Case Report

Quadrilateral suspensory and straight sesamoidean ligament calcifying desmopathy in an Arabian mare

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Summary

A 5-year-old Arabian mare was referred for the investigation of recent behavioural change, generalised stiffness, and owner-reported polyuria and polydipsia. Clinical examination revealed severe pain associated with palpation along the entire length of the suspensory ligament (SL) and palmar soft tissues of the pastern regions of both forelimbs. Radiographs of the distal forelimbs demonstrated marked radiopaque striations within the straight sesamoidean ligaments (SSL), and less severe but similar changes in the regions of the SL branches. Ultrasonography of both distal forelimbs revealed multifocal, hyperechoic lesions within the SSLs. The SL bodies and branches were enlarged and had mixed echogenicity on ultrasound scan. The horse was euthanased at the owner's request and submitted for post-mortem examination. Relevant gross findings were restricted to the SLs and SSLs of all four limbs, all of which contained severe mineralisation and irregularly arranged fibres. Histological examination revealed severe, multifocal to coalescing, dystrophic mineralization with cartilaginous and osseous metaplasia, which suggested a primary calcifying desmopathy affecting all four SLs and SSLs. Clinical findings in the case reported here were similar to that of degenerative suspensory ligament desmitis (DSLD) previously reported by many authors, but diagnostic imaging and histopathological findings were significantly different.

Introduction

Mineralisation (ossification or calcification) is one of several possible histological features of desmopathy and tendinopathy (O'Brien *et al.* 2012), but is rarely reported in equines. It is usually associated with a previous episode of trauma, which may be iatrogenic in nature (O'Brien *et al.* 2012). In humans, pain is reported to be associated with tendon mineralisation in the rotator cuff and other sites (O'Brien *et al.* 2012), and we assume the same would be the case for *equidae*.

Degenerative suspensory ligament desmitis (DSLD) is a debilitating condition of *equidae*, over-represented in Peruvian Pasos, regardless of age, sex, or athletic function. Affected horses can have multi-limb pathology, and often have pronounced lameness and pain on palpation of the SLs (Mero and Pool 2002; Mero and Scarlett 2005; Miller and Juzwiak 2010; Xie *et al.* 2010). Horses with DSLD are reported to have limited response to treatment, including analgesics, stall confinement and rest (Mero and Pool 2002).

DSLD has also been reported in older Arabians, American Saddlebreds, Quarter Horses, Thoroughbreds, and some European breeds that are intensively worked or have sustained a prior suspensory desmitis (Halper *et al*. 2006). The condition in these breeds is usually only bilateral (Dyson and Genovese 2011b). A single

case report by Miller and Juzwiak (2010) also described an unusual case of bilateral hindlimb DSLD in a 3-month old Standardbred foal with an acute SL rupture.

Histopathology of DSLD-affected SLs typically shows degeneration and swelling of collagen bundles within the ligaments, which gradually coalesce and fibrose with disease progression (Mero and Scarlett 2005; Halper *et al.* 2006).

This case report details an unusual case of suspected quadrilateral primary calcifying quadrilateral suspensory and straight sesamoidean ligament desmopathy, clinically similar to DSLD, but with different ultrasonographic, radiological and histological findings due to marked calcification and ossification within the affected ligaments.

Case details

Case history and physical examination

A 5-year-old Arabian mare, kept at grass, was referred with a history of polyuria (PU), polydipsia (PD), a change in temperament, and stallion-like behaviour. Marked stiffness was exhibited after rising from recumbency. The referring veterinary surgeon suspected a granulosa-theca cell tumour of the left ovary and the mare was referred for further investigation and possible ovariectomy.

On admission, the mare had a body condition score of 2/5 (Carroll and Huntington 1988) and normal vital parameters. Trans-rectal ultrasonographic examination of the uterus and ovaries revealed normal sized ovaries with multiple follicles of up to 2.5cm in diameter. Anti-Mullerian hormone levels were mildly elevated at 3.2 ng/ml (reference range 0.22 – 2.94 ng/ml), but this result was considered clinically insignificant, given the atypical history and normal ultrasonographic findings. It was therefore deemed unlikely that the mare had a granulosa-theca cell tumour, and further investigations were performed.

Water intake was measured over a three-day period and was found to be within normal limits, despite PU and PD being reported. Routine haematology and serum biochemistry showed no significant abnormalities. The mare had a pottery gait at walk, was reluctant to trot and was bilaterally lame in front (right > left). She was noted to be lying down for extended periods of time and was reluctant to move around the stable. Digital pulses were within normal limits and the mare showed a negative response to hoof testers. Palpation of the forelimb SL bodies, SL branches and soft tissues in the palmar pastern regions elicited severe pain responses. It was extremely difficult to draw meaningful conclusions from hind limb palpation because ofthe horse ‘s temperament. There was a marked increase in lameness after distal limb flexion of all four limbs.

