Some prenatal morphohistological studies on the developing human fourth rib with reference to ossification centers, cartilage vascular canals, in light of suret el Room8, Lukman11 and el thareate 21

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Abstract: Thirty five human fetuses aged 4, 5, 6, 7, full term and newborn infant: 4 months (13-16wks-CRL 9-14cm), 5 months old fetuses, (17 -20weeks) CRL 15-19cm, 6-months old fetus:(21 -24weeks) CRL 20-23cm, 7 months old fetus:(25-28weeks) CRL 24-27cm, besides full-term:(33-36 weeks- CRL 31-34cm) and newborn infant (37-38 weeks-(CRL 35-36cm) were studied in the present work.6 adult ribs were used for comparison. The fetuses were obtained from the miscarriage and spontaneous abortion from Gynecology and Obstetrics Department of Al -Zaharaa hospital-Faculty of medicine for girls –Al-Azhar University, Egypt (according to medical ethics). They were used to study the normal morphogenesis of the developing prenatal human fetal fourth rib. Adult corresponding ribs from new cadavers (male and female) were used. The cadavers were obtained from the Dissection room – Anatomy Department - Faculty of medicine for girls -Al Azhar University, Cairo -Egypt. The 4th ribs were dissected and the costochondral junction were cut off, decalcified, blocked and prepared for histological study using heamatoxyline and eosin stain, Malory triple stain and Masson trichrome stain. Photos for the histological study were taken by Olympus50xB Microscope, attached to Camera: OlympusDP72, and Connected to the computer Dell at King Fahd center-King Abdul Aziz. University-KSA-Jeddah, morphology photos by canon camera zoom were made. Measures of the length of subperiosteal bone collar SPBC, and estimation of the diameters of secondary ossification centers SOC, and the cartilage canals were done according to the scale on the microphotograph. It was found that, each fourth rib had anterior sternal end, shaft, and posterior vertebral end. The rib had an angle which divided it into posterior cylindrical 1/4 part and anterior 3/4 flattened part. The angle became more open with age progress. Each 4thrib had two borders; upper and lower and two surfaces; inner and outer. The lower border in the prenatal ages became sharper with age progress. Slight twist in the shaft of full term rib was first noted, whereas there was great twist in adult rib. The subcostal groove was detected in full term. The posterior end beard head, neck and tubercle. The tubercle was first noted at 6-month old age fetus:(21 -24weeks- 20-23cm), and became more prominent with age progress. The prenatal ribs increased in size and length with age progress. Histological examination of parts of radial, TS&LS serial sections of parts of the 4th developing prenatal human rib of 4,5, 6,7 aged fetuses, full term - and newborn infant, at the costochondral junction (CCJ), showed that the CCJ was formed of chondroblasts arranged in columns embedded in matrix, standing on sponge irregular bone. The epiphyseal cartilage growth plate was zones with no demarcation lines: the germinative, proliferative, hypertrophic zones. At 4month aged fetus, the cartilage growth plate was long with no provisional zone of calcification PZC, while the sponge area was short. The PZC was noted at 5 month aged fetus, and increased in surface area with age progress till full term. The cartilage growth plate of 9month aged fetus was short and thin, while the sponge bone increased in length due to added bone grown by endochondral ossification. The germinative (inert,reserve,stem cell zone) was formed of small cells within large amount of matrix. The proliferative zone started as small flat cartilage cells, with dividing cells, which increased in size and formed cell columns, between few matrix. The hypertrophic zone was formed of enormously enlarged cartilage cells regularly arranged in columns between threads of matrix, with final 2-3 ballooned cells bursting in the sponge bone of the metaphysis, which represented primary ossification center POC, causing rib appositional growth and elongation by endochondral ossification. The transitional complex area; cartilage -to- bone at CCJ, showed the presence of cartilage cell columns between threads of matrix, and chondroclasts gaint multinucleate cells, eroding the matrix, basophilic polarized osteoblasts on the surface of the newly formed matrix, besides blood cells in the sponge. Ossification corner forming primary ossification center POC was seen. The secondary ossification centers SOC, in the epiphysis after establishment of the POC of 4month aged fetus, showed the presence of cartilage cells columns between threads of matrix, chondroclasts, basophilic polarized osteoblasts and blood cells, in addition to Osteocytes in stages of formation present in eosin acidic field due to TRAP production from the chondroclasts. Enlarged cartilage cells around the SOC formed a morphological plate similar to the growth plate at the CCJ were seen. The differences between POC and SOC were that; the SOC center, was invaded by cartilage vascular canals, which incorporated, branched and distributed inside the SOC, and their walls transformed to basophil bone like cells. Osteoclasts were embedded between the matrix of the sponge bone, surrounded by, in the POC and also in the SOC. The periosteum and perichondriuem of 4month aged fetus were thin at the costochondral junction CCJ, and increased in thickness with

age progress. The periosteum was continuos with the perichondrium. The periosteum, at the CCJ of the developing prenatal human 4th rib of all ages studied, was formed of a fibrous sheath surrounded the outer surface of the bony part. It was composed of: outer layer of dense white fibrous tissue contained blood vessels, and an inner layer contained loose tissue and osteoblasts. The inner osteogenic or osteoblastic layer was the germinative layer which formed new bones. The sponge irregular bone was formed of irregular trabeculae appeared anastomosing. On the surfaces of the trabeculae, there were osteoblasts branched deep basophilic polarized cells formed a continues layer were seen, chondroclasts giant multinucleate cells were seen. The spaces between the trabeculea of the sponge at the CCJ increased with age progress. The thickness and density of the trabeculea increased with age progress. The perichondruin in 4months fetus, was formed of thin layers of CT, and mesenchyme` full of blood vessels, which increased in thickness with age progress. Three degrees of stain affinities were noted by Masson trichrome and Malory triple stain, in the perichondrium, sub perichondrium, and the hyaline cartilage of the developing prenatal human 4th rib, indicating the presence of different types of precollagen, collagen and tissue remodeling. The perichondrium stained strong deep, the sub perchondrium stained moderate and the hyaline cartilage had faint stain juxta the CCJ. The difference in stain affinities was obvious in all developing prenatal ages. At 4months aged fetus, the perichondria layers showed,special complex arrangement to allow pathway for cartilage canals and Spur contour arrangement of cartilage... cells beneath the complex arrangement in the epiphysis was seen by Mallory triple stain,and Masson trichrome stain.At 4,5,6,7 and 9 months aged fetuses, cartilage vascular canals surrounded by mesenchyme extended from the vascular perichondrium, were detected.Some canals at 4month aged fetus had mesenchyme and no vessels.Cartilage vascular canals varied in shape,size and content were noted close to secondary ossification centers SOC, aproched,anchored,incorporated and distributed inside the SOC in the epiphyseal cartilage in ALL prenatal ages studied. Cartilage canals number increased at full term. At the age of 6month aged fetus, some cartilage canals containing collagen with incomplete wall were noted. At the age of 7month fetus, some empty cartilage canals with conical ends and stripes of matrix around the canals were noted, besides obliterated occluded cartilage canals were seen. At 9month age fetus, cartilage canals with kinking course, small branches, reunited with terminal glomerulus were seen. Wedge shaped Groove of Ranveir (GR) full of newly formed cartilage cells, and subperiosteal bone collar SPBC or bone bark, which had rich vascular perchondrial supply were noted at the periphysis of 4month aged fetus and full term. The formed bone bark or SPBC, were formed by membranous ossification synchronous at the same time with endochondral ossification at the CCJ. That event, occurred to strengthen and support the rib and provided added cells to the growth plate, after endochondral ossification at the CCJ, to compansate and support cartilage cell loss after bursting in the sponge. That coincided with Quraan suret el ensane 28. which meant that Allah created the human and supported and strengthen his creation. The length of the SPBC of 4 month aged fetus was approximately 670um.and the length of the SPBC–of full term was approximately ranged 300-460um according to the scale on the microphotograph. At 4month aged fetus, Small secondary ossification centers coalesced to form large ossification center. Superficial vascular cartilage canals extending from the complex vascular perichondrium, invaded the secondary ossification center SOC At 6 and 9months aged fetuses, initial multiple secondary ossification centers were seen. The initial secondary ossification centers SOC were formed of collected enlarged cartilage cells aggregated in round areas, and surounded by vascular cartilage canals,which varied in shape size and contents.and some showed chondrolysis.Some vascular cartilage canals were occupied by one vessel.The secondary SOC occurred, AFTER first establishment of POC,coincided with Quran,suret al zomar 6and, suret Nooh14.Conclusion:Changes with age progress were noted in the prenatal developing human 4th rib at the costochondral junction CCJ: in the cartilage growth plate,cartilage vascular canals, tissue perichondrium, periosteum, grove of Ranvier,irregular cancelous spongy bone, bone trabecular –primary ossification center POC, secondary ossification center SOC.The changes were to accommodate for the function of the developing ribs as container and protection of the thoracic organs. The changes in the prenatal developing human 4th ribs with age progress illustrated some scientific, medical, and linguistic miraculous signs of suret el Room8 (Roman), Lukman11, el thareate21and fuselate 53. The Quran indicatd the presence of powerfull creator ALLAH.

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Key words: Developing prenatal human 4th rib- costochondral junction CCJ –Morphology-histology- Growth plate – cartilage canals – (primary –secondary ossification centers POC-SOC) - sponge bone- perichondrium- periosteum - grove of Ranvier –subperiosteal bone collar SPBC-

Introduction

The review of literature showed that the changes in the costal cartilage of children had involved in many diseases; rickets (Park, 1954; Shapiro & Boyde, 1987; Elisa et al., 2020), Achondrogenesis II-hypochondrogenesis (Borochowitz et al., 1986). Achondrogenesis-hypochondrogenesis (Harten et al., 1988). Achondrogenesis type II, (Horton et al., 1987), skeletal dysplasias (Sillence. et al., 1979), in dwarfism and achondroplasia (Harris and Russel, 1933), skeletal dysplasias (Yang et al., 1986), Goldenhar syndrome (Heffez anf Doku, 1984)

It was documented that the rib cartilage from the costochondral junction CCJ was used as grafts and transplants in surgical reconstruction of tempro mandibular joint TMJ, and the error in the graft might cause lateral facial asymmetry of children due to irregular cartilage cell growth (Copray et al., 1986, Daniels et al., 1987, Peltomaki, 1992, Peltomaki and Isotupa, 1991, Peltomaki and Ronning,1991; Siegel, 1958, Seinsheimer and Sledge 1981; Seinsheimer and Sledge, 1981, Copray and Jansen Duterloo, 1986).

It was reported that rib cartilage from the costchondral junction was used as cartilaginous fabrication in surgical reconstruction of microtia in Children (Andreoli et al., 2013; Yan et al., 2020).

The review of literature showed that the researches about the cartilage canals in the prenatal developing human rib were scarce and rare. The relation between the cartilage canals and secondary ossification centers was pointed out by few authors (Agrawal et al., 1984; Agrawal. 1986; Wilsman & Van sickle, 1970; Gruber et al., 1990).

Emery and Kalpaktsoglou (1967) established criteria for normality and abnormality of the costochondral junction for the foetus and newborn, and, after the study of about 500 costochondral junctions, their criteria were presented to the Pathological Society (Emery, 1957). Then, they examined over 5000 costochondral junctions from perinatal and child deaths. That had confirmed their original concepts of the normal appearances, and their conviction that the routine study of the costochondral junction was one of the most valuable examinations that could be carried out in perinatal pathology. Their paper concerned with three objectives. (1). A description of the appearance of the normal costochondral junction at birth and during the latter third of intrauterine life. They **summarized their histological** study of the costochondral junction, which had been carried out on a large series of perinatal deaths, that the normal and abnormal appearances of the costochondral junction of the older fetuses were described as follows: Two types of deformity were found, interpreted as due to growth arrest and to a bizarre form of growth. The evidence suggested that, of children dying during labour or in the neonatal period, approximately 75%0 showed evidence of **disordered growth in utero before labour had begun.** The histological study of the costochondral junction was an extremely valuable part of the study of any perinatal death.

Gruber et al. (1990) mentioned that knowledge of the structure of cartilage vascular canals was important for a more thorough understanding of the development of cartilage and the growth plate in the human neonate and growing child. They studied the costochondral junction of 6 normal neonates and 12 normal children (age 4 months-16 years) and utilized quantitative histomorphometry to define the percentage tissue area occupied by canals and the number of canals/mm2. They found that, both percentage canal area and the number of canals/mm2 were significantly greater in newborn vs. older children (percentage area: 0.42 +/- 0.15 (mean +/- S.E.M.) vs. 0.08 +/- 0.04, P = 0.003; number/mm2: 0.2 +/- 0.09 vs. 0.04 +/- 0.02, P = 0.02). They studied also eight newborn patients with achondrogenesis II-hypochondrogenesis. They found that, both percentage canal area and number were significantly elevated above normal (percentage area: 5.22 +/- 1.01, P less than 0.001; number/mm2: 1.45 +/- 0.26, P less than 0.001). Gruber et al., demonstrated that their results illustrated that: (i) quantitative differences in vascular canal area and numbers occurred during development; (ii) 10-fold increases in vascular canal area and number were present in achondrogenesis II-hypochondrogenesis. They suggested that data from normal subjects would provide normative values against which vascular abnormalities in other skeletal dysplasias could be compared.

Peltomäki (1994) pointed that costochondral grafts were used to restore dysplastic mandibular condyles. Despite their worldwide use, the growth of the condyle-ramus unit constructed with a costochondral graft was highly unpredictable, excess growth being the most common consequential problem. In a recent series of experiments on rats, it became evident that the amount of cartilage, especily the amount of germinative cells, in the rib graft had a direct bearing on its growth capacity. Peltomäki examined the histology of the human costochondral junction of growing individuals. It was implicated from the height of the proliferative plus hypertrophic cell zones of the junction that the grafts used clinically had always contained germinative cells, but in variable amounts. They cocluded that the reported growth variability of the condyle-ramus unit might be due to the amount of cartilage included in the grafts.

Craatz et al. (1999) made histological, histochemical and immunohistochemical investigations on pieces of rib cartilages of 34 persons

at the age of fourth fetal month up to 60 years they could regularly demonstrated cartilage canals containing blood vessels without any spatial or temporal relationship to degenerative changes in cartilage tissue. They obserced that many of those cartilage canals were located in the center of the rib cartilage. Blood vessels as well as neuronal structures in the connective tissue of cartilage canals were detected by means of antibodies against components of the vessel wall (Von Willebrand factor) and nerve fibers (PGP 9.5). They mentioned that nerves might have sensoric or vasomotoric functions as well, and they might influence cell differentiation and regeneration processes, respectively. Cartilage could not be regarded as vascularized like other tissues, but cartilage canals might have great functional importance for the metabolism of rib cartilage.

Yousefzadeh et al. (2008) studied gray-scale US and perfusion patterns of different cartilages in 42 normal neonates for the first time. Group A included the proximal femoral chondroepiphysis of 20 neonates as well as proximal humeral, distal femoral, and proximal tibial epiphyses of 8 others. Group B included the patellar cartilage of nine neonates and group C included the rib cartilage of five neonates. They found that early ossifying cartilages all had numerous echogenic columns on US. Rib cartilage was hypoechoic and amorphous at all ages. The blood supply was detectable in all cartilages except the ribs. Peak systolic velocities increased with age in the proximal femoral epiphysis. The patellar cartilage was less vascular than the distal femoral epiphysis at birth, but more vascular at 14-24 months of age. The rib cartilage did not have any discernable blood supply at any age. They concluded that cartilage blood flow correlated with the timing of its ossification. Normal cartilage blood flow might prognosticate normality of its growth and development potential.

Umlauf et al. (2010) reported that although great efforts had been made to investigate cartilage biology and osteoarthritis pathology, there was a lack of effective disease-modifying therapies.

Bots et al. (2011) studied in humans the link, of frequency of cervical ribs to stillbirths, other malformations and early childhood cancers. They analyzed the presence of skeletal anomalies in a series of 199 electively aborted fetuses, which were whole-mount stained with alizarin red specific for skeletal tissues. Their results showed that approximately 40% of the fetuses had cervical ribs, even though external congenital abnormalities such as craniofacial and limb defects were absent. They mentioned anomalies of the axial skeleton were known to be caused by a disturbance of early development, which alters Hox gene expression, but in their study the origin of the stress could not be verified as maternal medical data were not available. The co-occurrence of rudimentary or absent 12th ribs in 23.6% of the cases with cervical ribs indicated that in approximately 8% of the fetuses a homeotic shift occurred over a larger part of the vertebral column. Bots et al. (2011) suggested that the expression of multiple Hox genes might have been affected in those fetuses. Together, the high incidence of cervical ribs and also their co-occurrence with rudimentary or absent 12th ribs suggested that there might have been a disturbance of early development such that the studied fetuses were probably not informative about the general population.

Andreoli et al. (2013) mentioned that careful operative timing was required for children undergoing microtia repair using autologous costochondral grafting. That operation was performed as early as age 6 in efforts to treat children before school matriculation while allowing for sufficient rib growth. There remained a paucity of data regarding cartilaginous growth of the ribs and synchondrosis routinely harvested during microtia repair. That study employed CT imaging to generate normative costochondral growth characteristics in children. They made a population-based study, and performed. Setting in tertiary care children's hospital. They reviewed Chest CTs in 360 children ages 3 to 20 years. Measurements included: length of ribs 6, 7, and 8 and the height and width of the synchondrosis between ribs 6 and 7. Growth charts were presented for gender and laterality. They found that: at age 6: ribs 6, 7, and 8 measured 5.96 ± 0.69, 7.79 ± 0.84, and 6.33 ± 1.01 cm, respectively. In adulthood the mean length of ribs 6, 7, and 8 were 8.29 ± 1.00, 11.10 ± 1.19, and 8.95 ± 1.99 cm, respectively. The vertical height of the synchondrosis at years 6 and 20 were 2.42 ± 0.39 and 3.59 ± 0.53 cm, respectively. Ribs 6, 7, and 8 as well as the synchondrosis grew in a nearly linear fashion. They Concluded that: cartilaginous growth of ribs 6 to 8 during early childhood was nearly linear. Synchondrosis height approached adult auricle width at 8 years. Rib size was consistently larger in males and on the left side. These data were useful for the pediatric otolaryngologist and facial plastics and reconstructive surgeon performing microtia surgery.
أترابا:سورة ص52و الواقعة37 والنبأ33 -من بين الصلب والترائب الطارق7

Gelabert et al.,2014 identified patients with recurrent thoracic outlet syndrome TOS symptoms and regrown first ribs present between 1995 and 2012. Details regarding their presentation, evaluation, and treatment were gathered. They found eight patients (6 women and 2 men) presenting with recurrent TOS symptoms and regrown first ribs underwent 10 decompression surgeries. Prior surgeries included supraclavicular first rib resection (5), transaxillary first rib resection (5), scalenectomy (5), cervical rib

resection (1). The average period between initial surgery and reoperation was 4.7 years. Average age at current presentation was 40.8 years (range 29-52). All patients (8) represented with neurogenic symptoms and 1 patient with concomitant venous TOS symptoms. Presenting symptoms included pain (8), numbness and tingling (7), weakness (6), headache (2), and venous congestion (3). Initial treatment included physical therapy in all. Preoperative assessment included chest X-rays (8), magnetic resonance imaging (7), electrodiagnostic studies (8), venography (2), and anterior scalene muscle block (2). Surgical approach included transaxillary resection of the regrown first rib (10), neurolysis of brachial plexus (10), scalenectomy (5), and lysis of subclavian vein (1). After an average follow-up of 10.8 months, resolution of symptoms included 4 complete and 4 partial. They concluded that regrowth of the first rib was a rare event. There was a concordance between a regrown rib and TOS symptoms. Patients presenting with recurrent TOS symptoms and a regrown first rib had a high probability of improvement with resection of the regrown rib.21 وفي أنفسكم افلاتبصرون؟الذلريات

Kang et al. (2014) mentioned that the optimal age at which to initiate for auricular reconstruction was controversial. Rib cartilage growth was closely related to age and determined the feasibility and outcomes of auricular reconstruction. They developed a method to guide the timing of auricular reconstruction in children with microtia ranging in age from 5 to 10 years. The Rib cartilage and the healthy ear were assessed using low-dose multi-slice computed tomography. The lengths of the eighth rib cartilage and the helix of the healthy ear (from the helical crus to the joint of the helix and the earlobe were measured. Surgery was performed when the two lengths were approximately equal. They found that the preoperative eighth rib measurements significantly correlated with the intraoperative measurements ($P < 0.05$). From 5 to 10 years of age, eighth rib growth was not linear. In 76 (62.8%) of 121 patients, the eighth rib length was approximately equal to the helix length in the healthy ear; satisfactory outcomes were achieved in those patients. In 18 (14.9%) patients, the eighth rib was slightly shorter than the helix, helix fabrication was accomplished by adjusting the length of the helical crus of stent, and satisfactory outcomes were also achieved. Acceptable outcomes were achieved in 17 (14.0%) patients in whom helix fabrication was accomplished by cartilage splicing. In 9 (7.4%) patients with insufficient rib cartilage length, the operation was delayed. In one (0.8%) patient with insufficient rib cartilage length, which left no cartilage for helix splicing, the result was unsatisfactory. They concluded that, eighth rib cartilage growth was variable. Rib cartilage assessment relative to the healthy ear could guide auricular reconstruction and personalize treatment in young patients with microtia.

Yan et al. (2019) pointed that there was controversy over the optimal timing of microtia reconstruction. The eighth costal cartilage, which was used to shape the helix framework, could be one of the key factors determining surgical timing of microtia reconstruction. Nevertheless, it was difficult to predict the length of the eighth costal cartilage preoperatively. They discussed different methods of fabricating cartilaginous ear framework in children with microtia according to different lengths of the eighth costal cartilage. They mentioned that, based on the actual length of the eighth costal cartilage in microtia children, there were 2 methods to fabricate auricular framework. In method I, the eighth costal cartilage was divided into 2 parts. Part A was used to fashion the helix, while part B was used to protrude the antihelix, superior, and inferior crus. The seventh rib was used to form the main body and the sixth rib was used to form the base of the framework. In method II, the seventh costal cartilage was used to fashion the helix and extrude the antihelix, superior, and inferior crus as method I did. The sixth rib was used to form the main body and the eighth rib was used to form the base of the framework. They found, in a total of 68 microtia children underwent auricular reconstruction adopting the modified techniques between 2015 and 2016. The great majority of patients (66 patients) were satisfied with the reconstructed ears. Two patients were relatively satisfied with the reconstructed ears. Three cases had been selected to illustrate the favorable result achieved. They revealed that the helix, antihelix, superior crus, and inferior crus all appeared distinct and presented a favorable result of the contour of the reconstructed auricle. They concluded based on different lengths of eighth costal cartilage in children, different methods of fabricating ear framework made full use of the autogenous costal cartilage and elevated anatomical details, demonstrating that personalized treatment was necessary.

Sadler (2019) mentioned that the bony portion of each rib was derived from sclerotome cells that remained in the paraxial mesoderm and that grew out from the costal processes of thoracic vertebrae. Costal cartilage were formed by sclerotome cells that migrated across the lateral somatic frontier into the adjacent lateral plate mesoderm. Initially, there was a well-defined border between each somite and the parietal layer plate mesoderm called the lateral somatic frontier. That frontier separated two mesodermal domains in the embryo:1-The priaxial domain:that comprised the region around the neural tube and contained only somite-derived (paraxial mesoderm)cells. 2-The abaxial domain: that consisted of the parietal layer of lateral plate mesoderm together

with somatic cells that had migrated across the lateral somatic frontier. The lateral somatic frontier defined the lateral border between dermis derived from dermatomes in the back and dermis derived from lateral plate mesoderm in the body wall. It also defined a border for the rib development, such that the bony components of each rib were derived from priaxial sclerotome cells and the cartilaginous parts of those ribs that attached to the sternum and derived from sclerotome cells that migrated across the lateral somatic frontier (abaxial- cells).

Blumer 2021 pointed that, the bones were of mesenchymal or ectomesenchymal origin, formed the skeleton of most vertebrates, and were essential for locomotion and organ protection. As a living tissue they were highly vascularized and remodelled throughout life to maintaine intact. Bones consisted of osteocytes entrapped in a mineralized extracellular matrix, and they communicated with each other via their network of cytoplasmic processes, and with the (bone lining cells): cells on the bone surface. Bone tissue developed through a series of processes, and there were two modes of bone formation, the intramembranous or endochondral ossification. In intramembranous ossification, bones developed directly from condensations of mesenchymal cells, and the flat bones of the skull, the clavicles and the perichondral bone cuff developed via that process. The bones of the axial (ribs and vertebrae) and the appendicular skeleton (e.g. upper and lower limbs) formed through endochondral ossification where mesenchyme turned into a cartilaginous intermediate with the shape of the future skeletal element that was gradually replaced by bone. Endochondral ossification occurred in all vertebrate, and its onset involved differentiation of the chondrocytes, mineralization of the extracellular cartilage matrix and vascularization of the intermediate, followed by disintegration and resorption of the cartilage, bone formation, and finally – after complete ossification of the cartilage model – the establishment of an avascular articular cartilage. The epiphyseal growth plate regulated the longitudinal growth of the bones, achieved by a balanced proliferation and elimination of chondrocytes, and the question whether the late hypertrophic chondrocytes died or transformed into osteogenic cells was still being hotly debated. They pointed that the complex processes leading to endochondral ossification had been studied for over a century.

The aim of the present study is to study some morph histological observations on the developing prenatal human 4th rib with special reference to the costochondral junction CCJ, vascular cartilage canals, ossification centers and late hypertrophic chondrocytes and their relation to osteogenic cells. That is to fulfill suret el thareate ayah 21in Quran, suret fuselate ayah 53and and suret al najm3&4.

Material and Methods

35 Human (male and female) fresh fetuses aged 4(13-16wks-CRL 9-14cm), 5, 6, 7, and 9 months (full term 33-36 weeks) CRL 31-34cm) new born infant) 37-38 weeks) CRL 35-36cm) were used in this investigation. The fetuses were obtained from the miscarriage and spontaneous abortion with no apparent abnormalities or macerations, obtained from Gynecology and Obstetrics Department Al -Zaharaa hospital- Faculty of medicine for girls –Al-Azhar University. Nasr City -Cairo -Egypt (according to medical ethics). They were used to study the normal morphogenesis of the developing prenatal human fetal fourth typical ribs, and second rib. Adult corresponding ribs from new cadavers (male and female) were used for morphological study only. The cadavers were obtained from the Dissection room– Anatomy Department - Faculty of medicine for girls - Al Azhar University, Cairo -Egypt. Dissection of the developing and adult ribs was done according to Romanes (2000). The ribs were obtained after removal of the remains of serratus anterior and pectoral muscles from the upper ribs. Morphologic examination of the ribs was done by naked eyes, magnifying lenses and dissecting microscope. To illustrate the morphology of the developing ribs, photos were photographed by Canon camera zoom

Handling the rib.

The cartilage was severed at about 1 cm. from the rib bone. After the intercostal muscles had been divided, the fourth ribs from the age studied were cut with a pair of bone forceps at a distance of between 1 to 2 cm. from the cartilage, depending on the size of the fetuses. The specimens of the ribs were then fixed in 10% formal saline for10 days. The costochondral junction were cut off, the cut went from the cartilage to the bone. That exposed the marrow cavity and costochondral junction CCJ on both sides. The specimens of the ribs were then fixed for a week and then decalcified in 5% nitric acid. After decalcification, the shaft of the rib was trimmed off to a distance of approximately 0.06-or 1 cm. from the cartilage according to the size of the rib. The rib was processed and blocked on its cut surface. The stains used were Heamatoxilin and Eosin for general histological study, Malory triple stain, and Masson` trichrome for collagen (Drury and Wallington 1980).. TS and LS sections were made by the microtome at thickness of 7u m.

Photos for the histological study were taken by Olympus50xB Microscope, attached to Camera: OlympusDP72, and Connected to the computer Dell,

Magnification powerx40, x100, x200and x600 at the central lab, King Fahd center-King Abdul Aziz. University-KSA-Jeddah.

Some cartilage cells count of the hypertrophic Columns at the CCJ were estimated at the age of full term as. Emery and Kalpaktsoglou pitsa k. 1967. Columns with more than two staggered clusters of cells were not scored. Morphologic classification of chondrocytes followed that of Brighton (Brighton, CT 1984 The growth plate. Orthop Clin North Am 15:571-595) in which proliferative zone cells were flattened cells aligned in columns. The hypertrophic zone began when cells became spherical and enlarged. As noted by others (Kember NF, Sissons HA 1976 Quantitative histology of the human growth plate. J Bone Joint Surg 58B:426-434, repeated counts of specimens showed variability of less than 0.7 % in mean number of cells per column.

Measure of the length of subperiosteal bone collar SPBC, and estimation of the diameters of some secondary ossification centers SOC, as well as the cartilage canals were done according to the scale on the microphtograph.

The CRL of each fetus was obtained and then converted into weeks of menstrual prenatal ages according to tables of Streeter (1920) and (1949) and Sadler (2012)/ Estimation of the prenatal ages according Sadler (2012& 2019)

Results:

A-Histological Results:

- 4 months (13-16wks)-CRL 9-14cm aged fetu:figs1-20
- 5months (17 -20weeks) CRL 15-19cm aged fetus: figs21-22
- 6-months old fetus:(21 -24weeks) CRL 20-23cm:figs 23-30
- 7months old age fetus:(25-28weeks) CRL 24-27cm: figs 31-36
- 9month aged fetus 33-36 weeks- CRL 31-34cm) and newborn infant (37-38 weeks-(CRL 35-36cm) figs:37-66
- B-Morphological results: figs:67-68

A -Histological Results:

4month aged fetus (13-16wks-CRL 9-14cm:

Histological examination of part of LS of part of the 4th prenatal developing human rib at the costochondral junction CCJ of 4month aged fetus (13-16wks-CRL 9-14cm, showed in Fig. 1 the general appearance by Malory triple stain, of part of a long epiphyseal growth plate. formed of the germinative (reserve-stem cell-inert) zone, proliferative, and hypertrophic zones, and a short irregular area of sponge bone. The metaphesyeal bone formed of trabeculea, representing the site of primary ossification center (POC). The perichondrium was continuous with the periosteam

The groove of Ranvier was slightly wedge-shaped collection of cells which provided newly formed chondrocytes in the epiphysis, to share in the growth plate.

