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Microscopic study of chronic Charcot arthropathy foot bones contributes to understanding pathogenesis - a preliminary report

Salini Dharmadas[1], Harish Kumar[2], Minnie Pillay[1], Annie Jojo[3], P.J. Tessy[3,4], Thacho Sukumaran Mangalanandan[2], Lakshmanan Vivek[2], Valiyaparambil Pavithran Praveen[2] and Arun Bal[2]

[1]Department of Anatomy, [2]Department of Endocrinology, [3]Department of Pathology, Amrita School of Medicine, Amrita Vishwa Vidyapeetham and [4]Ernakulam Medical College, Kalamassery, Kochi, India

Summary. Introduction. Charcot arthropathy (CA) is non-infective, chronic destructive condition affecting the pes architecture of long standing diabetic patients with neuropathy. Even though several theories have emerged to disclose its pathogenesis, inflammatory cytokine induced osteoclastogenesis stands as the chief culprit. Studies on micro-architecture of foot bones of acute stage CA patients, describes mainly destructive phase of bone remodelling. Increased osteoclast cell activity is reported in all studies communicated. No study has to the best of our knowledge detailed the microscopic structure of chronic stage CA foot bones.

Aim. To study the microscopic structure of foot bones in patients with chronic CA.

Materials and methods. Foot bones were collected from the feet of chronic CA patients (six in number) who underwent corrective foot surgery in the department of Podiatric Surgery of a tertiary care hospital. Control samples were collected from the feet of age matched non-diabetic controls (2 in number). The samples were fixed in formalin, decalcified in 10% nitric acid, processed, sectioned and stained with haematoxylin and eosin. Histopathology and histomorphometry analysis were performed by two different pathologists.

Results. Trabeculae of chronic CA foot bones exhibited mainly a lamellar architecture, with reduced number of osteocytes and plenty of empty lacunae. Trabecular connectivity was lost and trabeculae showed considerable thinning. Trabecular osteoids lined by active osteoblast cells was a remarkable observation. Bone area was also considerably reduced in chronic CA foot bones.

Conclusion. Chronic stage CA foot bones presented features of both healing and fragile bone. The compromised bone quality may be due to thin and fragmented trabecular structure and reduced cellularity.

Key words: Histology, Histomorphometry, Chronic Charcot arthropathy, Lamellar architecture, Trabecular thinning

Offprint requests to: Dr Annie Jojo, Professor, Department of Pathology, Amrita School of Medicine, Amrita Vishwa Vidyapeetham, Kochi, 682041, Kerala, India. e-mail: anniejojo@aims.amrita.edu

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Introduction

Charcot arthropathy (CA) is a non-infective, chronic destructive condition affecting the pes architecture of long standing diabetic patients with accompanying neurological deficit. The onset of the disease is sudden and advances from an acute phase of development, through a stage of coalescence, to the final stage of reconstruction, in which consolidation of deformity and remodelling of bone occurs within itself, leaving a stable but deformed chronic CA foot (Rosenbaum and DiPreta, 2015; Dodd and Daniels, 2018). The healing process stretches from days to months, the duration of which fluctuates among patients. The treatment regimen varies depending on the stage and severity of disease. During

the acute stage of the disease complete offloading of the patient with total contact cast is recommended. Whereas during the chronic stage of CA, complicated by infectious wounds and fractures, surgery becomes unavoidable (Guven et al., 2013). In contrast to the fragile and soft bones encountered in acute stages of CA, in chronic stages they appear hard (La Fontaine et al., 2011). Even though several theories have emerged to detail the pathogenesis of CA, pro-inflammatory cytokines, especially the receptor activator of the nuclear factor-kB (RANK) ligand (RANKL) induced osteoclast proliferation, differentiation and maturation is considered to initiate the cascade of bone changes in CA (Larson and Burns, 2012). A concomitant increase in osteoclast cell activity with a decrease in osteoblast cell activity leads to excessive bone turnover rates in CA patients (Baumhauer et al., 2006). Increased bone turnover rates in turn cause decreased bone mineral density, increased bone fragility and increased fracture risk in CA foot (Larson and Burns, 2012).

