SOFT TISSUE CONTRACTURES IN CLUBFOOT: AN IMMUNOHISTOCHEMICAL STUDY

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SUMMARY

Introduction: To clarify the cytocontractile proteins of the medial, posterior and lateral capsular tissues of clubfoot, biopsy specimens were collected from 15 idiopathic clubfeet and studied with immunohistochemical method.

Material and Method: There were 7 boys and 3 girls with a mean age of 7.6 months (3 to 40). Five patients had bilateral and 5 had unilateral surgery, so the samples were received from 15 feet. No previous operations had been performed and all were diagnosed as idiopatic clubfoot. During the surgical procedure of complete subtalar release, samples from capsular tissues were collected from medial (talonavicular capsule), posterior (subtalar capsule) and lateral (calcaneocuboid capsule) sides. Monoclonal antibodies against cytocontractile proteins of vimentin, desmin and α-smooth muscle actin were used for immunohistochemical staining and each sample was assessed as either positive or negative.

Results: All specimens stained positively for vimentin and negatively for desmin. Eight specimens from medial, 3 from posterior and 3 from lateral side were stained positively for α-smooth muscle actin ($p<0.05$).

Discussion: Cells from medial capsule of clubfoot have more cytocontractile activity than cells from posterior and lateral capsules. But cells from all sides may play a role in clubfoot etiology and recurrences after the treatment.

Key Words: *Clubfoot, Capsule, Cytocontractile proteins, Immunohistochemistry.*

ÖZET

KONJENİTAL CLUBFOOT'DA YUMUŞAK DOKU KONTRAKTÜRÜ: İmmünohistokimyasal çalışma

Giriş: Konjenital clubfoot'lu ayakların medial, posterior ve lateralindeki kapsül dokularında sitokontraktil proteinlerin varlığını araştırmak için, 15 ayaktan alınan kapsül materyalleri immünohistokimyasal metod ile çalışıldı.

Gereç ve Yöntem: Ortalama yaşları 7.6 ay (3-40 ay) olan, 10 idiopatik konjenital clubfoot'lu çocuğun 15 ayağından örnekler alındı. Olguların 7'si erkek, 3'ü kızdı ve 5 olguda patoloji bilateraldi. Hiçbir ayağa daha önce ameliyat yapılmamıştı. Komplet subtalar gevşetme ameliyatı sırasında ayağın medial (talonaviküler kapsül), posterior (subtalar kapsül) ve lateral (kalkaneoküboid kapsül) yüzlerinden kapsül örnekleri alındı. Sitokontraktil proteinler olan vimentin, desmin ve α-düz kas aktin proteinlerini araştırmak için immünohistokimyasal yöntemle boyama uygulandı ve her örnek pozitif yada negatif olarak değer-lendirildi.

Bulgular: Tüm örnekler vimentin pozitif ve desmin negatif boyanma gösterdi. Ayak medialinden alınan 8, ayak posteriorundan alınan 3 ve ayak lateralinden alınan 3 kapsül örneği a-düz kas aktini ile pozitif boyanma gösterdi ($p<0.05$).

Tartışma: Konjenital clubfoot'da, medial kapsül hücreleri posterior ve lateral kapsül hücrelerine göre daha fazla sitokontraktil aktivite göstermekle beraber, ayağın tüm yüzlerindeki sitokontraktil aktivitenin, konjenital clubfoot etiyolojisinde ve deformitenin tekrarlamasında rolü olduğunu düşünüyoruz.

Anahtar Kelimeler: *Idiopatik, Clubfoot, Kapsül, Sitokontraktil proteinler,* İmmünohistokimya.

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INTRODUCTION

Although clubfoot is a common congenital deformity, the pathogenesis is controversial despite numerous hypothesis[1-4].

