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Histological study of the developing human femur

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SUMMARY

The development of the long bones at various gestational ages in the fetus has always been a subject of interest for many clinicians. Some morphometric parameters such as length, etc., are considered standard parameters for evaluation of the gestational age of the fetus. However, not much emphasis is laid upon morphometric parameters to assess the histological changes in these age groups. Therefore, the present study was undertaken to determine the histological changes occurring in a developing bone. 30 fetuses sent to the Dept. of Anatomy for routine fetal autopsy by the Dept. of Obstetrics and Gynaecology were selected for the microscopic study of the femur. Left femora were extracted, and transverse and longitudinal sections were taken and stained with hematoxylin and eosin. The epiphysis of the growing bone exhibited the formation and proliferation of different zones in different age groups. The formation and distribution of distinct cartilage canals has been evidenced as early as 13^{+2} weeks of gestation in the growing epiphysis. The appearance of a secondary centre of ossification in the distal femoral epiphysis was observed as early as 28^{+4} weeks. The diaphysis showed the formation of a cancellous bone with increasing trabeculae proliferating more on one side of the shaft. The above observations are discussed in the light of available literature.

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INTRODUCTION

Endochondral ossification is the process by which the embryonic cartilaginous model of most bones contributes to longitudinal growth and is gradually replaced by bone (Mackie et al., 2008). During normal endochondral ossification, the entire femur initially forms an avascular cartilaginous anlage at about the seventh week of gestation. At this stage no osteoblasts are produced by the cells in the chondrogenic layer of the developing perichondrium surrounding the cartilage model, because differentiation is taking place in an avascular environment (Hamilton et al., 1972; Burkus et al., 1993). The endochondral bone development depends upon neovascularization, and an early generation of vascularized cartilage canals is an initial event which precedes the formation of a secondary centre of ossification (Blumer et al., 2008). Cartilage canals are described as tubes of vascularized mesenchyme containing proliferating blood vessels and perivascular cells invading the epiphysis from the perichondrium (Blumer et al., 2005, 2006).

The literature stated previously contains several hypotheses regarding the mode of formation of cartilage canals. These perichondral invaginations of blood vessels and connective tissue have been found within the epiphysis prior to the appearance of a secondary centre of ossification in avian (Lutfi, 1970; Blumer et al., 2004a,b, 2005, 2006, 2007; Eslaminejad et al., 2006) and most mammalian

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(Stockwell, 1971; Wilsman and Van Sickle, 1972; Kneese, 1980; Cole and Wezeman, 1985; Visco et al., 1990; Burkus et al., 1993; Ganey et al., 1995; Roach et al., 1998; Rivas and Shapiro, 2002; Alvarez et al., 2005a,b: Pazzaglia et al., 2007) long bones. Cartilage canals have also been described in human short bones without epiphysis, i.e., talus, calcaneum, and vertebrae, and are present before the initial site of appearance of an ossification centre (Agarwal et al., 1984, 1986; Chandraraj and Briggs, 1988; Fritsch and Eggers, 1999; Fritsch et al., 2001). The histology of these canals have also been described in normal human rib cartilage, as well as neonates and children with achondrogenesis (Craatz et al., 1999; Gruber et al., 1990). The canals are seen to be even present within the human thyroid cartilage (Claassen et al., 1996).

The canals are considered to both nourish the chondrocytes (Wilsman and Van Sickle, 1970, 1972) and provide mesenchymal osteogenic cells to long bones (Lutfi et al., 1970; Kugler et al., 1979; Burkus et al., 1993; Ganey et al., 1995; Rivas and Shapiro, 2002; Morini et al., 2004; Alvarez et al., 2005a,b) as well as short bones (Agrawal et al., 1984, 1986; Chandraraj and Briggs, 1988; Fritsch et al., 2001). The structural features of cartilage canals are seen to be similar among vertebrates (Shapiro, 1998). Several vertebrate species undergo a process of chondrification, which is a physiological age-dependant process of regression of the canals as the animal gets older (Lutfi et al., 1970; Wilsman and Van Sickle, 1972; Cole and Wezeman, 1985; Ytrehus et al., 2004a,b).

Burkus et al. (1993) described the cartilage canals as acellular channels originating as small collections of pluripotent mesenchymal cells arising from the surrounding perichondrium and penetrating into the central portion of the chondroepiphysis. The proliferating canals were seen to develop during the tenth to twelfth week of gestation, and the mesenchymal cells formed vessels and supporting connective tissue elements with a thin, acellular, hyperchromatic boundary by twelve weeks. The canals were relatively evenly distributed throughout the epiphysis by fourteen weeks, and mesenchymal cells differentiated into endothelial cells forming distinct arterioles, capillaries, and veins over the next two weeks. Each canal contained at least one artery and vein separated by a highly organized fibrous tissue stroma by sixteen to twenty two weeks (Burkus et al., 1993). Stump (1925), Haines (1933), Hurrell (1934) and Lutfi (1970) thought that canals advanced by the breakdown of cartilage matrix. No canals originate along the articular joint surface and are not formed in the diaphysial region prior to development of primary centre of ossification.

