Glutamate, Cytokines & Matrix Metalloproteases are Elevated in Pathologic Posterior Tibial Tendons of Patients with Posterior Tibial Tendon Dysfunction

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Glutamate, Cytokines, and Matrix Metalloproteases are Elevated in Pathologic Posterior Tibial Tendons of Patients with Posterior Tibial Tendon Dysfunction

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My disclosure is in the Final AOFAS program book.

I have no potential conflicts with this presentation.
Posterior Tibial Tendon Dysfunction

- Posterior tibial tendon (PTT) function [1]
  - Most powerful inverter of the foot
  - Important dynamic stabilizer of the arch
- Insufficiency of PTT is painful
  - PTT dysfunction (PTTD), or adult-acquired flatfoot deformity
- ~3.3% of middle-aged women [2]

Anterior and posterior views of a patient with stage 2 PTTD. Note the loss of arch height, heel valgus, and the “too many toes” sign.

Images courtesy of Lew Schon, MD
Motivation & Objectives

- **Unknown etiology**
  - Tendon degeneration may be attributed to abnormal collagen, tenocytes, extracellular matrix turnover, vasculature, etc. [3]
    - Matrix metalloproteases (MMPs)
  - Inflammation and neurotransmitters produced in the tendon may contribute to pain symptoms [4,5,6,7].
    - Inflammatory cytokines, glutamate, substance P (SP), and calcitonin-gene-related-peptide (CGRP)
- Disease involvement of the PTT insertion is unclear

**OBJECTIVES**

1. To characterize the neurotransmitters, inflammatory cytokines and MMPs that may be the cause of pain in pathologic tendons in PTTD
2. Determine the involvement of PTT insertion
Sample Collection
- 21 patients (15 female, 6 males) undergoing flexor digitorum longus (FDL) tendon transfer for stage 2 PTTD that failed non-operative management
  - Average age: 64.3 yrs (range 53 to 76)
- Tissue samples collected intra-operatively as to-be-discarded surgical waste
  - Healthy FDL, PTT Insertion & Diseased PTT

Sample Processing
- Samples massed and incubated in DMEM media for 48 hours
- Media frozen at -80°C until analysis
- Tissue frozen sectioned for histology (8µm slices)

Cytokine, MMP & Glutamate Assays
- Cytokines and MMPs assayed using sandwich ELISAs
  - Cytokines: IL-1β, IL-6, IL-8, IL-10, IL-12p70, TNF-α, IFNγ
  - MMPs: MMP-1, MMP-2, MMP-3, MMP-9, MMP-10
- Glutamate concentrations determined by calorimetric assay

Histology
- IHC Targets: NMDAr1, SP, CGRP
- Hematoxylin & Eosin

Data Analysis
- Concentrations normalized by weight
- Differences amongst healthy FDL, diseased PTT and PTT insertion tested via repeated measures Friedman’s test (α = 0.05)
- Significant results further analyzed using Wilcoxon signed-rank post-hoc test with Bonferroni corrected α = 0.0167
Increased Inflammation in PTTD

- Diseased PTT and PTT insertions
- Significant cytokines:
  - IL-1β
  - IL-6
  - IL-8
  - IL-10
  - TNF-α
- Undetectable:
  - IL-12
  - IFNγ

**Notes:**

- $H = $ Healthy FDL
- $D = $ Diseased PTT
- $I = $ PTT Insertion
Increased Collagen Remodeling

- Diseased PTT and PTT Insertion
- Significant MMPs:
  - MMP-1
  - MMP-2
  - MMP-3
- Insignificant:
  - MMP-9
  - MMP-10
- Increased matrix turnover
  - Particularly of collagen types I to IV
- Elastin
- Laminin

*\[ p < 0.0001 \]

- \( H = \) Healthy FDL
- \( D = \) Diseased PTT
- \( I = \) PTT Insertion
**Increased Glutamate**

- **Diseased PTT** only
- **IHC** staining of 1 patient for NMDAr1 (20x).
  - Healthy FDL (A) stains negatively.
  - Diseased PTT (B) stains **positively** for NMDAr1
  - Hypercellularity
- May be contribute to pain symptoms

![Healthy FDL](image1)

![Diseased PTT](image2)

- *p ≤ 0.01
- **p < 0.001**

**Legend**
- H = Healthy FDL
- D = Diseased PTT
- I = PTT Insertion
Discussion

- Inflammatory cytokines, evidence of matrix remodeling and pain mediators are dramatically elevated in diseased PTT.
- PTT Insertion shows chronic degeneration *without* pain mediator expression.

**Ongoing & Future Work**
- Continue staining for NMDAr1, SP, CGRP, H&E and others.
- Associate cytokine production and neurotransmitter receptor staining to *patient reported outcomes*.

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References