CA mainly affects the short bones of the foot. The short bones of the foot have trabecular architecture with a greater rate of bone turnover compared to compact bone (Farber et al., 2002). Very few studies are reported in the literature on microstructure of CA foot bones. Among those reported most detail features of a destructive bone in the feet of acute stage CA patients (La Fontaine et al., 2011). No studies are reported to date on the microscopic structure of chronic stage CA foot bones. The aim of this study was to analyse the histology and histomorphometry of foot bones collected from amputated foot specimens of patients with chronic CA and compare it with that of age matched non-diabetic controls.

Materials and methods

Foot bones were collected from the feet of chronic CA patients (6) who underwent corrective foot surgery in the department of Podiatric Surgery of a tertiary care hospital. Control samples were collected from the feet of age matched non-diabetic donors whose bodies were donated to Anatomy department of the same Institution. Chronic CA was diagnosed by the presence of clinical and radiological parameters. Clinical parameters of chronic CA included a totally deformed foot without any redness, and swelling. (Fig. 1A) The difference in skin temperature between the two feet diminishes to less than 2°C. Radiological parameters include consolidation of deformity, joint arthrosis, fibrous ankylosis, smoothening of bone fragments (Papanas and Maltezos, 2013) (Fig. 2B). The institutional ethical committee clearance was obtained before conducting the study.

Foot bones were selected from the CA affected region of foot. For histopathological examination by light microscopy the samples were fixed in formalin, decalcified in 10% nitric acid. After dehydration and subsequent paraffin embedding, 4-6 µ sections were taken and stained with haematoxylin and eosin using standard staining procedure. Multiple sections of bone were studied from each bone sample randomly. Histomorphometry was analysed using Image J Software.

Bone area stands for bone volume over tissue volume (BV/TV) i.e. the percentage of area occupied by calcified bone in relation to the total area. Trabecular width or thickness (Tb.Wi) is the mean distance across individual trabeculae, given in micrometers (Vidal et al., 2012). Histomorphometry variables are named according

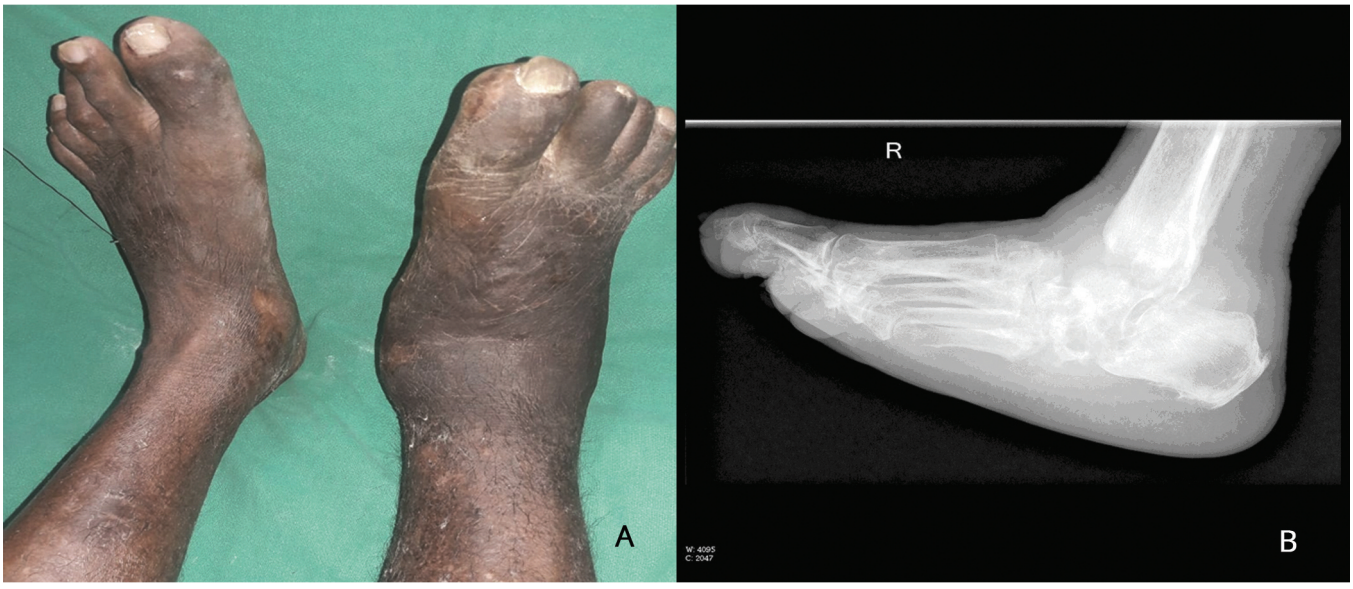

Fig. 1. A. Deformed chronic CA foot (right). **B.** Lateral view radiograph of chronic CA foot.

to the nomenclature scheme approved by the American Society for Bone and Mineral Research. Histopathology and histomorphometry analysis of bone microscopic structure was performed by two different pathologists.

