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Immunohistochemical investigation of nerve fiber presence and morphology in elderly cervical spine meniscoids

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Abstract

BACKGROUND CONTEXT: Innervation of anatomical structures is fundamental to their capacity to generate nociceptive impulses. Cervical spine meniscoids are hypothesized to be contributors to neck pain; however, their innervation is not comprehensively understood.
PURPOSE: This study aimed to examine the presence and morphology of nerve fibers within cervical spine meniscoids and adjacent joint capsules.
STUDY DESIGN: This is a cross-sectional study.
PATIENT SAMPLE: The sample consists of cervical hemispines of 12 embalmed cadavers (mean [standard deviation] age 82.9 [6.5] years, six female, six left). Either the right or the left half of the cervical spine (hemispine) of each cadaver was included in the sample. So six left sides and six right sides of the cadaver cervical spines made up the 12 hemispines that formed the sample.
METHODS: Cervical spine meniscoids and adjacent joint capsules were excised from lateral atlantoaxial and cervical zygapophyseal (C2–C3 to C6–C7) joints (n=67), then paraffin embedded. Meniscoids were sectioned sagittally (5 μm), slide mounted, and immunohistochemistry was performed using primary antibodies to neurofilament heavy (NF-H) and pan-neurofilament (Pan-NF) to identify nerve tissue. The study was supported by institutional graduate student funding. The authors have no conflicts of interest to declare.
RESULTS: Seventy-seven meniscoids (23 lateral atlantoaxial, 54 cervical zygapophyseal) were extracted and processed (154 sections in total). Sixty-four individual nerve fiber bundles were identified (26 NF-H positive, 38 Pan-NF positive) from 14 meniscoids. Nerves immunoreactive to both NF-H and Pan-NF were identified in 13 of 77 meniscoids (10 of 14 lateral atlantoaxial joint) from 11 joints (eight cadavers). Nerves were always located in joint capsules except three exclusively Pan-NF immunoreactive nerve fiber bundles from two adipose meniscoids.
CONCLUSIONS: The low nerve prevalence in elderly cervical spine meniscoids, with nerves only found in two adipose type meniscoids, suggests these structures may play a minimal role in cervical nociception generation in this demographic. The joint capsules, which were more frequently innervated, appear to be more likely generators of nociception in the elderly. Joint capsule nerves were mostly NF-H positive, indicating potential Aδ-fiber presence.

Keywords: Anatomy; Atlantoaxial joint; Cervical meniscoid; Cervical spine; Immunohistochemistry; Neck pain; Nerve; Nociception; Synovial fold; Zygapophyseal joint

FDA device/drug status: Not applicable.

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Introduction

Cervical spine meniscoids, also referred to as synovial folds, are invaginations of synovial membrane that lie between the articular surfaces of the lateral atlantoaxial and cervical zygapophyseal joints [1–7]. Meniscoid function has been hypothesized to improve the congruence of the articular surfaces and ensure the lubrication of the articular surfaces with synovial fluid [1,8]. These structures have been nominated as potential contributors to pain arising from spinal joints [1,8,9].

Cervical spine meniscoids are frequently found to be present in lateral atlantoaxial joints (100% [2,5,10]) and cervical zygapophyseal joints (77%–100% [1,3,4,11]). These structures are composed of adipose tissue, fibrous tissue, or a mix of both adipose and fibrous tissues [3–5,8]. The mechanism of meniscoid involvement in pathology remains unclear owing to the uncertainty about their innervation and therefore potential to generate nociceptive input [8], and it is unclear whether meniscoid composition has any relationship to the presence of innervation.