The present study was done to ascertain the histological changes in the human fetal femur with increasing gestational age. The structural changes in the origin and fate of the cartilage canals were assessed in both the proximal and distal epiphysis of the developing femur. An attempt was made to note the appearance of secondary ossification centres in the distal femoral epiphysis.

MATERIALS AND METHODS

The present study was carried out in the Department of Anatomy, Government Medical College and Hospital, Chandigarh, on 30 human fetuses from 12^{th} to 28^{th} week of gestational age. The specimens were provided by the department of Obstetrics & Gynecology for routine fetal autopsy. All fetal specimens were the result of intrauterine deaths or spontaneous abortions and care was taken to exclude the cases of congenital malformations of musculoskeletal system or visible skeletal defects. Consent to perform autopsy and relevant history from the parents was taken.

This study was conducted on the ethical guidelines for biomedical research on human subject as given in “Declaration of Helsinki” and by Central Ethics Committee on Human Research (CECHR) of ICMR, New Delhi and clearance was obtained from the Institutional Ethical-Committee. The femoa of left side were used for the present study and after the routine autopsy, were cautiously disarticulated in order to preserve articular cartilage. These were fixed in 10% formalin solution for 72 hours.

The fetuses were divided according to gestational age groups as follows:

Table 1. Gestational age groups and number of fetuses

GROUPS	GESTATIONAL AGE	NUMBER OF FETUSES
A	11-15 weeks	06
B	>15-20 weeks	11
C	>20-25 weeks	08
D	>25-30 weeks	05

The tissues were decalcified in acidic EDTA decalcifying solution comprising of 5.5 gms EDTA in 10% formalin for 3-4 weeks. The bones were cut into two halves at midshaft for longitudinal sections which could include epiphysis, metaphysis and disphysis both proximally and distally. The transverse sections of whole bones were taken at different levels. 8-10 µm thick paraffin sections were cut with rotary microtome and later stained with hematoxylin and eosin stain to assess the structure of the developing bone. The prepared slides were examined under an Olympus BX51 research microscope, and selected sections were photographed using DS-Fi1c Nikon camera attached to it.

RESULTS

The observations in both longitudinal and transverse sections are described in different age

groups as follows:

Group A (11-15 weeks) (Fig. 1)

Epiphysis- Chondroblasts were seen as an undifferentiated cluster in the transverse section of the whole epiphysis at both distal and proximal ends as early as 12 weeks of gestation [Fig. 1 (i)]. Chondrocytes were predominantly fusiform in shape with long axis along the long axis of bone in early gestations. A thick perichondrium with its both layers was visible, but was not seen lining the articular surface. An incipient epiphyseal plate was evidenced with formation of resting and proliferative zones. A hypertrophic zone was seen to appear by week 15 [Fig. 1 (iii)].

Cartilage canal formation was first evidenced in the proximal epiphysis at 58 mm, and in the distal epiphysis at 62 mm as stages of mesenchymal cells at the advancing tip of the canal to be organized in the centre. A hypertrophic cartilaginous matrix appears at the margins of the canals. There are no connective tissue elements or blood vessels in the canal yet [Fig. 1 (ii)]. Metaphysis is intact and clear, showing calcification changes and infiltration by numerous blood vessels between plates of calcified cartilage.

Diaphysis- Diaphysis of the immature bone at early gestations is seen to show a larger number of cells and doesn't display an organized lamellated appearance [Fig. 1 (iv)]. Periosteal collar formation is seen at 12^{+4}weeks and a well-formed thickened collar can be seen by 15 weeks of gestation. Formation of bony trabeculae is seen to predominate from one side of the shaft. Periosteum is not present on the articular surfaces of the bone, the sites of insertion of tendons and ligaments and at several other discrete sites such as the subcapsular area of the neck of the femur. Bone deposition is seen over the spicules of calcified cartilage [Fig. 1 (iii)].

Group B (>15-20 weeks) (Fig. 2)

Epiphysis- Well differentiated zones of reserve, proliferating and hypertrophic cartilage seen at both the proximal and distal epiphysis, and this zonation is more apparent on the distal epiphysis. Chondroblasts are seen to be fusiform in shape. The upper end still shows proliferating chondroblasts in early gestation, progressing to hypertrophic cartilage in the later part of gestational ages. In contrast to this, the lower end is full of hypertrophied chondroblasts even from the early ages, thereby implying that growth in the distal end precedes that of the proximal end [Fig. 2 (i)].