Several studies have implicated that the soft tissue structures on the medial side cause the deformity[3,5-11]. Hersh described the presence of a disc-like mass of fibrous tissue lying between the end of the navicular and the medial malleolus and recommended its excision[6]. Turco considered that the medial scar was caused by the posterior tibial tendon, talonavicular capsule, spring and deltoid ligaments[10]. Ippolito investigated specimens of fetal clubfoot histologically and described fibrous tissue penetrating muscle, fascia, ligament and the tendon sheath on the posterior and medial sides of the clubfoot and believed that retracting fibrosis causes the deformity[7]. Fukuhara et al. found myofibroblast-like cells and cells staining positive for desmin, a cytocontractile protein, in fetal clubfoot[5]. Zimny et al. used electron microscopy to study fascia from the medially contracted mass and found myofibroblast-like cells[11]. Sano et al. confirmed the presence of myofibroblast-like cells of the medial soft tissues along with vimentin and á-smooth muscle actin, cytocontractile proteins[9]. But, Khan et al. failed to reveal any myofibroblast-like cells in the clubfoot connective tissue[8].

To clarify the cytocontractile proteins of the medial, posterior and lateral connective tissue structures from clubfoot, biopsy specimens were studied with immunohistochemical method.

MATERIAL AND METHOD

During the surgical procedure of complete subtalar release, samples of capsular tissue were collected from medial (talonavicular capsule), posterior (subtalar capsule) and lateral (calcaneocuboid capsule) sides of the clubfoot were collected at the Department of Orthopaedic Surgery of University Hospital.

All patients had a history of failed conservative treatment of serial casting with to a maximum of 3 months. There were 7 boys and 3 girls with a mean age of 7.6 months (3 to 40). Five patients had bilateral and 5 had unilateral surgery. No previous surgery had been performed on these feet and all were diagnosed as idiopatic clubfoot.

The samples were fixed immediately in 10% neutralised formalin and embedded in paraffin. Sections 4 μm in thickness were cut, stained with haematoxylin and eosin, and assessed for the shape of the nucleus and the spatial arrangement of collagen fibres.

Monoclonal antibodies against vimentin (vimentin, Ab-2 (V9), MS-129-R7, NeoMarkers, Fremont, CA), desmin (desmin, Ab-1 (D33), MS-376-R7, NeoMarkers, Fremont, CA) and α-smooth muscle actin (actin smooth muscle, Ab-1 MS-113, NeoMarkers, Fremont, CA) were used for immunohistochemical staining. Sections were hydrated and then washed in TBS (5% M Tris-HCL Buffer). AEC (3% 3-amino, 9-ethylcarbazole) was applied and followed by counterstain with haematoxylin. Care was taken to examine only capsular tissue. Each sample was assessed as either positive or negative.

We used chi-square test to compare the results of the staining properties from medial, posterior and lateral sides of the clubfoot.

RESULTS

Forty-five specimens of 15 feet were examined by light microscopy. The capsular cells were arranged variying from regular to haphazard, and nuclei were spindle-shaped, plump or oval (Figure 1, Figure 2). The wavy pattern of the collagen fibres were sometimes clear (Figure 2).

Fifteen specimens from each sides with a total of 45 were examined immunohistochemically while specimens from all sides of the foot stained positively for vimentin (Figure 3) were negative for desmin. Eight specimens from medial, 3 from posterior and 3 from lateral were stained positively for α-smooth muscle actin (Figure 4) and

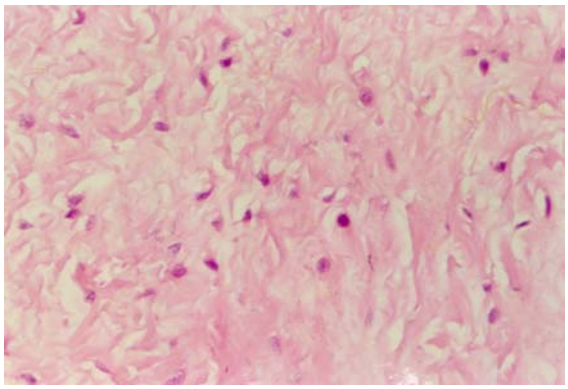

Figure 1: Capsular cells haphazardly arranged. The nuclei vary from spindle-shaped to plump. There is a disorganised arrangement of collagen fibres (haematoxylin and eosin x 40).

