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Identification of Tracheal Cartilage Canals in Camel

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Abstract:
Cartilage canals are vascular canals that commonly described in bone during embryonic development. They serve an important function in the ossification of the cartilage templates. Cartilage canals in permanent cartilage were uncommon. The current study identified the cartilage canal in the camel trachea. Tracheal samples were collected and processed for light microscopic examination. Cartilage canals were recognized in the camel trachea. Tracheal cartilage canals were confined to the peripheral region of the tracheal cartilage. Cartilage canals consisted of blood vessels and perivascular cells, some of which secrete cartilage matrix and transformed into chondrocytes. Thus, cartilage canals aim to provide the cartilage with chondrogenic potential cells to participate in the interstitial growth of the tracheal cartilage of camel. In conclusion, tracheal cartilage canals contain mesenchymal cells that participate in the interstitial growth of the tracheal cartilage. Future studies should investigate the role of the cartilage canals growth of the cartilage.
Keywords: Trachea, camel, cartilage canal, perivascular mesenchymal cells.

INTRODUCTION

Cartilage canals are vascular canals that commonly described in bone during embryonic development. They serve an important function in ossification of the cartilage templates. Cartilage canals are responsible for the formation of primary and secondary ossification centres during development of long bone. Two types of cartilage canals in embryonic and juvenile bone. Their description is based on the anatomical location in long bone. Each cartilage canal is formed in association with the vascular branch that innervates the long bone. Blood vessel gave rise to the main nutrient, the metaphyseal and the epiphyseal blood vessels (Delforge, 2002). Cartilage canals are associated with physeal and epiphyseal vasculature and their ramifications. The physeal branch penetrates the hypertrophic zone of the physeal growth plate directing toward the proliferating zone and forms the transphyseal cartilage canals. Epiphyseal cartilage canals developed in the epiphysis and direct toward the physis (SHAPIRO, 1998). Transphyseal cartilage canals from the primary ossification centre, while the epiphyseal cartilage canals are associated with the secondary ossification centre (Soliman, 2013).

Epiphyseal cartilage canals develop for the perichondrium as a connective tissue papillae (Gabner *et al.*, 2016). Cartilage canals contain blood capillaries of fenestrated type and the perivascular mesenchymal cells (Kobayashi *et al.*, 2008). Angiogenic factor, b-FGF (basic fibroblast growth factor) and matrix-degrading enzyme, MMP-9 (metalloproteinases gelatinase-
B) enhance vascular invasion into the chondroe piphysis and cartilage canal formation (Melton *et al.*). MMP-9, MMP-13 (collagenase-3), and MMP-14(membrane-type 1 metalloproteinase) are expressed in the perichondrial cells as well as the intrachondral canal .

Cartilage canals form vascular network in the epiphyseal cartilage and are implicated to provide the nutritional support of the developing cartilage and convey the osteogenic cells into the ossification centres (Brookes and Revell, 1998; Soliman, 2013). Cartilage canals are described in the developing cartilage of the irregular bone such as vertebrae (Uhthoff, 1990), flat bone including pelvic (Uhthoff, 1990), scapula (Nazir *et al.*, 2014) and cranial bones(Moss-Salentijn, 1975). Role of cartilage canal in bone formation is not limited to the long bone. They involve in osteogenesis and growth of the vertebral centra (Chandraraj and Briggs, 1988). Cartilage canals appear in the immature or developing stages and form a temporal vascular channel develops. Cartilage of adults is avascular tissue, receives nutrients from the vascular layer of the perichondrium. Existence of cartilage canals in the cartilage supporting the respiratory pathways of adult human and animal is uncommon. Cartilage canal has been documented in the adult skeleton such as hyaline cartilage of the laryngeal and costal cartilages(Brookes and Revell, 1998). The current study described for the first time existence of cartilage canals in cartilage of the camel trachea.

MATERIALS AND METHODS

Tracheal samples were obtained from 6 camels with different ages(adult age from 4-7 years) and processed for light microscopic examination. Sample processing was performed according to (Abd-Elhafeez and Soliman, 2016). Paraffin sections were stained by H&E (BANCROFT *et al.*, 2013) and combination of Crossman's modification and Wiegerts' resorcin fuchsin (Fath El-Bab, 1970). The stained sections were examined by Leitz Dialux 20 Microscope and Canon digital camera (Candison Powershot A95). Acridine Orange was used as a Fluorescent stain to detect lysosome activity. The staining procedure was performed according to (Abdel-Maksoud *et al.*, 2019; Hoff

et al., 1985). The stained sections were analyzed using a Leitz DM 2500 microscope with the external fluorescent unit Leica EL 6000.