Cartilage canals are distinct, more in number at the distal epiphysis. The canals now show appearance of a lining of single layered cells [Fig. 2 (ii)]. This lining further proliferates to being multi-

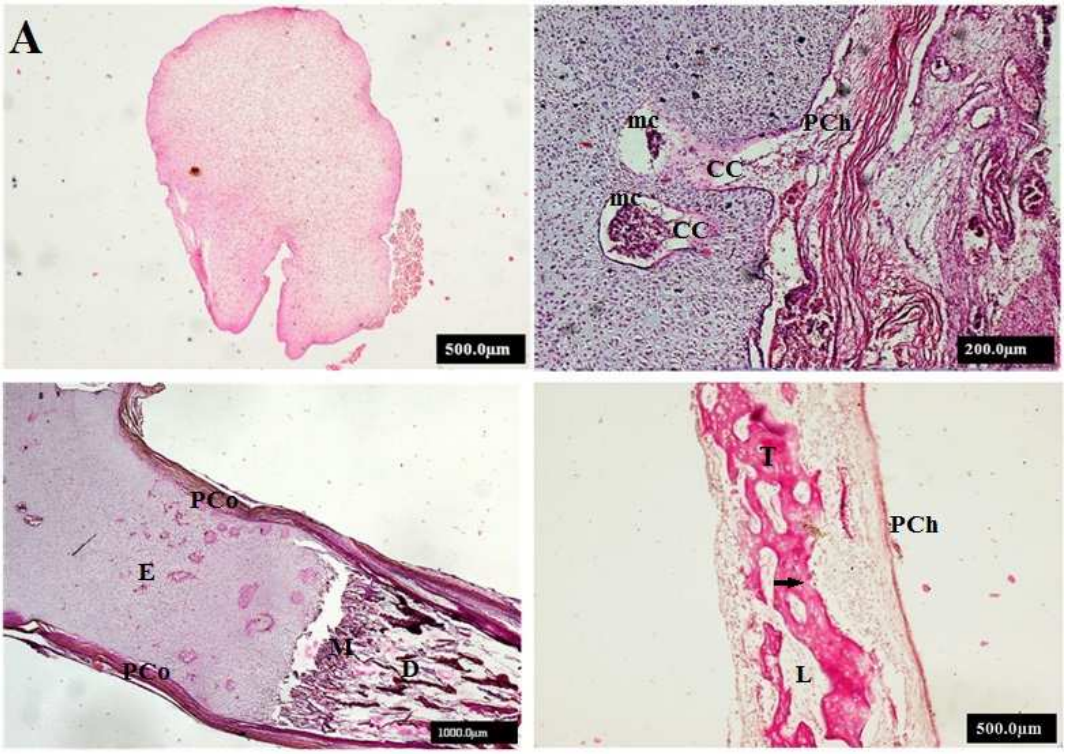

Fig. 1. Light microscopy- Group A (11-15 weeks): **i**) and **ii**)- Transverse (T.S) and longitudinal (L.S) section of the epiphysis at 12^{+1} and 13^{+2} weeks showing undifferentiated cluster of chondrocytes with absence of any cartilage canals and appearance of cartilage canals (CC) from perichondrium (PCh) containing cluster of mesenchymal cells (mc) at tip respectively. **iii**) L.S of bone at 15 weeks showing a well defined periosteal collar (PCo) and a clear cut distinction between epiphysis (E), metaphysis (M) and diaphysis (D). **iv**)-L.S Diaphysis at 13^{+4} weeks showing perichondrium (PCh) with invaginating trabeculae (T) more on one side of the shaft. The arrow points towards a Howship's lacuna.

