HISTOLOGICAL STUDY OF FIBULAR ANLAGE, THE EMBRYONIC TISSUE REMNANT IN TYPE II HEMIMELIA CASES

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Abstract

Background: Fibular hemimelia or fibular hypoplasia is the most common congenital longitudinal limb deficiency characterized by complete or partial absence of the fibula bone and it was described initially as a condition that is related to aplasia or hypoplasia of the fibula. Tethering effect of fibular anlage, the primordium of fibula which is frequently found with type II hemimelia may contribute to the deformities in the tibia and ankle. The etiology is unclear; the deformity is probably due to disruptions during the critical period of embryonic limb bud development. **Objective**: to find out and study the histological nature of the excised fibular anlage.

Patient and Method: From 40 limbs belonging to 32 patients (some were bilateral) who underwent surgical operation,19 fibular anlagen were found and excised as a first step of correction of lower limb deformity and bone elongation, the anlagen underwent fixation and tissue processing then the sections examined under light microscope.

Results: the histological examination revealed presence of hyaline cartilage, chondrocytes forming isogenous group and a lot of fibrous tissue with no sign of bone formation.

Conclusion: persistence of cartilaginous model of the fibular anlage confirms arrested growth of the limb bud and failure of endochondral ossification to commence.

Keywords: Hemimelia type II, fibular anlage, limb bud, endochondral ossification

Introduction

Fibular hemimelia or fibular hypoplasia or postaxial hypoplasia is a congenital longitudinal limb deficiency characterized by complete or partial absence of the fibula bone (Boaket et al.,1991) and it was described initially

as a condition that is related to aplasia or hypoplasia of the fibula. Fibular anlage, the primordium of fibula is found only in type II hemimelia cases may contribute to the deformities in the tibia and ankle (Conventry and Johnson, 1952, Achterman and Kalamchi, 1979). The etiology is unclear, the deformity is probably due to disruptions during the critical period of embryonic limb development, between 4th and 7th week of gestation (Amultz, 1972). Vascular dysgenesis, viral infections, trauma and environmental influences have been suggested as possible causes (Rodriguez et al., 2010). In human embryos, limb buds develop normally by the end of 4^{th} week with activation of mesenchymal cells in somatic lateral mesoderm, the upper limb buds appear on day 24 and the lower limb buds appear 1 or 2 days later, each limb bud consists of a mesenchymal core covered by a layer of ectoderm and Homeo box genes regulate patterning in the formation of the limbs (Moore et al., 2013). Long bones and the axial skeleton in the developing embryo are formed by endochondral ossification, namely, by remodeling of cartilage templates (Kronenberg, 2013). This process relies on the specialized morphoregulatory functions of hypertrophic chondrocytes (Noonan et al.,1998)Hypertrophic chondrocytes derive from the condensation of mesenchymal precursors and produce a type X collagen-rich avascular cartilaginous matrix. At the periphery of this cartilage tissue, the so-called "borderline" hypertrophic chondrocytes (Bianco et al., 1998) instruct surrounding mesenchymal cells to differentiate into osteoblasts, which results in the formation of a "bony collar." In parallel, chondrocytes in the central regions direct mineralization of the hypertrophic cartilage by initiating remodeling via the production of specific matrix metalloproteinases (MMP) and attract blood vessels by releasing vascular-endothelial growth factor (VEGF). The in-growing blood vessels deliver osteoblastic, osteoclastic, and hematopoietic precursors, which mediate resorption of the cartilaginous template and formation of vascularized bone containing the so-called stromal sinusoids, which provide the microenvironment for hematopoiesis (Kronenberg, 2013)·Chondroblasts at the core of the condensation are the first to secret cartilaginous matrix materials, which separate the cells again. When it is completely surrounded by cartilage matrix, a chondroblast is termed a chondrocyte. Peripheral mesenchyme condenses around the developing cartilage mass to form the fibroblast-containing dense regular connective tissue of the perichondrium (Moore et al., 2013).

Most of cases of hemimelia are sporadic; a family history has been reported in a small percentage of cases, however, chromosomal anomalies and autosomal dominant, autosomal recessive, and X-linked transmission have been reported(Lewin and Opitz, 1986) . Graham,1993 suggested that vascular or mechanical interference with limb bud function on embryonic

apical ectodermal ridge might lead to fibular hemimelia. Angiographic study has detected vasculature abnormalities including persistence embryonic vascular pattern, failure of plantar arch formation, absence of anterior tibial artery or absence of normal trifurcation of the popliteal artery at the level of the knee, and presence of one large posterior artery in the leg (Hootnick et al.,1980).