Radiographs

Standing lateromedial radiographs of the distal limbs revealed no osseous abnormalities; however, multiple radiopaque striations along the path of both SSLs in the forelimbs were visible. Less radiopaque changes were also noted in the area of the SL branches just proximal to the fetlock (**Fig 1**). Similar mineralisation was not seen in radiographs of the distal hindlimbs.

Ultrasound examination

A 12 mHz linear array transducer was used to examine the digital flexor tendons, SLs and SSLs of both forelimbs. There was mild enlargement of the SL bodies (maximal cross sectional area 1.4cm^2, reference range for Arabian horses 1.0 – 1.2cm^2) visible on transverse (**Fig 2a**), and longitudinal (**Fig 2b**) views. Numerous hypoechoic areas were present within the body of the SL with generalised hypoechogenicity and loss of fibre pattern involving large portions of the ligament (**Fig 2b**). Multifocal, hyperechoic foci were present in both left and right SSLs of the forelimbs (**Fig 3**).

Outcome

A non-steroidal anti-inflammatory drug (NSAID), meloxicam (Metacam 0.6 mg/kg SID PO)[1], was administered in an attempt to alleviate the significant musculoskeletal pain. No improvement was seen after five days and the owner elected to have the mare euthanased on humane grounds. The carcass was referred to the University of Nottingham Veterinary Pathology Service for post-mortem evaluation.

Post-mortem findings

Gross findings

Relevant gross findings were restricted to the SLs and SSLs of all four limbs. The affected ligaments had normal external surfaces, but changes could be seen on their cut surfaces. On transverse section, all four SLs (proximal part, body and branches) and SSLs felt gritty and showed irregularly arranged fibres. Multifocal pinpoint white foci of 0.5-1mm in diameter were seen on transverse incision, while the longitudinal incision revealed severe white striations (mineralization) within the ligaments (**Fig 4**). Examination of all 4 fetlock joints was unremarkable.

Histological examination

Representative tissue samples of liver, spleen, kidneys, ovaries, pituitary gland, SLs and SSLs were trimmed and routinely processed for histological examination. Relevant histological changes were observed primarily in the SLs and SSLs of all four limbs. Up to 90% of the SL and SSL were extensively mineralized, characterised by abundant, finely granular, extracellular deposits within the ligament fibres. These deposits

stained dark purple with haematoxylin and eosin (HE) and dark-brown to black with von Kossa stain, indicating mineralised granular deposition (**Fig 5**). Multifocally, islands of cartilaginous metaplasia with central bone formation (endochondral ossification) were also noted within the ligaments. The interfascicular connective tissue within the most severely affected areas showed minimal multifocal changes, characterized by low numbers of individual medium-sized arteries with intimal mineralization and mild multifocal vascular congestion of small blood vessels. Besides the above described mineral, cartilage and bone deposits, there was no evidence of any other extracellular matrix deposition (e.g. proteoglycans or fibrosis). Given the lack of inflammatory changes or evidence of trauma on any samples examined, the observed lesions were suspected to be of a degenerative nature.

Discussion

Ligament and tendon mineralisation may be due to calcification or ossification (O'Brien *et al*. 2012). The horse in this report had both abnormal calcium deposition and evidence of endochondral ossification in the SLs and SSLs. We believe that this type of severe quadrilateral mineralising desmopathy has not been described previously in equine literature. Whilst the mare described in this case showed many clinical similarities to DSLD, including multi-limb involvement, severe pain evoked by palpation of SLs, enlarged SLs, positive response to fetlock flexion tests, increased recumbency time, lameness, and reluctance to move (Mero and Pool 2002), diagnostic imaging and histopathological findings of the affected ligaments were different from the typical findings of DSLD (Mero and Pool 2002; Miller and Juzwiak 2010).

Aetiology of mineralisation of the SLs and SSLs in this case is unknown. The mare was a 5-year-old homebred pasture pet, and had not undertaken any significant athletic pursuit in her lifetime. Quadrilateral involvement and lack of inflammation seen on histopathological examination makes a traumatic aetiology unlikely, and a primary degenerative desmopathy is suspected.

Tendon mineralisation is reported to be a cause of pain and tendon weakness in both humans and equines (O'Brien *et al*. 2012; Dyson 2011a), and is associated with tendon failure, presumably because it impacts on the biomechanical function of the tendon (O'Brien *et al.* 2012; Dyson and Genovese 2011b). It was unclear how long the mineralisation process had been present, or how quickly it progressed, but the mare had been showing clinical signs for only 6 weeks. It would be possible that as she was turned out and not in regular work, that she may have had a level of undetected lameness for much longer than the reported time period. Diagnostic imaging and histopathological findings of the affected ligaments in this case were significantly different from the typical findings of DSLD (Mero and Pool 2002; Miller and Juzwiak 2010).

