cholesteatoma is cause of pediatric morbidity and death in countries with scarce hygiene and low possibility to access to advanced medical care (1). In order to understand cholesteatoma etiopathogenesis, we performed a SEM morphological analysis of 11 incus bones affected by cholesteatoma. Samples were fixed immediately upon recovery in 2.5% glutaraldehyde in PBS at 4°C for 48 h, then they were gently sonicated (to remove excess of keratinizing squamous epithelium, that would have prevented surface observation) and finally they were prepared with standard method for scanning electron microscopy observation. Five consecutive fields at 100X magnification aligned in 3 raws, the first one proximal and the last one distal to surgical removal point were analized. Images were obtained in secondary electron mode and in backscattering, bidimensional EDX analysis and mapping was also carried on. Incus bone surface analysis reveals the existence of an environment in which abnormal bone turnover takes place, in fact area of marked erosion were present together with areas of new bone formation. Resorbing bone surfaces with their characteristic lacunae were observed, resting surfaces (smooth and with collagen fibre bundles evident) were found and forming bone surface (collagen bundles in which calcium salts were just deposited) were also observed. Unusual flower-like apatite crystals rich in iron were uncovered in one sample. Iron presence may be due to cholesteatoma itself, being it made up of corneocytes that are iron-rich cells (2). Microvesicles of cellular origin, alone or clustered in groups or in about to fusing together, were found. Macrophages, lymphocytes osteoblast and osteoclast were observed in fully activated stage. The picture of these cell near to each other is the morphological representation of the complex cytochemical dialog existing among them. Taken all together our morphological results let us hypothesize that cholesteatoma creates an environment of chronic infection with peculiar biochemical characteristics that alters normal bone turnover on incus bone.

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## References

- [1] Kuo et al. (2015) Updates and knowledge gaps in cholesteatoma research. Biomed Res Int, 854024.
- [2] Milstone et al. (2012) Impact of epidermal desquamation on tissue stores of iron. J Dermatol Sci, 67(1), 9-14.

## Keywords -

Cholesteatoma; scanning electron microscopy; microvesicle; crystals.