Fibular hemimelia is the most common type of limb deficiency and is not an isolated malformation but rather a part of a spectrum of dysplasia of the entire lower limb. It is often associated with other anomalies of the lower limb, such as limb shortening, absent lateral rays of the foot, ball and socket ankle joint, tarsal coalition, hypoplasia of the lateral femoral condyle with knee valgus, all these are related to the fibular field of the lower limb bud(Monteagudo et al., 2006 and Tonbul et al., 2007). Congenital malformation of fibula results from alteration of musculoskeletal organogenesis that probably occurs at 4^{th}-7^{th} week of embryonic life the earlier the insult the more the involvement of proximal femur and fibula, later insult involves the fibula and foot to a great degree (Pappas et al.,1972). Prevalence of hemimelia is estimated at 1 in 40,000 birth, the case is apparent at birth and can vary from mild limb inequalities and asymmetry to sever shortening with wide range associated anomalies, bilateral involvement is not uncommon with a reported prevalence of 9-52% ,male to female ratio is 2:1, however some studies reported the incidence is more common in female (Lewin and Opitz, 1986, Stevens and Arms,2000, Mc Carthy et al., 2000, EL-Sayed et al 2010, Rodriguez et al., 2010,Birch et al., Naudie et al., 2011,) (and fibular anlagen are found in 60% of hemimelia that is only in type II and they are felt as a palpable posterior band which represents a fibrocartilagenous fibular remnant (Achterman and Kalamchi,1979).

Patients and method:

The study was carried out from 1/5/2013 to 1/6/2015 in Chammi Rezan and Shar teaching hospitals in Sulaimani city/ Kurdistan Region - Iraq, the total number of cases was 32 patients, the age of patients ranged from 3-16 years from the total 32 patients 24 were male and 8 were female, 40 limbs with fibular hemimelia as some cases were bilateral underwent reconstruction surgery of the ankle and foot with elongation of limb, 19 fibular anlagen were found and excised from hemimelia type II cases as a first step of operation to prevent recurrence of equinovalgus ankle and foot deformity. The absence of the lateral malleolus in fibular hemimelia causes subluxation and valgus deformity of the ankle joint due to lack of the lateral support at the ankle so excision of the fibular anlage and centralization of the ankle corrects the valgus deformity was

indicated(Hazem and Amin,2012).The excised anlagen were fixed in 10% formaldehyde then underwent tissue preparation and processing; the sections were stained by Hematoxylene and Eosin (H&E) stains and then examined under light microscope (Kim, 2012).Written consents were taken from parents of the patients regarding sending the specimens for histopathological examination.

Result

In this study, from the total 40 limbs belonging to 32 patients (bilateral limbs were involved in some patients) who underwent surgical operation, only 19 fibular anlagen were found and have been excised as a first step of surgical operation, grossly the mean size of anlagen/ the specimens was 1cm× 3cm (Figure 3), male to female ratio was 3:1.The result of histopathological study of the specimen showed presence of mature chondrocytes embedded within fibrous matrix, features of hyaline cartilage, no hypertrophied or proliferated chondrocytes were detected in addition there was no primary ossification centers were found (Figure 4&5)

Figure (1): A 5 years old girl with Type II fibular hemimelia, short right lower limb.

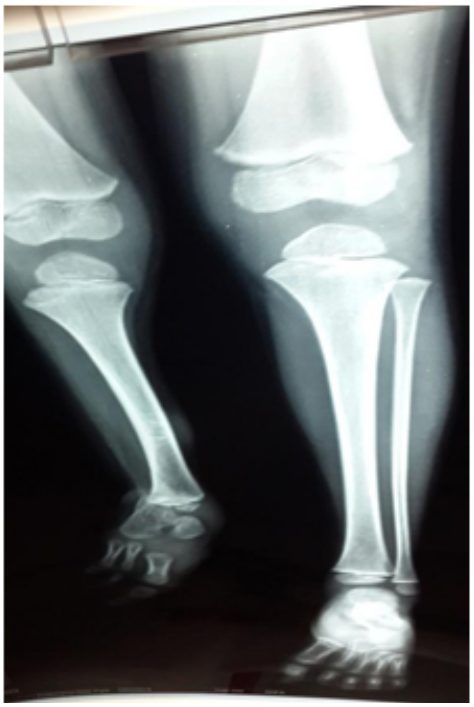

Figure (2): Plain radiograph of legs AP view, absent fibula& outer two digits.

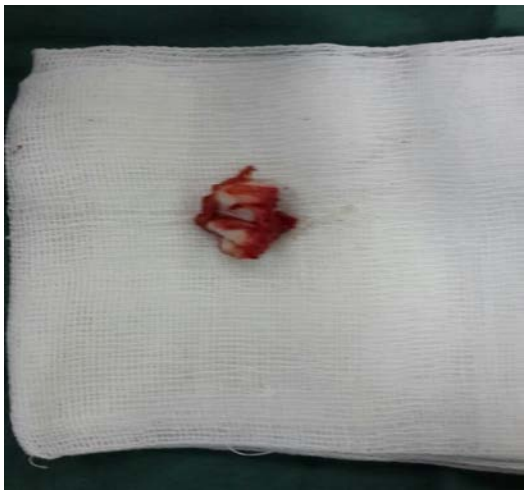

Figure (3): Gross Image of Fibular anlage

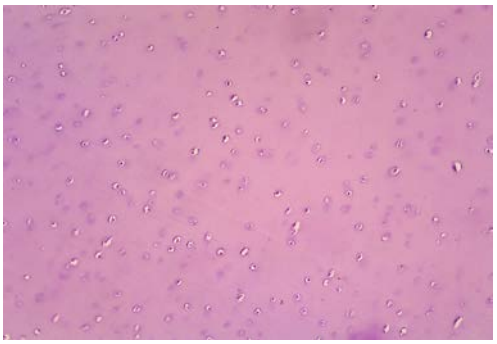

Figure (4): Section from Fibular anlage shows hyaline cartilage. H&E, X20.