Statistical analysis

Statistical analysis was performed using IBM SPSS version 20.0 software. Continuous variables are expressed as mean and standard deviation. To test the statistical significance of the mean changes of continuous variables between the two groups, chronic CA and control, Mann Whitney U test was used.

Results

Of the six diabetic patients with chronic CA selected for the study, five were males. The average age of these patients was 66.33±7.28 years. The average duration of diabetes was found to be 15.67±4.13 years. The patients had poor glycaemia control at the time of procedure, evidenced by HBAIC level of 8.27%. Pro-inflammatory cytokine C reactive protein (CRP) levels was above 35 mg/L in all subjects with an average value of 107.95 mg/L. Right foot was affected in two patients and left foot in four. Mid foot region was affected in five patients followed by hind foot region in one patient.

Histology

Results of the present study showed considerable difference between the chronic CA group and the controls. Microscopic structure of control foot bones showed a lamellar architecture with an abundance of osteocytes. Normal trabecular connectivity and thickness was observed. The inter trabecular space was filled with fatty marrow (Fig. 2A). The trabeculae of chronic CA foot bones also presented a lamellar architecture, but the normal trabecular connectivity or pattern was absent and the trabeculae showed considerable thinning. (Fig. 2B,C) Considerable reduction was seen in the number of osteocytes in CA bones and had plenty of empty lacunae. An abundance of trabecular osteoids with active osteoblast cell lining was a remarkable finding. (Fig. 2D,E) Osteoclast cells in Howship's lacunae suggestive of active bone resorption were seen in one of the