RESULTS

Blood vessels derived from the perichondrium underneath the serosa. These Blood vessels penetrated the tracheal cartilage to form the cartilage canals (Fig. 2A, B). Tracheal cartilage canals located at multiple sites and were confined to the peripheral region of the tracheal cartilage (Figure 1A, B). Cartilage canals consisted of blood vessels and perivascular cells, some of which secrete cartilage matrix and transformed to chondrocytes. Thus, cartilage canals aim to provide the cartilage with chondrogenic potential cells to participate in cartilage growth of came (Figure 1C-E). Some perivascular cells tend to secret cartilage matrix that exhibited less affinity to Aniline blue and higher affinity for Acid fuchsin-orange G (Figure 2 A, B).

Acridine orange was used to identify the degrading cells in the cartilage canals. Lysosomes stained positive for Acridine orange and appeared yellow, orange or red (Figure 3A-C).

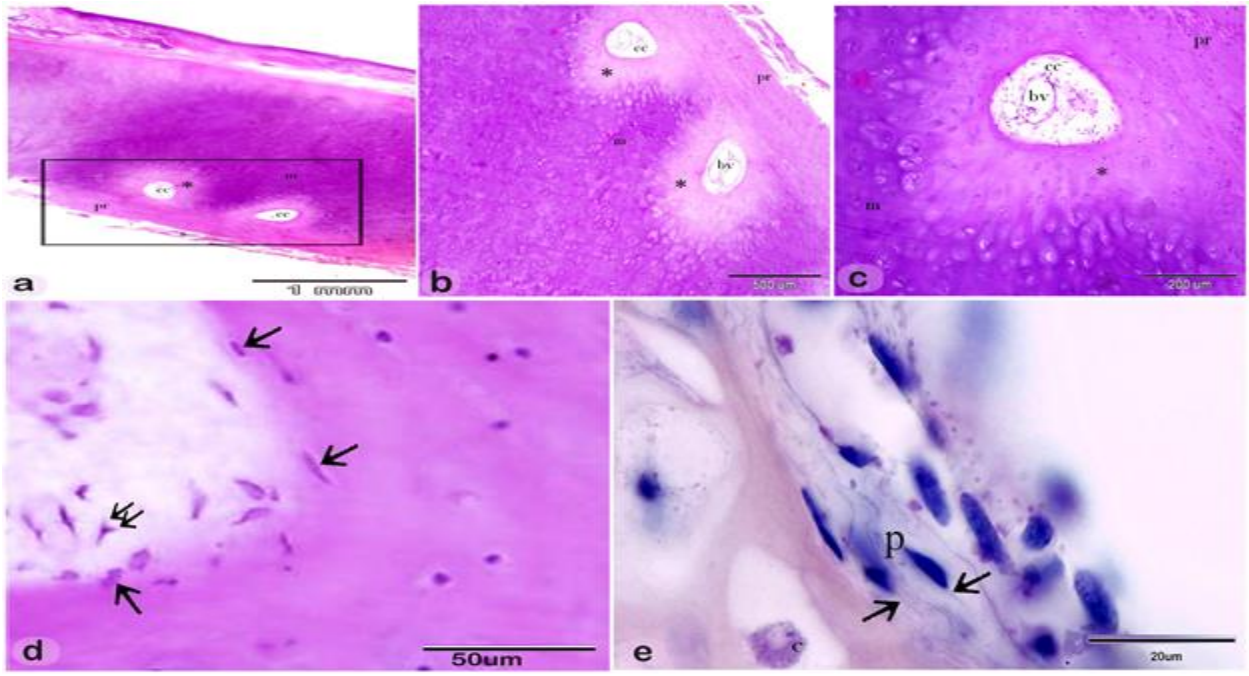

Fig. 1. Distribution of the tracheal cartilage canals.

Paraffin sections of the camel trachea stained by H&E. A, B: Cartilage canals (cc) in camel tracheal cartilage contained blood vessels and perivascular cells. Note perichondrium (pr), serosa (s) and cartilage matrix (m). Cartilage matrix that locates around the cartilage canals exhibited less affinity for hematoxylin (asterisks). C: high magnification of the cartilage canal. Note the perivascular cells had typical morphological features of mesnchymal cells that had a small cell body and cell processes (arrows). Note chondrocyte (c) had granular cytoplasm. D, E: Cartilage canals consisted of blood vessels (BV) and perivascular cells (P). perivascular cells (p) secrete cartilage matrix and transformed to chondrocytes (arrowhead).