The perichondrial ring PR provided mechanical support after loss of the cartilage cells bursting in the sponge. The intramembranous bone bark BB - subperiosteal bone collar (SPBC)-extended from the level of the proliferating zone to the primary ossification PO in the metaphysis. Part of the role of the PR was to lay down a thin layer of bone through **intramembranous ossification (bone bark),** which provided mechanical support to the growth plate and the metaphysis, and increased the width of the physis. That coincided with the Quraan –suret el ensane 28: Allah the most GRACEFULL the creator r had strengthen and fortified the human HE had created.

سورة الإنسان28 خلقناهم وشددنا اسرهم

The length of the subperiosteal bone collar SPBC–(bone bark) at that age was measured 670um according to the scale on the photomicrograph approximately.

Histological examination of part of LS of part of4th prenatal developing human rib at the costochondral junction CCJ, of 4 month aged fetus (13-16wks-CRL 9-14cm, showed Figs2-a&b and Figs3- a&b at the complex transitional zone ;cartilage to bone:the presence of multinucleate giant Chondroclast, osteoblasts on the surface of matrix which stained faint blue blood cells in the sponge.The hypertrophic cartilage cells with shrunken nuclei were noted at the CCJ(fig2-a).

Chondroclasts multinucleated giant cells, came from the fusion of mono-cytes/macrophages present in the bone marrow. They became activated when attached to the surface of mineralized cartilage via the sealing zone, and released tartrate resistant acid phosphatase(TRAP), also referred to as acid phosphatase5 tartrate resistant(Acp5)

Fig2- b- higher magnification of part of fig2- a, showed the transition zone was composed of mineralized cartilage and early bone matrix, stained faint blue by Malory triple stain and characterized by the presence of hypertrophic chondrocytes, chondroclasts, osteoblasts and blood vessels.

Figs- 3 a&b showed, part of the hypertrophic cartilage cells arranged in simple columns, and few terminal final cells bursting in the sponge.

Chondroclast in the sponge of irregular newly formed bone matrix and blood cells were between the trabecular spaces.

Fig3- b- higher magnification of part of fig 3-a, showed chondroclast in the irregular sponge bone of primary ossification center POC.

Deep basophilic osteoblast forming continuous layer on the surface of sealing newly formed matrix, capillary invasion of cartilage cells was noted

The Blood cells in bone marrow resulted from chondroclasts erosion of cartilage matrix.

Histological examination of part of LS of part of a prenatal developing human 4th rib at costochondral junction CCJ, of **4**months aged fetus **(13-16wks-CRL 9-14cm** showed Fig 4 A &fig 4-a by Masson trichrome stain, and fig.4b by Malory triple stain,the presence of:three degrees of stain affinity of the tissues: deep strong stain of perichondrium, moderate stain affinity of small subperichondrial area,weak stain affinity at the resting cartilage

The different stain affinities might indicate possible different types of collagen and tissue remodeling.

Fig4- d- showed part of the layers of the perichondrium with rich vascularity, Flat cells were arranged parallel between the collagen bundles.

The mesenchyme containied stem cells that might provide source of cartilage cells to the reserve zone of growth plate were noted.

The growing perichondrium of the 4th prenatal human rib showed the interstitial growth: growth from inside, and the appositional growth (growth from OUT side). In the growth from outside; the CT perichondrium: New layers of cartilage were added from the inner chondrogenic layer of the perichondrium: where undifferentiated mesenchyme cells UMCs formed chondrocytes (cartilage cells).

Histological examination of part of the jaxta costochondral junction CCJ of a prenatal human 4th rib of 4 months (13-16wks-CRL 9-14cm) aged fetus showed, by Mallory triple stain Fig5-a part of the resting cartilage and the covering perichondrium layers arranged in a complex manner to give pathway for precursors of three cartilage canal formation: The arterioles surrounded by mesenchyme were seen.

There were spur arrangement of cartilage cells formation started from subperichodreum area towards the center of the hyaline cartilage of the rib.

fig5-b: showed by Masson trichrome stain. part of the resting cartilage and the covering perichondrium layers arranged in a complex manner to allow pathway for precursors of cartilage canal formation: arterioles surrounded by mesenchyme

Brush border like at a small area of the perichondrium and mesenchyme tissue with capillaries between perichondrium were noted.

Histological examination of part of TS of part of a prenatal developing human4th rib of 4 **months (13-16wks-CRL 9-14cm aged fetus** at the costo chondral junction CCJ by Malory triple stain in Fig 6-a, and by HE stain Fig b-showed, the mesenchyme vascular tissue **perichondrium,** The vascular mesenchyme tended to extend to the primary ossification center.

The sponge irregular cancellous bone was formed of trabeculea and bone marrow

The growing perichondrium of the 4th prenatal human rib showed the interstitial growth: growth from inside, and the appositional growth from outside.

The interstitial growth from inside: showed a single cell had a capsule, when it divided into two, each daughter cell had its own capsule, the primary capsule disappeared and the two cells remained close to each (scale200um)

The Mesenchyme containing stem cells might provide source of cartilage cells to the growth plate and endochondral ossification, (and source of osteoblasts for later secondary ossification center SOC, besides the interstitial growth of cartilage.

Histological examination of part of TS of part of a 4th prenatal developing human rib juxta the costochondral junction CCJ, of 4 month aged fetus **(13-16wks-CRL 9-14cm** showed, Fig7the general morphology of part of the epiphysis, with small secondary SOC ossification center and one large secondary center SOC formed from fusion and coalescing of multiple small secondary ossification centers SOC.

Numerous superficial cartilage canals were around the center n=12 occupied a surface area represented 3% approximately from the resting cartilage

Vascular cartilage canal invaded the large secondary ossification center SOC.

Histological examination of part of of the jaxta the costochondral junction CCJ of prenatal human 4th rib of 4 **months (13-16wks-CRL 9-14cm)** aged fetus, showed in fig Fig. 8 - a three 3 vascular cartilage canals close to part of a secondary ossification center. SOC. The ossification center was invaded by cartilage canal containing large vessel and CT, which incorporated and continued inside the SOC ossification centerFigs.8-a&b

The wall of the canal had basophilic osteogenic like cells. Enlarged hypertrophic cartilage cells around the SOC ossification center, forming a plate, morphologically similar to the growth plate at the costochondral junction CCJ.was found.

The invading cartilage canal contained CT with stem cells that might represent source of osteoblasts sharing in the formation of the secondary ossification center SOC.

Histological examination of part of TS of part of 4th prenatal developing human rib juxta the costochondral junction, in the epiphysis of, 4 **months (13-16wks-CRL 9-14cm fetus** showed Figs. (9-a&b); in fig. 9-a superficial cartilage canal in with the intact wall of the cartilage canal, which contained mesenchyme with

Capillaries, Venule, Macrophages small arterioles and capillaries were present in the canals
Deep Stain of matrix around the cartilage canal was noted.
Fig.9- b: showed the relation between two superficial cartilage canals with intact wall of the canal, which contained mesenchyme. One cartilage canal showed chondrolyis (lacunae containing cells intimately associated with matrix and presence of granular debris.was noted.

Histological examination of part of LS of part of 4th prenatal developing human rib at costochondral junction CCJ, of 4 month aged fetus (13-16wks)-CRL 9-14cm) showed, Fig.10 part of secondary ossification center SOC contained deep basophilic polarized osteoblast on the surface of the newly formed bone matrix. Chondroclast multinucleate giant cells eroding the cartilage, produced eosin acidic field, due to TRAP production and contained particles was seen.

- Stages of bone cells formation preparing for bone remolding were seen

Osteoblasts bone forming cells, polarized cells formed continuous layer covering the newly formed matrix of bone were `seen. They were more basophilic (blue) cytoplasm due to excess RNA in the cytoplasm. Blood cells were noted.

Histological examination of part of LS of part of 4th prenatal developing human rib at costochondral junction CCJ, of 4 month aged fetus showed, (Figs.11-20), part of secondary ossification center SOC contained deep basophilic osteoblasts on the surface of newly formed matrix,newly formed bone cells present in eosin acidic field due to TRAP (tartrate resistant acid phosphatase) produced by chondroclast giant multinucleate. The TRAP enzyme was essential in bone remolding. Blood cells were present,after matrix erosion. Fig.12 showed: part of a secondary ossification center SOC, invaded by cartilage canal contained vessels, and the cell wall of the cartilage canal, which contained vesse, transformed to basophilic osteogenic like cells.

Group of osteocytes and osteoclasts embedded in the newly formed matrix, present in stages of formation for continuous remodeling, were found in eosin acidophilic field due to TRAP (released tartrate resistant acid phosphatase(TRAP), also referred to as acid phosphatase5 tartrate resistant(Acp5) produced by activated Chondroclasts, multinucleated giant cells.

Blood vessel in the secondary ossification centerSOC, with blood cells in the lumen were noted. Hypertrophic(late) cartilage cells burst in the center were seen.

Histological examination of part of LS of part of 4th prenatal developing human rib juxta the costochondral junction CCJ, of 4 month aged fetus showed, in Figs.12-17,part of secondary ossification center SOC in the epiphysis,illustrated: stages of new osteocyte formation embedded in the newly formed matrix,present within eosin stain acidic field due to TRAP formation (tartrate resistant acid phosphatase), which produsedby chondroclasts and that enzyme was necessary for bone development osteogenesis,remodling.

Blood cells and remnant **of eroded cartilage cells were noted.** Basophilic areas intermingled between the massive acidic field were seen (Figs.12-17).

Activated Chondroclasts multinucleated giant cells, WITH cytoplasmic extension Fig.16 was seen. Chondroclasts arising from the fusion of monocytes/macrophages present in the bone marrow, released tartrate resistant acid phosphatase (TRAP), also referred to as acid phosphatase5 tartrate resistant(Acp5) after attached to the surface of mineralized cartilage via the sealing.

Deep basophilic polarized osteoblasts were seen on the surface of the newly formed matrix (Figs.15-17)

Histological examination of part of LS of part of 4th prenatal developing human rib juxta costochondral junction CCJ, of 4 month aged fetus showed (Figs.18-20), part of a secondary ossification center SOC present in the epiphysis, illustrating hypertrophic enlarged cartilage cells at the edge of secondary ossification center SOC, bursting in the center. Ruptured enlarged cartilage cells at the edge of secondary ossification center SOC containing blood were noted cells.The late hypertrphic cartilage cells might die by apoptosis preprogrammed death,or transformed to osteoblast or Basophilic osteogenic cells as some workers claimed. That can occur as Quran mentioned the creation bytransformation. الخلق بالاستحالة كما ذكر في مفردات الفاظ القرآن للأصفهاني

Hypertrophic cartilage cell formed a plate around the SOC resembled morphologically just similar to the growth plate at the metaphysis at the costochondral junction CCJ was seen. Large spaces and areas in the large cartilage cells, after chondroclast eroded matrix leaving spaces and blood cells were noted (Figs. 18-20).

Osteoblasts basophilic polarized cells on the surface of newly formed matrix were seen Fig. 20. Stages of osteocyte formation (present in weak eosin field due to TRAP formation by chondroclasts giant multinucleate eroding the cartilage were seen. 5months (17 - 20weeks) CRL 15-19cm aged fetus

Histological examination of part of LS of part of 4th prenatal developing human rib of 5month aged fetus (17 -20weeks) CRL 15-19cm, in the epiphysis juxta the costochondral junction CCJ showed figs.21 a,b,c: in Fig 21a illustrated a large secondary ossification centers SOC,occupying approximately 75% of the surface area of the resting cartilage in the epiphysis.

Numerous cartilage canals invaded the SOC center, from all different sides, which might lead to the center expansion

Fig.21 -b-showed part of the large secondary ossification centers SOC, surrounded by cartilage canals, one large canal with absorbed material, and one anchoring canal to the SOC were seen. **Fig. 21-C-** higher magnification of part of fig. 21-a showed, one vascular cartilage canal was going to incorporate with the secondary ossification center SOC

Histological examination of part of TS of a developing prenatal human 4th fourth rib juxta the costochondral junctionCCJ of showed, figs. 22 a,b,c,d different cartilage canals at different developing ages present in the resting cartilage: fig.(a).. 4month aged fetus, fig. (b),5month aged fetus, fig.(c) 6month aged fetus, and fig. (d) full term The canals became complicated with age progress and contained blood vessels at the age of 6months and full term, capillaries, sinusoidal capillaries, and connective tissues.

6-months old fetus:(21 -24weeks) CRL 20-23cm

Histological examination of part of LS of part of the developing prenatal human 4th rib at costochondral junction CCJ **of 6-months old fetus:(21 -24weeks) CRL 20-23cm** showed Figs. 23 a,b,c,d in figs.23**a& b-** part of **the hypertrophic zone and cartilage cells of provisional zone of calcification PZC bursting in the sponge bone.**

Fig. 23c-part of the proliferative zone with dividing cells.

Fig.23 d- part of the resting (reserve -stem cell - inert zone)

Histological examination of part of TS of part of the prenatal human developing 4th rib, juxta the costochondral junction CCJ, **of 6month aged fetus** showed fig24-A the general appearance of a secondary ossification center SOC, surrounded by cartilage canals n=25. Two canals anchored the center, higher magnifications in figs. 24-a and b; of fig24- A, showed cartilage canals around a secondary ossification center SOC

Big branching cartilage canal with interrupted wall invaded the SOC center.

The canals which did not incorporate with the SOC degenerated and seen as ghosts

The large cartilage cells around the center which formed a plate similar morphologically to the growth plate at the costochondral junction CCJ

Fig24- b- higher magnification of part of the previous fig 24-b, showed cartilage canal with interrupted wall, contained central arteriole, venule, and mesenchyme CT

The long diameters approximately of the cartilage canal was 450um, and of the central arteroile was 210um,and the venule was 330um measured from the scale of the microphotograph

Histological examination of part of TS of part of a developing prenatal human fourth rib at the epiphysis,juxta the costochondral junction CCJ of **6-months old fetus:(21 -24weeks) CRL 20-23cm** showed Fig 25: a&b in fig.a the resting cartilage containing 4 vascular cartilage canals with different sizes,and content near a secondary ossification center SOC. The long diameter of the largest cartilage canal was approximately 200um, and the long diameter of the vessel was 170un measured from the scale of the photomicrograph.

Different stain affinity around the canals was observed

One cartilage canal contained absorbed substance and large one sinusoid occupied the whole diameter of the canal space, anchored the SOC

Fig b showed **ghost like atrophied canal were note**d. **The cartilage vascular canals were important in the development of epiphyseal ossification because the vessels with their associated mesenchyme cells served as the source for bone-synthesizing osteoblasts on the calcified cartilage**

Histological examination of part of TS of part of a developing prenatal human fourth 4th rib juxta the costochondral junction CCJ, of 6 months aged fetus:(21 -24weeks) CRL 20-23cm showed Fig 26:,two cartilage canals in the resting cartilage close to a secondary ossification center SOC. Chondroclasts Enlarged cartilage cells arranged in short columns, around the secondary ossification center a SOC, forming a plate similar to the growth plate at the CCJ were seen. Degenerated small cartilage canal were seen.

The big vascular cartilage canals had intact wall, contained branched arterioles, venules, sinusoidal capillaries, and loose perivascular connective tissue. Such canals provided nutrients to the cartilage and could serve as a source of cartilage stem cells for growth of the epiphysis. Vascular canals were essential for the development of epiphyseal ossification because the vessels with their associated mesenchyme cells could serve as the source for bone-synthesizing osteoblasts on the calcified cartilage.

Strong stain of the matrix around and in between the canal, indicating effect of the canals on the metabolic activity of the individual cartilage cells and the effect on the collagen of the matrix.

The diameter of the largest cartilage canal measured approximately 300um. and the diameter of the largest capillary sinusoids, measured approximately 150um. and the transverse diameter of the small cartilage canal measured approximately 160um. according to the scale on the microphotograph.

Histological examination of part of LS ofpart of 4th prenatal developing human rib of **6months** aged fetus**:(21 -24weeks) CRL 20-23cm**, showed in fig 27,

an oval vascular cartilage canal, contained central arteriole.and capillaries containing blood cells,and mesenchyme CT. Enlarged cartilage cells around the canal, few and dispersed. The wall of the canal was not complete. The Matrix around the canal was deeply stained.

The long and transverse diameter of the canal were 150umx130um

The long and transverse diameter of the central arteriole inside the canal were 60x50um,as the measurements from the scale on the microphotograph.

Histological examination of part of TS of part of a developing prenatal human 4th rib at the costochondral junction CCJ of 6month aged fetus:(21 -24weeks) CRL 20-23cm showed in Figs.28 –a&b ;fig a illustrated part of the resting cartilage containing cartilage canal had incomplete wall,containing collagen,mesenchyme, capillaries and lymph vessels surrounded by deeply stained matrix,which indicating the influence of the vessels of the canals on the metabolism of individual cartilage cells and the influence on the collagen of the matrix..

Fig28.- b showed part of the resting cartilage contained vascular cartilage canals contained capillaries and CT. Besides vascular cartilage canal with incomplete wall, contained capillaries and collagen.

Histological examination of part of TS of part of a developing prenatal human 4^{th} rib jaxta the costochondral junction CCJ, of **6month fetus** showed in Fig.29- a: vascular cartilage canals were close to the SOC and Chondoclast eroded the cartilage.

Enlarged cartilage cells around the secondary ossification center SOCwere seen.

Fig. 29-b showed,Chondoclast eroding the enlarged cartilage cells causing the presence of blood cells around the secondary ossification center. SOC

Vascular cartilage canals were close to the SOC.Deep stain of cartilage matrix around the canal,and the stain of the matrix of the secondary ossification center SOC differed in affinity were noted.

- Histological examination of part of TS of part of radial section of part of a developing prenatal human 4^{th} rib jaxta the costochondral junction CCJ of **6month fetus** showed Fig30 - A: Group of deep cartilage canals in the central area of the rib.
- fig 30 -B:showed part of the perichondrium with extension containing cartilage canals in the bud stage, and few superficial cartilage canals present in the resting cartilage
- Histological examination of part of TS of developing prenatal human 4^{th} rib at the costochondral junction CCJ of 9month fetus showed,adjacent areas in Figs 30-a& fig 30-b, in fig 30-a cartilage canals n =20, and in fig 30-b:larger vascular cartilage canals n =11 with different shapes,size and content present in the resting cartilage in surface area850x660um estimated from measuring the long and transverse diameter of the microphotograph.
- **The cartilage canal number increased with age PROGRSS till full term.There was deep stain affinity in the matrix areas close to the cartilage canals.**

7month aged fetus:(25-28weeks) CRL 24-27cm

Histological examination of part of radial section of part of the prenatal developing human 4^{th} rib of 7month aged fetus:**(25-28weeks) CRL 24-27cm** showed in Fig. 31 A - the cartilaginous tissue arranged in column, and Fig. 31 B- showed 3 deep obliterated occluded cartilage canals contained fibrous collagenous substance or empty areas. The long diameters of the canals approximately measured from the scale on the microphotograph were, canal a: 150um, canal b:75um, and canal c:100um

Fig.31 - c showed columns of chondroblasts, and deep two penetrating cartilage canals with chondrolysis at the ends of the canals.

A venule in canal a, was seen. A central arteriole, venule, and sinusoidal capillary,were present in cartilage canal **B**

The long diameter of the canal (A) was 15um, and the long diameter of the ballooned canal (B) was 100um approximately.

The transverse diameters of the sinusoisal capillary was 50um, and the central arteriole was12 um. as measured from the scale of the microphotograph

Histological examination of part of TS of part of a developing prenatal human 4^{th} rib at the costochondral junctionCCJ of **7month** aged fetus:**(25-28weeks) CRL 24-27cm** showed, Fig 32 branched deep cartilage canal containing deeply stained collagen,. Mesenchyme and small capillaries. The matrix around the canal was less deeply stained than the collagen inside the canal.and the cells around the canal were large and dispersed irregular with different shape and size.

- Three stain affinities were present by Masson trichrome stain; the deepest stain affinity was of the collagen inside the cartilage canal, the intimate matrix around the canal was moderately homogenously stained, and the matrix of the resting cartilage had the least weak stain affinity.
- Histological examination of part of LS of 4^{th} prenatal developing human rib at the costochondral junction CCJ, of 7month old age fetus**:(25-28weeks - CRL 24-27cm)** showed, Figs. 33-34: parts of numerous extended penetrating deep empty cartilage canals with conical bolus ends Fig. 33.Some canals had straight course, another canals were not straight having obliterated parts. Bubble like matrix around the canal was noted.

Histological examination of part of LS of part of a 4th prenatal developing human rib at costochondral junction CCJ of seven month aged fetus showed Fig.34 -a part of resting cartilage contained extended deep empty cartilage canals with intact wall,and bullous conical end. Closed and obliterated areas were seen.

The cartilage cells around the length of the canal were sparse, loosely packed, irregularly arranged flat and small.Bubble,flucuelent like appearance of areas of the matrix along the cartilage canal were seen.

The longest diameter of the conical end of the cartilage canal was approximately 150um measured from the scale of the microphotograph. The diameter of the canal width was 15 um.

Fig. 34-b, showed part of end of deep cartilage canal present in the resting zone, contained one sinusoidal vessel occupying the whole diameter of the canal with blood cells inside. The wall of the canal was intact. Stripe area of the matrix around the cartilage canal was noted. The cells around the canal had different size, some were enlarged, dispersed, some were flat, loosely irregularly packed.

Histological examination of part of LS of part of 4th prenatal developing human rib juxta the costochondral junction CCJ, of seven month aged fetus showed Fig.35: part of bullous end of deep empty cartilage canal with intact wall present in the resting zone

Stripe areas of the matrix deeply stained around the cartilage canal, and fluculent acellular area of matrix around the canal showed bubbles like appearance were seen.

The cells around the canal were different in size, some flat, loosely irregularly packed

Histological examination of part of LS of part of a4th prenatal developing human rib juxta the costochondral junction CCJ, of 7 months old age fetus, showed Fig. 36-a in fig36.a:part of vascular cartilage canal containing sinusoidal capillary, with interrupted wall containing blood cells and mesenchyme.Small capillary was noted

Fig. 36-b:showed, part of the lower blinded end of a complex cartilage canal, present in the resting zone containing;fibroblast,macrophage, vessels with one layer of endothelial cells. Capillary with relatively thick wall, contained blood cells. Sinusoidal capillary with blood cells.The cartilage cells around the canal showed chondrolysis:**(**lacunae containing cells intimately associated with matrix, and presence of granular debris).

9month aged fetus 33-36 weeks- CRL 31-34cm) and newborn infant (37-38 weeks-(CRL 35-36cm)

Histological examination of part of LS of part of a developing prenatal human 4th rib of at the costochondral junction of 9month aged fetus 33-36 weeks- CRL 31-34cm) and newborn infant (37-38 weeks-(CRL 35-36cm) showed, Figs 37 a,b,c,d and fig. 38

The growth plate was shorter and thinner than in the growth plate in the 4month aged fetus, and the previous ages. The sponge surface area was longer than that at 4month aged fetus and the previous ages. The growth plate was invaded by vessel that extended to the long spongy irregular bone. The part that surrounded the physis was "the ring of LaCroix," and it merged with the periosteum adjacent to the metaphysis and the perichondrium that surrounded the epiphysis. There was a subtle increase in diameter of the physis "the groove of Ranvier ",which extended from the junction of the epiphyseal cartilage and the zone between the proliferative and reserve zone of the growth plate. The perichondrial ring **of LaCroix** gave the bone bark, or the subperiosteal bone collar (SPBC),which was resistant to injury and providesd a protective mechanism. The (SPBC) extended from area of proliferative till the primary ossification center its length was 350um according the scale on the microphotograph in fig.37- a

Fig. 37- **b** showed branched vessel in the growth plate.and extending to the sponge Fig 37-c. **Fig. d** Showed the perichondrium with blood vessels, groove of Ranvier Gr and bone collar, bone bark or perichondrial ring of La Croix.

The perichondrium consisted of CT surrounded the cartilage was continuous with the periosteum.

Histological examination of part of LS of part of the resting cartilage of the prenatal human developing 4th rib of **9 month human full-term:(33-36 weeks) CRL 31-34cm)** AT The Costochondral junction CCJ showed Fig 38 part of the,primary ossification center,secondary ossification center POC &SOC

A similar morphological appearance of arrangement of cartilage cells close to the bone sponge at the costochondral junction CCJ around the formed primary ossification center POC, and secondary ossification center SOC was seen

The differences between POS and SOC were that the SOC center had close cartilage canals, which incorporate in the SOC and angiogenesis occurred via cartilage canals.

Histological examination of part of LS of part of a developing prenatal human fourth 4th rib at the costochondral junction CCJ of 9months aged fetus showed Fig39 the growth plate Figs 39-a&b the periphysis, encircling the metaphysis and a wedge-shaped groove of Ranvier.Thin layer of intramembranous bone (bone collar, bone bark or perichondrial ring of La CROIX were seen Fig-39 b

The length of the subperiosteal bone collar SPBC– formed by intramembranous ossification bone bark was approximately was460um according to the scale on the photomicrograph.The scale100um

Fig **39**c showed magnification of part of fig b, demostrating Grove of Ranvier GR contained newly formed cartilage cells,EXtenibg to form SPBCsubperiosteal bone collar..

Histological examination of part of LS of part of a developing prenatal human fourth 4th rib at the costochondral junction CCJ of 9month aged fetus, stained by Masson tr ichrome,showed Fig s40-42 parts of the zones of the epiphyseal cartilage growth plate: the proliferative and hypertrophic zones with the cartilage cells arranged in simple columns,and threads of matrix in between the columns in the hypertrophic zone. The perichondrial groove of Ranvier, and the subperosteial bone collae SPBC had rich blood supply from the perichondreum. The layers of the periosteum, and the perichondreum had rich blood supply,and were continous Fig.40

Three different stain affinities of the perichondriuem, subperchondreum and the matrix of the hyaline cartilage were noted.

Spur contour arrangement was found extended from subperichondreum beneath the grove of Ranveir and extended to the hyaline cartilage.

Figs. 41&42The SPBC projected beyond the metaphysis, extending along the physeal margin toward the epiphysis and ended near the junction of reserve and proliferative zones of the growth plate; and changed in length and thickness with age progress.

The periosteum continued with the perichondrium and the Sponge irregular bone was noted.

Fig.. 42-a showed part of the hypertrophic zone of the cartilage growth plate, with hypertrophic cartilage cells, arranged in simple one cell column, and mature terminal3-4 cells bursting cells in the sponge forming provisional zone of calcification PZC.

Chondroclasts giant multinucleate cells eroding matrix were seen in the transitional zone cartilage – bone –at the beginning of the sponge bone.

Bone cells were noted on the new matrix bone trabeculea, they were needed for remolding. The new added cells at the CCJ,represented part of, the primary ossification center POC.

Fig. 42-b, showed part of the growth plate: consisted of zones: the stem or reserve zone had had large amount of extracellular matrix. The proliferative zone, the cells began flatten, underwent cell division and became oriented into columns. In the last layer, the hypertrophic zone, had enlarged cells arranged in regular simple columns, mean cell count number in each column =9cells,cell division ceased and the chondrocytes began to terminally differentiate and enlarged, ballooned and were surrounded by reduced amounts of matrix.

the matured late ballooned ruptured cells in the irregular sponge bone of the growing rib. formed the provisional zone of calcification ZPC and included the terminal 3 – 5 hypertrophic chondrocytes surrounded by few threads hyaline calcified matrix.

Histological examination developing 4th rib of 9 month human full-term:(33-36 weeks) CRL 31-34cm) and newborn infant (37-38 weeks-(CRL 35-36cm) juxta the costochondral junction CCJ showed, Figs. 43a&b: superficial vascular cartilage canals extended from the rich vascular plexus network of the perichondrium.

The cartilage canals showed different length, and stages of formation, from bud till separation from the rich vascular perichondrium. Deep stain of the matrix around the canals was noted. The canals originated from the perichondrium and were composed of loose connective tissue with a terminal central arteriole.

Histological examination of part of TS of part of the resting cartilage of the prenatal human developing 4th rib of 9 month human full-term:(33-36 weeks) CRL 31-34cm) and newborn infant (37-38 weeks-(CRL 35-36cm) juxta the costochondral junctiom CCJ showed, Figs. 44a & b, and fig 45 two successive superficial cartilage canals, each canal contained one empty vessel, occupied the whole cartilage canal diameter, extending through mesenchyme tissue from the thick layers of the perichondrium.

Fig.45 showed part of the perichondrium layers,and extended stem from the perichondrium cobtained suspended superficial cartilage canals, containing one vessel, into the resting cartilage of the epiphysis. The vessel wall was interrupted.

The transverse diameter of the cartilage canal was 200um approximately, and the transverse diameter of the vessel inside the canal was 170um approximately. The long diameter of the canal was 280um and long diameter of the vessel was 260um approximately measured from the scale on the microphotograph.

Histological examination of part of TS of part of the prenatal human developing 4th rib juxta the costochondral junction of 9 month human full-term:(33-36 weeks) CRL 31-34cm) and newborn infant (37-38 weeks-(CRL 35-36cm): showed, Figs.-46 a &b ; part of the epiphysis contained collection of enlarged cartilage cells, forming initial Secondary ossification center SOC. Two cartilage canals around the initial secondary center SOC stared to incorporate with the SOC center were seen

Deeply stained matrix around and between the canals was seen.

The long diameter of the large cartilage canal was 2100um, and the transverse diameter was, 400um, approximately.

Histological examination of part of TS of part of the of the prenatal human developing 4th rib of human full-term:(33-36 weeks) CRL 31-34cm) and newborn infant (37-38 weeks-(CRL 35-36cm) juxta the costochondral junctiom CCJ showed Figs.47

a&b,illustrated part of secondary ossification center SOC in the epiphysis, and part of the vascular perichondrium, containing vascular canal, extends in the SOC center. Cartilage canals around the SOC center, and a blood vessel inside the SOC center were noted. The diameter of the secodary ossification center SOC were 2000umx2400um approximately measured from the scale of the microphotograph

Fig. 47 b showed a vascular canal close to the SOC containing sinusoidal capillary, central arteriole thin arteriole, capillary and CT. Macrophage, Fibroblast were seen.

The long diameter of the canal was 940um and transverse diameter was 560um measured from the scale on the microphotograph

The long diameter of the sinusoidal capillary was 400um, and the transverse was 370um.The transverse diameter of the central arteriole was approximately 100um.

Deep stain of the matrix around the canal and chondrolysis: lacunae containing cells intimately associated with matrix, and presence of granular debris) were noted

Histological examination of part of TS of part of the resting cartilage of the prenatal human developing 4th rib of 9 month human full-term:(33-36 weeks) CRL 31-34cm), showed Figs. 48 a&b and Fig. 49 part of a secondary ossification SOC contained large vessel.and surrounded by hypertrophic chondrocytes around the SOC center, forming a morphologically similar plate to the cartilage growth plate at the CCJ.