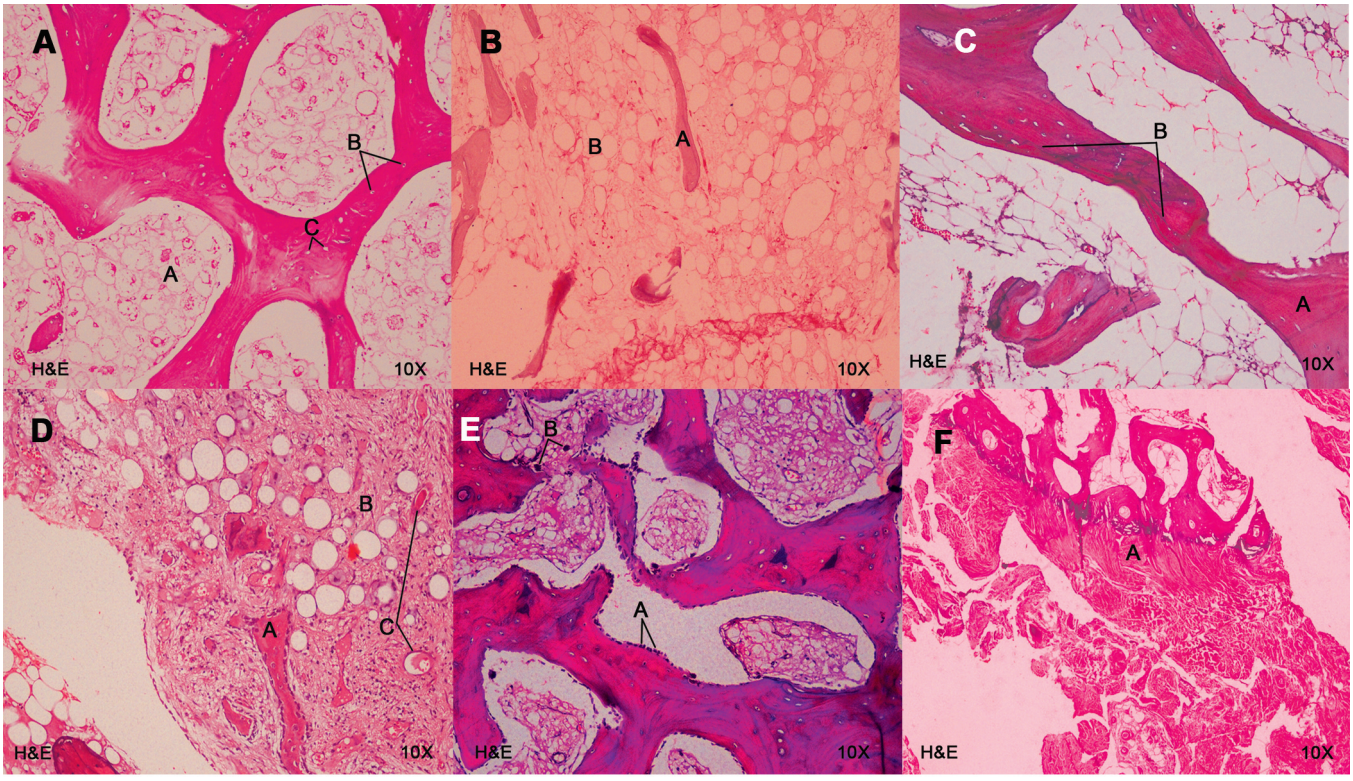

Fig. 2. A. Photomicrographs of normal bone showing a spongy appearance of lamellated trabeculae Mark A-intertrabecular space with fatty marrow, Mark B-osteocytes, Mark C- Empty lacunae. **B.** Photo-micrographs of CA foot bone showing Mark A-thin fragmented lamellated trabeculae without osteoblastic rimming, Mark B-fatty marrow. **C.** Photomicrographs of CA foot bone showing, Mark-A lamellated trabeculae, Mark B-prominent and irregular cement lines. **D.** photomicrographs of CA foot bone showing, Mark-A trabecular rims with osteoblastic rimming, Mark B- fatty marrow with inflammatory cells Mark-C- entrapped blood vessels. **E.** Photomicrographs of CA foot bone showing. Mark A-osteoblast cells lining trabeculae, Mark B-osteoclast cells. **F.** Photomicrographs of CA foot bone showing, Mark A-fibrocollagenous tissue. H&E.

Table 1. Histomorphometric parameters expressed as mean and standard deviation.

Index	Normals	Charcot patients	P value
BV/TV (%)	36.47±5.90	14.54±8.2	0.046
Tb.Wi (μm)	114.59±5.31	66.17±36.69	0.182

BV/TV, Bone volume/Trabecular volume; Tb.Wi, Trabecular width.

sections. (Fig. 2E) Another interesting finding was the presence of prominent and irregular cement lines giving a Pagetoid like appearance to chronic stage CA bones. (Fig. 2C) Inter-trabecular space was filled with fatty marrow and entrapped blood vessels. Irregularly arranged dense fibrocollagenous tissue was seen in abundance. (Fig. 2F) Few sections presented inflammatory cells with increased number of blood vessels.

Histomorphometry

Statistically significant difference was found in bone area [CA-14.54±8.2, control- 36.47±5.90, (P value-0.046)] between the two groups. The trabecular width (Tb.Wi) was considerably less in CA group compared to control. (Table 1)

Discussion

Bone is a dynamic tissue which goes through continuous remodelling all through life. Bone turnover is performed principally by bone-forming cells the osteoblasts and bone-resorbing cells the osteoclasts. Normal bone homoeostasis is retained by fine balance between bone resorption and bone formation, assuring new bone formation in proportion to bone removed (Feng and McDonald, 2011: Nakashima, 2013). This balance is regulated by physical parameters and polypeptides including hormones and cytokines. Disturbances in any of these parameters cause imbalance in the activity of these cells leading to bone metabolic disorders (Siddiqui and Partridge, 2014). An increase in this bone turnover is observed with ageing and in pathological conditions. This alteration in bone turnover rates leads to altered bone architecture and compromised bone strength independent of bone mineral density (Shetty et al., 2016).

CA is a chronic, progressive destructive process affecting architecture of foot bone, mainly in patients with a long history of diabetes and neuropathy. The exact cellular mechanism contributing to this insidious disorder is still largely unknown. Recent literature implicates inflammation induced alterations in OPG/RANKL/RANK pathway in the pathogenesis of CA. Inflammatory cytokines initiate bone metabolic changes ending in skeletal abnormalities characteristic of CA (Baumhaeur et al., 2006; Mascarenhas and Edward, 2014; Johnson-Lynn et al., 2018). Petrova et al. (2015) found increased concentration of inflammatory cytokines, and bone turn over markers in patients with acute stage CA. However bone mineral density is found reduced in both type 1 and type 2 diabetes patients with CA and is associated with an apparent loss of bone micro-architecture and marrow space (Kayanak et al., 2013).