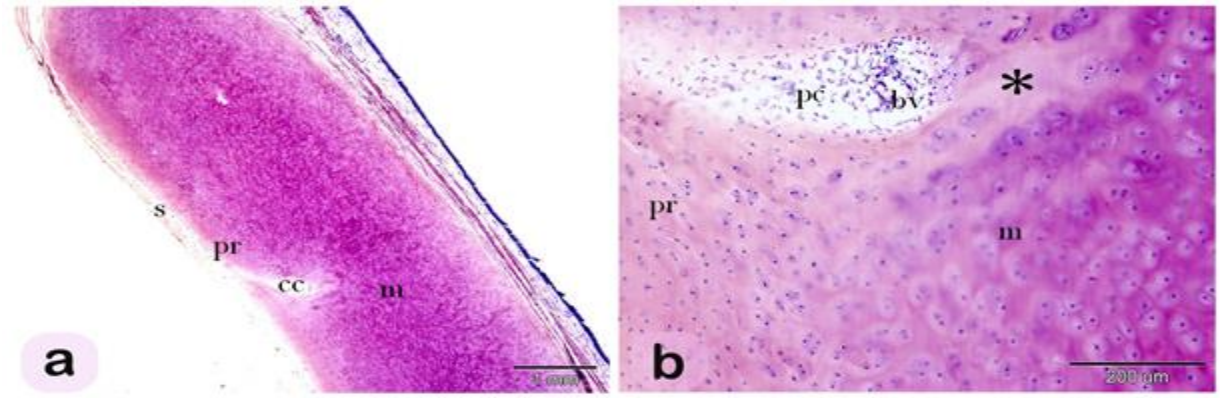

Fig. 2. Affinity of the tracheal cartilage canals for combined Crossman's modification and Wiegert's resorcin fuchsin.

Paraffin sections of the camel trachea stained by combined Crossman's modification and Wiegert's resorcin fuchsin stain. A, B: tracheal cartilage contained cartilage canals (cc) Cartilage canals continued with the perichondrium (pr) underneath the serosa (S). Cartilage canals contained blood vessels and perivascular cells (pc). Cartilage matrix (m) had strong affinity for Aniline blue except areas around cartilage canal had higher affinity for Acid fuchsin-orange G.

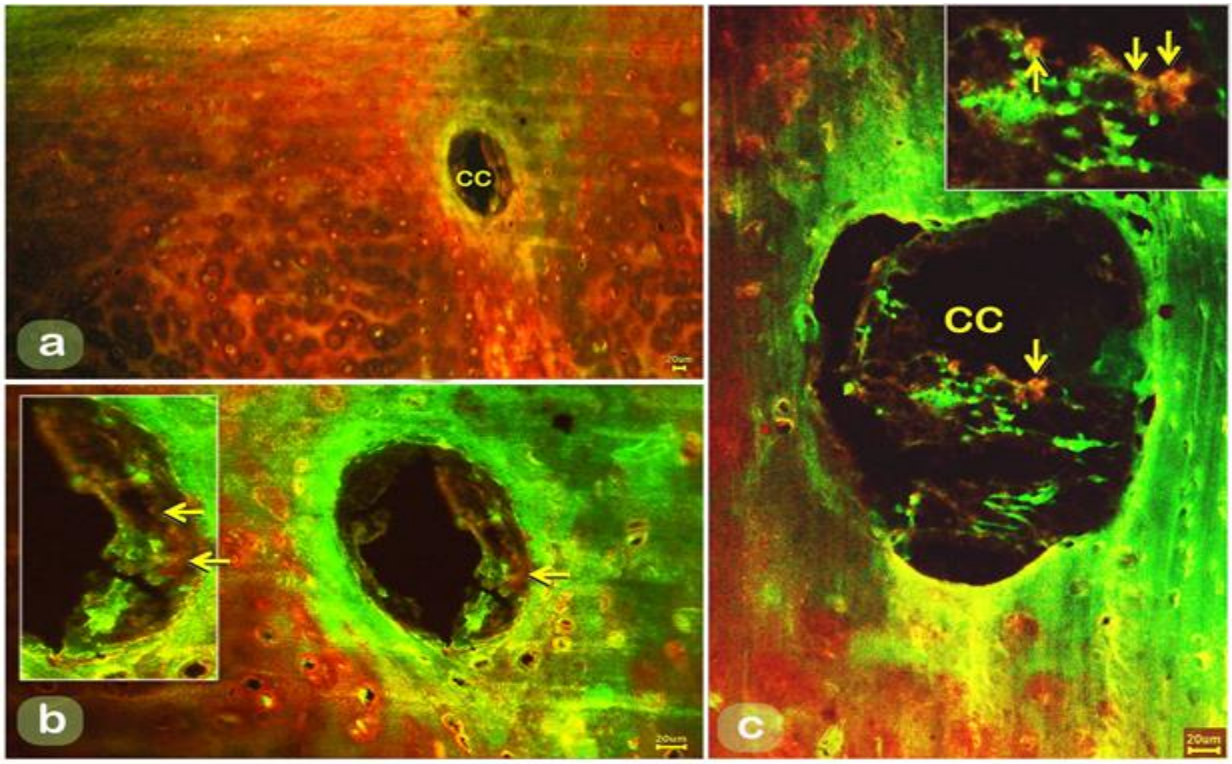

Fig. 3. Identification of lysosome-rich cells in cartilage canals using Acridine orange