Fig. 49 showed the vascular perichondrium was continuous with the secondary ossification center SOC, different stain affinities by Masson trichrome stain; in the perichondrium, the matrix, and the SOC center. The stain affinity of the perichondrium was strong, while the stain of the matrix was weak in areas and strong in another ares, while the stain of the matrix of the secondary ossification centers was moderate

Histological examination of part of LS of 4th rib of prenatal developing human 9 month human full-term:(33-36 weeks) CRL 31-34cm) and newborn infant (37-38 weeks-(CRL 35-36cm) showed in Fig. 50- a- part of radial section and in the center was the glomerulus end of some deep cartilage canals.

Fig. 50-b-: the entire cartilage canal bathed in plasma the cartilage canal was uniformly segmentally distributed, and alternative green and red segments were noted along the whole length of the canal. That indicated a metabolic exchange was facilitated throughout the entire length of the cartilage canal.The function of the capillary glomerulus was to decrease the velocity of blood flow thereby facilitating the percolation of plasma into the connective tissue of the cartilage canal.

Fig50- bb- the same cartilage canal, with the scale to illustrate the length of the canal was 500um approximately. The transverse diameter of the glomerulus was 80 um, the long diameter of the glomerulus was 77 um. The measurements were from the photomicrograph scale.

Histological examination of part of TS of part of a developing prenatal human 4th rib juxta the costochondral junction CCJ of **9month fetus** showed Fig.51 part of the layers of the perichondrium with deep stain and the flat cartilage cells beneath it.

Superficial cartilage canals, extending from the perichondrium.

Deep cartilage canals completely surrounded by matrix in the resting cartilage, with different shapes and contained course arterioles, venules and sinusoidal capillaries that were important for nutrition and also loose perivascular connective tissue which served as a source of stem cells for bone-synthesizing osteoblasts on the calcified cartilage.

Different stain affinities of the perichondruem, subperichondruem and the matrix of the hyaline cartilage were seen. Strong, moderate, and weak. That might indicte different types of collagen

Deep stains of matrix areas of the resting cartilage round the canals were seen

Histological examination of part of TS of part of a developing prenatal human 4th rib juxta the costochondral junction of 9month fetus showed Fig.52-a cartilage canal with two venules and capillaries, surrounded by deeply stained matrix Two sinusoidal capillaries, each had one endothelial layer, their transverse diameters were 90um&110um approximately measured from the scale on the microphotograph

The cartilage cells around the canal were large, dispersed and few.

The matrix around the canal had deep stain.

Fig. 52-b: showed part of the resting cartilage tissue contained cartilage canal full of capillaries of different size, and CT mesenchyme.

The transverse diameter of the canal was 100um, and the long diameter was 300um approximately according to the scale on the microphotograph. The transverse diameter of the capillaries inside the cartilage canal ranged between 50um, 40um, and 10 um.

There was different deep stain affinity of the matrix around the canal, which might indicate the effect of the canal on the incividual cartilage cell metabolism.

Histological examination of part of TS of part of a developing prenatal human 4th rib juxta the costochondral junction CCJ of 9month fetus (Figs.53-61) showed Fig.53 (n=4) vascular cartilage canals, present in the resting cartilage with different in diameter, shapes, size.Cartilage canals contained

course arterioles, venules and sinusoidal capillaries that were important for nutrition, Cartilage canals contained also perivascular loose connective tissue which was a source of stem cells.
There was deep different stain affinity of the matrix close to the cartilage canals.
The arterioles divided to two or more capillaries
Regularly loosely arranged cartilage cells around and between the Cartilage canals were seen. The diameter of the canals range as measured from the scale of the microphotographs: were approximately 30um, 40um, 70um, 130 um.
Histological examination of part of TS of part of a developing prenatal human 4th rib jaxta the costochondral junction CCJ of 9month fetus showed Fig.54-a vascular cartilage canals with no particular anatomical relation present, in the resting cartilage having different size, shapes and contents.
The vascular cartilage canals contained two or three distinct capillary–sinusoidal vessels interspersed with connective tissue and many small discrete vessels, some canals were close to each other seen as one cartilage unit canal, giving triangle appearance. Some branching capillaries inside the canals were seen.
Enlarged cartilage cells around the canals were noted.
Areas of chondrolysis were noted at the tips and between the close canals of the canals. The canal wall was frequently not intact. Some large regularly loosely arranged cartilage cells between the close cartilage canals were noted.
The variations of cartilage canals, were according to the degree of capillary anastomoses among the arteriole, venule, and capillaries of the glomerulus, at the end of cartilage canal. The simple glomeruli were observed on the ends of short canals or on the end of the first branches of a long canal. Other glomeruli were formed when the arteriole divided into more than two capillaries
Deep stain of matrix between the canals might indicate effect of the canal on the metabolism of the individual cartilage cells and the effects of cartilage canals on the collagen of the matrix.
Fig.54- b: magnification of part of the previous fig 54a, showed one vascular cartilage canal triangle in shape surrounded by regularly arranged enlarged different sized cartilage cells and flat cells. The smallest and largest transverse diameters of the canal were approximately 60um &260 um and the longest diameter was 380um, according the scale on the microphotograph: The diameter of the largest sinusoidal capillary was -95um.
Active chondrolysis was noted at the end of the canal (lacunae containing cells intimately associated with matrix, and presence of granules.
Histological examination of part of TS of part of developing prenatal human 4th rib juxta the costochondral junction CCJ in the resting cartilage of 9month fetus showed Fig.55: three complex cartilage canals close to each other seen as one cartilage unit canal, giving triangle appearance,surrounded by different sized cartilage cells and flat cells, as well as large matrix around the canals with deeper stain.
The cartilage canal contained numerous course arterioles, venules and sinusoidal capillaries, and also connective tissue which served as a source of stem
The smallest and largest transverse diameters of the canal were approximately 200um&350 um and the longest diameter was 400um. According the scale on the microphotograph: The diameter of the largest sinusoidal capillary was -95um.
Chondrolysis was noted between the canals (lacunae containing cells intimately associated with matrix, and presence of granular debris).
Histological examination of part of TS of part of a developing prenatal human 4th rib at the costochondral junction of 9month fetus showed Fig. 56 two cartilage canals and the cartilage cells at the ends of the canals show chondrlysis.
The matrix around the canals was deeply stained. Spotted deep stain of matrix was seen around and among the vascular canals. The arterioles divided to two or more capillaries
The canals contained arterioles, capillaries, venules, sinusoidal capillaries and CT
The cartilage cells around the canals were enlarged dispersed loosely packed in irregular manner. Some large irregular cells were seen between the close cartilage canals
chondrolysis wae seen.
Fig. 57 –a&b two vascular cartilage canals with different shapes and size.fig.57 a. demonstrated deep stain of matrix around the canals and Chondrolysis of cells around the canals. chondrolysis was noted at the end of the canal: lacunae containing cells intimately associated with matrix, and presence of granular debris)
One canal was elongated and had extended extension, and satellite canals small around.
Fig. 57 -b showed - one vascular cartilage canal surrounded by different sized cartilage cells and flat cells. Deep matrix stain around the canals was seen.
The cartilage canal contained numerous course arterioles, venules and sinusoidal capillaries that were important for nutrition, and also connective tissue which served as a source of stem cells.
There were three distinct capillary–sinusoidal vessels interspersed with connective tissue and many small discrete vessels
The transverse diameter of the largest 3 vessels. according the scale on the microphotograph: were100um- 90um-70um.

chondrolysis was noted (loss of metachromasia, lacunae containing cells intimately associated with matrix, and presence of granular debris)

Histological examination of part of TS of part of a developing prenatal human 4th rib jaxta the costochondral junction CCJ, of 9month fetus showed Fig. 58: vascular cartilage canals in the resting cartilage with different shapes, size and contained CT, present in spotted differently stained matrix with different stain affinities.

One canal had extension and showed chondrolysis.

Fig. b magnification of part of fig.58 a

Histological examination of part of TS of part of a developing prenatal human 4th rib at the costochondral junction CC J of 9month aged fetus showed Figs. 59a&b numerous cartilage canals had different shapes, size and content basically arterioles,capillaries, venules andCT,and close small canals seemed as satellite might be detached from the big canals. Some canals seem having tail like, seen as ghost.

Deep spotted stain of matrix around and between the canals was seen.

The unique vascular anatomy, a single vessel filled the entire diameter of the canal was noted. Usually two or three distinct capillary–sinusoidal vessels interspersed with connective tissue and many small discrete vessels were noted.

Histological examination of part of TS of part of a developing prenatal human 4th rib jaxta the costochondral junction CCJ of **9month fetus** showed Fig.60 cartilage canals in the resting cartilage with different shapes, size and contents.

Deep stain of the matrix around and between the vascular cartilage canals was noted.

Chondrolysis was noted at the end of the canals (lacunae containing cells intimately associated with matrix, and presence of granular debris) were noted

Each cartilage canal, had two or three distinct capillary–sinusoidal vessels interspersed with connective tissue and many small discrete vessels.

Histological examination of part of TS of a developing prenatal human 4th rib juxta the costochondral junctionCCJ of 9 months old age fetus showed Fig. 61 five cartilage canals with different shapes,size and Their transverse diameter: range: 10um, 80um. 100om, 120um according to the scale on the photo micrographe.

Cartilage canals contained distinct numerous course arterioles, venules and sinusoidal capillaries that embedded in loose connective tissue which served as a source of stem cells. The cartilage canals were important for nutrition, and metabolism of individual cartilage cells. Might serve as a source of cartilage stem cells for growth of the rib epiphysis. Deep stain in the matrix around the cartilage canals that might indicate the effects of the canals on the individual cartilage cells and matrix collagen

One cartilage canal contained large one sinusoid capillary occupied the whole cartilage canal space was seen.

Histological examination of part of LS of developing prenatal human 4th rib at the costochondral junction CCJ of 5,6,**9month aged fetuses** showed in Figs. 62 a,b,c part of The cartilage growth plate was formed of the hypertrophic, proliferative.germinative zones and became shorted.thinner with age progress.The irregular sponge bone and trabeculea were loose in 5month aged fetus, and dense in 6month aged fetus and less dense in 9 month aged fetus with spaces,respectively Three stain affinities of collagen: the deep stain of rib perichondrium, less stain affinity in the sub perichondrium,and faint stain in the hyaline cartilage were seen.

The periosteam was fibrous sheath surrounded the outer surface of the prenatal developing human 4th rib. It composed of two layers: an outer layer made of dense white fibrous tissue consisted of blood vessels, and an inner layer consisted of loose tissue containing osteoblasts. The inner layer was the osteogenic or osteoblastic layer which formed new cells

Histological examination of part of LS of part of the 4th prenatal developing human rib at the costochondral junction CCJ of 4month aged fetus Fig. 63 a, 6months aged fetus Fig 63-b and full term fig 63 -c illusted the relation between the growth plate and the developing sponge bone by endrochondral ossification.The developing prenatal 4th rib showed the growth plate decreased and became thinner with age progress,while the sponge increased in length and surface area and grew due to added cartilage cells which burst in the sponge, and endochondral ossification and appositional growth,which was a surface phenomenon, That coincided with the Quraan suret al baqara 259 and 259سورة البقرة-كيف ننشزها العظامcontinued remodeling during the prenatal rib development the shortening of the growth plate with age progress,while increased leng th of the growing bone(Figs.63- a,b,c).

The growth plate was formed of the germinative, proliferative, hypertrophic zones.

The irregular cancellous sponge of metaphesyeal bone formed of trabeculea

The perichondrium was continuous with the periosteam.

Histological examination of part of LS of part of the prenatal human developing 4th rib of 9 month human full-term:(33-36 weeks) CRL 31-34cm) and newborn infant (37-38 weeks-(CRL 35-36cm) showed in fig. 64a parts of the growth plate, the primary ossification centerPOC and sponge.

Figs.64 -c,d,e magnification of parts of the sponge of fig.64 a,
Fig.64- b showed osteoblasts on the surface of matrix and osteocladsts inside the matrix of the sponge.
The sponge bone was formed of irregular trabeculae and appeared as anastomosing. The trabeculae were covered with the small branched osteoblasts which were, bone forming cells. They formed continues layer covering the trabecular of cancellous bone.they were POLARISED on the surface of the trabeculae,meanwhile osteoclasts were between the matrix,surrounded by it.Osteoclasts could not divide.osteoblsts were more basophilic(bule)cytoplasm due to excess RNA in the cytoplasm.
Histological examination of part of TS of part of a developing prenatal human 4th rib at the costochondral junction CCJ, of 9month aged fetus showed Figs. 65&66 part of, the irregular sponge cancellous bone of the developing rib,was formed of irregular trabeculae appeared as anastomosing with each other.The osteoblasts formed a continues layer of the branched, bone forming cells. They were on the surface of the trabeculea, meanwhile osteoclasts had embedded in the matrix, surrounded by it.Osteoclasts could not divide. Osteoblasts were more basophilic (blue) cytoplasm due to excess RNA in the cytoplasm. The sponge at CCJ formed the primary ossification center POC.Blood cells were seen.
Fig. 66,showed, part of the Primary ossification center POC, and the sponge bone, was formed of irregular trabeculae appeared anastomosing.The trabeculae were covered with the osteoblasts which found on the surface of the trabeculae, meanwhile osteoclasts were between the matrix surrounded by it.
A trapped osteocyte was noted embedded in the bone matrix.

B-Morphological Results: figs 67&68:(Figs. A, B, C)

Morphological examination of Prenatal developing human right fourth 4th typical rib showed Fig A: the morphology and angle of the ages: 4,5,6,7 months fetuses, and full term superior view of (surface-anterior) and (inferior posterior
Each rib had anterior and posterior end, and body.The posterior end had a tubercle and articular facet for the body of vertebra which developed at full term.The Costal groove was just clear at full term.
Increase size and length of the ribs with age progress was seen,
The tubercle of the posterior end of the rib appeared at the age of 6 months aged fetus.
Fig.B (a&b) showed anterior view of the Prenatal developing human Second Rib(a):,and fourth rib(b) to illustrate the morphology and angle, of the ages: 4,5,6,7 months fetuses, and full term
Each rib was composed of anterior sternal end, and posterior vertebral end, and haft.
The lower border of the developing prenatal human fourth rib was sharper compared to the upper border. The sharpness increased with age progress.
Fig C: showed The second and fourth adult ribs, showed the sternal end.and the vertebral end which consisted of head, tubercle, neck, and angle.
There was twist in the 4th rib adult, and no twist in the second rib,
The lower border was sharper than the upper border in the fourth adult rib. The sub costal grove was clear.

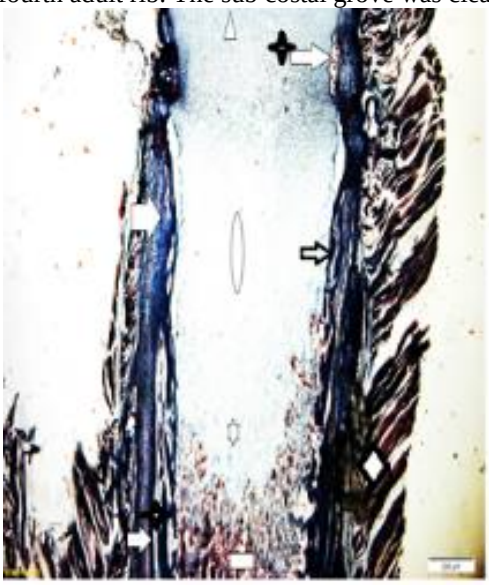


Fig 1: Photomicrograph of part of LS of part of the 4th prenatal developing human rib at the costochondral junction CCJ of 4month aged fetus (13-16wks-CRL 9-14cm, showing the general appearance of part of a long epiphyseal growth plate. formed of the germinative (reserve-stem cell-inert) zone (triangle), proliferative (oval), and hypertrophic (star) zones
Part of the intercostal muscles is noted (diamond)
Note the short irregular developing sponge bone of metaphesyeal bone (box) formed of trabeculea, representing the site of primary ossification center (POC).
Note the perichondrium (thick white arrow) is continuous with the periosteam (short white arrow)
Note the groove of Ranvier is slightly wedge-shaped collection of cells which provides chondrocytes newly formed cells (arrow star) in the epiphysis
To provide the growth plate, with cells for the longitudinal growth. The perichondrial ring (PR) provides mechanical support.
The intramembranous bone bark extends from the level of the proliferating zone to the primary ossification in the metaphysis. Part of the role of the PR is to lay down a thin layer of bone through intramembranous ossification (bone bark), which

provides mechanical support to the growth plate and the metaphysis, and increases the width of the physis. That coincided with the Quraan –suret el ensane 28: Allah the creator, strengthen and fortifies what is created.

سورة الانسان28 خلقناهم وشددنا اسرهم

The length of the subperiosteal bone collar SPBC– bone bark is 670um according to the scale on the photomicrograph (empty arrow) approximately

Malory triple stainx40

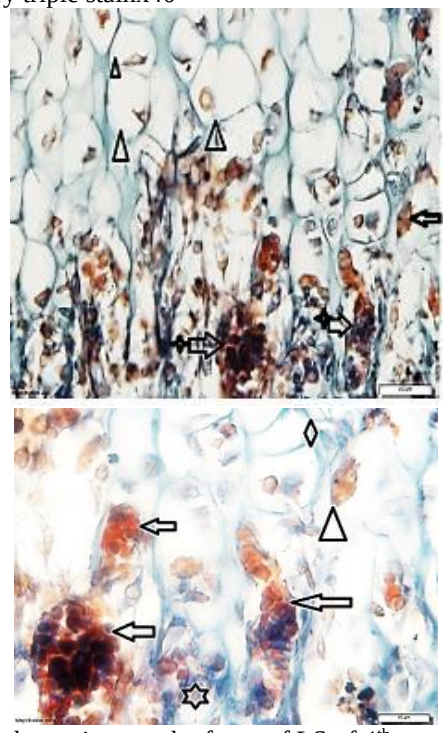


Fig2: photomicrograph of part of LS of 4th prenatal developing human rib at the costochondral junction CCJ, of 4 month aged **fetus (4 months (13-16wks-CRL 9-14cm**, showing)

- Multinucleate giant Chondroclast (arrowstar), osteoblasts (arrow)on the surface of matrix stained faint blue.
- Note the hypertrphic cartilage cells with shrunken nuclei(arrow head)
- Note the blood cells (star)in the sponge
- Chondroclasts are multinucleated giant cells, arising from the fusion of mono-cytes/macrophages present in the bone marrow. Once attached to the surface of mineralized cartilage via the sealing zone they become activated and release tartrate resistant acid phosphatase(TRAP), also referred to as acid phosphatase5 tartrate resistant(Acp5)
- During longitudinal growth, not only the POC but also the bone cuff expands from the centre of the diaphysis towards the cartilaginous endings (epiphysis). A thickening and persistent remodelling of the bon trabeculae governed by a balanced mutual action of osteoclasts and osteoblasts accompanies the growth. Osteoclasts have the same origin and molecular,and ultrastructural characteristics of chondroclasts but break up the bone matrix onto which they adhere
- in the complex sequence of cartilage degradation, bone apposition and growth the original cartilage model is replaced by bone,and these critical events occur at the chondro-osseous junction of the epiphyseal growth plate (roach, 2000; for review)

FIg b- higher magnfication of part of fig a: shows the transition zone is composed of mineralized cartilage and **early** bone matrix (diamond stained faint blue) and characterized by the presence of hypertrophic chondrocytes, chondroclasts(arrow), osteoblasts)head arrow)and blood vessels(star

Malory triple stain x200

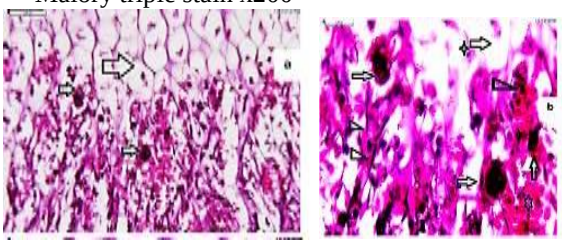


Fig 3Photomicrograph of part of LS of part of 4th prenatal developing human rib at costochondral junction CCJ, of four month aged fetus (4 months (13-16wks-CRL 9-14cm aged fetus fig3-ashowing, part of the hypertrophic cartilage cells arranged in simple columns, and few terminal final cells bursting in the sponge(big arrow) chondroclast (small arrow)in the sponge of irregular cancelloius newly formed bone matrix.(box)

Note the blood cells and spaces between the trabecular. HEX

In the complex sequence of cartilage degradation, bone apposition and growth, the original cartilage model is replaced by bone, and these critical events occur at the chondro-osseous junction of the epiphyseal growth plate (Roach, 2000; Shapiroet al., 2005). This transition zone is composed of mineralized cartilage and early bone matrix and characterized by the presence of hypertrophic chondrocytes, chondroclasts, osteoblasts and blood vessels.

Fig 3-b- higher magnirfication of part of fig a, showing chondroclast (arrow) in the irregular sponge bone of primary ossification center(POC).

Deep basophilic osteoblast forming continuous layer (head arrow)on the surface of sealing newly formed matrix,. Note the capillary invasion of cartilage cells (big triangle) Note the Blood cells in bone marrow (star), result from chondroclasts erosion of cartilage matrix. HEX200

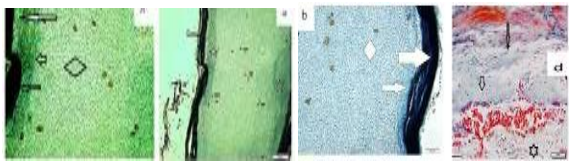


Fig 4 -photomicrograph of part of LS of part of prenatal developing human 4th rib at costochondral junction CCJ, of **4**months aged fetus (4 ***months (13-16wks-CRL 9-14cm*** showing,in fig,A: and fig 4-a:three degrees of stain affinity deep strong stain of perichondrium(long arrow)
less stain affinity of subperichondrial (short arrow). least weak stain affinity at the resting cartilage (diamond)
The different stain affinity of the perichondrium (triangle in fig a)) and the matrix OF subperchondrium in the **of hyaline cartilage** of the rib indicating the possible different types of collagen.Masson trichrome x100
Fig b-Three degrees of stain by Mallory triple stain of the perichondrium strong deeply stained(big arrow) the subperdhondruim less strong stain affinity (small arrow) the hyaline matrix of cartilagenous tissue week stain)diamond Malory triple stain).
Fig d- shows part of the layers of the perichondrium with rich vascularity, (short arrow)
Note the flat Cells (long thin arrow)are arranged parallel between the collagen bundles.
Note the mesenchyme (star) containing stem cells that may provide source of cartilage cells to the reserve zone of growth plate.
The growing perichondrium of the 4th prenatal human rib shows the interstitial growth:growth from inside, and the appositional growth (growth from outside ;the CT pericondrium:New layers of cartilage are added from the inner condrogenic layer of the perichondrium: where undifferentiated mesenchymal cells UMCs forms chondrocytes(cartilage cells), Masson trichrome x 200.

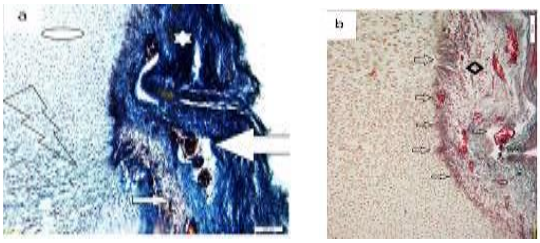


Fig5:Photomicrograph of part of the jaxta costochondral junction CCJ of a prenatal human 4th rib o f 4 ***months (13-16wks-CRL 9-14cm aged fetus*** showing part of the resting cartilage (oval)and the covering perichondrium(star) layers arranged in a complex manner to give pathway for precursors of three cartilage canal formation: arterioles surrounded by mesenchyme.(big arrow)
Note the spur arrangement of cartilage cells formation (zigzag arrow).started from subperichodreum.
Note the mesenchyme tissue with capillaries(small arrow)between perichondrium. **scale200um**
Mallory triple stainx40
Fig b:showing part of the resting cartilage and the covering perichondrium(arrows) layers arranged in a complex manner to give pathway for precursors of cartilage canal formation: arterioles surrounded by mesenchyme.(big arrow) Note the brush border like at the small area of the perichondrium (thick arrow)
Note the mesenchyme tissue with capillaries(diamond)between perichondrium. Masson trichrome X100

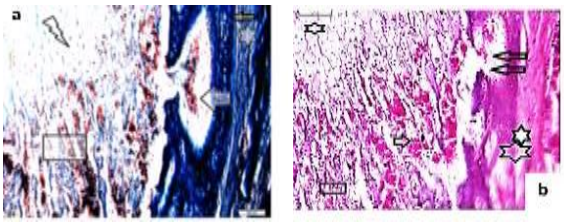


Fig6a:photomicrograph of part of TS of part of a prenatal developing human4th rib of four month (4 *months (13-16wks-CRL 9-14cm aged fetus* of a costo chondral junction CCJ showing Precursor of formation of cartilage canal from the perichondrium(star).: vascular tissue is surrounded by mesenchyme,starts extension (arrow).
Vascular mesenchyme is seen, which serve for new cartilage cell formation in interstitial growth of the rib (big arrow)
Note the sponge irregular cancellous bone (box) formed of trabeculea and bone marrow
The mesenchyme tend to extend to the primary ossification center and ossification corner.
Note the growing perichondrium of the 4th prenatal human rib shows the interstitial growth:growth from inside, and the appositional growth from outside ; the CT pericondrium:New layers of cartilage are added from the inner condrogenic layer of the perichondrium: where undifferentiated mesenchymal cells UMCs formed chondrocytes(cartilage cells).
Note that in the interstitial growth(thin arrow):growth from inside: single cell has a capsule, when it divides into two,each daughter cell has its own capsule, the primary capsule disappears and the two cells remained close to each.
Mallory trIple stain x40, scale200um
Fig b-shows: part of cartilage cells in columns from the hypertrophic zone (star) ossification corner primary ossification center POC(arrow)
Metaphysis sponge irregular cancelous bone(box), Mesenchyme tissue in the perichondrium starts to extend to form cartilage canal (double arrow).
Mesenchme containing stem cells that may provide source of cartilage cells to the growth plate and

endochondral ossificaion.(and source of osteoblasts for later secondary ossification center SOC, besides the interstitial growth of cartilage. Periosteum(double star), HEX200.

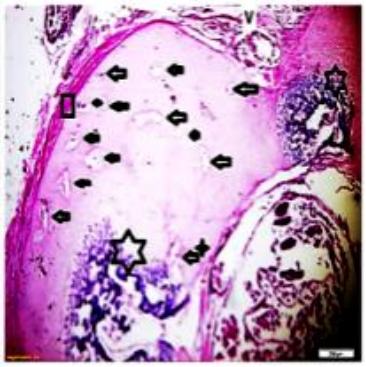


Fig7: photomicrograph of part of TS of part of 4th pre8tal developing human rib juxta the costochondral junction CCJ, e of 4 month aged fetus *(13-16wks-CRL 9-14cm aged fetus* showing, the general morphology of part of the epiphysis, with two secondary ossification centers:one large center formed from fusion and coalescing of multiple small secondary ossification centers (big STAR),
Small center is seen) small STAR)

Note the numerous superficial cartilage canals are around the center (arrow) n=12 in surface area represents 3% approximately from the resting cartilage
One canal is invading the large center (arrow star)
Note the vascular cartilage canal (arrowstar)invading the coal icing secondary ossification center.
Note the covering layers of perichondruem(box). Withrich blood vessels(V)
Note the basophilic stain of the sponge bone of the secondary ossification center SOC, ADD
Note Vascular canals large extended from subperchondruen invading the the big and the small SOC and ANASTOMSE AND BRANCH IN NTHE SOC
Large spaces are inside the SOC, scale200um, HEX40

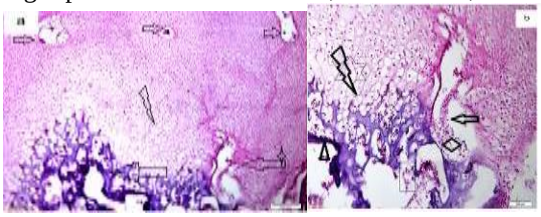


Fig 8:Photomicrograph of part of ES OF part of the jaxta the costochondral junction CCJ of prenatal human 4th rib of (4 ***months (13-16wks-CRL 9-14cm*** aged fetus,showing in fig a three 3 vascular cartilage canals(arrows) close to part of a secondary ossification center. SOC (box)

Note that the ossification center is invaded by cartilage canal (arrow star) containing large vessel and CT,which incorporates and continues inside the SOC ossification center
Note the enlarged hypertrophic cartilage cells around the ossification center(zigzag arrow),forming a plate,morphologically similar to the growth plate at the costochondral junction CCJ., HEX40
fig b –shows higher magnification of part of the previous of an invading cartilage canal to the secondary ossification center**SOC**,**incorporated with the center, distributed in it, and leads to transform of the walls of the invading vessels TO basophilic osteogenic like cells.(head arrow)**

Note the invading canal contains CT in which the stem cells may represent source of osteoblasts sharing in the formation of the secondary ossification center(arrow).
Note the enlarged chondrocytes arranged in small columns close to the secondary ossification center.SOC, and the final cells burst in the center in basophilic field(ZICZAZ), HEX40.