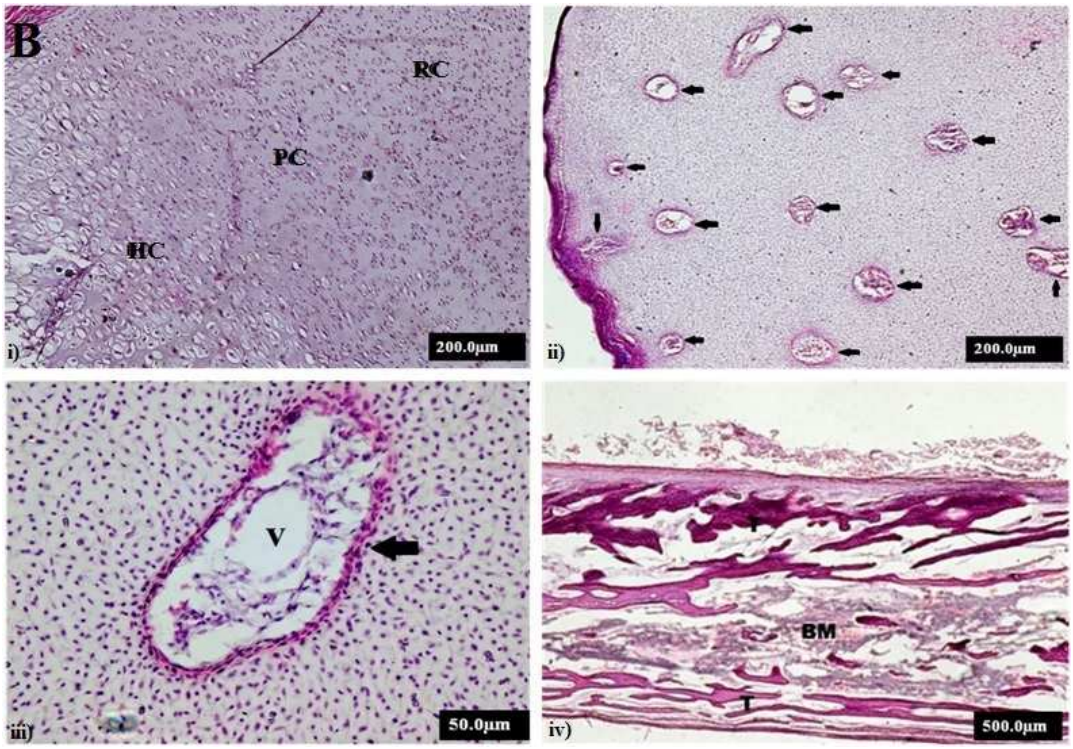

Fig. 2. Light microscopy- Group B (>15-20weeks): **i)-ii)** L.S Epiphysis at various developmental stages showing well differentiated zones of resting (RC), proliferating (PC) and hypertrophic (HC) cartilage at 15^{+6} weeks (i) and increasing no. of cartilage canals (shown with arrows) with a lining of single layered cells at 16^{+2} weeks (ii). **iii)** shows a higher magnification of a cartilage canal with a multilayered lining membrane and evidence of vasculogenesis (v) at 17^{+3}weeks. The canal is surrounded by fusiform shaped chondrocytes. **iv)** L.S Diaphysis at 17^{+3} weeks showing developing trabeculae (T) more on one side of the shaft with bone marrow (BM) in between.

layered, and mesenchymal clusters of cells begin to arrange themselves in circular patterns and show vascularization [Fig. 2 (iii)].

Diaphysis- The periosteum of the bone clearly has two layers, viz. inner cellular and outer fibrous, and the periosteal bone continues to form after the formation of periosteal collar [Fig. 2 (iv)]. The region of the diaphysis approximating the metaphysis shows the characteristics of woven bone, whereas, on moving farther away from the metaphysis, the formation of cancellous bone with scattered trabeculae is seen. This formation of bony trabeculae starts replacing the calcified cartilage spicules, and is seen to be more prominent on one side of the shaft. An irregular primary marrow cavity is seen, which is narrow in the centre of the shaft, and broader with more blood vessels towards the end of the diaphysis.

Group C (>20-25 weeks) (Fig. 3)

Epiphysis- The epiphysial plate as gestation increases is more developed and easily apprecia-

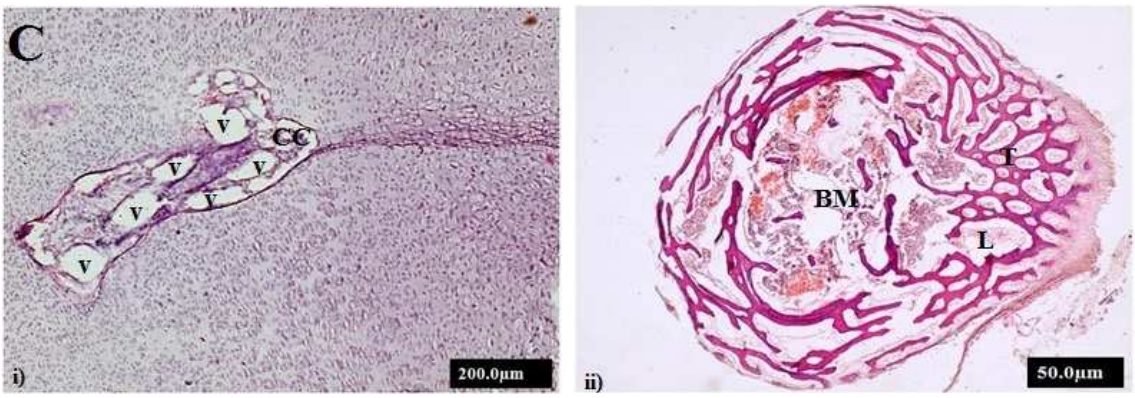

Fig. 3. Light microscopy- Group C (>25-30weeks): **i)** L.S epiphysis at 20^{+4} weeks showing differentiation of mesenchymal cells in cartilage canal (CC) into distinct vascular channels (v). **ii)** shows T.S Diaphysis at 21^{+5} weeks showing well defined trabeculae (T) and lacunae (L) more on one side of the shaft with bone marrow (BM) in the center.