Few studies have examined the innervation of meniscoids in any region of the spine to determine their potential to generate nociceptive input [8,12,13]. In the lumbar spine, Giles and Harvey [14] demonstrated the presence of substance P (SP) reactive nerve fibers in lumbar zygapophyseal joint meniscoids, and Giles and Taylor [15] reported small, myelinated fibers coursing through lumbar spine meniscoid tissue; both findings are suggestive of nociceptive potential [16]. Gronblad et al. [17] however suggested that protein gene product 9.5 immunoreactive nerve fibers observed in lumbar spine meniscoids were more likely to be responsible for local vasoregulation, rather than nociception, owing to their proximity to blood vessels. In the cervical spine, there has only been one study investigating innervation of meniscoids. Inami et al. [18] described small nerve fibers immunoreactive to antibodies to SP, calcitonin gene-related peptide (CGRP), β-III tubulin, and protein gene product 9.5 in 10 cervical spine meniscoids excised from five patients (mean age 53 years) during laminoplasty for cervical spine pathology, suggestive of innervation by C-fibers with likely nociceptive and vasoregulatory functions [19–21].

A detailed understanding of the innervation of cervical spine meniscoids is important to effectively underpin evidence-based clinical management of cervical spine pain. The only previous investigation of cervical spine meniscoid innervation examined a small sample of meniscoids in surgical patients [18], and it is therefore unclear whether meniscoid innervation is similar in a population that does not have obvious cervical pathology that requires surgery. Further, the existence of different nerve *types* in cervical spine meniscoid innervation is unknown. Both large (Aδ-) and small (C-) nerve fibers serve nociceptive functions [22,23], and maladaptive function of both these nerve fiber types has been implicated in chronic pain pathophysiology as contributors to hyperalgesia and allodynia [24–26]. Understanding if and how meniscoids are innervated, and by what type of nerve, is important in regard to targeted therapeutic intervention for different mechanisms of nociception [25].

Given the potential clinical significance of cervical spine meniscoids [1,8] and the substantial social burden of musculoskeletal neck pain [27–29], the aim of this study was to explore the presence and morphology of innervation in cervical spine meniscoids to facilitate greater understanding of how these structures may contribute to spinal pain and pathology.

Materials and methods

Cervical spine meniscoids from elderly cadavers were removed and processed using immunohistochemistry to identify nerve presence and morphology; meniscoid morphology was determined using histology [4,5]. Antibody to neurofilament heavy (NF-H) was used to identify the presence of myelinated nerve fibers [30,31], consistent with the presence of Aδ- or Aβ-fibers that conduct information on pain and kinesthesia, respectively [32]. Antibody to neurofilament (Pan-NF) was used as a pan-axonal marker to identify any nerves present. Pan-neurofilament antibodies have previously been shown to identify both unmyelinated and myelinated nerve fibers [33–36].

Ethics statement

Ethical approval to undertake this study was granted by The University of Newcastle Human Research Ethics Committee. Bodies were bequeathed in accordance with the Human Tissue Act (2008) of New Zealand, and written informed consent was obtained from donors before death. The study was undertaken in accordance with the ethical standards of the Declaration of Helsinki. No conflicts of interest or associated biases were applicable to this study.

Dissection

The cervical spines of 12 cadavers were sourced from the University of Otago Department of Anatomy (mean [standard deviation] donor age 82.9 [6.5] years, six female). Cadavers were embalmed using ethanol- and water-based solutions as per departmental protocol, and were included for assessment if their lateral atlantoaxial and cervical zygapophyseal joints were intact; exclusion criterion included previous surgery to the region that was macroscopically apparent.

Overlying skin and muscles were removed from each specimen to expose the skull and vertebral column and allow the occiput to be disarticulated from the atlas. A dental burr (Beaver Ace Dental Micro Engine, Osada Electric Co. Ltd., Tokyo, Japan) was used to drill through the posterior arch of the atlas at either side of the posterior tubercle to allow removal of the posterior aspect of the bone. A bone saw was then used to cut along the longitudinal axis of the vertebral column through the laminae and pedicles, such that the cervical articular pillars could be separated from the vertebral bodies. Through a process of random allocation, either the left or the right articular pillar of each specimen was selected for inclusion in the study, such that an even number of left and right sides made up the sample.

Lateral atlantoaxial and cervical zygapophyseal (C2–C3 to C6–C7) joints were dissected using a surgical microscope (Op-Mi 6, Carl Zeiss, Jena, West Germany) by carefully