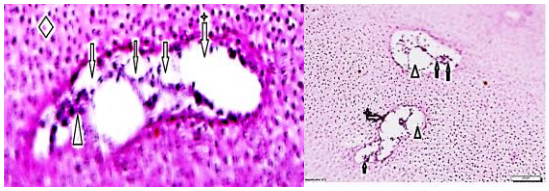


Fig 9 a&b:photomicrograph of part of TS of part of 4th prenatal developing human rib juxta the costochondral junction,in the epiphysis (diamond) of, 4 ***months (13-16wks-CRL 9-14cm aged fetus*** showing in fig a superficial cartilage canal in with the intact wall of the cartilage canal,which contain mesenchyme, Note the Capillaries (arrow), Venule (arrow star), Macrophages (head arrow)

Note the deep Stain of matrix around the cartilage canal. HEx200

Fig 9-b:showing the relation between two superficial cartilage canals with intact wall of the canal,which contain mesenchyme(arrow)
small arterioles(arrow) and capillaries(head arrow) are present in the canals

Note the arrow star points to end of ONE cartilage canal with chondrolyis **(lacunae containing cells intimately associated with matrix, and presence of granular** small arterioles and capillaries are present (head arrow)in the canals HEx200-scale50um

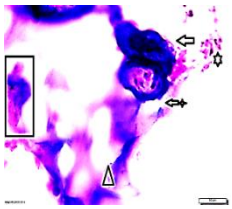


Fig10 photomicrograph of part of LS of part of 4th prenatal developing human rib at costochondral junction CCJ, of 4 *months (13-16wks)-CRL 9-14cm aged* fetus,showing, part of secondary ossification center SOC contains deep basophilic polarized osteoblast on the surface of the newly formed bone matrix(arrow). and Chondroclast multinucleate giant cells eroding the cartilage (arrowstar)
Stages of bone cells formation and remolding (box) osteoblasts which are bone forming cells, small branched polarized cells. They formed continues layer covering the newly formed matrix of bone. They found on the surface of the trabeculea osteoblasts are more basophilic(blue)cytoplasm due to excess RNA in the cytoplasm, Note the blood cells (star)
Stages of cell bone formation(box)present in eosin field due to TRAP formation from the chondroclast.
Chondroclasts are multinucleated giant cells, arising from the fusion of mono-cytes/macrophages present in the bone marrow. Once attached to the surface of mineralized cartilage via the sealing zone they become activated and release tartrate resistant acid phosphatase(TRAP), also referred to as acid phosphatase5 tartrate resistant(Acp5)
During logitudinal growth, not only the POC but also the bone cuff expands from the centre of the diaphysis towards the cartilaginous endings(epiphysis). A thickening and persistent remodelling of the bony trabeculae governed by a mutual action of osteoclasts as well as osteoblasts accompanies the growth. Osteoclasts are embeded in the bone matrix and have the same origin and molecular as well as ultrastructural characteristics as chondroclasts but break up the bone matrix onto which they adhere, HEX1000.

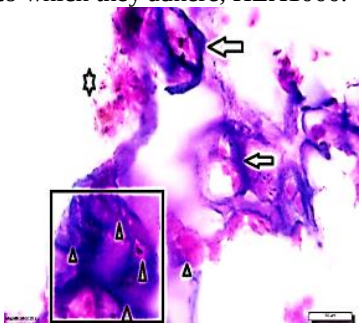


Fig11 photomicrograph of part of LS of part of 4th prenatal developing human rib at costochondral junction CCJ, of **4 *months (13-16wks)-CRL 9-14cm aged* fetus,** showing, part of secondary ossification center SOC contains Deep basophilic osteoblasts(arrow) on the surface of newly formed matrix
Note the newly formed bone cells in eosin acidic field due to TRAP(tartrate resistant acid phosphatase) formation from the chondroclast (small arrowheads inside box), TRAP enzyme is essential in bone remolding, blood cells (star), HEX 200-scale50um

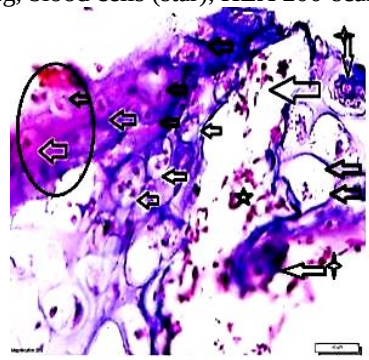


Fig12: photomicrograph of part of LS of part of 4th prenatal developing human rib at costochondral junction CCJ, of 4 *months (13-16wks)-CRL 9-14cm aged* fetus,showing, Part of a secondary ossification center SOC, invaded by vessels (large arrow) and the wall of the vessel transformed to basophilic osteogenic like cells
Note the group of Osteocytes and osteoclasts embedded in the newly formed matrix (oval),in stages of formation for continuous remodeling.the cells present in eosin acidophilic field due to TRAP production (circle -oval) produced by Chondroclasts, multinucleated giant cells, arising from the fusion of mono-cytes/macrophages present in the bone marrow
Note the Blood vessel in the secondary ossification center(big arrow),with blood cells in the lumen.
Note the Chondroclast(arrow star)giant multi nucleate cell eroding the cartilage. they become activated and release tartrate resistant acid phosphatase(TRAP), also referred to as acid phosphatase5 tartrate resistant(Acp5), Hypertrophic cartilage cells burst in the center (double arrow, HEX200

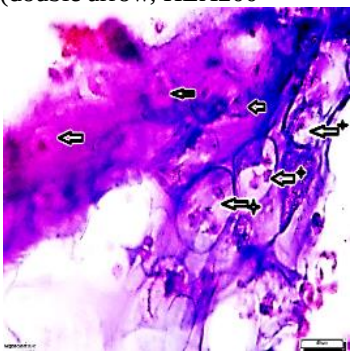
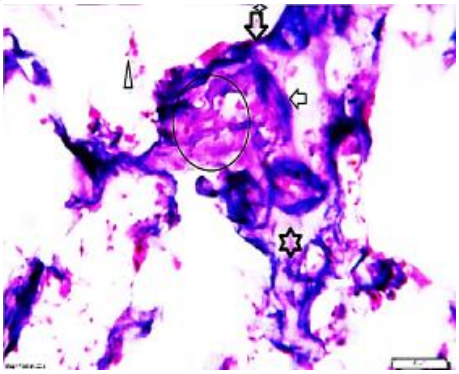
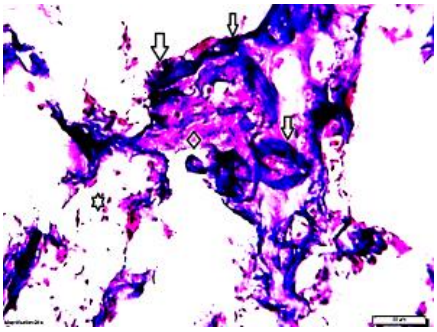


Fig13: photomicrograph of part of LS of part of 4th prenatal developing human rib juxta the costochondral junction CCJ, of **4 *months (13-16wks)-CRL 9-14cm***

***aged* fetus,**showing, Part of secondary ossification center SOC in the epiphysis,showing:
Stages of new osteocyte formation embedded in matrix (arrow) within masseosin stain acidic field due to TRAP formation (tartrate resistant acid phosphatase),which is necessary for bone osteogenesis,remodling and development Ive Note the blood cells (arrow star)
Basophilic areas intermingled between the acidic field., HEX200



Fi g 14: photomicrograph of part of LS of part of 4th prenatal developing human rib juxta the costochondral junction CCJ, of 4 *months (13-16wks)-CRL 9-14cm aged* fetus showing, part of secondary ossification center SOC present in the epiphysis,illustrating: stages of osteocytes formation embedded in eosin field (circle)due to production of TRAP (tartar acid phosphate from the chondroclasts.TRAPis needed for bone development,osteogenesis and remodeling.
Note the intermingling of acidic field within basophilic field and Chondroclasts are multinucleated giant cells, arising from the fusion of monocytes/macrophages present in the bone marrow. Once attached to the surface of mineralized cartilage via the sealing zone they become activated and release tartrate resistant acid phosphatase(TRAP), also referred to as acid phosphatase5 tartrate resistant(Acp5)
Deep basophilic polarized osteoblasts are seen on the surface of the newly formed matrix(arrow).
Blood cells(arrow head), Remenant of cartilage cells (star), HEX200-scale 50



Fi g 15: photomicrograph of part of LS of part of 4th prenatal developing human rib juxta the costochondral junction CCJ, of 4 months (13-16wks)-CRL 9-14cm aged fetus, showing, part of secondary ossification center SOC containing basophilic Osteoblast(arrow) on surface of new matrix present in acidic field (diamond), Note the blood cells star, HEx200

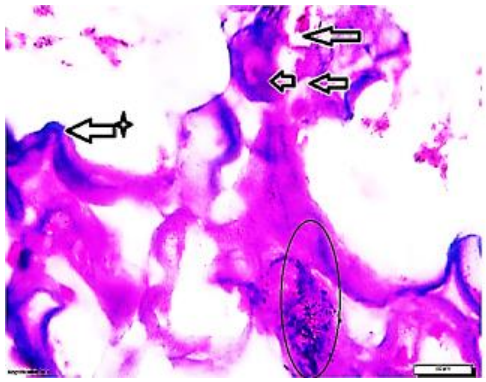


Fig16: photomicrograph of part of LS of part of 4th prenatal developing human rib juxta costochondral junction CCJ, **4 *months (13-16wks)-CRL 9-14cm aged* fetus,**showing, Part of secondray oss sification center SOC,in the epiphysis.
NOTE THE stages of osteocyte cell formation embedded in matrix within eosin media (arrow) due to TRAP formation needed for bone remodeling and development.

Note the chontroclast(circle)giant cell multinucleate with cytoplasmic. extension
Chondroclasts are multinucleated giant cells, arising from the fusion of mono-cytes/macrophages present in the bone marrow. Once attached to the surface of mineralized cartilage via the sealing zone they become activated and release tartrate resistant acid phosphatase (TRAP), also referred to as acid phosphatase5 tartrate resistant(Acp5) the field around the giant cell is eosinic,which Indicating it is most probably chodroclast.
During longitudinal growth, not only the POC but also the bone cuff expands from the centre of the diaphysis towards the cartilaginous endings(epiphysis). A thickening and persistent remodeling of the bony trabeculae controlled and governed by a mutual balanced action of osteoclasts and osteoblasts accompanies the growth. Osteoclasts have the same origin and molecular as well as ultrastructural characteristics as chondroclasts but break up the bone matrix onto which they adhere, HEX200.

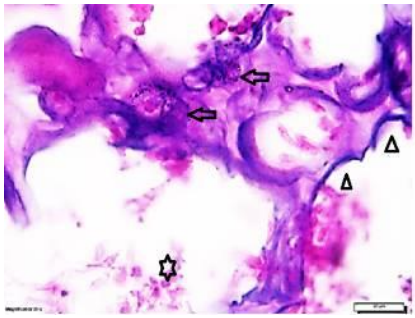


Fig17:photomicrograph of part of LS of part of 4th prenatal developing human rib juxta costochondral junction CCJ, 4 *months (13-16wks)-CRL 9-14cm aged* fetus, showing, part of a secondary ossification center SOC present in the epiphysis, illustrating: the acidic field,eosin stain due to Chondroclasts multinucleated giant cells, (arrow star) –which arising from the fusion of mono-cytes/macrophages present in the bone marrow-. Once attached to the surface of mineralized cartilage via the sealing zone they become activated and release tartrate resistant acid phosphataseTRAPalso referred to as acid phosphatase5 tartrate resistant(Acp5),which caue the acidic field,essential for bone formation and remodeling

Notice the basophilic POLARIZD flat osteoblasts (head arrow)on the surface of the newly formed matrix

Note the Blood cells (star)

During longitudinal growth, the POC but also the bone cuff expands from the center of the diaphysis towards the cartilaginous endings (epiphysis). A thickening and persistent remodeling of the bony trabeculae controlled and governed by a balanced mutual action of osteoclasts and osteoblasts accompanies the growth. Osteoclasts have the same origin and molecular and ultrastructural characteristics as chondroclasts but break up the bone matrix onto which they adhere, HEX200

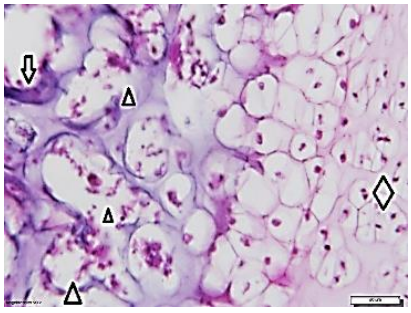


Fig18:photomicrograph of part of LS of part of 4th prenatal developing human rib juxta the costochondral junction CCJ, of 4 months (13-16wks)-CRL 9-14cm aged fetus, showing, part of a secondary ossification center SOC present in the epiphysis, illustrating enlarged cartilage cells at the edge of secondary ossification center SOC (diamond)

Ruptured enlarged cartilage cells at the edge of secondary ossification centerSOC (head arrow) with bloos cells, Basophilic Osteoblast (arrow)
HEX2000

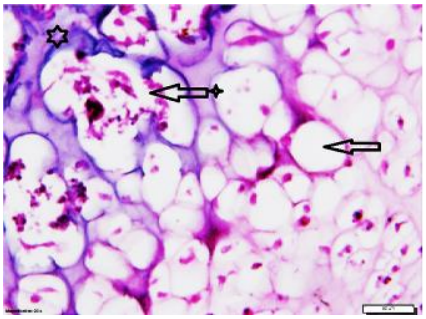


Fig19: photomicrograph of part of LS of part of 4th prenatal developing human rib juxta the costochondral junction CCJ, of **4 months (13-16wks)-CRL 9-14cm aged fetus,**showing, Part of secondary ossification center SOC present in the epiphysis

Note the hypertrophic cartilage cells(arrow) around the center bursting in the center, which may die by apoptosis preprogrammed death,or may transform to osteoblast osteogenic cells as some workers claimed

Note: chondroclast eroding (arrow star) matrix (star),leaving spaces and blood cells.

Hypertrophic cartilage cell (arrow) around the SOC resemble morphologically the growth plate at the metaphysis at the costochondral junction CCJ, Part of secondray ossification center SOC,illustrating: SOC,at the edge,hypertrophic cartilage cells bursting in the center,and blood cells are between the bursting cartiage cells, HEX200

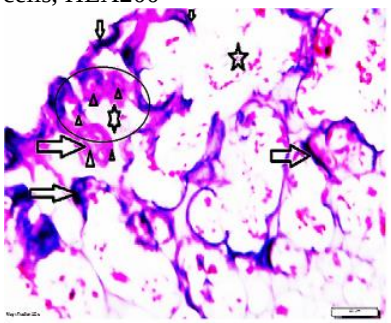


Fig 20: photomicrograph of part of LS of part of 4th prenatal developing human rib juxta the costochondral junction CCJ, of **4 *months (13-16wks)-CRL 9-14cm aged* fetus,**showing, part of a secondary ossification center SOC,illustrating: large chondrocytes at the edge of secondary ossification center bursting in the center and full of blood cells (asterisks)

Osteoblasts BASOPHILIC polarized cells on the surface (small arrow) newly formed matrix

stages of osteocyte formation (arrowhead inside oval)present in weak eosin field due to TRAP formation by chondrocladts giant multinucleate eroding the cartilage needed for bone formation and

remodeling. Note the Chondroclast (arrow),which are giant multinucleate cells present eroding the cartilage (star),, HEX200-SCALE50um.

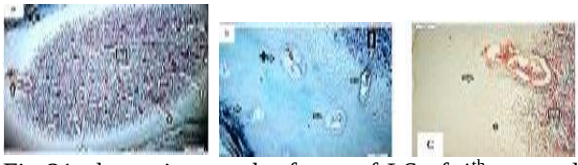


Fig 21 photomicrograph of part of LS of 4th prenatal developing human rib of 5months *(17 -20weeks) CRL 15-19cm* aged fetus,in the epiphysis juxta the costochondral junction CCJ showing in fig a-large secondary ossification centers SOC(box),occupying aproximately 75% of the surface area of the resting cartilage (diamond)

Note the numerous cartilage canals invading the center(arrow), from different sides of the center,which lead to the SOCcenter expansion.

Note the perichondrium (star).

Fig b- part of the large secondary ossification center SOC (box)surrounded by cartilage canals,one large canal with absorbed material(arrowstar) and one anchoring canal (arrow) to the SOC

Fig C- higher magnification of part of fig a showing One vascular cartilage canal(arrow) is going to incorporate with the secondary ossification center SOC(box), Masson trichrome x40-scale200um in fig a&b

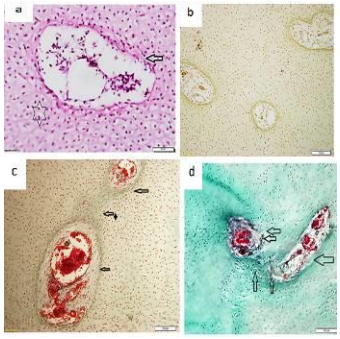


Fig 22 photomicrograph of part of TS of developing prenatal human 4th fourth rib juxta the costochondral junctionCCJ,showing different cartilage canals in different developing ages: (a)..4month aged fetus,(b),5month (c) 6month –aged fetus, (d)a full term the canals are simple at 4and 5months fetus,,or contain CT,no vessels are seen. The canals become complicated with age progress and contain blood vessels at the age of 6months and full term. full of blood (branched)sinusoidal capillaries and vessels and connective tissues

Note the different shape,size and contents. Fig-a: HEx100, Figs b,c,d:Masson trichrome stai x100

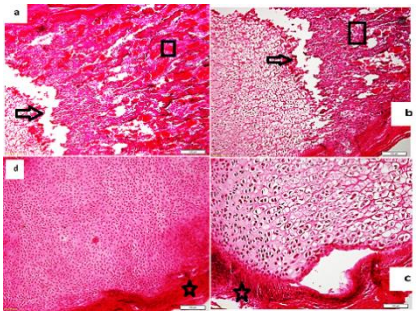


Fig 23 a,b,c,d:photomicrograph of part of LS of part of the developing prenatal human 4th rib at costochondral junction CCJ of *6-months old fetus:(21 -24weeks) CRL 20-23cm* showing in a-and b- part of the hypertrophic zone and cartilage cells of provisional calcification zone (arrow)

PZC bursting in the sponge bone.(box), Fig c-part of the proliferative zone with dividing cells, Fig d- part of the resting reserve (stem cell –inert) zone

Note the perichondrium(asterisks), HEx100

Fig24:photomicrograph of part of TS of part of the prenatal human developing 4th rib,juxta the costochondral junction, **of 6month aged fetus** showing in fig A the general appearance of a secondary ossification center(box) sounded by cartilage canals (line)n=25,two canals (arrow)are anchoring the center.

figs a & b higher magnifications of part of fig a.showed cartlage canals (arrow)around a secondary ossification(box) center SOC(box)

Big branching cartilage canal (arrowstar) with interrupted wall is invading (oval)the SOC center.

the canals which do not incorporate with the SOC degenerated and seem as ghosts (arrow)

Note the large cartilage cells around the SOC center form plate similar morphologically to the l growth plate at the costochondral junction CCJ

Fig b- higher magnification of part of the previous FigA,showing cartilage canal with interrupted wall arrow star),and extension (oval).

invading secondary ossification center SOC (box)

Note the canal contains central arteriole(long arrow), capillaries (short arrow), venule (headarrow, and mesenchyme CT (diamond).

Note the hypertrophic cartilage cells (zigzag)around the SOC center, similar morphologically to the growth plate at the costochondral junction CCJ at the metaphysis'.

The long diameter of the cartilage canal is 450um, The diametre of the central arteroile is approximately 210um, The long diameter of the venule is

approximately 330um measured from the scale of the microphotograph, Masson trichrome x100.scale100 علقة

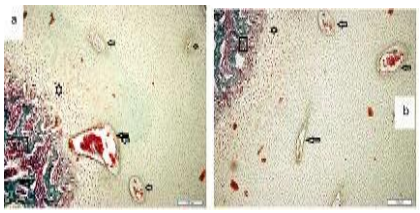


Fig 25: a&b:Photomicrograph of part of TS of part of a developing prenatal human fourth rib at the epiphysis,juxta the costochondral junction CCJ of 6-months old fetus:(21 -24weeks) CRL 20-23cm showing in fig a the resting cartilage containing 4 vascular cartilage canals with different sizes,content near a secondary ossification center (box),

Note the long diameter of the largest cartilage canal is approximately 200um, and the long diameter of the vessel is 170um measured from the scale of the photomicrograph. (double arrow)

Note the different stain affinity around the and between canals

Note that one cartilage canal contained absorbed substanceand large one sinusoid occupies the whole canal space (double arrow). is anchoring the SOC

Note the LARGE cartilage cell columns.(star) around the secondary ossification center (box).

Fig b showing vascular cartilage canals close to a SOC (box) and ghost like atrophied canal (long arrow

The cartilage Vascular canals are important in the development of epiphyseal ossification because the vessels with their associated mesenchymal cells serve as the source for bone-synthesizing osteoblasts on the calcified cartilage, Masson trichromex100

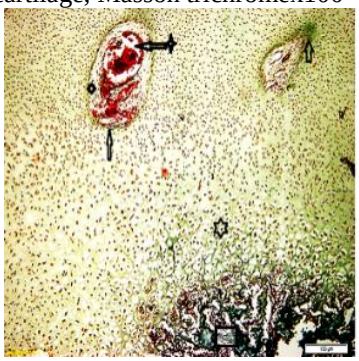


Fig 26:Photomicrograph of part of TS of part of a developing prenatal human fourth 4th rib juxta the costochondral junction CCJ, OF 6 months aged fetus*:(21 -24weeks) CRL 20-23cm* showing,two cartilage canals in the resting cartilage close to a secondary ossification center SOC(box).

Note the enlarged cartilage cells arranged in short columns (star),around the secondary ossification center aSOC (box)forming a plate similar to the growth plate at the CCJ.) Note the degenerated SMALL cartilage canal (short arrow)

The big vascular cartilage canals has intact wall, contains Branched arterioles (arrow star), venules, sinusoidal capillaries, and loose perivascular connective tissue. Such canals provide nutrients to the cartilage and can serve as a source of cartilage stem cells for growth of the epiphysis. Vascular canals are essential for the development of epiphyseal ossification because the vessels with their associated mesenchyme al cells serve as the source for bone-synthesizing osteoblasts on the calcified cartilage

strong stain around the canal, different from the resting tissue indicating effect of the canals on the metabolic activity of the individual cartilage cells and effect on the collagen of the matrix.

The diameter of the largest cartilage canal measured approximately 300um. and the diameter of the largest capillary sinusoids in it measured approximately 150um. and the transverSE diameter of the small cartilage canal measured approximately 160um.(arrow)

Note the matrix around the canals and in between has deeper stain than the resting cartilage (arrow star), Massson trichrome x100

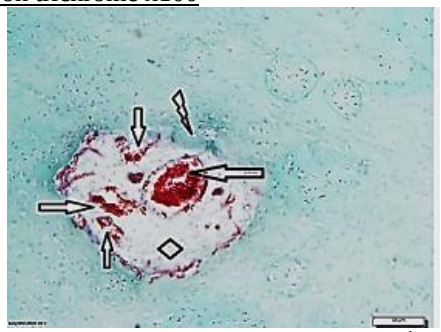


Fig 27 photomicrograph of part of LS of 4th prenatal developing human rib of 6months aged fetus***:(21 -24weeks) CRL 20-23cm***, showing, oval vascular cartilage canal,contains central arteriole (long arrow)and capillaries containing blood cells(small arrows),and mesenchyme CT) (diamond).

Note the cartilage cells around the canal are enlarged, few and dispersed. And the wall of the canal is not complete (zigzag

Note the matrix around the canal is deeply stained., The long and transverse diameter of the canal are 150umx130um

The long and transverse diameter of the central arteriole inside the canal are 60x50um,as the measurements from the scale on the microphotograph. Masson trichrome x200-scale50um

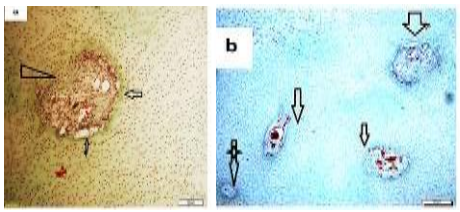


Fig28 a:Photomicrograph of part of TS of part of a developing prenatal human 4th rib at the costochondral junction CCJ of 6month aged fetus*:(21 -24weeks) CRL 20-23cm* showing part of the resting cartilage containing round cartilage canal (arrow) contains CT and surrounded by chondroylsis (arrowstar) and deep stain of matrix (arrow)
Note the Cartilage canal with not complete intact wall (triangle,containing collagen,mesenchyme and capillaries and lymph vessels.

Note the stain of the matrix is deep around the cartilage canal, indicating influence of the vessels of the canals on the metabolism of individual cartilage cells and the collagen of the matrix. Masson trichromex100
Fig b photomicrograph of part of LS of part of the prenatal developing human 4th rib jaxta the costochoeral junction CCJ, of 6 month aged fetus, showing part of the resting cartilage contains vascular cartilage canals (arrow) contain capillaries and ct. besides vascular cartilage canal with incomplete wall, contain capillaries and collagen, (Big arrow). one small atrophied canal (arrow star)Is noted.
Big arrow points to cartilage canal with incomplete ete wall contains collagen, Masson trichromex100

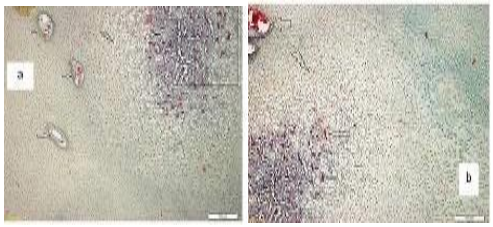


Fig 29: Photomicrograph of part of TS of part of a developing prenatal human 4th rib jaxta the costochondral junction of **6month fetus** showing in fg al Chondoclast(arrrow) in the rectangle)eroding the cartilage. Note the enlarged cartilage cells(zigzag) around the secondary ossification (box)center. SOC
Note the vascular cartilage canals are close to the SOC(ARROW)iMasson trichrome x100
n fg bChondoclast(arrrow) eroding the enlarged cartilage cells(zigzag) causing the presence of blood cells around the secondary ossification (box)center. SOC
Note the vascular cartilage canals are close to the SOC
Note the deep different stain of matrix cartilage and the stain of the matrix of the secondary ossification center SOC, Masson trichrome x100

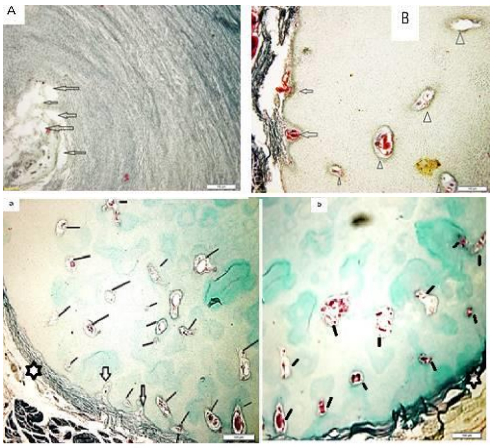


Fig30 A,B:Photomicrograph of part of radial section of part of a developing prenatal human 4th rib jaxta the costochondral junction of 6month fetus showing in fig
A: Group of deep cartilage canals (arrows)
fig B: part of the perichondrium with extension containing cartilage canals in the bud stage (arrow)and cartilage canals present in the resting cartilage (arrow head), Masson trichrome

Fig 30,a,b:Photomicrograph of part of TS of developing prenatal human 4th rib at the costochondral junction CCJ of 9month fetus showing in fib a,n =20cartilage canals (line)with different shapes,size and content present in the resting cartilage in surface area850x660um estimated from measuring the long and transverse diameter of the microphotograph.
Note the superficial canals (arrows),extending from the perichondrium (star)., Note the different stain affinity in the matrix areas close to the canals.
Fig b:showing adjacent field. The with larger canals n =11, note the increased in canal concentration with age progrss, Masson tricromex100

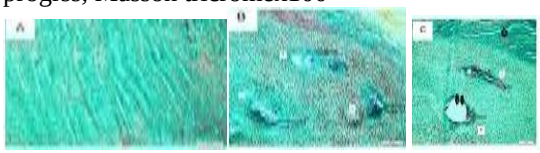


Fig 31: Photomicrograph of part of radial section of part of the4th prenatal developing human 4th rib of 7month aged fetus***:(25-28weeks) CRL 24-27cm*** showing in FIG A - the cartilaginous tissue arranged in columns – showing in FIG B- Three deep obliterated oculuded cartilage canals with fibrous collagenous substance or empty areas(arrow) the long diameters approximately measured from the scale on the microphotograph for, canal a: 150um, canal b:75um c:100um showing in Fig ccolumns of chondroblasts(star) and deep two penetrating cartilage canals with chondrolysis at the ends of the canals (arrow):

Note the venule(arrow star) in canal aThe central arteriole in canal **B**(triangle), Note the sinusoidal capillary (double arrow) cartilage capillary(line)
Venule (double arrow)in canal **B**, sinusoidal capillary) double arrow in canal **B**)
The long diameter s of the long thin canal (A)is 15um,and the ballooned canal (B)long Diameter is 100um approximately as measured from the scale of the microphotograph, The transverse diameter of the sinusoisal capillary is 50um, the arterole central 12 um Masson trichromex200.