Paraffin sections of the camel trachea stained by Acridine orange. Cartilage canals (cc) contained Lysosome-rich cells (arrows) that stained yellow to orange.

DISCUSSION

Cartilage canal is a temporal vascular structure, forms during bone development and plays an important role in ossification process. In the current study, cartilage canal was identified in the permanent hyaline cartilage of camels. They continued with the blood vessels of the peripheral perichondrium that located underneath the serosa. Tracheal cartilage canals located at multiple sites and were confined to the peripheral region of the tracheal cartilage. Cartilage canals have been described in the adult skeleton such as hyaline cartilage of the laryngeal and costal cartilages (Brookes and Revell, 1998). Thyroid cartilage is devoid of cartilage canals during childhood and early adolescence, while cartilage canals begin to develop about the end of the second decade in both sexes. Cartilage canals primordia is marked by detection of the IV collagen, belonging to blood vessels in cartilage canals. Thyroid cartilage canal is limited to the dorsal part of the thyroid cartilage of male and in the ventral half of female thyroid cartilage (Claassen *et al.*, 1996).

Tracheal cartilage canal contained mesenchymal cells that transformed to chondrocytes and secrete cartilage matrix. Thus, mesenchymal cells participate in interstitial growth of the tracheal cartilage. Tracheal cartilage didn't exhibit hypertrophic chondrocytes and signs of ossification. Contribution of mesenchymal cells in interstitial growth of cartilage has been previously described (Soliman, 2018; soliman and Abd-Elhafeez, 2018a; Soliman and Abd-Elhafeez, 2018b; Soliman *et al.*, 2017a; Soliman, 2014; SOLIMAN and ABD-ELHAFEEZ, 2014; Soliman and Abd-Elhafeez, 2016a; Soliman and Abd-Elhafeez, 2016b; Soliman *et al.*, 2017b; Soliman *et al.*, 2017c). Unlike the thyroid cartilage of humans, the authors suggested that in male, thyroid cartilage exhibits the differential sequences of chondrocytes that undergo hypertrophy. Thyroid cartilage canal has a critical role in cartilage mineralization and ossification while female thyroid cartilage has negative immunostaining for type IV collagen and not undergoes ossification. Function of the thyroid cartilage canal has been estimated based on the immunoreactivity for specific antibodies against type I, II, pro-III and IV collagen. The authors suggested that thyroid cartilage canal provides the cartilage by fibroblastic and chondrogenic cells (Claassen *et al.*, 1996). Epiphyseal cartilage canal contained type I collagen expressing osteogenic cells that form endochondral bone (Blumer *et al.*, 2004).

Acridine orange is a one of the cationic dyes. It is specific for detection of the low pH membranous compartments particularly lysosomes (Nadrigny *et al.*, 2007). We used Acridine orange to detect Lysosome-rich cells which contributed in degradation of cartilage matrix and formation of cartilage canals. Matrix metalloproteinase have a significant role in induction of cartilage canals formation including MMP-3, MMP-9 and mmp-13 (Alvarez *et al.*, 2005; Melton *et al.*, 2006; Soliman *et al.*, 2019).

The current study showed that the cartilage canals of camel trachea as a permanent structure. Fate of epiphyseal cartilage canal depends on the type of bone and development of the secondary ossification centre. Not all long bone develop a secondary ossification center. In this type of bone, cartilage canal undergo chondrifcation thus known as chondrifying canals. While long bone forms a secondary ossification center, cartilage canals associated with endochondral ossification (Soliman, 2013).

CONCLUSION

Cartilage canal could be a permanent vascular structure in adult skeleton. Tracheal cartilage canals located at peripheral regions of the tracheal cartilage of camels. They contain mesenchymal cells that participate in interstitial growth of the tracheal cartilage. Future studies should investigate role of the cartilage canals growth of the cartilage.

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ETHICAL APPROVAL

The method of consent and the animal work were approved by the ethics committee of Assiut University, Egypt.

CONFLICT OF INTEREST

All authors read and approved the final manuscript. The authors declare that they have no competing interests.

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