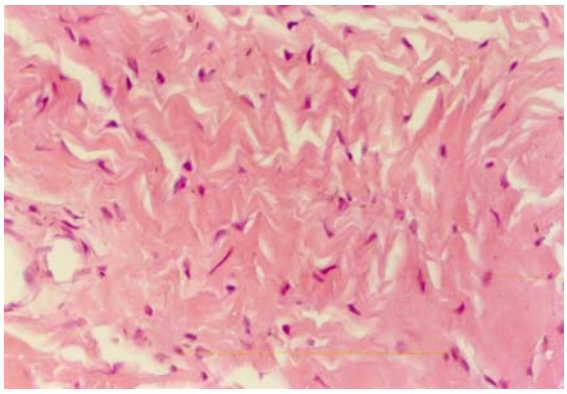

Figure 2: Capsular cells have a regular spatial arrangement and most nuclei are spindle-shaped or oval. There is a wavy pattern of the collagen fibres (haematoxylin and eosin x 40).

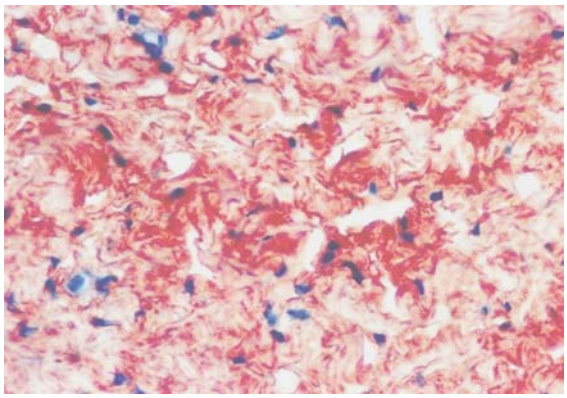

Figure 3: Positive staining for vimentin (x 40).

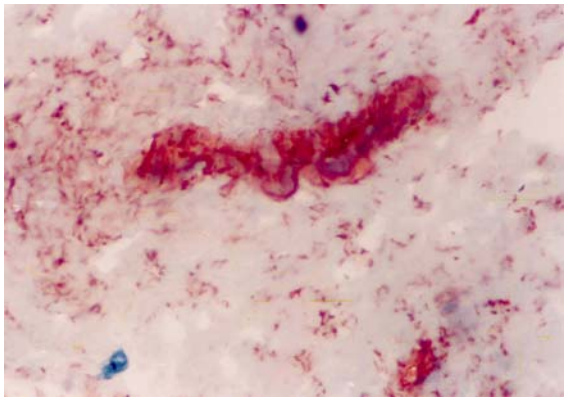

Figure 4: Positive staining for α-smooth muscle actin (x 40).

statistically significant difference was found between the medial and other sides ($p<0.05$) (Table I).

Myofibroblasts has four main phenotypes; expressing vimentin (V cell), co-expressing vimentin and desmin (VD cell), co-expressing vimentin and α-smooth muscle actin (VA cell), and co-expressing vimentin, desmin and α-smooth mucle actin (VAD cell) (12). VA/V cells ratio was 1.14 in medial side, 0.25 in posterior and 0.25 in lateral (Table II).

Table I
Comparison of the Immunohistochemical Staining Properties From Different Sides of Idiopatic Clubfoot

	Medial (n: 15)	Posterior (n: 15)	Lateral (n: 15)	P value
Vimentin	15	15	15	p>0.05
Desmin	0	0	0	p>0.05
α-smooth muscle actin	8	3	3	p<0.05

Table II
Cell Types of the Different Sides of Clubfoot

	VA cell	V cell	VA/V cell ratio
Medial	8	7	1.14
Posterior	3	12	0.25
Lateral	3	12	0.25