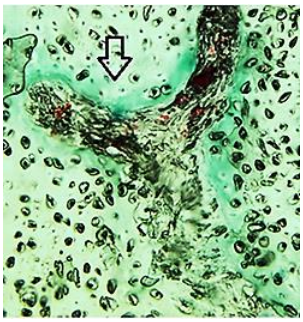


Fig 32:Photomicrograph of part of TS of part of a developing prenatal human fourth rib at the costochondral junctionCCJ of **7month** aged fetus:***(25-28weeks) CRL 24-27cm*** showing branched deep cartilage canal containing collagen (arrow)deeply stained. mesenchyme and small capillaries.
The matrix around the canal is homogenously less deeply stained than the collagen inside the canal and the cells around the canal are large and dispersed irregular with different shape and size.
Three stain affinities are presentl; of the collagen of the canal, intimately around the canal,and the matrix of the resting cartilage.Masson trichrome

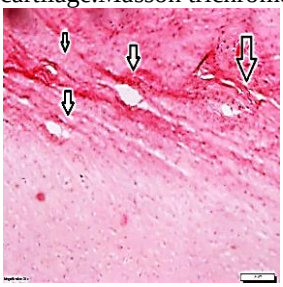


Fig 33:photomicrograph of part of LS of 4th prenatal developing human rib at the costochondral junction CCJ, of 7months old age fetus***:(25-28weeks) CRL 24-27cm***, showing parts of numerous extended penetrating deep empty cartilage canals with conical bolus ends(arrow).Note not straight course of some canals with obliterated parts (Big arrow), Note the bubble like matrix around the canal (long arrow), tissue around the canal, HEX200

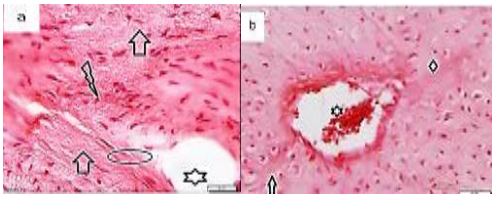


Fig34 photomicrograph of part of LS of part of 4th prenatal developing human rib at costochondral junction CCJ of seven month aged fetus***:(25-28weeks) CRL 24-27cm,*** showing in fig a part of resting zone containing extended deep empty cartilage canals with intact wall, and bullous conical end (star)
closed and obliterated sites(ovalare seen.
The cartilage cells around the length of the canal are sparse, loosely packed, irregularly arranged flat and small(zigzag arrow)
Bubble,flucuelent like appearance of areas of matrix along the cartilage canal (arrow) the longest diameter of the conical end of the cartilage canal is average 150um measured from the scale of the microphotograph. The diameter of the canal width is 15 um.
Fig b-showing part of end of deep cartilage canal intact wall present in the resting,contains one sinusoidal vessel occupying the whole diameter of the canal with blood cells (star), Note the stripe area of the matrix around the cartilage canal (diamond)
Note the cells around the canal are different in size, some are enlarged, dispersed,some are flat,loosely irregularly packed (arrow), HEX200

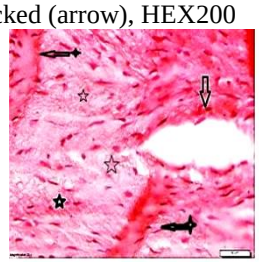


Fig35:Higher magnification of part of the previous photomicrograph of part of LS of part of 4th prenatal developing human rib juxta the costochondral junction CCJ, of seven month aged fetus***:(25-28weeks) CRL 24-27cm,*** showing part of bullous end of deep empty cartilage canal with intact wall present in the resting zone (arrow)
Note the stripe areas of the matrix around the cartilage canal (arrow star)
Note the cells around the canal are different in size, some flat,loosely irregularly packed (arrow), Note part of the matrix is deeply stained
Note the fluculent acellular area of matrix around the canal shows bubble like appearance (asterisks), HEX200

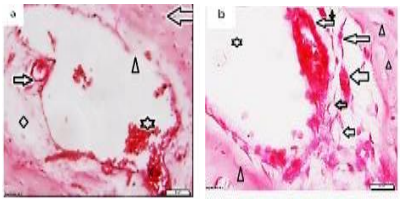


Fig 36:photomicrograph of part of LS of 4th prenatal developing human rib juxta the costochondral junction CCJ, of 7 months old age fetus***:(25-28weeks) CRL 24-27cm***, showing in fig a:part of vascular cartilage canal (arrow)containing sinusoidal capillary, with interrupted wall (head arrow)containing blood cells (star). Small capillary is noted (small arrow), The cartilage vascular canal contains also mesenchyme (diamond), HEx20

Fig b:showing, part of the lower blinded end of a complex cartilage canal, present in the resting zone containing: Fibroblast(long thin arrow)

Macrophage (thick arrow), vessels with one layer of endothelial cells (small arrow), Capillary with relatively thick wall,contains blood cells.(arrow star)

Sinusoidal capillary(star)with blood cells

The cartilage cells around the canal show chondrolysis:**(lacunae containing cells intimately associated with matrix, and presence of granular debris)**

(arrow head), HEX 200

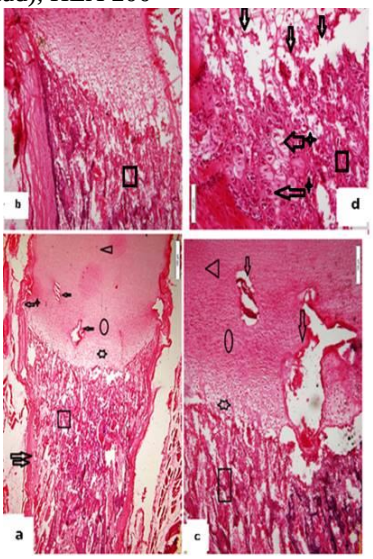


Fig 37s **a,b,c,d**:Photomicrograph of part of LS of part of a developing prenatal human 4th rib of at the costochondral junction of **9month** aged fetus ***33-36 weeks- CRL 31-34cm) and newborn infant (37-38 weeks-(CRL 35-36cm)*** showing in **fig a** part of the growth plate and invading vessel in the growth plate (oval), the long spongy irregular bone(box), and bone marrow between bone trabeculea. The part that surrounds the physis **" the ring of LaCroix**, " and it merges with the periosteum adjacent to the metaphysis and the perichondrium that surrounds the epiphysis. A subtle increase in diameter of the physis is called " the groove of Ranvier ". The ring **the ring of LaCroix** gives rise to a circular rind of " bone bark "— the subperiosteal bone collar (SPBC) resistant to injury

Grove of Ranveir(arrow) and the subperiostealbone collar demonstrated by ZONE extending from area of proliferative till the primary ossification center its length is 350um according the scale on the microphotograp

HEX100

Fig **b** shows branched vessel in the growth plate, HEX100

Fig c skows branched vessel in the growth plate extending to the sponge (arrow)

Fig: d- shows the perichondrium with blood vessels(star), groove of Ranvier and bone collar, bone bark or perichondrial ring of La Croix) (arrow)

The perichondrium consists of CT surrounded the cartilage. figs:a& c:HEX 40, Figs:,b,d:HEx100

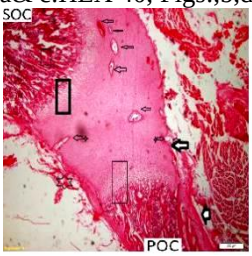


Fig 38photomicrograph of part of LS of part of the resting cartilage of the prenatal human developing 4th rib of **9 month *human full-term:(33-36 weeks) CRL 31-34cm) and newborn infant (37-38 weeks-(CRL 35-36cm) at the***costochondral junction CCJ, showing part of the primary ossification center, POC and secondary ossification center SOC

Note the similar MORPHOLOGICAL appearance of arrangement of cartilage cells forming the epiphyseal growth plate close at the CCJ(thin rectangular), and the arrangement of Hypertrophic cartilage arrangement forming a plate around he secondary ossification center SOC(thick rectangular): The zones forming the growth plate at the CCJ zones: reserve(germinative),proliferative.hypertrophic zones (asterisks)

The differences between POC&SOC:are the center is round and,thepresence of close cartilage canals(arrow) to the SOC, as the canals associate in the osteogenesis and incorporate in the SOC.

Note the Perichondrium (long white arrow is continuous with the periosteum(short white arrow)

Secondary ossification center cartilage canals in the resting around(arrow) SOC (box)

Superficial cartilage canals extending from the perichondruem.(arrowstar), HEX40

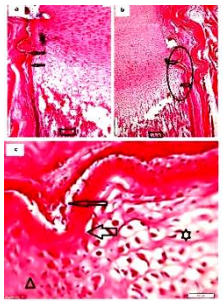


Fig39:Photomicrograph of part of LS of part of a developing prenatal human fourth 4th rib at the costochondral junction CCJ of 9month aged fetus 33-36 weeks- CRL 31-34cm) and newborn infant (37-38 weeks-(CRL 35-36cm) showing the growth plate
Figs a&b the periphysis, encircling the metaphysis and depicting the wedge-shaped groove of Ranvier (arrowstar)and the thin layer of intramembranous bone (bone collar, (arrow)bone bark or perichondrial ring of La CROIX
Fig- b The length of the subperiosteal bone collar SPBC–bone bark is (arrow)approximately 460um according to the scale on the photomicrograph in HE scalex100
Fig c magnification of part of fig b,showing Grove of Ranvier with cartilage cells newly formed (thin arrow) EXtenibg to form subperiosteal bone collar. SPBC. Note the thin layer of intramembranous bone (bone collar, Note the SPBC (thick arrow) is formed by intramembranous ossification, HEX100

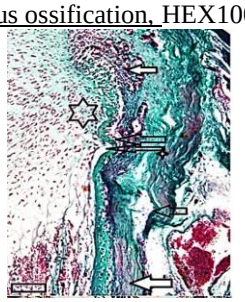


Fig 40:Photomicrograph of part of LS of part of a developing prenatal human fourth 4th rib at the costochondral junction of **9month** aged fetus showing parts of the ***zones of the epiphyseal cartilage growth plate: the proliferative and hypertrophic zones with the cartilage cells arranged in simple columns,and threads of matrix inbetween.***, Note the perichondrial groove of Ranvier(arrow star)
Note the subperosteial bone collae SPBC,and Bone bark and bone collar with rich blood supply in the perichondreum behind (white small arrow)
Note the perichondrial groove of Ranvier(arrowstar) and The ring **the ring of LaCroix** gives rise to a circular rind of " bone bark **intramembranou**s"— the subperiosteal bone collar (SPBC)(arrow).
Note the layers of the periosteum(white big arrow),with the rich blood supply. perichondreum is continuous with the periosteum(long white arrow
Note THE three different stain affinities of the perichondriuem,subperchondreum and the matrix of the hyaline cartilaGE
Note the spur contour arrangement: (star), Note the sponge Bone trabeculea(box),, Masson trihromex200

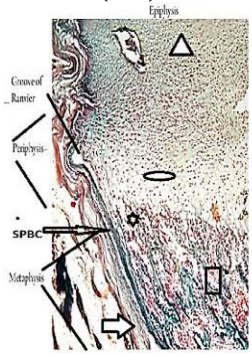


Fig 41 photomicrograph of part of ls of developing prenatal human 4th rib at the costochondral junction CCJ of 9 month fetus showing
the groove of Ranvier ". The appearance of the groove varies with age progress.
The SPBC projects beyond the metaphysis, extending along the physeal margin toward the epiphysis and ends near the junction of reserve and proliferative zones of the growth plate, Note that the Ranvier and LaCroix
zones are a single structure depicting the thin layer of intramembranous bone at the periphery
of the growth plate and metaphysis. The fibrous zones and Ranvier and LaCroix are richly supplied with blood from several perichondrial
Note the Sponge irregular bone of the rib(box), Note that the periosteum continues with the perichondrium, Masson trichromex100

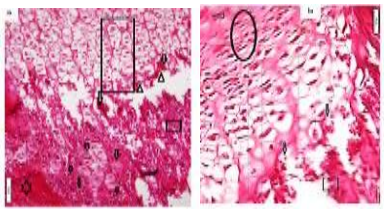


FIG 42:Photomicrograph of part of LS of part of a developing prenatal human 4th rib jaxta the costochondral junction CCJ **of 9months** aged fetus showing in fig -a part of the hypertrophic zone of the cartilage growth plate, with Hypertrophic cartilage cells, arranged in simple one cell columns (rectangular) with mature terminal3-4 cells bursting cells in the sponge (head arrows), forming provisional zone of calcification PZC
Note the chondroclasts giant multinucleate cells eroding matrix (arrow) are seen in the transitional zone cartilage –bone -beginning of the sponge bone.

New bone cells (small arrow)on the new matrix (arrow star) bone trabeculea,needed for remolding and new added cells and bone marrow (box), forming primary ossification center POC, HEX100

Fig b part of the growth with a large amount of extracellular matrix(triangle) the stem reserve zone (triangle) is the proliferative zone (oval), the cells begin to flatten, undergo cell division and become oriented into columns(oval). In the last layer, the hypertrophic zone)the enlarged cells arranged in regular simple columns (rectangular), mean cell count number in each column =9cells,cell division ceases and the chondrocytes begin to terminally differentiate and enlarge, ballooned and are surrounded by reduced amounts of matrix.

the matured late ballooned ruptured cells (arrow).

burst in the irregular sponge bone(box) of the growing rib.forming the provisional zone of calcification ZPC and includes the terminal 3 – 5 (arrow)hypertrophic chondrocytes in which the surrounding hyaline matrix is calcified. Some authors consider the histologic ZPC a fourth zone (instead of a subdivision within the zone of hypertrophy) and refer to it as " the zone of mineralization " or " the zone of calcifying cartilage, HEX1000.

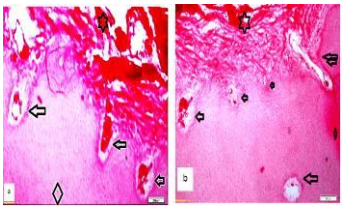


Fig 43:photomicrograph of part of TS of part of the resting cartilage of the prenatal human developing 4th rib of nine 9 month ***human full-term:(33-36 weeks) CRL 31-34cm) and newborn infant (37-38 weeks-(CRL 35-36cm)*** juxta the costochondral junctiom CCJ showing 3superficial VASCULAR canals (arrow)extending from the rich vascular PLEXUS OF thick perichondrium (star) extending to the resting cartilage (diamond).

The canals show different length, and stages of formation, from bud till separation from the rich vascular perichondrium(star_

Note the deep stain of the matrix around the canals.

The canals originate from the perichondrium and are composed of loose connective tissue with a central arteriole terminating HEX40

Fig b-One elongated extension from the perichondriuem is seen (double arrow)

Note the Deep canal(long arrow) present in the resting cartilage(diamond)

Note the deep stain of the cartilage close to the canals.(diamond)

stages of formation, from bud till separation from the perichondrium. Note the deep cartilage canals,(big arrow)

Occasionally, at the origin of a cartilage canal, a perivascular capillary network was observed which consisted of a dense capillary network surrounding both the arteriole and venule, HEX40

Figs44a and b:photomicrograph of part of TS of part of the resting cartilage of the prenatal human developing 4th rib of nine 9 month ***human full-term:(33-36 weeks) CRL 31-34cm) and newborn infant (37-38 weeks-(CRL 35-36cm)*** juxta the costochondral junctiom CCJ showing**,** two successive superficial canals, each canal contains one empty vessel, occupying the whole cartilage canal (arrow)extending through mesenchyme tissue from the thick layers of the perichondrium (star).

Note the other suspended superficial cartilage canals canal(arrow star) extends also from near area of the perichondrium in the resting cartilage.(diamond)

Note the deep matrix stain around and between the canals.

Deep vascular canal is seen (short arrow)

Fig b-: higher magnification of the prevous photomicrograph of part of TS of part of the resting part of the previous,showing cartilage Canal(thin arrow)) extending from the thick perichondrium (star)stem mesenchyme CT tissue (thick arrow).to the resting cartilage (diamond)

the vessel wall was interrupted. HEX40

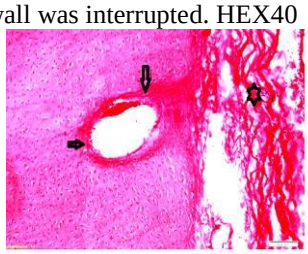
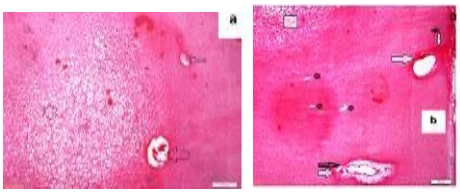


Fig45:Photomicrograph of part of TS of part of the resting cartilage of the prenatal human developing 4th rib of **9 month *human full-term:(33-36 weeks) CRL 31-34cm) and newborn infant (37-38 weeks-(CRL 35-36cm)*** rib juxta the costochondral junction showing part of the perichondrium layers with suspended superficial cartilage canals(, containing one vessel(arrow). Extending by a stem from the perichondrium) into the resting cartilage of the epiphysis contains One large empty vessel occupies the whole cartilage canal. (arrow.) **The unique vascular anatomy of the canal**

Note some cartilage cells around the canal are sparse,large, within deeply stained matrix
the transverse diameter of the canal is 200um transverse diameter of the vessel inside the canal is 170um approximately,measured from the scale on the microphotograph n ote the and long diameter of the canal is 280um and long diameter of the vessel is 260um approximately measured from the scale on the microphotograph, note the vessel wall was interrupted. HEX40



Figs 46 a and b:Photomicrograph of part of TS of part of the prenatal human developing 4th rib juxta the costochondral junction of **9 month *human full-term:(33-36 weeks) CRL 31-34cm) and newborn infant (37-38 weeks-(CRL 35-36cm):fig a*** showing part of the epiphysis contains collection of enlarged cartilage cells (star), forming initial Secondary ossification center SOC.
Note the two cartilage canals(arrow) around the initial secondary center SOC start to incorporate with the SOC center (big arrow)
Note the deep stain of matrix around the cartilage canals., HEX200
Fig b showing part of collected cartilage cells (box)in the resting cartilage forming initial secondary ossification center (box)
Note the superficial cartilage canal (big arrow) extending from the perichondrium by a stalk (arrow star).The canal is close to collection of cartilage cells forming initial secondary ossification center SOC (box)
Note the deeply stained matrix around and between the canals.
Note the large deep cartilage canal (double arrow) the long diameter of the canal is 2100umx and the transverse diameter is, 400um
Many small canals are seen (small arrow), HEx40

Fig47:photomicrograph of part of TS of part of the of the prenatal human developing 4th rib of *human full-term:(33-36 weeks) CRL 31-34cm) and newborn infant (37-38 weeks-(CRL 35-36cm*) juxta the costochondral junctiom CCJ
Showing in fig a: part of secondary ossification center SOC in the resting cartilage and part of the vascular perichondrium (star), containing vascular canal (big arrow), extends in the SOC center, Cartilage canals around the center(small arrows)
and blood vessel inside the SOC center are seen
The diameter of the secodary ossification center are 2000x2400umum approximately measured from the scale of the microphotograph, HE X
Fig b magnification of part of previous microphotograph showing a vascular canal containing sinusoidal capillary (arrow star), central arteriole thin (arrow) arteriole, capillary (arrow head)and CT(diamond)
Macrophage (line) and Fibroblast(zigzag)
The long diameter of the canal is 940um and transverse 560um measured from the scale on the microphotograph
The long diameter of the sinusoidal capillary is 400um, and the transverse is 370um.The transverse diameter of the central arteriole is approximately 100um.
Note the deep stain of the matrix around the canal. The top of the canal (white arrow) shows chondrolysis: lacunae containing cells intimately associated with matrix, and presence of granular debris), HEX40

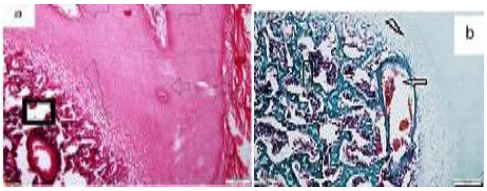


Fig 48:Photomicrograph of part of TS of part of the resting cartilage of the prenatal human developing 4th rib of **9 month *human full-term:(33-36 weeks) CRL 31-34cm) and newborn infant (37-38 weeks-(CRL 35-36cm)*** showing Part of a secondary Ossification SOC(box),surrounded by hypertrophic chondrocytes around the center (star)
Note the arrangement of cartilage cells in columns in a similar morphologic way as the cartilage growth plate at the CCJ close to the primary ossification center POC. Note the cartilage canals around the secondary ossification center SOC
HE X:40-scale200

Fig b:showing Large vessel (arrow) in the secondary ossification center SOC (box).
Note the large chondrocytes(zigag) around the SOC (box) serving as growth plate simulating the growth plate at the CCJ at metaphase of the primary ossification center POC.
Note the different stain o the matrix of the SOCand the stain of the resting cartilage, Masson trichrome x40-scale200

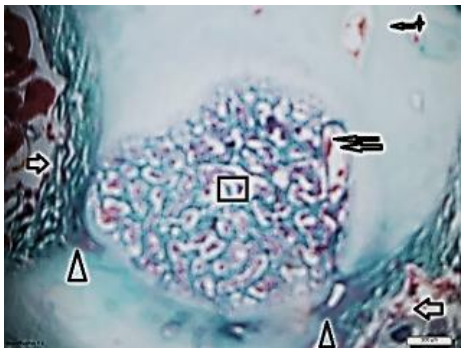


Fig 49:Photomicrograph of part of TS of part of the resting cartilage of the prenatal human developing 4th rib of **9 month *human,showing*** the vascular perichondrium (arrow)is continuous (head arrow) with the secondary ossification center SOC(box)
Note the stain affinity of the perichondrium is strong, while the stain of the hyaline cartilage matrix is weak and strong in areas,and the stain of the matrix of the secondary ossification centers is moderate, Note the blood vessel in the secondary ossification center (double arrow)
Note the cartilage canal (arrow star), Masson trichrome x 40 scale200um

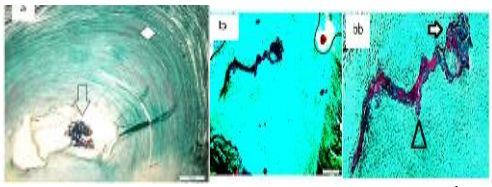


Fig 50 Photomicrograph of part of LS of 4th rib of prenatal developing human **9 month *human full-term:(33-36 weeks) CRL 31-34cm) and newborn infant (37-38 weeks-(CRL 35-36cm)*** showing **in fig a**in fig a- part of radial section and in the center is the glomerulus end of deep cartilage canals (arrow)
Fig b-: the entire cartilage canal is bathed in plasma and that metabolic exchange is facilitated throughout the entire length of the cartilage canal.. The cartilage canal is **uniformly** segmentally distributed,and alternative green and red segments are noted in the whole length of the canal(arrow head) The function of the capillary glomerulus **is to decrease the velocity of blood flow thereby facilitating the percolation of plasma into the connective tissue of the cartilage canal**.
Fig bb- the same cartilage canal,with the scale to illustrate the length of the canal is 500um
The transverse diameter of the glomerulous is 80 um the Long diameter of the glomerulus is 77 um
The measurements are from the photomicrograph
long cartilage canal with terminal **complex Glomerulous(double arrow)** The **variations are according to** the degree of capillary anastomoses among the arteriole, venule, and capillaries of the glomerulus, at the end of cartilage canal
The simple glomeruli are most frequently observed on the ends of short canals **or on the end of the first branches off a long canal**. Other glomeruli are formed when the arteriole divided into more than two capillary, Masson trichrome x

Fig51 Photomicrograph of part of TS of developing prenatal human 4th rib juxta the costochondral junction of 9month fetus showing part of the layers of the perichondrium with deep stain and the flat cartilage cells beneath it(star)
Note the superficial cartilage canal s(big arrow) extending from the perichondrium(star.)
Note the deep cartilage canals completely surrounded by matrix in the resting (arrow)with different shapes and containing course arterioles, venules and sinusoidal capillaries that are important for nutrition, and also loose perivascular Connective tissue which serves as a source of stem cells serve as the source for bone-synthesizing osteoblasts on the calcified cartilage.
Note the strong,moderate, and weak.different stain affinities of the perichondruem,subperichondruemnd,a matrix respectively.
Note the deep matrix stain of areas of the resting cartilage around and close the canals
Note the perichondrium consists of CT with blood vesselssurrounded the cartilage.The cartilage cells underneath the perichondrium are flat,parallal to the surface mostly single. Masson trichrome x100

Fig 52: Photomicrograph of part of TS of developing prenatal human 4th rib juxta the costochondral junction of **9month fetus** showing cartilage of the prenatal human developing 4th rib of **9 month *human full-term:(33-36 weeks) CRL 31-34cm) and newborn***

infant (37-38 weeks-(CRL 35-36cm) showing deep cartilage canal surrounded by deeply stained matrix (zigzag)with simple two venules(arrow) and capillaries
Two sinusoidal capillary(head arrow) one endothelial layer, their transverse diameters are 90&110um measured from the scale pn the microphotograph
Note the cartilage cells around the canal are large,dispersed and few(.
Note the matrix around the canal, Masson trichrome x100-scale 100um
Fig b:showing part of the resting cartilage tissue(diamond) contains cartilage canal full of capillaries of different size(arrow star),and mesenchyme(arrow)
The transverse diameter of the canal is 100um,and the long diameter is 300um approximately according to the scale on the microphotograph. The transverse diameter of the capillaries inside the cartilage canal ranged between 50um,40um,amd 10 um.
Note the different stain affinity of the matrix around the canal, Masson trichromex100-scale 100um

Fig53:Photomicrograph of part of TS of part of a developing prenatal human 4th rib juxta the costochondral junction of 9month fetus showing (n=4) vascular cartilage canals,present in the resting cartilage with different in diameter, shapes, size.Cartilage canals contain course arterioles, venules and sinusoidal capillaries that are important for nutrition, Cartilage canals contain also perivascular loose connective tissue which is a source of stem cells.
Note the deep different stain affinity of the matrix close to the cartilage canals (arrows)
The arterioles divided to two or more capillaries
Note the regularly arranged cartilage cells around the Cartilage canals,Some large irregular cartilage cells loosely arranged are seen between the close cartilage canals)double arrow), The diameter of the canals range as measured from the scale of the microphotographs: 30-40-70- -130 um

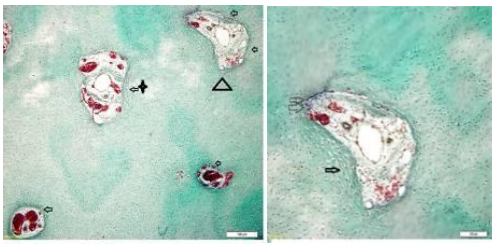


Fig54 Photomicrograph of part of TS of developing prenatal human 4th rib jaxta the costochondral junction CCJ of 9month fetus showing vascular cartilage canals in the resting cartilage having different size, shapes and contents.
Note that no particular anatomical relation is present.
The vascular cartilage canals contain two or three distinct capillary–sinusoidal vessels interspersed with connective tissue and many small discrete vessels, arterioles, capillaries, venules, sinusoidal capillaries that are important for nutrition, besides, connective tissue which serves as a source of stem cells.
Note there are three canals close to each other seen as one cartilage unit canal, giving triangle appearance.(arrow star) Note the some branching capillaries inside the canals.
Enlarged cartilage cells around the canals are noted.
Areas of chondrolysis are noted at the tips and between the close canals of the canals (arrow star)
the wall of the canal is not intact.
Note the relation of the canals to each other and the concentration of the canals in relation to the resting cartilage.
Note the deep stain of matrix between near the canals indicating effect of the canal on the metabolism of the individual cartilage cells and the influential effects of cartilage canals on the collagen of the matrix.
Note the regularly arranged cartilage cells around the Cartilage canals, Some large irregular cartilage cells loosely arranged are seen between the close cartilage canals (double arrow)
Fig b is magnification of part of fig a,showing one vascular cartilage canal triangle in shape surrounded by regularly arranged enlarged different sized cartilage cells and flat cells (arrow) The smallest and largest transverse diameters of the canal are approximately 60 &260 um and the longest diameter is 380um. according the scale on the microphotograph: The diameter of the largest sinusoidal capillary is -95um.
chondrolysis (double arrow)is noted at the end of the canal (lacunae containing cells intimately associated with matrix, and presence of granular, Masson trichrome x100.

Fig55: Photomicrograph of part of TS of part of developing prenatal human 4th rib juxta the costochondral junction CCJ in the resting cartilage of **9month fetus** showing three complex cartilage canals close to each other seen as one cartilage unit canal, giving triangle appearance OF one cartilage canal triangle in shape surrounded by different sized cartilage cells and flat cells (arrow) and large amount of matrix around the canals with deeper stain at the periphery of the canal (arrows)

Note cartilage canal contains numerous course arterioles, venules and sinusoidal capillaries thatare important for nutrition, and also connective tissue which serves as a source of stem and T.The transverse The smallest and largest transverse diameters of the canal are approximately 2**00um&350** um and the longest diameter is 400 um. according the scale on the microphotograph: The diameter of the largest sinusoidal capillary is -95um.

chondrolysis (arrow)is noted at the end of the canal (lacunae containing cells intimately associated with matrix, and presence of granular debris)

The **variations of cartilage canals,are according to** the degree of capillary anastomoses among the arteriole, venule, and capillaries of the glomerulus, at the end of cartilage canal

The simple glomeruli are most frequently observed on the ends of short canals **or on the end of the first branches off a long canal**. Other glomeruli are formed when the arteriole divided into more than two capillary, Masson trichrome x100

Fig 56 Photomicrograph of part of TS of part of a developing prenatal human 4th rib at the costochondral junction of 9month fetus showing two cartilage canals and the cartilage cells at the ends of the canals show chondrolysis (short arrows).

Note the matrix around the canals is deeply stained. The arterioles divided to two or more capillaries

The canals contain arterioles, capillaries, venules, sinusoidal capilareis.CT

The cartilage cells around the canals are enlarged dispersed loosely packed in irregular manner.

Some large irregular cells are seen between the close cartilage canals (double arrow)

chondrolysis (loss of metachromasia, lacunae containing cells intimately associated with matrix, and presence of granular debris) similar to

Similar to CHAPPARD, D., ALEXANDRE, C. & RIFFAT, G. (1986). Uncalcified cartilage resorption in human fetal cartilage canals. Tissue and Cell 18, 701-707.

The variations of cartilage canals,are according to the degree of capillary anastomoses among the arteriole, venule, and capillaries of the glomerulus, at the end of cartilage canal

The simple glomeruli are most frequently observed on the ends of short canals or on the end of the first branches off a long canal. Other glomeruli are formed when the arteriole divided into more than two capillary.

Spotted stain of matrix was seen, Masson trichrome x100

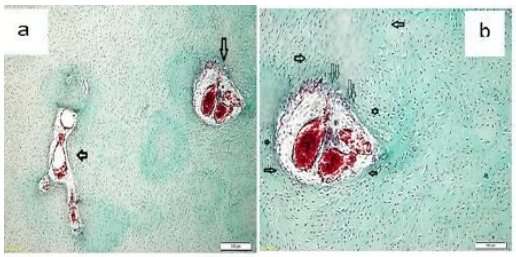
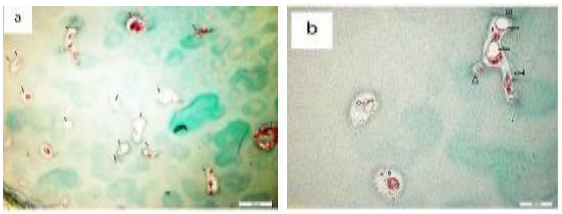


Fig 57 Photomicrograph of part of TS of art of a developing prenatal human 4th rib IN THE EPIPHYSIS JXTA THE the costochondral junction

ccj of 9month fetus showingin fig a showing TWO VASCULAR cartilage canals with different shapes and size(arrows).
Note the deep stain of matrix around the canals.
Note the chondrolysis of cells around the canals. chondrolysis (long arrow)is noted at the end of the canal (loss of metachromasia, lacunae containing cells intimately associated with matrix, and presence of granular debris)
One canal is elongated(short arrow_ and has extended extention, and satellite canals small around
Fig b shows - one vascular cartilage canal surrounded by different sized cartilage cells and flat cells (arrow)
Noise the large amount of matrix deep in stain around the canal (star).
Note cartilage canal contains numerous course arterioles, venules and sinusoidal capillaries that
are important for nutrition, and also connective tissue which serves as a source of stem cells.ca pillary sinusoids with different shapes and size embedded in CT.
There are three distinct capillary–sinusoidal vessels interspersed with connective tissue and many small discrete vessels
The transverse diameter of the largest 3 vessels. according the scale on the microphotograph: are100-90-70um.
chondrolysis (double arrow)is noted (loss of metachromasia, lacunae containing cells intimately associated with matrix, and presence of granular debris)
The variations of cartilage canals,are according to the degree of capillary anastomoses among the arteriole, venule, and capillaries of the glomerulus, at the end of cartilage canal
The simple glomeruli are most frequently observed on the ends of short canals or on the end of the first branches off a long canal. Other glomeruli are formed when the arteriole divided into more than two capillary, M asson trichrome x100.