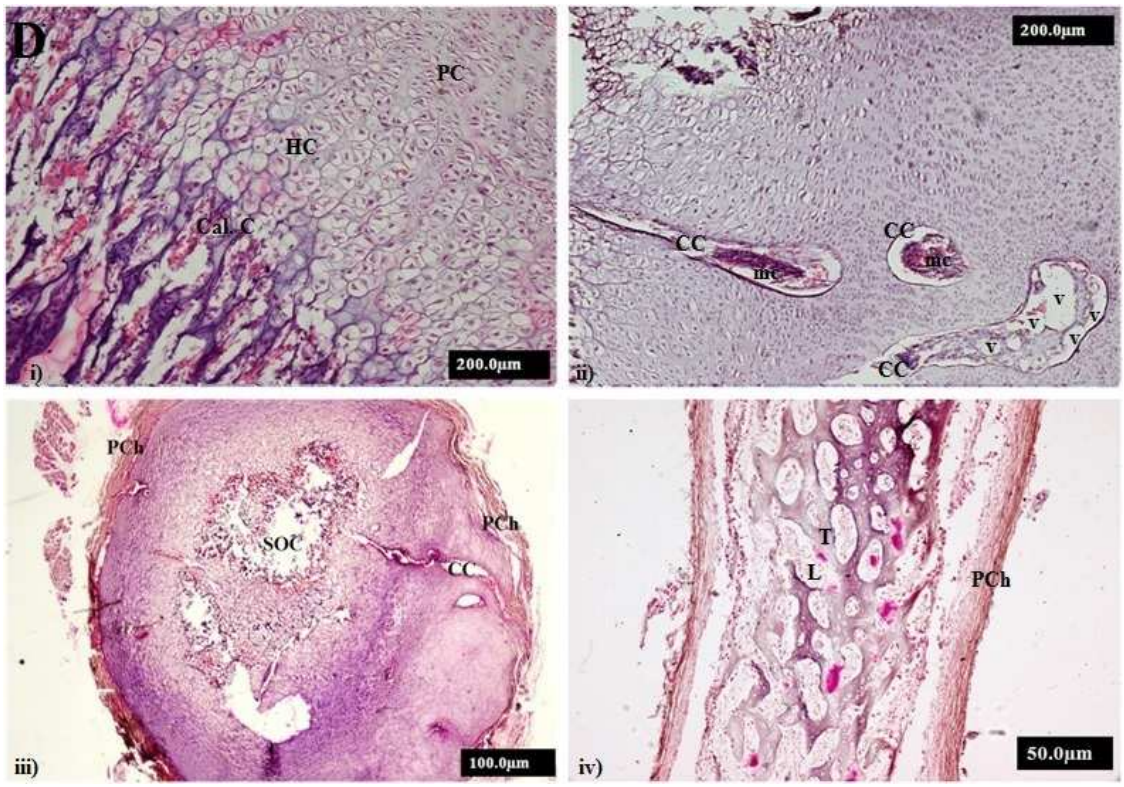

Fig. 4. Light microscopy- Group D (>25-30weeks): **i)**- LS Epiphysis at 25^{+3} weeks of gestation showing intact metaphysis with zones of proliferating (PC), hypertrophic (HC) and calcified cartilage (Cal. C) as well as cartilage canals in **ii)** at 25^{+6} weeks of gestation showing cartilage canals approximating the hypoertrophic cartilage (HC) with dedifferentiation of well defined vascular and connective tissue elements into aggregates of mesenchymal cells. **iii)** shows the presence of secondary centre of ossification (SOC) within the central portion of T.S Epiphysis at 28^{+4} weeks of gestation, an advancing cartilage canal from perichondrium (PCh) to centre is visible. L.S Diaphysis at 29weeks in **iv)** shows perichondrium (PCh) with well defined developing trabeculae (T) and lacunae (L).

ble at a lower magnification at distal epiphysis. The proliferation of cartilage to hypertrophic zone at later gestations in group B at the proximal epiphysis is now well developed with increasing age. At higher magnification, the epiphysial growth plate shows a progression of morphological changes from resting, proliferative and hypertrophic to calcified cartilage approximating the diaphysis. This change is clearly seen to precede in the distal epiphysis rather than the proximal. The cartilage canals are observed to predominate the distal epiphysis and are seen to contain the blood vessels differentiating into distinct arterioles and venules [Fig. 3 (i)]. The width of the metaphysis is more at this gestation with much increased calcification, blood vessels and the bone shows the changes in the formation of woven bone.