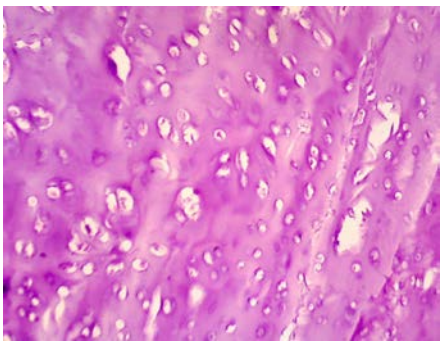

Figure (5): Fibular anlage shows hyaline cartilage, chondrocytes forming isogenous groups. H&E, X 40.

Discussion:

Congenital absence of the fibula is the most common deficiency of long bone deficiencies (Caskey and Lester, 2002)The limb bud development starts at the end of the 4th week of embryonic life as an outpocketings from the ventrolateral body wall including a mesenchymal core which is derived from somatic layer of lateral plate mesoderm from which bone and connective tissues of the limb will be formed and it is covered by a layer of ectoderm(Thomas, 2010). Early development of the vertebrate skeleton depends on genes that pattern the distribution and proliferation of cells from cranial neural crest, sclerotomes, and lateral plate mesoderm into mesenchymal condensations at sites of future skeletal elements. Within these condensations, cells differentiate to chondrocytes or osteoblasts and form cartilages and bones under the control of various transcription factors (Bjorn and Anthony, 2000), multiple interactions take place between these tissues so the definitive shape of a structure could depend on only one of its

component (Thomas,2010).. The development of the vertebrate limb skeleton is initiated when multipotent mesenchymal cells in the limb bud aggregate to form mesenchymal condensations which prefigure the skeletal elements. Cells within the pre-chondrogenic condensation up-regulate cell adhesion mechanisms and begin to synthesize specific extracellular matrix molecules, and the condensations expand through a combination of proliferation and recruitment of surrounding mesenchyme. Mesenchymal condensations creates an environment which is conductive to chondrogenic differentiation (Zhitnikov, 1979, Saldanha and Nayagam, 2011).

As the cells differentiate into chondrocytes, they synthesize a framework of cartilage matrix, known as an anlage, in the approximate shape of the future bone. Chondrocytes in the centre of the anlage proceed through a series of discrete developmental stages that include proliferation, maturation and hypertrophy (DeLise et al., 2000, Hall and Miyak, 2000, Saldanha and Nayagam,2011) .The hypertrophic cartilage is first calcified and then, following vascular invasion, replaced by primary bone that is subsequently remodeled to form secondary bone. This process radiates outwards from the centre of the anlage with the development of highly ordered growth plates that separate the cartilaginous epiphyses from the bony diaphysis, inspite of all these facts in fibular hemimelia type II the cartilaginous model or anlage fails to grow and progress in the current study the histological findings showed no sign of hypertrophy and proliferation of chondrocytes and no primary ossification center was detected.

Histology of fibular anlage has been studied and showed replacement of bone tissue by mature collagen bundles surrounded by fibroconnective tissue (Sham and Nuckollas, 2002). in current study, the histology of the excised anlagen were thoroughly studied and revealed presence of mature hyaline cartilage, the chondrocytes were found forming isogenous groups embedded in a fibrous connective tissue matrix, presence of mature chondrocytes in the sections mean that the process of chondrogenesis has taken place successfully but the growth has been arrested at that point which probably occurred before 8th week and as there was no sign of osteogenesis or ossification signs this explains the defect in initiation of bone formation which in limbs occurs by endochondral ossification (Caskey and Lester, 2002, Moore et al., 2013), as the primary ossification centers in long bones appear by the 8^{th} week of development and it is normally influenced by several factors which play important role in initiation the endochondral ossification process that starts first by appearance of primary ossification centers in long bones (Moore et al., 2013). It was stated that intensity of chondrogenesis and growth of bones are affected by several processes: intensity of chondrocyte multiplication, the rate of their repeated division in the proliferative zone, the velocity with

which the cells transfer into the state of hypertrophy and the rate of the periostal bone formation at the border-line of metaphysis and diaphysis (Zhitnikov,1979) also morphogenesis of the vertebrate limb bud depends upon reciprocal interactions between epithelial and mesenchymal tissues a characteristic limb vascular pattern is essential for normal limb outgrowth (Pizette and Niswander,2000)

Conclusion:

Persistence of cartilaginous model of the fibular anlage confirms arrested growth of the limb bud and failure of endochondral ossification to commence.

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