Ultrasonographic changes observed within a DSLD-affected SL include enlargement of the ligament and diffuse loss of echogenicity and fibre pattern (Mero and Scarlett 2005; Miller and Juzwiak 2010). In the case reported here, the SLs had a diffuse loss of echogenicity and fibre pattern with numerous focal hypoechoic areas within the ligaments. In addition, widespread hyperechoic foci were seen within the SSLs. Some of these foci created shadowing artifact, indicating mineralization of the ligaments.

Heterotopic ligament mineralisation is an uncommon feature of DLSD, where the hallmark histological findings are abnormal proteoglycan (PG) accumulation in SLs, and in other connective tissues including the superficial and deep digital flexor tendons, patellar and nuchal ligaments, aorta, coronary arteries and sclerae (Halper *et al*. 2006). An alternative aetiology was proposed by a more recent study by Schenkman *et al*. (2009), who concluded that abnormal PG deposition in DSLD-affected ligaments likely developed as a result of disease progression and was not the cause. Small foci of cartilage calcifications are occasionally found in the SLs of advanced cases of DSLD (Halper *et al.* 2006), but the SLs in the case reported here were extensively affected. The SL and SSLs of all limbs in this case were extensively mineralised with frequent cartilaginous metaplasia and islands of endochondral ossification; these changes appeared to be progressive and irreversible, and were associated with overt lameness and marked pain on palpation.

There was no evidence of abnormal PG deposition in the tendons, ligaments, or other anatomical structures examined histologically in this case report, and as such, this was dissimilar to previously described cases of DSLD. Given the range of changes observed within the affected ligaments, the term 'calcifying desmopathy' would best describe the case reported here.

Whilst unilateral or bilateral tendon injury is a very common disorder in horses, tendon or ligament mineralisation is a rare event. In contrast, tendon mineralisation is found relatively commonly (2.7 - 22% prevalence) after rotator cuff injury in human beings (Oliva *et al*. 2011). Other less commonly affected sites include the Achilles tendon, bicep brachii tendon, extensor pollicis longus tendon, quadriceps tendon, anterior cruciate ligament and medial collateral ligament (O'Brien *et al*. 2012). In humans, tendon mineralisation also arises after surgical trauma, but can also occur as a feature of a primary tendinopathy or desmopathy (O'Brien *et al*. 2012; Lafuente *et al*. 2009). In horses, tendon mineralisation is reported anecdotally after injection of corticosteroids either into the digital flexor tendon sheath (Dyosn 2011a) or directly into the tendons themselves (usually for treatment of core lesions). Several reports have also documented the development of biceps brachii tendon calcification or ossification in cases of chronic biceps brachii tendinitis in horses (Gillis and Vatistas 1997; Meagher *et al.* 1979). Seignour *et al*. (2011) reported

that there was an association between fibrosed, mineralized palmar or plantar distal digital annular ligament and chronic ligament injury. The horse in this case had no previous history or histological evidence of trauma or inflammation, and similarly no history of iatrogenic intervention that could have induced the changes seen at post-mortem.

A study by Baird and Kang (2009) demonstrated that NSAID administration reduced the development of approximately 60% of heterotopic ossification in traumatized human tissue. This indicated that inflammation had an important role in heterotopic ossification development. Other mechanisms for heterotopic ossification within tendons (without necessarily having pre-existing injury or inflammation) that are proposed in human medicine and could potentially be relevant to this case include tendon underuse, genetic factors, tissue hypoxia, hormonal, and endocrine disorders (O'Brien *et al*. 2012). All presumably affect bone morphogenetic protein (BMP) production within affected tendons (O'Brien *et al*. 2012).

Ingestion of plants containing toxic levels of vitamin D-like compounds, such as day jasmine (*Cestrum diurnum*) in Florida (Krook *et al.* 1975), or nightshade (*Solanum glaucophyllum*) in Argentina and Brazil (Worker and Carrillo 1967), were reported to cause hypercalcaemia and widespread metastatic tissue calcification in horses. The mare in this case report had normal blood calcium levels, and did not show any evidence of metastatic calcification in any of the organs and tissues examined histologically. The ligament abnormalities observed in this case could have been due to a toxic insult, but of course this cannot be ruled in or out definitively.

Given the severe and extensive changes seen at post-mortem and on histological examination, we believe that euthanasia was the correct decision for this case. Although the duration of attempted medical treatment was quite short, the mare was frequently recumbent and showed significant signs of distress after rising from recumbency; as such her quality of life was deemed to be poor by both the owner and ourselves. There is currently no cure for equine DSLD. It is a progressively debilitating disease with a poor prognosis, and many cases are similarly euthanased on humane grounds.

Manufacturer's address

[1]Boehringer Ingelheim Limited, Bracknell, Berkshire, United Kingdom

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