

Tuberous sclerosis: histological analysis with confocal laser scanning microscope of gingival angiofibromatosis

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Key words

Tuberous Sclerosis Complex • Confocal Laser Scanning Microscopy • Diffuse Angiofibromatosis • Diode Laser

Summary

Introduction. Tuberous sclerosis (TS) is an autosomal dominant neuro-cutaneous syndrome characterized by multiple hamartomas in various organs, especially on skin and central nervous system. The most common features of TS include facial angiofibromas, hypomelanotic cutaneous macules, shagreen patches in the lumbar area, cerebral cortical tubers, sub-ependymal nodules, sub-ependymal giant cell astrocytomas, cardiac rhabdomyomas, and renal angiomyolipomas. Frequently oral manifestations such as fibrous hyperplasia, angiofibromas and dental enamel pitting are also observed.

The aim of this case report was to describe the histological aspects of oral diffuse hyperplastic angiofibromatosis, never reported in the English literature and analyzed by Confocal Laser Scanning Microscope (CLSM), and to highlight the surgical implications of these aspects such as use of Diode Laser.

Case report. A 14-years-old female patient with TS diagnosis came to our attention for diffuse gingival hyperplasia on the mandible. Clinical examination highlighted epidermal hamartomas on the whole body, especially on the face and scalp.

Pathologic hyperplastic tissue was removed by pulsed diode laser at the power of 5-6W, and the surgical samples were sent for conventional and CLSM histopathological examination. After laser excision, wounds healed quickly without complications. At CLSM examination collagen fibres, showing intense fluorescence and with variable spatial orientation, and variably sized blood vessels were noticed suggesting the diagnosis of gingival angiofibromatosis, a still unreported finding in TS patients.

Conclusions. CLSM analysis allows to highlight some unusual histopathological features of TS; diode laser is very effective for the treatment of gingival angiofibromatosis.

Introduction

Tuberous sclerosis (TS), also known as Epiloia or Bourneville-Brissaud syndrome, is a rare multisystemic disease characterized by hamartomas in various organs, mainly affecting skin and central nervous system¹. Most studies suggest that the prevalence of TS is 1 in 6000-10000 new-borns².

TS develops as an abnormal growth of ectodermal and mesodermal cells, producing benign tumours of the head, heart, brain and kidneys, such as sub-ependymal giant cell astrocytoma, orofacial angiofibroma, hypomelanotic macules, periungueal fibromas, epidermal hamartomas and renal angiomyolipomas. The classical clinical triad consists of angiofibromas, epilepsy and de-

velopmental delay³, and the most common causes of mortality and morbidity are renal and neurological complications¹.

Oral manifestations include gingival fibromas and angiofibromas, affecting about 46% of adults, and are rarely found in children. They are fleshy or erythematous well-delimited lesions, mainly located on the anterior gums. Furthermore, enamel pits can also be present. The histological aspects of these oral manifestations have not been fully characterized as yet².

The aim of this report was to describe the pathogenesis and the histological aspects of a new TS feature, diffuse oral hyperplastic angiofibromatosis, analyzed by Confocal Laser Scanning Microscope (CLSM) and never reported in the English literature, and to highlight the

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surgical implications of these aspects such as the use of Diode Laser.

Case report

A 14-years-old female patient, who had been previously diagnosed with TS at 7 months of age, was referred to the Complex Operating Unit of Odontostomatology of the University of Bari. The diagnosis of sporadic TS had been formulated based on the patient's clinical history showing seizures, mental retardation, and cardiac anomalies. Phenobarbital, Levetiracetam and Rufinamide were administered to control epileptic seizures.

General clinical examination highlighted epidermal hamartomas on the whole body, especially on the forehead and scalp (Fig. 1), while intra-oral examination showed poor oral hygiene and diffuse mandibular gingival enlargement; the hyperplastic gingival tissue involved not only anterior sector, as generally reported in literature, but the molar region as well. The hyperplastic gums appeared pale pink, and covered the corresponding teeth until the occlusal surface or the incisal margins. Orthopantomographic X-rays showed a radiolucent lesion in the region of 3.6 (Fig. 2).

Pathologic hyperplastic tissue removal was made under general anaesthesia with concurrent local infiltration of mepivacaine, using a diode laser in pulsed mode (t-on 300ms/ t-off 400ms), at the power of 5-6W; no sutures were applied on the treated areas (Fig. 3). The surgical samples were formalin-fixed, paraffin-embedded, stained with hematoxylin-eosin and Pricosirius red, and examined using a Nikon Eclipse E600 microscope (Nikon Corporation, Tokyo – Japan), equipped with Argon-ion and Helio-Neon lasers, emitting at 488- and 543 nm wavelengths, which allows both optical and confocal laser scanning analyses. The Nikon EZ C1 software (Nikon Corporation, ver. 2.10 Coord Automatisering) was used for bi-dimensional image processing.

The post-operative course was uneventful and gingivectomy allowed for better oral hygiene and prevented

Fig. 1. Epidermal hamartomas on the forehead: typical cutaneous lesions with fibrous and vascular components.