Diaphysis- The longitudinal section through the diaphysis is seen to show calcified cartilage spicules on which the bone has deposited and at some places the spicules have already grown to create bony trabeculae. These initial trabeculae still contain remnants of calcified cartilage. A transverse section at the distal end of the diaphysis and at the level of mid shaft of the femur also supports the prominence of growth at one end of the bone with a larger number of trabeculae on one side of the shaft [Fig. 3 (ii)].

Group D (>25-30weeks) (Fig. 4)

Epiphysis- The presumptive epiphyseal plate region between the epiphysis and the diaphysis has the zones of resting cartilage, proliferation, hypertrophy, calcification and ossification adjoining the region of metaphysis which is adjacent to the diaphysis. Some of these zones may "overlap" and this is frequently seen in the case with the zones of calcification and ossification [Fig. 4 (i)].

Simultaneous changes were seen to occur in the central chondrocyte cells and in the adjacent, centrally-located cartilage canals. The chondrocytes begin to hypertrophy and show regression changes with dedifferentiation of well-defined vascular and connective tissue elements into aggregates of mesenchymal cells within the cartilage canals [Fig. 4 (ii)]. This dedifferentiation is not observed to be predominating the proximal epiphysis yet. The appearance of a secondary centre of ossification within the central portion of the distal femoral epiphysis was evidenced at 28^{+4} weeks. The presence of covering periosteum and all zones of epiphyseal plate from periphery to centre were visible. A vascular channel is seen arising from the perichondrium and penetrating the ossified mass [Fig. 4 (iii)].

Diaphysis- The formation of bony trabeculae is

seen to be increased more with increasing gestation. Some remains of the calcified cartilage are seen in trabeculae even at this stage. The intertrabecular spaces of the bone are filled with bone marrow containing the primitive stem cells from which all the cellular elements of bone are derived [Fig. 4 (iv)].

DISCUSSION

The development of cartilage and bone in the primary centres of the skeleton has been the subject of a very large number of researches, and the morphological aspect of the various phases is now known in great detail. The process of endochondral ossification occurring for the longitudinal growth of the long bones comprises sequential changes of mesenchymal condensations, chondrocyte proliferation and hypertrophic differentiation and terminal replacement with bone. This multistep process is regulated by a complex network of signalling systems and the factors responsible for the same have been studied by various researchers (Pines and Hurwitz, 1991; Putz, 1996; Babarina et al., 2001; Vortkamp, 2001; Milz et al., 2002; Higashikawa et al., 2006; Mackie et al., 2008).

The development and structure of the epiphysis remains a keystone for the development of the long bones. Putz (1996) studied the structure of the epiphysis of the fetal long bones and described the characteristic series of events occurring in the individual epiphysis and the factors associated with them. The sequential changes in the chondrocytes behaviour have been described to be regulated by both systemic and locally secreted factors (Mackie et al., 2008; Milz et al., 2002; Ortega et al., 2004). Various studies have elaborated the molecular mechanism of chondrocyte differentiation and highlighted the interactions of a variety of growth factors, cytokines and signal molecules. The process of mesenchymal condensations, chondrocyte differentiation and proliferation has been shown to be regulated by bone morphogenetic proteins (BMPs) and Sry-box 9. The rate of cartilage differentiation is modulated by parathyroid hormone related peptide (PTHrP) and Indian hedgehog (Ihh), while fibroblast growth factor receptor 3 inhibits proliferation of chondrocytes and promotes hypertrophic differentiation (Babarina et al., 2001; Vortkamp, 2001; Higashikawa et al., 2006; Kitoh and Ishiquro, 2007).

The present study documented the presence of an undifferentiated cluster of chondroblasts and their further differentiation with the increasing gestational age, and noted the characteristic features of distinct cartilage canals with increasing gestational ages. Cartilage canals have been widely investigated as to their mode of formation, morphologic distribution, function and fate. The cartilage canals as described by Stump (1925) are the collection of arteriole, venule and the capillary plexus between them lying within the matrix of connective tissue. They originate as small collections of pluripotential mesenchymal cells from surrounding perichondrium and contribute to osteogenesis in a secondary ossification centre (Wilsman and Van Sickle, 1970; Kugler et al., 1979; Agarwal et al., 1984; Burkus et al., 1993; Ganey et al., 1995; Shapiro, 1998; Fritsch et al., 2001; Rivas and Shapiro, 2002). The anatomy of the human fetal cartilage canals was described by Haines (1933) from serial sections and classified as branched and unbranched, simple-, double- and multiple rooted, tunnel, divided, communicating, nutrient and centrifugal canals.