DISCUSSION

There is a strong evidence that localised soft-tissue contraction is involved in the pathogenesis of clubfoot[5,7,9,11]. Hersh, Turco and Ippolito have implicated the soft tissue structures on the medial side as the cause of clubfoot[6,7,10]. Fukuhara et al. collected ligamentous tissue from the medial side of the fetal clubfoot and found myofibroblast-like cells and cells staining positive for desmin, and sometimes positive for vimentin and α-smooth muscle actin, a cytocontractile proteins[5]. Similar findings were confirmed by Zimny et al[11]. They obtained samples from medial and lateral plantar fascia and found myofibroblast-like cells only on the medial side with electron microscopy (EM) and postulated that the degranulation of mast cells provided histamine that was the stimulus for the myofibroblast-like cell contraction[11]. Sano et al. collected ligamentous tissue from the medial side of the foot and found myofibroblast-like cells on the medial soft tissue with EM and cells staining positive for vimentin and a-smooth muscle actin[9]. But, Khan et al. collected specimens from medial and lateral capsule and failed to reveal any

myofibroblast-like cells in the clubfoot connective tissue structures with EM[8].

Myofibroblast have been described in Dupuytren's and Ledderhose's disease, infantile desmoid-type fibromatosis and in healing wound. All these authors postulated that myofibroblasts caused the contractile changes in these[13-15].

Cytocontractile proteins and myofibroblasts are present during soft-tissue contraction[12-15]. Myofibroblasts are known to have phenotypic characteristics of both fibroblasts and smooth muscle cells and immunohistochemistry has shown that they have differing combinations of the staining properties of desmin, vimentin and of a-smooth muscle actin[12-16].

Desmin is believed to be a marker of general differentiation of smooth and skeletal muscle, while vimentin is a marker of mesenchymal differentiation[17]. The contractile protein actin is more ubiquitous than desmin and vimentin[17].

Myofibroblast-like cells have been described only on the medial side of the clubfoot[5,9,11]. We examined the medial, posterior and lateral side capsular specimens of the clubfoot with immunohistochemical method and compared them. We did not find any study which compared the medial, posterior and lateral connective tissues of the clubfoot.

Fukuhara et al. studied clubfeet from fetuses and most of the specimens stained for desmin and some of them stained for vimentin[5]. Sano et al. reported that all specimens stained positive for vimentin and negative for desmin[9]. Like Sano et al., we studied clubfeet from children and determined that all specimens stained positive for vimentin and negative for desmin. Kocher et al. reported that cells which had migrated into the intima contained decreased amounts of desmin and increased amounts of vimentin at 15 days after endothelial injury of the rat aorta[16]. Shum and McFarlane observed desmin-positive cells in proliferative Dupuytren's nodules, which decreased significantly in fibrous phase of the disease[18]. The cytocontractile activity in the ligamentous tissue of clubfoot during intrauterine period may differ from that in the postnatal period. Increasing vimentin and decreasing desmin seem to reflect this change.

Fukuhara et al. and Sano et al. compared clubfoot and normal foot specimens, and found that staining for a- smooth muscle actin distributed equally in normal foot and clubfoot and they stressed that it cannot be used as a marker of a specific differentiation between normal foot and clubfoot[5,9]. In our study, 8 of 15 specimens from medial, 3 of 15 from posterior and 3 of 15 from lateral sides of the clubfeet stained positive for a-smooth muscle actin and there was a statistically difference between the medial side and other sides (Table I). During wound healing, VA cells reverts to a quiescent form, V cells when the wound is closed[12]. In our study, VA/V cells ratio was greater in medial side than posterior and lateral sides, in other words medial capsular cells had more cytocontractile activity than posterior and lateral capsular cells. According to us, a-smooth muscle actin can be used as a marker of metabolic activity level of the different sides of the clubfoot.

This study shows that the cells from the medial, posterior and lateral capsules of clubfoot have myofibroblastic characteristics. So that;

1. Cells from medial capsule of clubfoot have more cytocontractile activity than cells from posterior and lateral capsules.
2. Medial, posterior and lateral soft-tissue contractions may play a role in clubfoot etiology.
3. Some recurrences after conservative and surgical treatments may be attributed to the cytocontractile activity of cells from the medial, posterior and lateral capsules of clubfoot.

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