Fig. 2. Diffuse Angiofibromatosis: pre-operative clinical aspect associated with inadequate oral hygiene.



Fig. 3. Gingivectomy with Diode Laser: post-operative clinical aspect of treated areas.

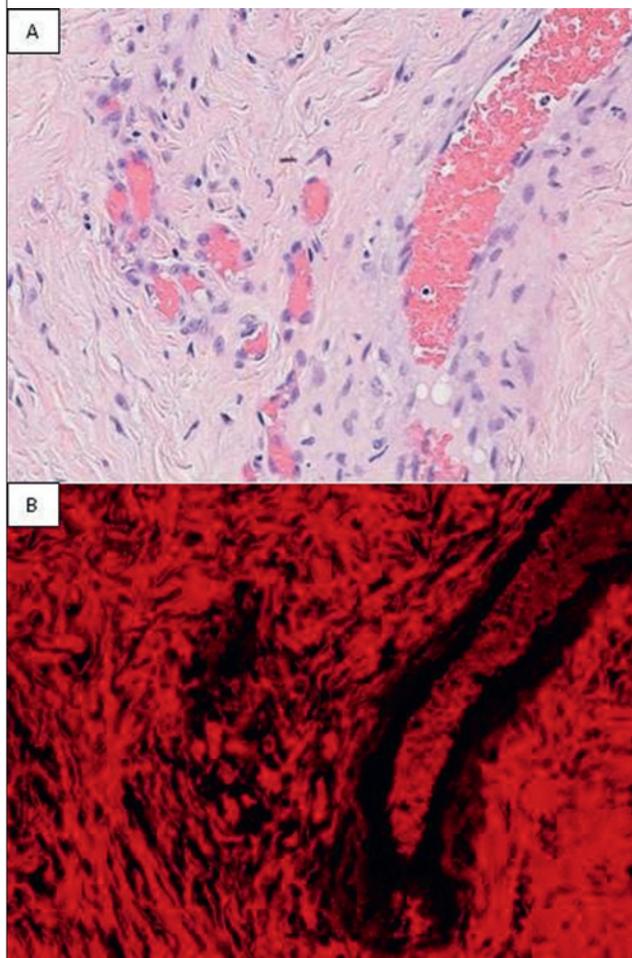


complications such as bleeding, dental inclusions and retentions.

Conventional histological examination showed thickened and acanthotic epithelium with elongated rete ridges, densely packed, whirly collagen fibres, fibroblasts, and variably sized vascular structures, without chronic inflammatory cells. At CLSM examination, the collagen fibers, showing intense fluorescence, also manifested variable spatial orientation, due to cross-links among the bundles, typical of fibromatosis. Also, variably sized blood vessels and large and polygonal interstitial cells displayed fluorescence of lower intensity were noticed. The vascular component consisted of small groups of venous-like structures, frequently showing dilated lumina, thin walls and plump endothelial lining (Fig. 4). These findings were consistent with periodontal angiofibromatosis.

The parents of the patient released informed consent for the use of such data for scientific publication, and on di-

Fig. 4. (A) The vascular component consisted of small groups of venous-like structures, frequently showing dilated lumina, thin walls and plump endothelial lining. (B) Confocal Scanning Laser Microscopy: collagen fibers, showing intense fluorescence, manifested variable spatial orientation, typical of fibromatosis. Variably sized blood vessels and large and polygonal interstitial cells displayed fluorescence of lower intensity.



agnostic and therapeutic procedures; this study was performed in accordance to the principles of the Declaration of Helsinki and has been approved by our institution ethical committee (Study n° 4576 – Prot. 1443/C.E.).

Discussion

TS is an autosomal dominant neurocutaneous syndrome, generally diagnosed in the first 15 months of life, which manifests with great phenotypic variability. This disease was first described by von Recklinghausen in 1862 and more recently, by Bourneville, Pringle, and Vogt⁴.

TS is caused by inactivating mutations of Tuberous Sclerosis Complex 1 (TSC1) and Tuberous Sclerosis Complex 2 (TSC2), which encode for “hamartin-tuberin complex” acting as tumour suppressor genes. One-thirds of TS cases are inherited from an affected parent and de novo mutations have been implicated in two-thirds of

all cases. Affected patients with familial TS may show phenotype variability due to genetic mosaicism².

The TSC phenotype is widely variable with some pathognomonic features present at birth and others developing later during the patient’s life.

The second International Tuberous Sclerosis Complex Consensus Conference (Washington 2012)² revisited the clinical diagnostic criteria published subsequent to the first International TS Consensus Conference in 1998⁵⁻⁷.

The Table I reports the major and minor TS diagnostic criteria; the diagnosis is *definite* when 2 major features or 1 major feature with ≥ 2 minor features are present or after the identification of a TSC1 or TSC2 pathogenic mutation; *possible* when 1 major feature or ≥ 2 minor features². Ventricular tachycardia, paroxysmal arrhythmia, Wolff-Parkinson-White syndrome, epileptic crises, autism, learning disorders, abnormal behaviour, dyspnea and spontaneous pneumothorax are all possible consequences of the presence of the lesions in different organs⁸⁻¹⁰.