The presence of cartilage canals has also been assessed in avian as well as other mammalian studies. Several studies done on the tibial and femoral epiphysis of chicken showed that the changes in avian cartilage canals is comparable to that of mammals (Lutfi, 1970a,b; Domenech-Ratto et al., 1999; Blumer et al., 2004a,b). Ytrehus et al. (2004a,b) in their study showed that cartilage canals regress with age in the distal epiphysis of the femur in pigs. Cartilage canals are evidenced in human proximal metacarpal and distal phalyngeal epiphysis without the presence of an ossification centre (Soames, 1995). Cartilage canals may also be involved in perichondral (intramembranous) bone formation (Gray and Gardner, 1969; Chandraraj and Briggs, 1988). Several studies conducted in marsupials and rats showed that cartilage canals appear to be absent in the femoral head during early development, thus stating that cartilage canals are not a prerequisite for endochondral ossification (Thorp, 1990; Morini et al., 1999; Reno et al., 2006). The vascular canal area and number was estimated in human ribs and findings were compared in normal neonates and children and in achondrogenesis II- hypochondrogenesis by Gruber et al. (1990). Both the canal area as well as the number showed a 10 times increase in achondrogenesis II- hypochondrogenesis

Blumer et al., in various studies on distal limbs of chicken (*Gallus gallus*) by light microscopy, electron microscopy and immunohistochemistry with VEGF, type I and type II collagens, stated the structure, formation and the role of cartilage canals and laid emphasis upon neovascularisation for the endochondral bone formation (Blumer et al., 2004a,b, 2005, 2007, 2008). Burkus et al. (1993) established the formation of cartilage canals in distal epiphysial centres of the femur in their study on human fetuses from 6th week to 32nd week of gestation. The beginning of formation of the canals was seen as early as eight to ten weeks of gestation and the mesenchymal tissue elements were seen to fill and differentiate into complex network of vascular and connective tissue elements in the canal by 14th week. By the 16th to 20th week the canals were shown to have distinct differentiation into arterioles and venules.

The present study showed the similar structure of the cartilage canals in the longitudinal sections of the femur from the 11^{th} to the 30^{th} week of gestation. Both the proximal and the distal epiphysis have been studied in the present study in contrast to only distal epiphysis studied by Burkus et al. (1993). The earliest appearance of the cartilage canal was seen at 13^{+2} weeks in the proximal epiphysis, and showed the mesenchymal tissue advancing towards the tip of the canal. The mesenchymal tissue in the canal was seen both proliferating towards the tip and organized in the centre at 14^{+4} weeks in the present study. The mesenchymal tissue was seen to be organized in the centre of the canal by 15^{th} week of gestation. The canals were seen to show a lining of single layered cells and the initiation of differentiation into vascular tissue by 15^{+4} weeks. This lining was seen to proliferate into a multiple-layered lining by 17^{+1} weeks. The beginning of formation of distinct arteriole and venule within the canal was seen by 21^{+3} weeks. The cartilage canals were seen to be distinct and more in number at the distal end. The resting chondrocytes at the tip of the cartilage canals were classified by Blumer et al. (2007) into a light staining viable and a dark chondrocyte showing changes of degeneration. Anderson and Matthiessen (1966), using only light microscopical and histochemical techniques, described collections of multinucleate histiocytes at the end of the cartilage canals. Kugler et al. (1979) observed the presence of chondroclasts as multinucleated giant cells containing large numbers of mitochondria, varying numbers of vesicles and a ruffled border. These were seen to be in contact with the cartilage matrix responsible for removing the intervening cartilage to allow the osteoblasts to proliferate within the secondary centre.

Chondrification changes were seen in the present study by 27^{+2} weeks of gestation. In this process the vessels degenerate and the mesenchymal cells are converted into matrix-producing chondrocytes, occluding the lumen of the canals making them unavailable for bone formation (Lutfi et al., 1970; Wilsman and Van Sickle, 1972; Cole and Wezeman, 1985; Ytrehus et al., 2004a,b). The canals in the present study adjacent to the central region of hypertrophying cartilage showed similar features of dedifferentiation of vascular elements and clumps of mesenchymal tissue elements by 27^{+2} weeks. This regression was not seen in developing chicken femur up to the second week post hatching where preosteoblast cells are recruited which differentiate osteoblasts which were seen to express type I collagen (Blumer et al., 2005).