This study on microscopic structure of chronic CA foot bones is unique in that none of the previous studies on microstructure of CA have mentioned the phase of CA as either acute or chronic, or/and had a combination of samples from both acute and chronic stages of CA, or/and had sections from compact bones other than trabecular bones. To the best of our knowledge this is the first report on microscopic structure of chronic stage CA foot bones. CA mainly affects the short bones of the foot. The short bones of the foot are trabecular in nature and have a greater rate of bone turnover compared to compact bone. According to modified Eichenholtz classification, the natural history of CA progress from initial pre stage -1 or prodromal stage to stage I of development and stage II of coalescence to the terminal stage III of reconstruction (Gouveri and Papanas, 2011). However according to clinicians CA has two stages, acute and chronic. In the acute/active stage, the foot is hot, swollen and erythematous. Pain is insignificant compared to patients without neuropathy and similar degree of inflammation. A temperature difference greater than 2°C may be observed in the affected region of the foot compared to similar region on the contra lateral foot (Rogers et al., 2011). Surgeons come across soft bones in this stage of CA (La Fontaine et al., 2011). In chronic stage of CA, signs of local inflammation disappear, and the difference in skin temperature between the two feet diminishes. The bones become hard and the foot progressively becomes stable but deformed (Papanas and Maltezos, 2013). The progression period from acute to chronic stage ranges from patient to patient and may vary from weeks to even months.

However only very few studies are reported in the literature on microscopic structure of CA foot bones and all these studies detail CA foot bone in its acute stage. La Fontaine et al. (2011) in his study on CA foot bones observed woven bone architecture illustrative of immature and disorganised bone structure. They had samples from both acute (5 in number) and chronic stages (3 in number) of CA. Woven bone is composed of loosely and randomly arranged collagen bundles and is typical of embryonic skeleton. It also appears in pathological conditions featured by rapid bone resorption, bone growth and replacement or increased bone turnover (Malluche and Faugere, 1986). Baumhauer et al. (2006) explained sections of lamellar bone with a disproportionate increase of osteoclast to osteoblast cells in his study on acute stage CA bone. Lamellar bone is secondary bone created by remodelling of woven bone. In lamellar bone, the collagen fibres are arranged in parallel layers and are typical of adult

skeleton (Standring, 2016). In the present study, a preponderance of lamellar bone lined by osteoblast cells was observed demonstrating the presence of mature bone in chronic stages of CA. Irregularity in trabecular pattern with reduced thickness similar to our findings is reported by Sinha et al. (1972) in their study on CA bones. Considerable difference in cellularity is described in all previous studies on CA foot bones. Osteocytes play a crucial role in the bone-remodelling process by maintaining connections between bone surface, osteoblasts and osteoclasts, and other osteocytes through an extensive canalicular network. A reduced number of osteocytes can considerably alter the bone remodelling process (Graham et al., 2013). The presence of trabecular osteoids with active osteoblast cell lining is suggestive of new bone formation or progression of healing process in chronic stages of CA (Delaisse, 2014). In spite of increased presence of active osteoblast cells bone trabeculae appear thin and fragmented in chronic stages of CA. The molecular mechanisms that mediate this impaired bone formation despite the presence of active osteoblast cells are poorly understood and need to be elucidated. Such an understanding may pave the way for new modalities or approaches to the treatment of chronic CA. Prominent and irregular cement lines denote changes in mineralization and quality of bone tissue deposited (Milovanovic et al., 2018). Irregular and prominent cement lines may be secondary to multiple attempts of fracture repair in an already weak diabetic neuropathic foot. Absence of woven bone sections and dramatic reduction of osteoclast cells in Howship's lacunae shows absence of bone destruction in chronic stages of CA. Increased presence of fibrocollagenous tissue represents fusion of bones in chronic stages of CA leading to abnormally rigid and consolidated joints.

Conclusion

This is the first study on the microscopic structure of chronic CA foot bones to date. Within the limitations of the sample size, the present study shows that chronic CA foot bone has a mature lamellar architecture. Despite the abundance of active osteoblast cells the trabeculae in chronic stage CA foot bone appear thin and fragmented the pathophysiology of which has to be elucidated. A more detailed study with large sample size may help in further understanding the pathogenesis of this debilitating disorder.

Conflict of interest. The authors declare that there is no conflict of interest associated with this manuscript.

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