Fg 58:Photomicrograph of part of TS of part of a developing prenatal human 4th rib jaxta the costochondral junction CCJ of **9month fetus** showing THE DENERAL APPEARANCE OF THE EXCESS cartilage canals different shapes, size and contain CT (diamond)
Present within the differtly spotted stained matrix at the resting cartilage with Capillary (arrow).

Note one canal has extension and shows chondrolysis (double arrow), Fig b is magnification of part of fig 58a. Masson trichrome x100

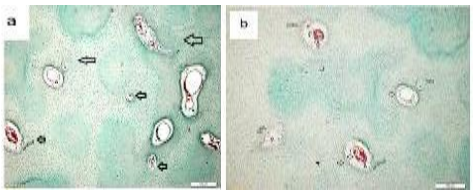


Fig 59:Photomicrograph of part of TS of part o a developing prenatal human 4th rib at the costochondral junction CC J of 9month aged **fetus** showing numerous cartilage canals and close small canals seemed as satellite may be detached from the big canals,(arrow).
Note the deep stain Of matrix around and between the canals.
Note the cartilage canals with different shapes, size and content basically arterioles,capillaries, venules andCT,
Some canals seem having tail like,seem as ghost. (big arrow)
The unique vascular anatomy of the canals, a single vessel rarely filled the entire diameter of the canal is noted. Usually two or three distinct capillary–sinusoidal vessels interspersed with connective tissue and many small discrete vessels wide similar to ***Jaramillo et al.,2004***
Masson trichrome x100
Fig b showing five cartilage canals(arrow)
Note that some canals contain central capillary (head arrow)and CT(diamond),some canals contain only CT (double arrow)
Masson trichromex100-scale 100um

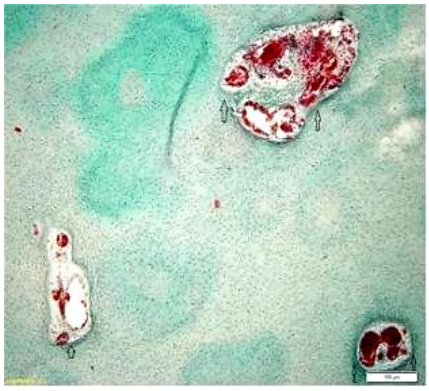


Fig 60: Photomicrograph of part of TS of part of a developing prenatal human 4th rib jaxta the costochondral junction of 9month fetus showing cartilage canals in the resting cartilage with different shapes, size and contents.
Note the deep stain of the matrix around and between the vascular cartilage canals

Note the chondrolysis (arrow)is noted at the end of the canals (lacunae containing cells intimately associated with matrix, and presence of granular debris) are noted
Notice in each cartilage canal,there are two or three distinct capillary–sinusoidal vessels interspersed with connective tissue and many small discrete vessels, Masson trichrome x100

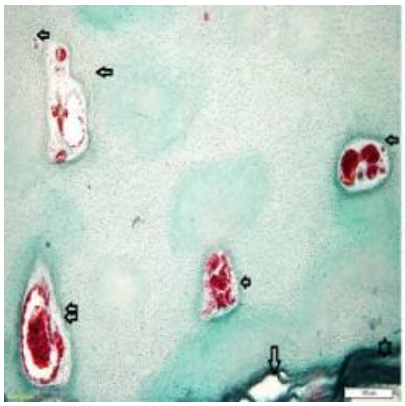


Fig 61 Photomicrograph of part of TS of a developing prenatal human 4th rib juxta the costochondral junctionCCJ of 9 months old age fetus showing five cartilage canals with different shapes,size and Their transverse diameter: range: 10um,80um.100om,120um (arrows) according to the scale on the photo micrographic slides
Note that the cartilage canals contain distinct numerous course arterioles, venules and sinusoidal capillaries that embedded in loose connective tissue which serves as a source of stem cells
The cartilage canals are important for nutrition, and metabolism of individual cartilage cells. may serve as a source of cartilage stem cells for growth of the rib epiphysis
Note the deep stain in the matrix around the cartilage canals that may indicate the effects of the canals on the individual cartilage cells and collagen of matrix.
Note that one cartilage canal contains large one sinusoid capillary occupying the whole canal space (double arrow).
Note the deep stain of the perichondrium(star)., Masson trichrome x100

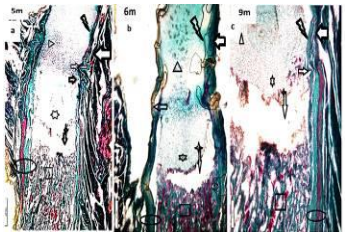


Fig 62: a,b,c Photomicrograph of part of LS of developing prenatal human 4th rib at the costochondral junction of 5,6,9month fetus showing deep stain of the rib perchondrium(white thick arrow) less stain affinity in the subperichondrium (zigzag),and faint stain in the hyaline cartilage
Note the cartilage growth plate IS SHORTED WITH AGE PROGRESS: the hypertrophic (arrowstar), proliferative (star).germinative (traingle).
Note the periosteum (oval)with blood vessels; The periosteam is fibrous sheath surrounded the outer surface of the prenatal developing human 4th rib. It composed of two layers: an outer layer made of dense white fibrous tissue consisted of blood vessels, and an inner layer consisted of loose tissue containing osteoblasts. The inner layer was the osteogenic or osteoblastic layer which formed new cells
Note the irregular sponge bone (box)and trabeculea are loose in 5months fetus, and dense in 6months fetus and less dense in 9months fetus with spaces
Masson trichrome x200

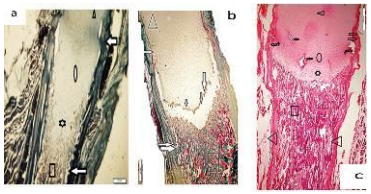
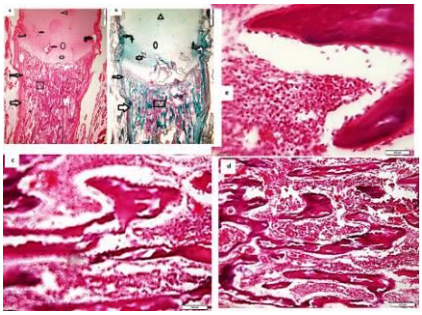


Fig 63 a,b,c: Photomicrograph of part of LS of part of the 4th prenatal developing human rib at the costochondral junction CCJ of 4month aged fetus, showing in fig a the long epiphyseal growth plate.and the short trabecular sponge bone.
The growth plate is formed of the germinative (triangle), proliferative(oval),hypertrophic (star) zones
Note the irregular cancellous sponge of metaphesyeal bone(box)formed of trabeculea
Note the perichondrium(short arrow) is continuous with the periosteam(long arrow)
Masson trichrome x40
Fig b-Photomicrograph of part of LS of part of a developing prenatal human 4th rib at the costochondral junction CCJ OF 6-months old fetus:(21 -24weeks) CRL 20-23cm, showing part of the cartilage growth plate: the germinative zone (triangle)
the hypertrophic zone with final cells bursting in bursting in the sponge bone(box), and the provisional zone of calcification PZC (arrows)
Masson trichrome x40
Fig c: Photomicrograph of part of LS of part of a developing prenatal human fourth 4th rib at the costochondral junction CCJ of 9month aged fetus *human full-term:(33-36 weeks) CRL 31-34cm) and newborn infant (37-38 weeks-(CRL 35-36cm)* showing the Short zones of the cartilage growth plate,as the cartilage cells decrease in number after their burst in the sponge causing elongation of the rib by endochondral ossification.
The epiphyseal growth plate is thin,while the sponge bone grows and elongated.HEX



Figs 64:a,b,c,d Fig photomicrograph of part of LS of part of the prenatal human developing 4th rib of 9 month *human full-term:(33-36 weeks) CRL 31-34cm) and newborn infant (37-38 weeks-(CRL 35-36cm)* showing in fig a parts of the growth plate,the primary ossification center and sponge (box)
figs c,d,e magnification of parts of the sponge of fig a, Fib b showing osteoblasts ON THE SUFAXE OF matrix and osteoclasts inside the matrix of the sponge. The sponge bone is formed of irregular trabeculae and appears as anastomosing with each other.The trabeculae are covered with the osteoblasts which are bone forming cells, small branched cells. They formed continues layer covering the trabecular of cancellous bone.they are POLARISED on the surface of the trabeculae,meanwhile osteoclasts are between the matrix,surrounded by it.osteoclasts can not divide.osteoblsts are more basophilic(*bule*)cytoplasm due to excess RNA in the cytoplasm Fibs a,c,d,e:HEx100
Fig b-masson trichromex100.

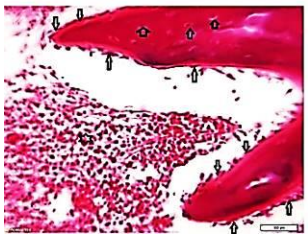


Fig 65:Photomicrograph of part of TS of part of a developing prenatal human fourth 4th rib at the costochondral junction CCJof 9month aged fetus showing The irregular spongne cancellous bone of the is formed of irregular trabeculae appeared as anastomosing with each other.They are covered with the osteoblasts (thin long arrows)which are bone forming cells, small branched cells. They form continues layer covering the trabecular of cancellous bone. They are on the surface of the trabeculea, mean while osteoclasts are between the matrix(short thick arrow), surrounded by it.Osteoclasts can not divide.osteoblasts are more basophilic(blue)cytoplasm due to excess RNA in the cytoplasm. the sponge at CCJ formed the primary ossification center POC of The matrix (star) blood dells (asterisks), HEX100

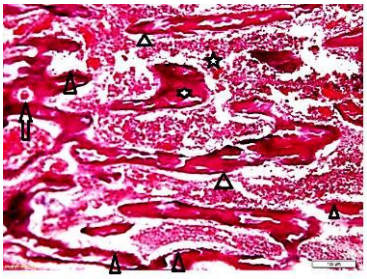


FIG 66: photomicrograph of part of LS of part of the developing prenatal developing full term:(33-36 weeks) CRL 31-34cm) and newborn infant (37-38 weeks) CRL 35-36cm) human rib close to the costochondral junction showing The sponge bone, which represent part of the Primary ossification center POC, is formed of irregular trabeculae appeared anastomosing.The trabeculae are covered with the osteoblasts which are bone forming cells, small branched cells.They formed continues layer covering the trabecular of matrix of cancelous bone (arrowhead). they found on the surface of the trabeculae, meanwhile osteoclasts are between the matrix surrounded by it.osteoclasts could not divide.osteoblsts are more basophilic(bule)cytoplasm due to excess RNA in the cytoplasm a trapped osteocyte (arrow) is noted embedded in the bone matrix (star), Blood cells (asterixks), HEx100

Fig 67 A: Photograph of Prenatal developing human right fourth 4th typical rib showing the morphology and angle. of the ages: 4,5,6,7 months, and full term superior view of (surface-anterior) and (inferior posterior)
Note that each rib has anterior and posterior end and body, The posterior end has a tubercle and articular facet for the body of vertebra) which developed at full term (double arrow, Note that the Costal groove is just clear at full term., Note the increase in size and length of the ribs with age progress,
Note the appearance of tubercle at posterior end of the rib at the age of 6 months(arrow) aged fetus.

Fig 68:B. (a&b) Photograph anterior view of the Prenatal developing human rib Second Rib(a):,and fourth(b) showing the morphology and angle. Of the ages: 4,5,6,7 months, and full term

The arrows point to the vertebral ends of the ribs.

Note that the lower border of the developing prenatal human fourth rib is sharper compared to the upper border. The sharpness increases with age progress.

C– Photograph of adult second and fourth ribs,showing the sternal end (triangle).and the vertebral end WHICH consists of head(arrow), tubercle(star), neck(double arrow), (and the rib angle(arrow star).

Note the twist in the 4th rib, and no twist in the second rib,

lower border is sharper than the upper border in the fourth adult rib. The sub costal grove is clear.

Discussion

Growth plate -Primary ossification center POC-secondary ossification center SOC

In the present work, histological examination of serial LS&TSsections of parts of prenatal developing human 4th rib at the costochondral junction CCJof 4,5,6,7, and 9, months aged fetuses, showed that, the transitional complex area cartilage-to bone was formed of columns of hypertrophic cartilage cells present between matrix, standing of the sponge bone. There were chondroclasts eroding the matrix, polarized basophilic osteoblasts on the surface of the newly formed matrix, and blood cells.

The growth plate was formed of layersranged in basically three zones;the germinate zone with small cartilage cells and large amount of matrix,the proliferative zone, started with flat cells,and dividing cells arranged in columns, and the hypertrophic zone, consisted of enormously enlarged non dividing cartilage cells having wide perinuclear space, continously increased in size towards the sponge,the terminal hypertrophic cells of the final 3-4 rows bursting in the sponge. The presence of provisional zone of calcification PZ C as separate zone from the hypertrophic zone was well defined at the age 5months and increased in surface area.with age progress.

Some workers claimed the death of the hypertrophic terminal cells by apoptosis, preprogrammed death, or autophagy and transformation of the terminal hypertrophic cells into osteoblast that shared the sponge bone (Blumer2021).

In the present work, histological examination of the prenatal developing human 4th rib at 4month aged fetus,showed that the epiphyseal growth plate area was long,and the sponge had small area, meanwhile at the age of full term the growth plate was short thin that continued to decrease in length and the sponge had large area,.That was due to loss of cartilage cells bursting in the sponge,and formation of new added bone by endochondral ossification, and by appositional growth.That agreed with EL Rakawy1971,who mentioned appositional growth occurred only of long bone by surface phenomenon.

That agreed with Quraan, suret el baqara (the cow) 259 (ننشزها سورة البقرة)

The replacement of the non vascular cartilage tissue to the living vascular bone was mentioned in Quraan: another creations suret el moemenoon 14 سورة المؤمنون خلقا آخر14. the explanation of wards of Asfahani,mentioned also the change and replacement of creation to another creations b(estehala)

The results of the present work agreed with (Roach et al., 2003; Shapiro et al., 2005).Blumer 2021.who pointed the final stage of hypertrophic chondrocyte differentiation, and death the and ultimate fate, was either by the induction of programmed cell death (apoptosis) or self-destruction (autophagy)

Blumer 2021 mentioned that, the epiphyseal growth plate function was regulated by molecules such as the nuclear receptor Retinoid X Receptor (RXR) which had important roles in development. A prolonged RXR signalling in rats, lead to a reduced cell proliferation and shortened long bones compared with the controls (Dupuis et al., 2019; Lui et al., 2014).

The results of the present work agreed with BluHmer 2021 who pronounced the epiphyseal growth plate comprised three layers with different structural organization: in the resting zone the chondrocytes were irregularly scattered, whereas in the proliferative and hypertrophic zone they were arranged in columns, parallel to the bone long axis. The longitudinal growth was the fast proliferation of chondrocytes associated with large secretion of extracellular matrix, and, hypertrophy (enlargement) of the cells. The growth plate function was regulated by various molecules and, also by the nuclear receptor Retinoid X Receptor (RXR) which had important roles in development. A prolonged RXR signalling in rats, lead to a reduced cell proliferation and shortened long bones compared with the controls (Dupuis et al., 2019; Lui et al., 2014).

With age, the growth plate thinned and was finally replaced by bone, achieved by a continuous elimination of late hypertrophic chondrocytes at the cartilage-to-bone transition zone.

In the present work, histological examination of the prenatal developing human 4th rib at **4month aged fetus**, showed that the late hypertrophic chondrocytes at the edge of secondary ossification centres SOC and the cell wall lining of the cartilage canals that

incorporated in the SOC, had basophilic osteogenic like cells.That meant that some cartilage cells coiuld be treansformed to bone like cells.

However, Blumer2021 mentioned that it was still the predominant view that hypertrophy was the final stage of chondrocyte differentiation, and death their ultimate fate, either by the induction of programmed cell death(apoptosis) or self-destruction (autophagy) (Roach et al., 2003; Shapiro et al., 2005).. In the hypertrophic cartilage programmed cell death could occur in response to mineralization besides an insufficient supply with nutrients and oxygen. That also agreed ith El Rakawwy 1971 Blumer 2021 mentined that,experimental evidence for apoptotic cells was shown in several studies using different techniques such as caspase3 immunohistochemistry, TUNEL assay and transmission electron microscopy (Allerstorfer et al., 2010; Farnum and Wilsman, 1989; Erenpreisa and Roach, 1998). Autophagy acted as a cell protective mechanism, prevented apoptosis and occurred in response to an enhanced tissue stress (Park et al., 2015). The sudden increased of oxygen levels in the late hypertrophic cartilage due to the ingrowth of blood vessels could trigger that process in the chondrocytes. 14 خلقا آخرسورة المؤمنونUltrastructural findings revealed some morphological characteristics of apoptotic cells but also vacuoles with a double membrane indicating the presence of autophagolysosomes. In the process of self-digestion, energy was generated serving to help the cells complete their lifecycle (Park et al., 2015; Shapiro et al., 2005).In opposition to the concept of chondrocytes' death, several authors claimed that late hypertrophic chondrocytes could survive and transforne to share in the sponge trabeculaea. Clear evidence was lacking,although some data were in accordance with that mechanism, Roach, 1997; Roach and Clarke, 1999; Shapiroet al., 2005). Bulmer 2021 explained that recent studies using transgenic mouse models and cell-specific markers made it possible to trace labelled chondrocytes in developmental time, shedding more light upon their fate. The use of immunohistochemical and in situ hybridization experiments had unambiguously indicated that a substantial amount of osteoblasts derived from hypertrophic chondrocytes(chondrocyte-derived osteoblasts) and directly contributed to trabecular bone formation in foetal as well as early postnatal stages.Once committed to the osteogenic lineage, they turned into osteocytes, contributing to the adult bone (Park et al., 2015; Yang et al., 2014; Zhou et al., 2014). In the present work,the replacement of cartilage to bone at the complex transitional area cartilage –to bone at the CCJ and the presence of newly formed bone cells in **the primary ossification center** POC as well as the secondary ossification center SOC might be what mentioned in Quraan SURET ELkeyama38 38 (القيامة:ثم كان علقة **فخلق فسوي**sawa)

The results of the present work agreed with Quran SURET ELMoomenoon14 **سورة المؤمنون 14 خلقا آخر** , ذكر في مفردات الفاظ القران للأصفهاني في معني الخلق الخلق بالاستحالة والتحول – كما تحول بالاستحالةa into osteoblasts.

The results of the present work agreed with Blumer 2021who pointed that in the development of the POC, there was clear evidence that the pool of osteoblasts originated from two distinct sources: the periosteum, from where they invaded the diaphysis as osteoprogenitor cells along with blood vessels, and the epiphyseal growth plate. The chondrocyte-derived osteoblasts could evolve from a subpopulation of late hypertrophic chondrocytes that displayed morphological features of autophagy. However, apart from those cells transforming into osteoblasts distinct fraction of hypertrophic chondrocytes was destined to die through apoptosis (Park et al., 2015; Yang et al., 2014). It was conceivable that the latter processes also held true for the hypertrophic cartilage which surrounded the SOC.

In the present work, histological examination of the prenatal developing human 4th rib at 4month aged fetus and full term showed that, at the transitional complex area at CCJ; cartilage-to-bone, there were chondroclasts giant multinucleate cells(formed from fusion of monocyte and macrophage) eroding the cartilage matrix,osteoblast cells on the surface of the matrix and blood heaomopoitic cells. That agreed with workers who claimed that osteoblast were polarized cells secreted only towards the bone surface.Some workers mentioned the similarity in structure and same gene regulating of Chondroclasts and osteoclasts,(Blumer2021),but the chondroclats eroded cartilage matrix,while the osteoclast broke the bone.

In the present work, histological examination of the prenatal developing human 4th rib showed that, at 4month aged fetus till full term, the shaft of rib was composed of the diaphysis and the above metaphysis, where the primary ossification center occurred. The physis had a subtle wedge shaped grove of Ranvier full of newly formed cartilage cells, increasing the width of the rib, and extended membranous periosteal bone collar, SPBC by membranous ossification, SPBC was formed by the ring of La croix, which provided mechanical support after cartilage cells lost, burting in the sponge bone.Bone growth by endochondral ossification occurred synchronously at the same time of formation of spbc by membranous ossification. In the present work.the length of SPBC was measured approximately from the scale on the slide at the age of 4month aged fetus, and full term, and was shorter in full term. The length of SPBC extended from the zone of the area between the reserve and proliferative till the beginning of sponge bone. These events happened to

provide mechanical support, strengthen and fortify the rib, after cartilage cells lost burting in the sponge. That result of the present work coincided with the Quraan surert el ensane28 (the human) سورة الإنسان خلقناهم وشددنا أسرهم 28. ALLAH created the human and supported His creation

In the present work, histological examination of the prenatal developing human 4th rib all ages studied, showed that the epiphysis, was the site of formation of secondary ossification center, SOC,after the establishment of the primary ossification center POC. That coincided with the Quraan suret that creation occurred in steps and stages.NOOH 14` خلقكم اطوارا and suret el zomar 6 خلقا من بعد خلق

In the present work, histological examination of the prenatal developing human 4th rib at the epiphysis of 4month age there were multiple secondary ossification centers SOC, which coalesced to form one large center. Some vascular cartilage canals invaded the SOC, hypertrophic cartilage cells between matrix were present around the SOC. That arrangement was similar morphologically to the growth plate at the metaphase at the CCJ.The results of the present work agreed with Blumer 2021

Initial Cartilage canals and secondary ossification center SOC:

In the present work histological examination of part of the 4th prenatal developing human rib juxta the CCJ of 4,6and9months age fetus showed the presence of collection of enlarged cartilage cells found in specific round area of the epiphysis,which formed an initial secondary ossification center.SOC.Many vascular cartilage canals were around the Initial SOC,Some canals invaded and incorpoirated with SOC.. Enlarged cartilage cells around the SOC were similar morphology to the growth cartilage plate at the CCJ were noted. Some chondroclasts multinucleate were seen eroded the cartilage cells, at the edge of the SOC.and inside the SOC Polarized basophilic osteoblasts were on the surface of newly formed matrix. Different stages of osteocyte formation were seen embedded in the matrix present in eosin acidic field, due to TRAP (tartrate resistant acid phosphatase) production from the chondroclasts. TRAP could facilitate the matrix disintegration.Blood cells were noted inside the SOC.

The results of the present work and the presence of eosinic field due to TRAP and the possible transformation **of** the late hypertrophic Cartilage cells to bone cells at the primary ossification center POC, and secondary ossification center SOC, and that they had similar fate agreed with Blumer (2021).

In the present work histological examination of part of the 4th prenatal developing human rib at the CCJ of 9months age fetus showed that POC and SOC were similar in the presence of plate of enlarged cartilage cells between the matrix at the edge of the centers,but the SOC differed in that,there were vascular cartilage canals around the SOC centers,which invaded, incorporated, distributed, branched inside the SOC, and caused its expansion. That agreed with Blumer 2021 who mentioned that once the POC was established, the secondary ossification centre (SOC) developed in the epiphysis., the cellular and molecular mechanisms leading to the formation SOC centers in the epiphysis, resembled those took place in the diaphysis; however, the differences were. First, vascularization occurred prior to the development of an osseous cuff, hypertrophy and mineralization of the cartilage, thus indicating that mineralization was not a prerequisite for vascular invasion. Second, angiogenesis occurred via cartilage canals. Third, resting but not hypertrophic chondrocytes released VEGF to attract the vessels (Allerstorfer et al., 2010; Alvarez et al., 2005; Roach et al., 1998 (Blumer et al., 2008b),

VASCULAR Cartilage canals CC

In the present work, histological examination of TS&LS sections of parts of the prenatal developing human 4th rib at epiphysis,at the 4,5,6,7,9 months aged fetuses, showed that,there were cartilage canals CC extending from the perichondrium contained mesenchyme CT and central arteriole.Some canals were empty and contained only mesenchyme at the age of 4month aged fetus,some CC canals had collagen and absorbed substances at the age of 6 and 7 months aged fetus, Some vascular CC canals contained capillaries,venulles and sinusoidal capillaries,Some vascular CC canals were occupied by only one vessel.Some cartilage canals CC had straight course.while others had tortuous course.Some CC canals invaded the SOC,branched and incorporated with the SOC center,caused its expansion,the walls of the invading vascular CC canals transformed to basophilic bone like cells. The vessels in the cartilage canals branched and distributed inside the SOC. At 9month aged fetus, cartilage canals terminated by COMPLEX glomerulus were noted.

In the present work, histological examination of TS&LSsections of parts of the prenatal developing human 4th rib at epiphysis,at the 4,5,6,7,9 months aged fetuses, showed that,some cartilage canals CC showed active chondrolysis especially increased at 9months aged fetus at their tips:manifested as lacunae containing cells intimately associated with matrix,and presence and some of the cells around the tips had granules at their tips. Cartilage cells around the canals CC were enlarged.dispeared,.Some canals at their ends had macrophages,fibroblasts,besides the blood cells in the vessels. Some canals CC had simple vessels,other canals had branched anastomosed capillaries. Some canals CCat the age of 7month aged fetus were

obliterated with incomplete wall and contained collaged and fibroid material. The presence of active chondrolysis around at the vascular cartilage canals tips, in addition to the extensions of the vascular cartilage canals from the vascular perichondriuem might indicate that the vascular cartilage canals developed by both ways: passive inclusion and active chondrolysis. The presence obliterated cartilage canals with incomplete wall WHICH contained collaged and fibroid material,might coincide with suret yaseen 68which meant that the creation undrerwent regression after growth. يس68:و من نعمره نكسه في

The results of the present work were similar to Chappard et al.,1986 who mentioned that, in the human fetus, epiphyses appeared as a solid avascular cartilaginous mass until the eleventh week of development. Around the third fetal month of development, vascular canals coming from the perichondrium were recognized in the mineralized epiphyseal cartilage. Whether cartilage canals developed by passive inclusion or active chondrolysis was still a matter of controversy. They studied the relationships between the intracanalar cells and the surrounding matrix on human fetal epiphyses embedded in glycol methacrylate. At the blind end of canals both stellate fibroblast-like cells and vacuolated macrophages were observed. These cellular foci showed all characteristics of active chondrolysis (loss of metachromasia, lacunae containing cells intimately associated with matrix, and presence of granular debris). Their observations supported the view that chondrolysis due to both fibroblasts (of mesenchymal origin) and macrophages was the basic mechanism for cartilage canal development

The results of the present work were similar to Craatz et al., (1999) who,mentioned that, the hyaline rib cartilage took up a special position due to its size, shape and the kind of mechanical stress. Those facts influenced the metabolism of rib cartilage. In their histological, histochemical and immunohistochemical investigations on pieces of rib cartilages of 34 persons at the age of fourth fetal month up to 60 years they demonstrated cartilage canals containing blood vessels without any spatial or temporal relationship to degenerative changes in cartilage tissue. They mentioned that many of those cartilage canals were located in the center of the rib cartilage. Blood vessels as well as neuronal structures in the connective tissue of cartilage canals were detected by means of antibodies against components of the vessel wall (Von Willebrand factor) and nerve fibers (PGP 9.5). They explained that nerves might have sensoric or vasomotoric functions, and they might influence cell differentiation and regeneration processes, respectively. Cartilage could not be regarded as vascularized like other tissues, but cartilage canals might have great functional importance for the metabolism of rib cartilage..

Summary and conclusion

In the present work,histological examination of parts of LS&TS sections of the prenatal developing human 4th rib,at the 4,5,6,7,9,months aged fetuses,showed changes with age progress in the pirary ossification centers POCin the metaphysis, secondary ossification centers SOC and cartilage canals in the epiphysis, as follows; At 4months aged fetus: At the costochondral junctionCCJ; at the complex transitional area cartilage-bone;there were enlaged chondrocytes arranged in columns,.Chondroclasts eroding the cartilage, and osteoblast on the newly formed matrix, as well as blood cells were noted.Primary ossification center POC formed later at that site.

In the epiphyses, of 4months aged fetus: multiple small secondary ossification centers SOC were noted, small centers coalesced to form lager expanded SOC center, cartilage canals were around the SOC, some Cartilage canals incorporated with the secondary ossification center SOC. Mesechyme from the perichondrium containing vessels were seen invading the SOC, distributing inside the SOC causing its expansion. that lead to the presence of large SOC a the age of 5month aged fetus occupied more than 75%of the epiphysis surface area.

In the present work the SOC composed of enlarged hypertrophic cartilage cells at the edge of the center. Chondroclasts giant cells eroded the hypertrophic cartilage cells matrix, and formed acidic eosin field due to TRAPby chondroclasts (tartartecoid phosphatae) production, were seen.Basophilic osteoblasts on the newly formed matrix surface were noted.Stages of osteocytes within acidophilic field, eosin stain were noted.Intermingled areas of basophilic and eosin field -due to (tartrate resistant acid phosphate)TRAP production by the chondroclasts-,and basophil areas were seen. TRAP was needed for bone remodeling and growth. Osteoclasts were surrounded in the matrix and embedded in it.The wall of the invading cartilage canals with vessel, inside the SOC transformed to basophilic flat bone like cells. At the edge around the SOC, hypertrophic cartilage cells arranged in small columns between matrix were noted, morphologically similar to the epiphyseal growth plate at the CCJ.

At the age of 6month aged fetus,an initial SOC was seen, formed of collection of enlarged cartilage cells,surrounded by numerous vascular cartilage canals which invaded the SOC. The canals occupied more than 4% from the surface area of the resting cartilage. Cartilage canals which did not incorporate with the SOC atrophied,and were similar to ghosts.Some canals had incomplete wall and

contained collagen, Some canals were large contained arterioles, capillaries,venules,CT.Some cartilage canals contained only one large sinusoidal capillary, invading the SOC and its tip showed chondrolysis,and the canal lumen contained absorbed substance.

At 7month aged fetus: perforating spring of empty cartilage canals with conical ends, surrounded by martrix having stripe appearance were noted.Some canals were toursous and obliterated contained collagen masses stained deep green with Masson trichrome stain. Bubble like matrix was noted around the course of caetilage canals.

At the age of 9month aged fetus,an initial SOC was formed of collection of enlarged cartilage cells,surrounded by numerous vascular cartilage canals starting to invade the SOC. Cartilage canals in different stages of formation ;bud stage. Cartilage canals with diifferent length extending approximately, at equal regular distance from the complex vascular perichondium, to the resting cartilage were seen. Perichondrium extensions of mesenchyme contained vessels approached the SOC were noted. The canals originated from the perichondrium and were composed of loose connective tissue with a central arteriole terminating in a capillary glomerulus from which a single venule passessed back to the origin. The matrix was deeply stained around and between the cartilage canals,indicating the influence of the canals on the cartilage cell metabolism and the collagen of the matrix. Enlarged cartilage cells around the canals were large in areas and small flat in concentrically arranged or randomly loosely packed in some sites around the canals

Some measurement were held for the diameters of the cartilage canals and the vessels they contained, the space between some superficial cartilage canals were measured, in some ages using the scale from the microphotograph, and the spaces were approximately equal.