Apart from type I collagen, these preosteoblasts express other bone-specific proteins such as periostin and TNAP (tissue-non-specific alkaline phosphatase). Periostin is a specific marker for preosteoblasts and its levels are seen to decrease with further differentiation of preosteoblasts into osteocytes as osteocytes do not synthesize periostin (Horiuchi et al. 1999; Allen et al., 2004). Periostin expression ceases in preosteoblast/preosteocyte cell line MLO-A5, MLO-C2, MLO-D1, MLO-D6 and MLO-Y4 thus indicating that these cells represent a later stage of differentiation similar to osteoblast to osteocytes respectively. Blumer et al. (2006) established the localization of periostin by immunohistochemistry and mRNA *in situ* hybridization. The expression of periostin mRNA was seen to be high in canals outside the secondary centre of ossification within the perichondrium.

At 28^{+4} weeks the appearance of a secondary ossification centre was evidenced in the transverse section within the central portion of the distal femoral epiphysis. Epiphysis grows in transverse diameter by peripheral extension of epiphyseal endochondral ossification with regressive changes and bone formation similar to that in the diaphysial cartilage. The presence of covering periosteum and all zones of epiphysial plate from periphery to centre was visible. A thin zone of hyaline cartilage always remains at the surface as the articular cartilage (Wheater et al., 1987). The appearance of a secondary centre of ossification in the present study supports the radiographic documentation of an ossification centre of distal femoral epiphysis by 29-30 weeks of gestation (Davies et al., 1974).

The development and proliferation of cartilage canals was seen to be larger in the distal epiphysis than in the proximal, though the formation was noted earlier at the proximal epiphysis. The features of the canals described in the present study have been described in favour of the contention that the canals provide osteogenic cells and blood vessels for the formation of the secondary centre of ossification well before the centre is formed. The ossification centre for the distal epiphysis of femur appears just before birth in 9^{th} month of gestation, whereas the three ossification centres for the proximal epiphysis arise at 6 months, 4 and 12 years of life. The cartilage canals in the present study were seen at both the proximal and distal ends but varied in their number. Increasing and more proliferating canals were seen to predominate in the distal epiphysis in favour of the earliest appearance of its centre by as early as 29^{+3} weeks of gestation (Davies et al., 1974). The features of the canals seen in proximal epiphysis at 24^{+6} weeks were seen to be similar to those seen in distal epiphysis at 18^{+4} weeks of gestation.

Gilmore and Palfrey (1987) studied the histology of human femoral condylar articular cartilage in both infantile (26, 30 weeks, 1, 6 days and 2 years) and mature (5, 6, 31 and 35 years) pattern. The infantile pattern showed characteristic fusiform cells with long thin nuclei, while the mature pattern showed more rounded cells. Also in the present study the chondrocytes visible were predominantly fusiform with long thin nuclei and long axis of the cell along the long axis of the bone. In another

study, they studied the distribution of chondrocytes throughout the total thickness and estimated the cell density in infantile, children and adult specimens (Gilmore and Palfrey, 1988). The distribution of chondrocytes in the articular cartilage was not assessed in the present study.

Quantitative data on metaphyseal bone histology during early human development are scarce. A study was conducted by Salle et al. (2002) on the proximal femoral metaphysis by histomorphometry, and showed that trabecular thickening was seen to occur at an estimated rate of 3µm/day in areas close to the growth plate. Gardner and Gray (1970) studied the prenatal development of the human femur on forty pairs from fetuses of 26 to 342 mm crown-rump length. They stated that a primary bone collar was present before the end of the embryonic period, and cartilage canals were noted to first appear in the proximal epiphysis at 57 mm and in the distal epiphysis at 61 mm. Trabeculation of the bony collar was first noted at 37 mm. The cartilage canals in the present study were also seen to appear first in proximal epiphysis at 58 mm and distal epiphysis at 62 mm.

The characteristic features of the diaphysis of the prenatal femora from the 11^{th} to the 30^{th} week of gestation showed the presence of both cellular and fibrous layers of the periosteum at 13^{+4} of gestation in the present study. Periosteal collar formation was seen at 12^{+4} weeks and a thick well defined collar was evidenced by 15weeks. With increasing gestation, the region of diaphysis approximating the metaphysis showed the characteristics of woven bone, whereas on moving farther away from metaphysis, the features of cancellous bone with scattered trabeculae were seen. The periosteal collar and bony trabeculae were seen to be more prominent on one side of the shaft in most of the specimens.

Not much literature could be gathered on the histological changes occurring in the diaphysis of the fetal femur. The present study noted the proliferation of the primary centre of ossification which starts in the middle of the shaft by 8 weeks of gestation. It was seen that the ossification started on one side of the shaft and then propagated throughout. Although work has been done on the structural changes occurring in the epiphysis and the metaphysis, the present study could not be compared to the changes in the diaphysis.

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