TS oral manifestations are quite frequent and consist of benign, well-delimited, fibrous nodules of the anterior gingiva, lips, palate, or tongue, variably known as gingival fibromas, oral angiofibromas, or oral fibrous papules^{2,11}. These lesions tend to appear in late childhood, can be normal-colored, as gingival fibromatosis, or red, suggesting a reactive lesion such as pyogenic granuloma.

Although a prevalence of 11% has been reported, the real frequency of these lesions may be significantly higher. Lygidakis and Lindenbaum¹² found oral fibromas in 46% of TS patients, while Araujo et al⁴ detected angiofibromas of the anterior region of the gums, dorsal tongue, buccal mucosa, and lip in 5 patients.

It has been suggested that oral fibromas might not be directly related to TS but rather resulting from drugs ad-

Tab. I. Tuberous Sclerosis Diagnostic Criteria².

Genetic diagnostic criteria:
The identification of either a TSC1 or TSC2 pathogenic mutation in DNA from normal tissue
Clinical diagnostic criteria:
Major features
1. Hypomelanotic macules (≥ 3 , at least 5-mm diameter)
2. Angiofibromas (≥ 3) or fibrous cephalic plaque
3. Ungual fibromas (≥ 2)
4. Shagreen patch
5. Multiple retinal hamartomas
6. Cortical dysplasias
7. Subependymal nodules
8. Subependymal giant cell astrocytoma
9. Cardiac rhabdomyoma
10. Lymphangiioleiomyomatosis (LAM)
11. Angiomyolipomas (≥ 2)
Minor features
1. “Confetti” skin lesions
2. Dental enamel pits (>3)
3. Intraoral fibromas (≥ 2)
4. Retinal achromic patch
5. Multiple renal cysts
6. Nonrenal hamartomas

ministration for the neurological manifestations of the disease. At this regard, phenytoin, often administered to control epileptic seizures in such patients, is a well known inducer of gingival hyperplasia. Lygidakis¹² found oral fibromas in up to 46% of patients with TS, many of whom had never received phenytoin or any other type of anti-seizure medication. Consequently, gingival fibromas have been thereafter considered as typical clinical signs of TS, especially when affecting multiple sites, such as the tongue, palate or lips¹¹.

Damm et al. reported maxillary bone involvement with the presence of fibrous tumors in TS¹³. Furthermore, dental enamel pitting is observed in up to 100% of patients with TS. Dental pits can be observed in the general population, but at lower frequencies and with fewer lesions than in TS⁴.

In our study, the patient showed pale pink enlargements of anterior and posterior lower gums and, in view of her therapeutic regimen including Phenobarbital, Levetiracetam and Rufinamide but not phenytoin, her gingival hyperplasia was considered unrelated to treatment.

In the current case, gingival outgrowth caused teeth displacement, spacing, bleeding and bad oral hygiene; consequently, gingivectomy and gingivoplasty were performed by pulsed diode laser that also allows effective haemostasis and coagulation without bleeding, as well as an excellent incision, due to its penetration in depth, estimated around 0.5–3 mm¹⁴. The clotting capability of the diode laser provided a clear view of the surgical site and a precise extension of the incision. Hence, this procedure may be considered very effective for oral soft tissue surgery. Moreover, other advantages are reduced necessity of anaesthesia and faster wound healing, with less discomfort for the patient. Furthermore laser excision does not induce tissue artifacts, retractions, deformation or detachment.

The surgical specimen underwent conventional histological examination that showed thickened and acanthotic epithelium with elongated rete ridges, densely packed, whirly collagen fibres, fibroblasts, and variably sized vascular structures, without chronic inflammatory cells.

CLSM analysis showed variably sized blood vessels and large and polygonal interstitial stem-like cells displaying fluorescence of lower intensity which may represent a distinctive intra-oral alteration of TS. The vascular component consisted of small groups of venous-like structures with dilated lumina among the collagen fibres with variable spatial orientation, typical of fibromatosis, thus suggesting the diagnosis of hyperplastic angiofibromatosis. The presence of the polygonal interstitial stem-like cells in association with the lack of chronic inflammation confirmed that angiofibromatosis could not be considered a reactive event, but a structural feature of the disease. This pathologic vascular component suggests the use of diode laser photocoagulation in order to reduce lesions dimensions before laser excision.

Conclusions

This case report represents a never-reported clinical finding of TS, periodontal angiofibromatosis, that could help to achieve the correct diagnosis of TS in patients with an equivocal clinical presentation. The diagnosis was obtained thanks to CLSM histological examination allowing the identification of additional features, such as low fluorescence areas and a typical vascular component. The presence of the vascular component confirmed the effectiveness of diode laser for the treatment of such lesions in view of cutting precision, clotting capability, without intra- or post-operative complications and faster wound healing.

ABBREVIATIONS

TS, Tuberous Sclerosis; TSC, Tuberous Sclerosis Complex; CLSM, Confocal Laser Scanning Microscopy.

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