The results of the present work agreed with Lutfi 1970 and EL RAKAWY 1971,Blumer2021

Bumer2021 who mentioned that primary ossification Centre (POC) developed in the diaphysis, followed by the secondary ossification Centre (SOC)in the proximal and the distal epiphysis.in development of the long bones, once the bone cuff was formed around the middle region of the shaft (diaphysis). The metaphysis was located and contained the growth plate between the epiphysis and the diaphysis.Its activity controlled the longitudinal growth of the bone.Dissolution and disintegration of the cartilage matrix occurred,in formation of the primary ossification centre (POC),before the onset of bone formation, induced by the differentiation of the chondrocytes that divided, matured and finally underwent a tremendous enlargement or hypertrophy. Hypertrophic chondrocytes were active cells and had the ability to express type-X collagen, a hallmark for the calcification or mineralization of the adjacent extra cellular matrix. They then released the vascular endothelial growth factor (VEGF) to attract endothelial cells, triggering the ingrowth of vessels from the periosteum (covered the bone cuff) into the Centre of the diaphysis. Vascular invasion also depended on a local proteolytic rearrangement of the cartilage matrix, and metallo-proteinases (MMPs) such as MMP 9 and 13 as well as MT1-MMP,a membrane-bound MMP, shared in that process owing to their ability to cleave the cartilage. MMP knockout mice, had severe skeletal defects,indicating the crucial role of that enzyme in bone development.Mice deficient in individual MMPs showed a delayed angiogenesis and an altered endochondral ossification MT1- (Carlevaro et al., 2000; Carpio et al., 2017; Gerber et al., 1999;Holmbeck et al., 1999, 2003; Maes et al., 2004; Petersen et al., 2002;Stickens et al., 2004; Vu et al., 1998 Zelzer et al., 2002, 2004; Zhouet al., 2000). Chondroclasts and hematopoietic cells were provided, with the vessels containing osteoprogenitor cells (early osteoblasts), Chondroclasts were multinucleated giant cells, arising from the fusion of mono-cytes/macrophages present in the bone marrow. After attachment to the surface of mineralized cartilage via the sealing zone they became activated and released tartrate resistant acid phosphatase(TRAP), also referred to as acid phosphatase5 tartrate resistant(Acp5). At the attachment sites the chondroclasts' cell membrane was highly folded (ruffled border) and created an acid milieu (pH∼4.5) to demineralize and then resorbed the cartilage matrix. That process, at the same time, made the organic network more accessible, and cathepsin K seemed to be responsible for the degradation of the type-II collagen fibrils (Hollberg et al., 2002, 2005; Minkin, 1982; Proff and Romer, 2009). The chondroclasts, formed shallow depressions or deep lacunae in the underlying cartilage. The TRAP enzyme was an important in development, since TRAP −/− mice displayed an aberrant endochondral bone formation due to a defective cartilage turnover, resulting in skeleton abnormalities reflected by a fore shortening of the long bones and an osteopetrotic phenotype (Blumer et al., 2012; Hayman and Cox,2003; Hayman et al., 1996).The continuous erosion of the diaphyseal cartilage caused the marrow cavity to be filled with blood vessels and hematopoietic cells and a scaffold areas of mineralized cartilage upon which osteoblasts deposited osteoid. Osteoblasts originated from osteoprogenitor cells, and transcription factors such as the runt related transcription factor two (RUNX2) and osterix (OSX) were essential for their differentiation. Osteoid

mineralized through alkaline phosphate activity and formed trabeculae of woven bone, leading to the establishment of the POC (Maruyama et al., 2007; Proff and Romer,2009; Roach, 2000; Salhotra et al., 2020).

Blumer 2021 added that during longitudinal growth, the POC and also the bone cuff expanded from the center of the diaphysis towards the cartilaginous endings(epiphysis). A thickening and persistent remodeling of the bony trabeculae controlled by a balanced mutual action of osteoclasts and osteoblasts accompanied the growth. Osteoclasts had the same origin and molecular besides ultrastructural characteristics of chondroclasts but broke up the bone matrix onto which they adhere. Recently, it had been shown that the Schlafen2 (Slfn2)gene regulated osteoclast precursor cells and loss of function ofSlfn2 apparently resulted in a developmental defect of osteoclasts,leading to an increased volume of the trabecular bone (Omar et al.,2018). Blumer 2021 pointed that, in the process of new bone formation it was still unclear in which direction osteoblasts released the collagen fibrils, and by which mechanism they were encased (Nefussi et al., 1991; Franz-Odendaal et al., 2006). Studies in mice, provided evidence that osteoblasts were polarized synthesizing osteoid only from the surfaced which faces the bone matrix Blumer et al., 2007.

Blumer 2021, reported that,it remained unclear whether osteoblasts were buried into the osseous tissue by the following generation, or by themselves.Once the POC was established, the secondary ossification centre (SOC) developed in the epiphysis. The cellular and molecular mechanisms leading to its formation resembled those took place in the diaphysis; the differences were: First, vascularization occurred prior to the development of an osseous cuff, hypertrophy and mineralization of the cartilage,thus indicating that mineralization was not a prerequisited for vascular invasion. Second, angiogenesis occurred via cartilage canals. Third, resting but not hypertrophic chondrocytes released VEGF to attract the vessels (Allerstorfer et al., 2010;Alvarez et al., 2005; Roach et al., 1998). (Blumer et al., 2008b),

In the present work, histolgical examinationof part of the 4th prenatal developing human rib, at the CC,showed at the age of 4month aged fetus and full term,there were multiple secondary ossification centers. Initial secondary ossification centers were seen at full term,formed of aggregated enlarged cartilage cells,surrounded by cartilage canals that originated from the perichondriuem. In some areas of the perichondrium,speial arrangement of perichodrial layers` was seen to allow formation, passage of cartilage canals ingrowth and advancement.Some secondary ossification SOC centers were close to the perichondriuem and the perichondriuem containing vessels,extended in the center. The number of cartilage canals increased, at full term.the canals were increased enormously numerous like flood, formed complex network. The cartilage canals shared in nourishment of rib cartilage,and recently researches indicated that they had osteogenic potential to enable the formation and the expansion of the SOC.

The results of the present work agreed with Sadler 2019 who mentioned that,in endochondral bone formation,the mesenchyme occurred as the cells began to condensate and differentiate into chondrocytes, then chondrocytes formed a cartilaginous model of the prospective bone.Blood vessels invaded the center of cartilaginous model,bringing osteoblasts.and restricting proliferation of chondrocyte cells to the ends of the bones,Chondrocytes towards the shaft side diaphysis underwent hypertrophy and apoptosis as they mineralized the surrounding matrix. Osteoblasts bound to the mineralized matrix and deposited bone matrix. Later, as,blood vessels invaded the epiphyses, secondary ossification centers formed. Growth of the bones was maintained by proliferation of chondrocytes in the growth plate.

The results of the present work agreed with Blumer 2021 who reported that in the formation of the secondary ossification Centre (SOC),the first step in epiphyseal ossification was the early development of the cartilage canals. They occurred in birds and mammals having the same structural organization but, had not yet been described in amphibians, reptiles and bony fish. The canals originated from the perichondrium and were composed of loose connective tissue with a central arteriole terminating in a capillary glomerulus from which a single venule passessed back to the origin. Blumer 2021 added that cartilage canal occurred at different times in the species; e.g. in chicken they developed long before hatching, (Lotfi1970) in marines shortly after birth. Similar to the events in the diaphysis, initially MMPs and after mineralization chondroclasts broke up the cartilage matrix, thus clearing a path for the canals' ingrowth and advancement. During epiphyseal growth the number of canals increased, and just before the onsetof ossification they constituted a complex network (Blumer et al.,2004a, 2007; Chappard et al., 1986; Eslaminejad et al., 2006; Finnoyet al., 2017; Haines, 1933; Hellings et al., 2017; Holmbeck et al., 1999; Kugler et al., 1979; Kuettner and Pauli, 1983; Lutfi, 1970;Melton et al., 2006; Shapiro, 1998; Wang et al., 2015; Wilsman andVan Sickle, 1972).The primary function of the vascularized canals was the nourishment of the growing cartilage which would, otherwise, depend exclusively on the vessels' plexus of the perichondrium (Kuettnerand Pauli, 1983; Wilsman and Van Sickle, 1972).Recent findings had

demonstrated that the canals' perivascular cells had the osteogenic potential to enable the formation and the expansion of the SOC. Adjacent to the developing ossification centre, several layers of hypertrophic and proliferating chondrocytes acted as a surface growth plate. Its activity regulated the longitudinal and radial growth of the epiphysis,and also formed the immature articular cartilage (Alvarez et al.,2005; Blumer et al., 2008b; Hunziker et al., 2007)

Blumer 2021 pointed that in elasmobranch fishes (sharks, skate and rays) the vascularized cartilage canals also occurred in several skeletal pieces during development and growth, although their skeleton remained cartilaginous throughout life (Marconi et al., 2020). Elasmobranchs showed no signs of endochondral ossification, however, reinforced their endoskeleton by a layer of mineralized tiles (tesserae) covering the unmineralized cartilage core of each skeletal element (Deanand Summers, 2006; Seidel et al., 2016, 2017).In the complex sequence of cartilage degradation, bone apposition and growth the original cartilage model was replaced by bone,and these critical events occurred at the chondro-osseous junction of the epiphyseal growth plate (Roach, 2000; Shapiroet al., 2005).

In the present work histolgical examination, of parts of serial sections TS&LS of the prenatal developing human 4th rib,,showed at the age of 4month aged fetus and full term,there were hypertrophic chondrocytes, chondroclasts, osteoblasts and blood vessels occurred at trasisional zone cartilage –to-bone-the chondro-osseous junction That agreed with (Park et al.,2015; Shapiro et al., 2005; Yang et al., 2014; Zhouet al., 2014),who pointed that,the transition zone was composed of mineralized cartilage and early bone matrix and characterized by the presence of hypertrophic chondrocytes, chondroclasts, osteoblasts and blood vessels. Blumer 2021 added that In particular, the role of the late (terminally differentiated) hypertrophic chondrocytes had been debated over decades, and their fate was still a matter of opposing.

In the present study,histological examination of parts of the 4th prenatal developing human rib at the CCJ, at 4month aged fetus, establishment of the POC occurred. and SOC was formed after POC establishment. Late hypertrophic cartilage cells were seen bursting, ruptured in the POC and SOC centers. The transformation of some LATE hypertrophic cartilage cells to osteocytes might be possible.and coincided with the Quraan:that pointed at the another creation suret al moomenon 14.suret al zomar 6,suret NOOH 14 خلقا أخر -المؤمنون 14و/سورة الزمر 6 خلقا من بعد خلق,,سورة نوح خلقكم أطوارا

Blumer 2021 mentioned that. recently, two types of endochondral bone formation had been described in the zebrafish. The first type was similar to mammalian endochondral ossification, whereas the second differed in some aspects.

Blumer 2021 concluded that,in intramembranous ossification, a mesenchymal membrane directly differentiated to bone, whereas in the more complex process of endochondral ossification a cartilaginous intermediate served as a bone precursor.The long bones of the appendicular skeleton developed through endochondral ossification, and after the formation of the bone cuff (intramembranous ossification), the blood vessels, osteoblasts and chondroclasts originating from the periosteum infiltrated the mineralized hypertrophic cartilage of the middiaphysis. Chondroclasts broke up the cartilage matrix and osteoblasts layed down bone matrix and transformed into osteocytes. In a coordinated process of bone deposition (osteoblasts) and removal (osteoclasts) the bone trabeculae were continuously remodelled and gain in thickness during bone growth. Once the POC and the marrow cavity had been established, the SOC formed in the epiphysis, initiated by the invasion of vascularized cartilage canals. The epiphyseal growth plate mediated longitudinal growth and comprised layers of differentiating chondrocytes with hypertrophy as their final state. At the transition zone of mineralized cartilage and early bone, numerous late hypertrophic chondrocytes died while the growth plate got thinner with age. A fraction of those cells survived and differentiated into osteoblasts, having an impact on bone formation.Thus, the osteoblasts in endochondral bone formation derived from two sources: the periosteum and the growth plate. After the longitudinal growth. The results of the present work agreed with Quraan suret el bagara (the cow259)259 خلقا آخر– العظام كيف ننشزها()سورة البقرة المؤمنون 14(Suret al moemenoon14)14.

. Marconi et al. (2020)had suggested that the canals contributed to interstitial growth of the cartilage but further data were needed to strengthen these findings.

- In the present work,during longitudinal growth,of the prenatal developing 4th human rib,in all ages studied, the POC and also the bone cuff expanded from the center of the diaphysis towards the cartilaginous endings(epiphysis). A thickening and persistent remodeling of the bone trabeculae was controlled by a balanced mutual action of osteoclasts and osteoblasts accompanied the growth.The second ossification was formed after establishment of POC multiple SOC appeared in the epiphysis at the age of 4month aged fetus,and at 6month age fetus and full term fetuses,
- SPBC was formed by membranous ossification at the same time of endochondral ossification at the CCJ.

- The results of the present work agreed with Sadler 2019 who mentioned that ossification of the bones of the extremities,endochondral ossification began by the end of the embryonic period.Primary ossification centers were present in all long bones of the limbs ossification by the 12th week of development,From the primary center in the shaft or diaphysis of the bone,endochondral ossification gradually progressed toward the ends of the cartilaginous model.At birth the diaphysis of the bone was usually completely ossified,but the top ends, the epiphysis, were still cartilaginous. Shortly then after, however, ossification centers arised in the epiphysis,Temporarily a cartilage plate remained between the diaphyseal and epiphyseal ossification centers. That plate,the epiphysial plate,played an important role in growth in the length of the bones.Endochondral ossification proceeded on both sides of the plate.When the bone had acquired in full length,the epiphesial plates disappeared, and the epiphysis united with the shaft of the bone.In long bones, an epiphysial plate was found on each extremity, in smaller bones,such as phalanges,it was found only at one extremety,and in irregular bones,such as the vertebrae,one or more primary centers of ossificatioon and usually several secondary centers were seen
- The results of the present work agreed with Blumer 2921 who reported that osteoclasts had the same origin and molecular,and ultrastructural characteristics of chondroclasts but broke up the bone matrix onto which they adhered
- In the complex sequence of cartilage degradation, bone apposition and growth,the original cartilage model was replaced by bone,and these critical events occurred at the chondro-osseous junction of the epiphyseal growth plate.
- The results of the present work agreed with El Rakawy 1971. who mentioned that the mechanism of ossification was that some UMCs in the presence of rich blood supply differentiated and transformed to bone forming cells:the osteoblasts. Osteoblasts secreted phosphatase, that would cause precipitation of salts and formed the bone matrix. When all osteoblasts turned into osteocytes, the bone stopped to grow.
- El rakawy 1971 mentioned that bone developed as a result of 2 balanced mutual phenomena under normal condition: Bone formation on one of the surfaces of the bone and bone absorption on the another surfaces of the bone. the two phenomena were balanced. Meanwhile ; The mechanism of Cartilaginous Ossification was as follows:
- 1-The small model of Cartilage had to be destroyed and absorbed. 2-
- and replaced by bone: Ossification. 3- The bone had to grow in length.and had to4- grow in width diameter5- The bone had to change its shape consciously and gradually as the bone grew which was called remodeling. The Cartilage model destroyed and absorbed by calcification of matrix, when the cartilage cells matured and enlarged they secreted alkaline phosphatase which caused calcification of the matrix around them. That would prevent nutrition of cartilage cells and their death.
- cells death caused dissolution of the calcified cartilage matrix and disappearance of the piece of cartilage. EL RAKAWY 1971 pointed that Ossification started from the perichondrium in two ways: formation of collar of bone around the middle third of the shaft,called the periosteal collar of bone. PBC2- formation of primary center of Ossification POC: Blood invaded the center of the shaft(diaphysis). In the formation of the sub periosteal bone collar SPBC; Some UMCs from the inner layer of perichondrium divided and transformed into osteoblasts which laid down a cylindrical (collar) of bone around middle third of the shaft. 1- (membranous ossification) worked as mechanical support to fortify and strengthen the shaft after the bursting of cartilage cells in the sponge.
- The results of the present study agreed with the Quraan suret el ensane (the human) 28, خلقناهم وشددنا أسرهم سورة الانسان 28which meant: Allah mntioned that He created the human and fortified their built)
- The results of the present work agreed with EL RAKAWY 1971, who mentioned that after birth bone grew in length by the epiphyseal secondary center of Ossification SOC. The epiphyseal secondary center of ossification SOC was responsible of ossification of epiphysis except two parts of epiphysis remained cartilaginous and not Ossified: the articular cartilage and the epiphyseal disc which was a cartilaginous transverse disc between the bone of epiphysis and bone of diaphysis. The epiphyseal disc grew by interstitial growth: the cells divided and gave new small cartilage daughter cells. the interstitial growth of the epiphyseal disc was responsible for the growth in length of shaft diaphysis as follows: a-the epiphyseal disc sent new small cartilage daughter cells towards the diaphysis and the disk itself retracted away from the center of the diaphysis.b- the small cartilage daughter cells died after their secretion of phosphatase and calcification of cartilage matrix.Destruction of the cartilage cells and disappearance's- bone from the diaphyseal.Ossification center grew to replace the died cartilage. Although there was continuous processes of interstitial growth in the epiphyseal disc,the disk did not increase in thickness. Because the older cartilage

cells (the cells towards the diaphysis)matured,died and became replaced by bone.

- El rakawy1971 explained that,In the epiphyseal disc,there was battle between 2 mechanisms:the a-interstitial growth of cartilage of the epiphyseal disc and b- maturation,death,and replacement of cartilage by bone on the diaphyseal side of the disk.in a way,it was a battle in which the epiphyseal discs on either sides of the bone which caused the shaft to increase in length i.e interstitial growth of the epiphyseal cartilage,did not increased thickness of the epiphyseal disc,but increased the length of the diaphysis.
- The results of the present work were similar to Wilsman& Van Sickle (1970) who mentioned that the Initial osteogenesis of secondary centers of ossification in the humeral head of the dog was studied with serial sections, histochemistry, radiography and vascular injections. At birth this chondroepiphysis was found to be well vascularized by a network of cartilage canals. On the second day after birth the first morphological evidence of the secondary center of ossification was seen. That was in the form of multiple foci of calcification. Each focus of calcification occurred immediately adjacent to the glomerular end of a cartilage canal and not in an avascular matrix. The capillaries of the glomerulus were modified in the process and persisted as the blood supply to the secondary center of ossification. By four days of age the individual foci had coalesced into a single larger focus of calcification. That process of ossification was found to possess morphological similarities to that occurring at the metaphyseal side of the epiphyseal plate.
- The results of the present work agreed with Chappard et al., (1986). Who pointed that in the human fetus, epiphyses appeared as a solid avascular cartilaginous mass until the eleventh week of development. Around the third fetal month of development, vascular canals coming from the perichondrium were recognized in the mineralized epiphyseal cartilage
- Whether cartilage canals developed by passive inclusion or active chondrolysis was still a matter of controversy. They studied the relationships between the intracanalar cells and the surrounding matrix on human fetal epiphyses embedded in glycol methacrylate. At the blind end of canals both stellate fibroblast-like cells and vacuolated macrophages were observed. Those cellular foci showed all characteristics of active chondrolysis (loss of metachromasia, lacunae containing cells intimately associated with matrix, and presence of granular debris). Similar resorptive foci had been observed in the pannus of rheumatoid joints and in the embryonic chick growth plate composed of uncalcified cartilage. They concluded that a cellular cooperation (fibroblast/macrophage) necessary for uncalcified cartilage breakdown. In the human fetus, monocytes/macrophages had been recognized in the peripheral blood as early as the twelfth week of gestation. Their observations supported the view that chondrolysis due to both fibroblasts (of mesenchymal origin) and macrophages was the basic mechanism for cartilage canal development.
- The results of the present work agreed with Gilroy 2013 who mentioned that the clavicle and some skull bones developed by membranous ossification,in which bones were directely formed from ossification of mesenchy template down during the embryonic period.
- Many bones including long bones of the limbs were from mesenchyme in the fetal life,then in the second decade ossification replaced most of the cartilage of the embryonic life were developed from cartilagenous template formed Endochondral ossification.

The periosteum, the perichonmdiuem and sponge bone at the costochondral junction CCJof the prenatal developimg human 4th rib:

- In the present study histological examination of parts of the periosteum of the 4th prenatal developing human rib of 4,5,6,7,9,months aged fetuses at the costochondral junction CCJ showed that, the periosteum covered the surfaces of the rib and increased in thickness and layers with age progress. The layers of the periosteum continued with the perichondrium. The sponge bone at the costochondral junction CCJ,was formed of irregular trabeculae,which appeared as anastomosing with each other.The newly formed matrix of the prenatal developing ages studied were covered with the basophilic polarized osteoblasts which were, small branched bone forming cells. They formed continues layer covering the trabecular of matrix of the developing bone. They found on the surface, meanwhile osteoclasts embedded between the matrix. Osteoclasts could not divide. Osteoblasts had more deep basophilic (blue) cytoplasm due to excess RNA in the cytoplasm. The spaces between the trabeculae of bone were filled with blood cells, which increased with age progress and formed bone marrow. The periosteum was fibrous sheath surrounded the outer surface of the prenatal developing human 4th rib. It composed of two layers: an outer layer made of dense white fibrous tissue consisted of blood vessels, which were developed at the age of 5month aged fetus,and increased in prominence with age progress. The inner layer consisted of loose tissue containing osteoblasts, which formed the germinate osteogenic or osteoblastic layer that formed new cells.

The results of the present work coincided with El Rakawy (1971), who mentioned that the periosteum OF adult long bone was composed of two layers: an outer layer made of dense white fibrous tissue consisted of blood vessels, and an inner layer consisted of loose tissue containing osteoblasts. The inner layer was the germinate osteogenic or osteoblastic layer which formed new cells. The periosteum in adults was more adherent to under lying bone where tendons became inserted in the bone. At these sites the coarse collagenous fibers extended from the periosteum to enter the bone and acted as nails to fix them together.

The perichondrium

In the present study histological examination of parts of serial sections TS& LS the perichondrium of the 4th prenatal developing human rib of 4,5,6,7,9,months aged fetuses at the costochondral junction CCJ, showed that the perichondrium covered the cartilaginous part of the rib at the costochoondral junction CCJ,and showed interstitial growth.The layers of the perichondrium continued with the periosteum.The perichondrium stained strong deep with Masson trichrome and Malory triple stain. Small araes of the subperichonduom stained less deep than the perichondrium. The hyaline matrix, stained faint; That three different stain affinity might indicate different types of collagen in each area and tissue remolding of the 4th prenatal developing human rib.

In the present work histological examination of parts serial sections TS& LS of the 4th prenatal developing human rib,juxta the CCJ, at the epiphysis,stained by Mallory triple stain and Masson trichrome stain,showed that the layers of the perichondrium had special arrangement to prepare for cartilage canal formation,surrounded by mesenchyme.Spur formation was noted adjacent in subperchondrium. That three different stain affinity might indicate different types of collagen in each area, and tissue remolding of the 4th prenatal developing human rib.

There was continuous extensions of the perichondrium and secondary ossification centers SOC, at the age of 4month, and 9month aged fetus. Different stain affinities were present In the the stain affinity of the perichondrium was strong,while the stain of the matrix of perichondrium, the secondary ossification centers SOC,and the resting cartilage was weak,and the stain of the matrix of the secondary ossification centers SOCwas moderate.

The results of the present work were similar to Claassen , Kampen, Kirsch. 1995 who investigated by immunofluorescence staining with specific antibodies in order to obtain a better understanding of tissue remodelling during the development of first rib cartilage. In childhood and early adolescence type I collagen. They found that that the localization of fibrillar type I and II collagen was found to be restricted to the perichondrium of first rib cartilage, while type II collagen was localized in the matrix of hyaline cartilage. However, in advanced age type I collagen was also found in the territorial matrix of intermediate and central chondrocytes of first rib cartilage. The matrix of subperichondrial chondrocytes was negative for type I collagen. That suggested that some chondrocytes in first rib cartilage underwent a modulation to type I collagen-producing cells. The first bone formation was observed in rib cartilages of 20- to 25-year-old adults. The ossification began peripherally, adjacent to the innermost layer of the perichondrium where areas of fibrocartilage had developed. The newly formed bone matrix showed strong immunostaining for type I collagen. Fibrocartilage bordering peripherally on bone matrix revealed only a faint staining for type I collagen, but strong immunoreactivity to type II collagen. The interterritorial matrix of the central chondrocytes failed to react with the type II collagen antibody, in both men and women, from the end of the second decade. These observations indicated that major matrix changes occurred at the same time in male and female first rib cartilages. They mentioned that their findings indicated that ossification in human first rib cartilage did not not follow the same pattern as that observed in endochondral ossification of epiphyseal discs or sternal cartilage.

In the present work The growing perichondrium of the 4th prenatal human rib, showed the interstitial growth: growth from inside, and the appositional growth (growth from outside.The present results agreed with El Rakawy (1971) who mentioned that the perichonmdiuem consisted of CT surrounded the cartilage.The cartilage cells underneath the perichondrium were flat,parallal to the surface mostly single. The cartilage cells In the center of the rib hyaline cartilage were found in group called cells nests. The cartilage cells were found in spaces called lacunae and surrounded by a capsule stained darker than that of the intercellular substance. The intercellular substance stained deeper because it contained chondroitin sulphoric acid, especially more in the center.

In the interstitial growth:growth from inside:each single cartilage cell had a capsule, when it divided into two,each daughter cell had its own capsule,the primary capsule disappeared and the two cells remained close to each.In the appositional growth (growth from outside ;the CT perichondrium: New layers of cartilage were added from the inner chondrogenic layer of the perichondrium: where undifferentiated mesenchymal cells UMCs formed chondrocytes (cartilage cells).

However Kampen et al.(1995) mentioned that Ossification was not directly correlated with the

invasion of blood vessels and could be classified as one of the classical concepts of intramembranous or endochondral osteogenesis.The results of the present work were similar to Kampen et al. (1995) who mentioned that mineralization and osteogenesis in the human first rib cartilage were studied radiologically and by means of normal and polarized light microscopy. Onset of mineralization occurred at the end of puberty and was located directly beneath the perichondrium. Bone was formed in a typical spur-like pattern, arising medially from the upper edge of the manubrium sterni and laterally from the caudal rim of the bony part of the rib. From the middle of the second decade, large cartilage canals with several blood vessels and loose perivascular connective tissue were seen in central areas of the first costal cartilage. These parts were the last to be mineralized and ossified in old age. The type of osteogenesis could be classified according to common patterns. In spite of the subperichondral localization it could not be intramembranous, because the new bone was separated from the perichondrium by a layer of mineralized cartilage. Osteogenesis could not be called endochondral compared with the epiphyseal plate for the following reasons: there were no hypertrophied chondrocytes; immunoreactivity for collagen type X was missing; areas where bone was formed directly on hyaline cartilage could be proved. Vascularization and onset of osteogenesis were separated in time and localization. Mineralization and osteogenesis in human first rib cartilage were physiological age-related changes, which could be regarded as degenerative processes.

The relation of Cartilage canals CC and secondary ossification center SOC in prenatal developing human fourth 4th rib:

In the present study histological examination of parts of serial sections TSand LSof the prenatal developing human fourth 4th rib of all studied aged fetuses 4,5,6,7, and full term, at the CCJ costochondral junction, showed the presence of cartilage canals, which continued with the perichondial vesenls,and contained capillaries,sinusoidal capillaries,venultles,arteriole, loose perivascular Connective tissue CT, and mesencchyme. Cartilage canals were important for nutrition, and the perivascular Connective tissue might be a source of stem cells that provided a source for bone-synthesizing osteoblasts on the calcified cartilage.

At 7, month aged fetus Straight, empty canals with conical ends were noted in the 4th prenatal developing human rib, some obliterated canals contained collagen were noted Some atrophic cartilage canals were seen. The absorbed contents were seen in the canals close to the ossification center. At 9month aged fetus, there were numerous vascular cartilage canals in cartilage of the epiphysis.. Some cartilage canals were close to a secondary ossification center SOC.Some canals invaded the secondary ossification center, incorporated into the growing secondary ossification center, and the blood vessel contained blood distributed in the SOC. Most VASCULAR cartilage ended with terminal complicated glomerulus and increased in number at full term.At the age of full term long canals with glomerulus were seen.some canals had incomplete walls and showed chondrolysis at their tips.

The results of the present work were similar to Jaramillo et al., 2004 who studied age-Related Vascular Changes in the Epiphysis, Physis, and Metaphysis: Normal Findings on Gadolinium-Enhanced MRI of Piglets.They pointed out that vascular cartilage canals were important in the development of epiphyseal ossification because the vessels with their associated mesenchymal cells served as the source for bone-synthesizing osteoblasts on the calcified cartilage. However, Jaramillo et al., 2004 mentioned that the number and size of the canals decreased with maturity, particularly after the appearance of the secondary center of ossification SOC. The concentration of vascular canals varied throughout the epiphysis. They were particularly numerous in a band adjacent to the reserve zone of the physis and around the secondary center of ossification.

Grove of Ranvier (G R) and subperiosteum bone collar SPBC, or ring of LaCroix

In the present study, histological examination of of part of LS&TS of part ofa 4th prenatal developing human rib showed at the ages 4,and,9,months aged fetuses, showed the presence of grove of Ranveir GR that surrounded the cartilaginous part of the growth plate (or physis) and a thin layer of intramembranous bone (bone collar, bone bark or perichondrial ring of LaCroix). The grove of Ranvier (GR) was full of newly formed cartilage cells,and continued with the subperiosteal bone bollar SPBC.They both were supplied by heavy plexus of perichondrial vessels,and seemed as one continuous structure. GR was` wedge shaped subtle area caused increased width of the rib and formed subperiosteal bone collar SPBC,by membranous bone ossification,that occurred at the same time synchronous with the endochondral ossification that was at the chostochondral junction CCJ.of the prenatal developing human 4th rib. Membranous ossification of SPBC, occurred to provide mechanical support of the growth plate and to fortify the rib strengthen the rib after the loss of cartilage cells busting in the sponge.That results Of the present study coincided with Quraan suret el ensane 28: which meant,that Allah the most gracefull created

the human and fortified his creation. suret el enane 28.
خلقناهم وشددنا أسرهم سورة الانسان 28
In the present work SPBC was noted extending from the sponge till area between proliferative and resting zone. Subperiosteal bone collar SPBC, or ring of LaCroix) was short cylindrical osseous sheath, oriented parallel to the longitudinal axis of the bone, encircling the periphery of the costo chondro- junction CCJ region. Under the grove of RnveirGR. there was appearance of a spur (or "step off").
The length SPBC of 4month and w9months fetus were estimated according the scale on the microghroph of the histological sections, and decrcreased in length with age progress.
The results of the present work agreed with Elisa and Suma et al.,2020 who mentioned that the post mortem (PM) examination protocol in their institution included the removal of the right5th and/or 6th for assessing the child's previous health; a practice which was introduced by John Emery. (Emery and Kalpaktsoglou1967, da Cunha Castro et al., 2006) Elisa and Suma et al. (2020) mentioned that the rib was the most rapidly growing long bone in infants and demonstrated growth arrest at onset of the insult (Silberberg and Silberberg 1940, Emery1967, Emery JL, da Cunha Castro2006).
The results of the present work were similar to Elisa and Suma et al. (2020) who anounced that the periphysis, was a fibrochondro- osseous structure that encircled the metaphysis and the adjacent CCJ of the tubular bones in infants and young children (Ostreich AE, Ahmad1992, Brighton1984, Schollmeier1999). The periphysis consisted of a wedges shaped cellular zone (groove of Ranvier) that surrounded the cartilaginous part of the growth plate (or physis) and a thin layer of intramembranous bone (bone collar, bone bark or perichondrial ring of LaCroix) (Brighton1984, Schollmeier et al., 1999, Ayoub et al., 2015). They reported that the Ranvier and LaCroix zones were a single structure represented the thin layer of intramembranous bone at the periphery of the growth plate and metaphysis. The fibrous zones, Ranvier and LaCroix had rich blood supply from perichondrial arteries, contrasting with the naturally avascular hypertrophic zone of the fully developed growth plate (Brighton 1984). The principal effect of the periphysis was on the metaphyseal collar (Laval-Jeantet et al., 1968) the groove of Ranvier was wedge-shaped collection of cells which provided chondrocytes for the longitudinal growth of the growth plate or physis and the perichondrial ring (PR) provided mechanical support to it (Fenichel et al., 2006) Under the GR. there was appearance of a spur (or "step off"). It should not be mistaken for a child abuse fracture or a manifestation of rickets.
Elisa and Suma et al. (2020) hypothesized that young infants with histologicalfeatures of VDD/MBD present in the growth plate (Cohen et al., 2013, Oppenheimer et al., 1980. Scheimberg et al., 2014) would associate with a simultaneous thickening of the periphysis (groove of Ranvier and PR of LaCroix). They envistigated that phenomenon as a compensatory mechanism to stabilize the rib. Elisa and Suma etal.,2020 recorded that PM histological abnormalities at the rib growth plate in vitamin D deficiency (VDD) /metabolic bone disease (MBD) had been well defined by them (Cohen etal, 2013) and other reports (Oppenheimer et al., 1980 Scheimberg et al., 2014). Elisa and Suma et al. (2020) mentioned that the experimental studies demonstrated that the cells from the groove of Ranvier were pushed into the reserve and proliferative regions of the growth plate in order to supply cells for the reserve layer of chondrocytes. That caused an expansion of the diameter of the growth plate and served as cartilaginous stem cells, thereby playing a major role in endochondral ossification (Fenichel et al., 2006. Shapiro et al., 1977)
Elisa and Suma et al., 2020 reported that the Indian hedgehog (Ihh), member of the hedgehog family, was a key molecule coordinating those processes (Olsen et al., 2000 Try fonidou et al., 2010). Ihh was also part of the vitamin D pathway: it stimulated chondrocyte proliferation in the growth plate, it prevented chondrocyte hypertrophy and regulated bone formation in the perichondrial collar and in the trabecular bone below the growth plate (Olsen et al., 2000 Tryfonidou et al., 2010). Elisa and Suma et al. (2020) reported that, much attention had been given to describe how differentiation of the hypertrophic chondrocytes in the growth plate and the subsequent calcification of the matrix were impaired in VDD leading to the flaring of the ends of the long bones (Bikle 2012). The bone bark in foetuses could be identified after 10 weeks' gestational age, and at 16 weeks all the structures in the periphysis became clearly defined (Schollmeier et al., 1999).
Elisa and Suma et al. (2020) pointed that it should be noted that the bone bark of the PR was more prominent in the developing bones in which there was extensive metaphyseal remodelling (Shapiro et al., 1977).At the metaphyseal end, the intramembranous bone bark was overlaid by the periosteum and at the same time in intimate contact with the cartilage matrix underwent endochondral ossification in the zone of primary bone trabeculae (Schollmeier et al., 1999; Shapiro et al., 1977)
Elian and Suma eta., 2020reported that the membranous bone produced by the periphysis showed variation in thickness in different parts of the long bones (Schollmeier etal,1999) and could adopt the appearance of a spur (or "step off"). It should not be

mistaken for a child abuse fracture or be considered in itself a manifestation of rickets (Ostreich and, Ahmad 1992). The rapid bone growth and remodeling seemed to be responsible for subperiosteal new bone formation, a common finding in infants between 1 and 4 months of age (Kwon et al.,2002). During the active stage of rickets, the impaired mineralization of the zone of provisional calcification and the PR resulted in hypertrophy of the chondrocytes and accumulation of osteoid and unmineralised matrix, which could be exuberant in some infants. Elisa and Suma et al. (2020) mentioned that experimental studies in dogs had demonstrated VDD induced proliferation of exuberant cartilaginous tissue (Hjertquist 1961) and osteoid deposition in the exuberant cartilage and proliferation of capillary vessels in the healing stage of VDD could closely mimic a healing fracture to those unaware of that process. Similarly, the bone bark might be radiologically visible at the edge of the physis at the wrist of infants and children up to 12 ½ years and should not be mistaken for fracture or rickets (Oestreich, 2014; Elisa and Suma et al., 2020) Concluded that the histological changes in the perichondrial ring were significantly associated with histological changes of VDD /MBD at the rib growth plate with an Odds Ratio of 3.04.

Discussion of the morphological results

In the present work, morphological examination of the developing prenatal human 4th rib, at the ages of 4,5,6,7,9,months fetuses, showed that each rib had anterior and posterior end and a body forming the shaft (diaphysis). The posterior end had a tubercle and articular facet to articulate with the vertebra body, which developed with age progress till full term.

There were increase in size, length and thickness of the prenatal developing 4th rib with age progress.of. The Costal groove of the shaft was just seen very shallow at full term fetus. There was no twist in the 4th prenatal developing human ribs, and no twist in the second the prenatal developing human rib. There was twist in the 4th adult rib, and no twist in the second adult rib. That twist was to fit for the biomechanics characterization of rib geometry, for the supportive and respiratory functions of the ribs. The posterior tubercle of the prenatal developing human 4th ribs, appeared clear at 6 month aged fetus

Morphological examination of prenatal developing human Second rib, and fourth, showed the morphology and angle of the ages: 4,5,6,7 months aged fetuses, and full term

Each rib was formed of body shaft, vertebral posterior end, and anterior sternal end.

The lower border of the developing prenatal human fourth rib was sharper compared to the upper border. The sharpness increased with age progress.

Morphological examination of the adult second and fourth ribs, showed that each rib was formed of body shaft, anterior sternal end, and the posterior vertebral end. The posterior end consisted of head, tubercle, and neck. The rib angle of each rib was noted.

There was great twist in the 4th adult rib, and no twist in the second adult rib.

The lower border was sharper than the upper border in the fourth adult rib. The sub costal grove was clear.

The results of the present work were similar to bastir et al., 2013 who mentioned that adult-like thoracic shape was achieved early, by the end of the second postnatal year, with the circular cross-section of the newborn thorax transforming into the ovoid shape of adults; and that the ribs became inclined such that their anterior borders came to lie inferior to their posterior. They studied growth changes using geometric morphometrics applied to extensive landmark data taken from the ribcage. They digitized 402 (semi) landmarks on 3D reconstructions to assess growth changes in 27 computed tomography-scanned modern humans representing newborns to adults of both sexes. They found a curved ontogenetic trajectory, resulting from different ontogenetic growth allometries of upper and lower thoracic units. Adult thoracic morphology was achieved later than predicted, by diverse modifications in different anatomical regions during different ontogenetic stages. Besides a marked increase in antero-posterior dimensions. They noted an increase in medio-lateral dimensions of the upper thorax, relative to the lower thorax. That transformed the pyramidal infant thorax into the barrel-shaped one of adults. Rib descent was produced by complex changes in 3D curvature. Developmental differences between upper and lower thoracic regions related to differential timings and rates of maturation of the respiratory and digestive systems, the spine and the locomotor system. Their findings showed changes in the relative rates of growth of these systems and structures impacted on the development and evolution of modern human body shape.

Sandoz et al. (2013) defined the 3D geometry of children's rib cages: including sternum, ribs and costal cartilage. Three-dimensional reconstructions of 960 ribs, 518 costal cartilages and 113 sternebrae were performed on thoracic CT scans of 48 children, aged 4 months to 15 years. The geometry of the sternum was detailed and nine parameters were used to describe the ribs and rib cages. A "costal index" was defined as the ratio between cartilage length and whole rib length to evaluate the cartilage ratio for each rib level. For all children, the costal index decreased from rib level 1 to 3 and increased from level 3 to 7. For all levels, the cartilage accounted for 45-60 % of the rib length, and was longer for the first years of life. The mean costal index decreased by 21 % for subjects over 3-year old

compared to those under three ($p < 10(-4)$). The volume of the sternebrae was found to be highly age dependent. Such data could be useful to define the standard geometry of the pediatric thorax and help to detect clinical abnormalities.

Kindig et al. (2013) mentioned that, while a number of studies had quantified overall ribcage morphology (breadth, depth, and kyphosis/lordosis) and rib cross-sectional geometry in humans, few studies had characterized the centroidal geometry of individual ribs. In their study, a novel model was introduced to describe the centroidal path of a rib (i.e., the sequence of centroids connecting adjacent cross-sections) in terms of several physically-meaningful and intuitive geometric parameters. Surface reconstructions of rib levels 2-10 from 16 adult male cadavers (aged 31-75 years) were first extracted from CT scans, and the centroidal path was calculated in 3D for each rib using a custom numerical method. The projection of the centroidal path onto the plane of best fit (i.e., the "in-plane" centroidal path) was then modeled using two geometric primitives (a circle and a semiellipse) connected to give C1 continuity. Two additional parameters were used to describe the deviation of the centroidal path from that plane; further, the radius of curvature was calculated at various points along the rib length. That model was fit to each of the 144 extracted ribs, and average trends in rib size and shape with rib level were reported. In general, upper ribs (levels 2-5) had centroidal paths which were closer to circular, while lower ribs (levels 6-10) tended to be more elliptical; further the centroidal curvature at the posterior extremity was less pronounced for lower ribs. Lower ribs also tended to exhibit larger deviations from the best-fit plane. The rib dimensions and trends with subject stature were found to be consistent with findings previously reported in the literature. They reported that their model addressed a critical need in the biomechanics literature for the accurate characterization of rib geometry, and could be extended to a larger population as a simple and accurate way to represent the centroidal shape of human ribs.

The results of the present work agreed wth Standring et al., 2016 who reported that,the ribs were of elastic arches., each consisting of highly vascular trabecular bone containing large amount of red marrow enclosed in a thin layer of compact bone,the ribs articulated posteriorly with the vertebral column and front the greater part of thoracic skeleton. Their number might be increased by cervical or lumbar ribs or reduced by the absence of the twelfth pair. The first seven (true) ribs connected to the sternum by costal cartilages; whilst the remaining lower five false ribs either joined the superjacent costal cartilage (8-10)or float free at their anterior ends as relatively small and delicate structures tipped with cartilage (11-12).The tenth rib might also floated;the incidence varied from 35% to 70% depending on ancestry..They mentied that the costal element of the seventh cervical vertebra might be a mere epiphysias on its transverse process was long enough but more often it had neck and tubercle. When a shaft was present, it was of variable length and extended anterolaterally into the posterior triangle of the neck, where it might end or join the first rib,its costal cartilage or even the sternum. Cervical rib might be partly fibrous but its effects were not related to its osseous part. If it was long enough.its relations were those of first thoracic rib: the brachial plexus and sub clavian vessels were superior and apt to suffer compression in narrow angle between the rib and scalenus anterior.Hence. Cervical ribs might first be revealed by neurovascular symptoms. Particularly those caused by pressure on the eighth and first thoracic spinal nerves. A cervical rib (pleurapophysis) might show synostosis or diarthrosis with either the anterior (parapophysial) or the posterior (diapophysial) roots of the called seventh cervical transverse process or.more usually with both.

The results of the present work coincided with Beresheim et al. (2020) who mentioned that there was considerable variation in the gross morphology and tissue properties among the bones of human infants, children, adolescents, and adults. They used 18 known-age individuals ($n_{female} = 8$, $n_{male} = 9$, $n_{unknown} = 1$; birth to 21 years old), from a well-documented cemetery collection, Spitalfields Christ Church, London, UK, their study explored growth-related changes in cortical and trabecular bone microstructure. Micro-CT scans of mid-shaft middle thoracic ribs were used for quantitative analysis. Their results were compared to previously quantify conventional histomorphometry of the same sample. They found: Total area (Tt.Ar), cortical area (Ct.Ar), cortical thickness (Ct.Th), and the major (Maj.Dm) and minor (Min.Dm) diameters of the rib demonstrated positive correlations with age. Pore density (Po.Dn) increased, but age-related changes to cortical porosity (Ct.Po) appeared to be non-linear. Trabecular thickness (Tb.th) and trabecular separation (Tb.Sp) increased with age, whereas trabecular bone pattern factor (Tb.Pf), structural model index (SMI), and connectivity density (Conn.D) decreased with age. Sex-based differences were not identified for any of the variables included in their study. Some samples displayed clear evidence of diagenetic alteration without corresponding changes in radiopacity, which compromised the reliability of bone mineral density (BMD) data in the study of past populations. Cortical porosity data were not correlated with two-dimensional measures of osteon population density (OPD). They mentioned that unfilled resorption spaces

contributed more significantly to cortical porosity than dId the Haversian canals of secondary osteons. Continued research using complementary imaging techniques and a wide array of histological variables would increase the understanding of age- and sex-specific ontogenetic patterns within and among human populations.

However Schlager et al., (2022) reported that the morphology of the rib cage affected both the biomechanics of the upper body's musculoskeletal structure and the respiratory mechanics. That became particularly important when evaluating skeletal deformities, as in adolescent idiopathic scoliosis (AIS). They identifed morphological characteristics of the rib cage in relation to the lung in patients with non-deformed and scoliotic spines. Computed tomography data of 40 patients without any visible spinal abnormalities (healthy group) and 21 patients with AIS were obtained retrospectively. All bony structures as well as the right and left lung were reconstructed using image segmentation. Morphological parameters were calculated based on the distances between characteristic morphological landmarks. Those parameters included the rib position, length, and area, the rib cage depth and width, and the rib inclination angle on either side, as well as the spinal height and length. They determined the left and right lung volumes, and the area of contact between the rib cage and lung. Differences between healthy and scoliotic spines were statistically analysed using the t-test for unpaired data. They found that the rib cage of the AIS group was significantly deformed in the dorso-ventral and medio-lateral directions. The anatomical proximity of the lung to the ribs was nearly symmetrical in the healthy group. By contrast, within the AIS group, the lung covered a significantly greater area on the left side of the rib cage at large thoracic deformities. Within the levels T1-T6, no significant difference in the rib length, depth to width relationship, or area was observed between the healthy and AIS groups. Inferior to the lung (T7-T12), these parameters exhibited greater variability. The ratio between the width of the rib cage at T6 and the thoracic spinal height (T1-T12) was significantly increased within the thoracic AIS group (1.1 ± 0.08) compared with the healthy group (1.0 ± 0.05). No statistical differences were found between the lung volumes among all the groups. While the rib cage was frequently strongly deformed in the AIS group, the lung and its surrounding ribs appeared to be normally developed. The observed rib hump in AIS appeared to be formed particularly by a more ventral position of the ribs on the concave side. Furthermore, the rib cage width to spinal height ratio suggested that the spinal height of the thoracic AIS-spine was reduced. They concluded that their results indicated that the spine would gain its growth-related height after correcting the spinal deformity. Those were the important aspects to consider in the etiology research and orthopaedic treatment of AIS.

Summary and Conclusion

In the present study histological examination of serials sections TS and LS, of parts of perenatal developing human 4th rib, at the costochondral junction CCJ,showed changes with age progress in the structures found in the rib; cartilage growth plate,cartilage vascular canals, (primary and secondary ossification centers **POC-SOC**) - sponge bone, the perichondrium, the periosteum, grove of Ranvier GR,andsubperiosteal bone collar SPBC.

References

[1]. Agrawal, P., Atre, P. R. & Kulkarni, D. S. (1984). The role of cartilage canals in the ossification of the talus. Acta anatomica 119, 238-240.

[2]. Agrawal, P., Kulkarni, D. S. & Atre, P. R. (1986). The participation of cartilage canals in the ossification of the human fetal calcaneum. Journal of Anatomy 147, 135-142.

[3]. Andreoli S M · Mills J C, Kilpatrick L A, White D R, Patel. 2013 K GCT measured normative cartilage growth in children requiring costochondral grafting Otolaryngol Head Neck Surg

[4]. Allerstorfer, D., Longato, S., Schwarzer, C., Fischer-Colbrie, R., Hayman, A.R., Blumer, M.J., 2010. VEGF and its role in the early development of the long bone epiphysis.J. Anat. 216, 611–624.

[5]. Alvarez, J., Costales, L., Serra, R., Balbin, M., Lopez, J.M., 2005. Expression patternsof matrix metalloproteinases and vascular endothelial growth factor duringepiphyseal ossification. J. Bone Miner. Res. 20, 1011–1021. Aszodi, A.,

[6]. Ayoub D, Hyman Ch J. Misinterpretation of birth-related growth plate injuries as classic metaphyseal lesions. Letter to the editor. J Forensic Radiol Imaging 2015; 3: 67-9.

[7]. Bastir M , García Martínez D, Recheis W, Barash A, Coquerelle M, Rios L, Peña- A Melián, F García Río, P O'Higgins 2013 Differential growth and development of the upper and lower human thorax eCollection Sep 20;8(9):e75128.- doi: 10.1371/journal.pone.0075128. 2013PMID: 24073239 PMCID: PMC3779208 DOI: 10.1371/journal.pone.0075128

[8]. Beresheim A C , Pfeiffer S· Grynpas M2020: Ontogenetic changes to bone microstructure in

an archaeologically derived sample of human ribs J Anat Mar;236(3):448-462.doi: 10.1111/joa.13116. Epub 2019 Nov 15. PMID: 31729033- PMCID: PMC7018627- D OI: 10.1111/joa.13116

[9]. Bikle 2012DD Vitamin D and bone. Curr Osteoporos Rep.; 10: 151–9.

[10]. Blumer, M.J., 2021 Bone tissue and histological and molecular events during development of the long bones Annals of Anatomy 235 (2021) 151704 Contents lists available at ScienceDirec

[11]. Blumer, M.J., Fritsch, H., Pfaller, K., Brenner, E., 2004a. Cartilage canals in the chickenembryo: ultrastructure and function. Anat. Embryol. (Berl.) 207, 453–462.

[12]. Blumer, M.J., Longato, S., Fritsch, H., 2004b. Cartilage canals in the chicken embryoare involved in the process of endochondral bone formation within the epiphy-seal growth plate. Anat. Rec. A 279, 692–700.

[13]. Blumer, M.J., Longato, S., Richter, E., Perez, M.T., Konakci, K.Z., Fritsch, H., 2005. Therole of cartilage canals in endochondral and perichondral bone formation: arethere similarities between these two processes? J. Anat. 206, 359–372.

[14]. Blumer, M.J., Schwarzer, C., Perez, M.T., Konakci, K.Z., Fritsch, H., 2006. Identificationand location of bone-forming cells within cartilage canals on their course intothe secondary ossification centre. J. Anat. 208, 695–707.

[15]. Blumer, M.J., Longato, S., Schwarzer, C., Fritsch, H., 2007. Bone development in thefemoral epiphysis of mice: the role of cartilage canals and the fate of restingchondrocytes. Dev. Dyn. 236, 2077–2088.

[16]. Blumer, M.J., Longato, S., Fritsch, H., 2008a. Localization of tartrate-resistant acidphosphatase (TRAP), membrane type-1 matrix metalloproteinases (MT1-MMP)and macrophages during early endochondral bone formation. J. Anat. 213,431–441.

[17]. Blumer, M.J., Longato, S., Fritsch, H., 2008b. Structure, formation and role of cartilagecanals in the developing bone. Ann. Anat. 190, 305–315.

[18]. Blumer, M.J., Hausott, B., Schwarzer, C., Hayman, A.R., Stempel, J., Fritsch, H., 2012.Role of tartrate-resistant acid phosphatase (TRAP) in long bone development.Mech. Dev. 129, 162–176.

[19]. Bots J, Wijnaendts L C D, DelenS, Dongen S V, HeikinheimoK, and GalisF 2011Analysis of cervical ribs in a series of human fetuses Published online 2011 Jun 20. doi: 10.1111/j.1469-7580..01400.x PMCID: PMC3171776 PMID: 21689099

[20]. BOROCHOWITZ, Z., ORNOY, A., LACHMAN, R. & RIMOIN, D. L. (1986). Achondrogenesis II-hypochondrogenesis: Variability versus heterogeneity. American Journal of Medical Genetics 24, 273

[21]. Brighton, CT 1984 The growth plate. Orthop Clin North Am 15:571-595)

[22]. Carlevaro, M.F., Cermelli, S., Cancedda, R., Descalzi Cancedda, F., 2000. Vascularendothelial growth factor (VEGF) in cartilage neovascularization and chondro-cyte differentiation: auto-paracrine role during endochondral bone formation.J. Cell Sci. 113 (Pt 1), 59–69.

[23]. Carpio, L.R., Bradley, E.W., Westendorf, J.J., 2017. Histone deacetylase 3 suppressesErk phosphorylation and matrix metalloproteinase (Mmp)-13 activity in chon-drocytes. Connect. Tissue Res. 58, 27–36.

[24]. Claassen H, Kampen WU, Kirsch T Localization of type I and II collagen during development of human first rib cartilage..Anat Embryol (Berl). 1995 Oct;192(4):329-34. doi: 10.1007/BF00710102.PMID: 8554166

[25]. Chappard, D., Alexandre, C., Riffat, G., 1986. Uncalcified cartilage resorption inhuman fetal cartilage canals. Tissue Cell 18, 701–707. PMID: 3775757 DOI: 10.1016/0040-8166(86)90071-6

[26]. Cohen MC, Offiah A, Sprigg A, Al-Adnani M. 2013Vitamin D deficiency and sudden unexpected death in infancy and childhood: a cohort study. Pediatr Dev Pathol.;16:292-300

[27]. Cole, A.A., Cole, M.B., 1989. Are perivascular cells in cartilage canals chondrocytes?J. Anat. 165, 1–8.

[28]. Cole, A.A., Wezeman, F.H., 1985. Perivascular cells in cartilage canals of the devel-oping mouse epiphysis. Am. J. Anat. 174, 119–129.

[29]. Copray jvcm, Jansen HWB, Duterloo HS (1986) Growth and growth pressure of mandibular condylar and some primary cartilages of the rat in vitro. American Journal of Orthodontics and Dentofacial Orthopedics 90, 19-28

[30]. Craatz S, Weiss J, Schmidt W 1999 Histologic-histochemical and immunocytochemical investigations of cartilage canals in human rib cartilage] Ann Anat1999 Jul;181(4):359-63.. PMID: 10427373DOI: 10.1016/s0940-9602(99)80128-9

[31]. da Cunha Castro EC, Popek EJ. 2006Osteochondral junction lesions in stillborn fetuses and their relationship to autopsy diagnoses. Early Hum Dev; 83: 593-9
[32]. Daniels S, Ellis E, Carlson DS (1987) Histological analysis of costochondral and sternoclavicular grafts in the TMJ of the juvenile monkey. Journal of Oral and Maxillofacial Surgery 45, 675-682.
[33]. Davoli, M.A., Lamplugh, L., Beauchemin, A., Chan, K., Mordier, S., Mort, J.S., Mur-phy, G., Docherty, A.J., Leblond, C.P., Lee, E.R., 2001. Enzymes active in the areasundergoing cartilage resorption during the development of the secondary ossifi-cation center in the tibiae of rats aged 0–21 days: II. Two proteinases, gelatinaseB and collagenase-3, are implicated in the lysis of collagen fibrils. Dev. Dyn. 222,71–88.
[34]. Dean, M.N., Summers, A.P., 2006. Mineralized cartilage in the skeleton of chon-drichthyan fishes. Zoology (Jena) 109, 164–168.
[35]. Di Giancamillo, A., Andreis, M.E., Taini, P., Veronesi, M.C., Di Giancamillo, M., Modina, S.C., 2016. Cartilage canals in newborn dogs: histochemical and immunohisto-chemical findings. Eur. J. Histochem. 60, 2701.
[36]. Drury R.A.B. and Wallington EA (1980): Carlatons's Histological Technique. 5th edition. Published by Oxford University press, London, New York, Toronto.
[37]. Dupuis, H., Pest, M.A., Hadzic, E., Vo, T.X., Hardy, D.B., Beier, F., 2019. Exposure to theRXR agonist SR 11237 in early life causes disturbed skeletal morphogenesis in arat model. Int. J. Mol. Sci. 20, 5198.
[38]. Elisa G, Suma U, Vázquez Z, Zainun R,Khairu C, Marta C 2020 Abnormalities of the rib growth plate and the periphysis of previously healthy infants and toddlers dying suddenly and unexpectedly medRxiv preprint doi: https://doi.org/10.1101/2020.06.25.20139832; this version posted June 26, 2020.
[39]. Emery JL. 1967:Evidence from bone growth that most of the infants dying in the neonatal period had been ill before birth. Acta Paediatrica Scandinavica; 172: 55-9.
[40]. Emery JL Kalpaktsoglou PK. 1967: The costochondral junction during later stages of intrauterine life, and abnormal growth patterns found in association with perinatal death. Arch Dis Child; 42:1-13.
[41]. El Rakawy M T:1971: illustrative Histology FIRST edition Printed in UNITED Abab republics
[42]. EL Rakhawy (2000): Illustrative anatomy thorax and heart. The Mew Egyptian Office. Cairo, Egypt.
[43]. Erenpreisa, J., Roach, H.I., 1998. Aberrant death in dark chondrocytes of the aviangrowth plate. Cell Death Differ. 5 (1), 60–66.
[44]. Eslaminejad, M.R., Valojerdi, M.R., Yazdi, P.E., 2006. Computerized three-dimensional reconstruction of cartilage canals in chick tibial chondroepiphysis.Anat. Histol. Embryol. 35, 247–252.
[45]. Farnum, C.E., Wilsman, N.J., 1989. Condensation of hypertrophic chondrocytes at the chondro-osseous junction of growth plate cartilage in Yucatan swine: relation-ship to long bone growth. Am. J. Anat. 186, 346–358.
[46]. Fenichel I, Evron Z, Nevo Z. 2006The perichondrial ring as a reservoir for precartilaginous cells. In vivo model in young chicks' epiphysis. Int Orthop.;30:353-6.
[47]. Franz-Odendaal, T.A., Hall, B.K., Witten, P.E., 2006. Buried alive: how osteoblastsbecome osteocytes. Dev. Dyn. 235, 176–190.
[48]. Gelabert H A , JaboriS , BarlebenA , Kiang S , O'Connell J , Jimenez J C , Derubertis B , Rigberg D 2014Regrown first rib in patients with recurrent thoracic outlet syndrome Ann Vasc Surg May;28(4):933-8. doi: 10.1016/j.avsg.2014.01.004. Epub 2014 Jan 23. PMID: 24462650 DOI: 10.1016/j.avsg.2014.01.004
[49]. Gerber, H.P., Vu, T.H., Ryan, A.M., Kowalski, J., Werb, Z., Ferrara, N., 1999. VEGF couples hypertrophic cartilage remodeling, ossification and angiogenesis during endochondral bone formation. Nat. Med. 5, 623–628.
[50]. Gilroy AM2013: Anatomy an Essential Textbook.Thieme.New York: Stuttgart
[51]. Copray JVCM, Jansen HWB, Duterloo HS (1986) Growth and growth pressure of mandibular condylar and some primary cartilages of the rat in vitro. American Journal of Orthodontics and Dentofacial Orthopedics 90, 19-28
[52]. Gruber, H. E. & Rimoin, D. L. (1989). Quantitative histology of cartilage cell columns in the human costochondral junction: Findings in newborn and pediatric subjects. Pediatric Research 25, 202-204
[53]. Gruber H E , LachmanR S , Rimoin D L 1990: Quantitative histology of cartilage vascular canals in the human rib. Findings in normal neonates and children and in achondrogenesis II-hypochondrogenesis J Anat Dec;173:69-75. PMID: 2074231 PMCID: PMC1256081

[54]. Harten, Van Der, H. J., Brons, J. T. J., Dukstra, P. F., Niermeyer, M. F., Meijer, C. J. L. & Van Gien, H. P. (1988). Achondrogenesis-hypochondrogenesis: The spectrum of chondrogenesis imperfecta. A radiological, ultrasonographic and histopathologic study of 23 cases. Pediatric Pathology 8, 571-597.

[55]. Hansen, H., 2003.MT1-MMP-dependent, apoptotic remodeling of unmineralized cartilage: a crit-ical process in skeletal growth. J. Cell Biol. 163, 661–671.

[56]. Hansen, H., 2003.MT1-MMP-dependent, apoptotic remodeling of unmineralized cartilage: a crit-ical process in skeletal growth. J. Cell Biol. 163, 661–671.

[57]. Harris HA, Russel AE 1933 The atypical growth in cartilage as a fundamental factor in dwarfism and achondroplasia. Proc R Soc Med 26:779-787

[58]. Heffez L, doku hc (1984) The Goldenhar syndrome: diagnosis and early surgical management. Oral Surgery 58, 2-9.

[59]. Hayman, A.R., Cox, T.M., 2003. Tartrate-resistant acid phosphatase knockout mice.J. Bone Miner. Res. 18, 1905–1907.

[60]. Hayman, A.R., Jones, S.J., Boyde, A., Foster, D., Colledge, W.H., Carlton, M.B., Evans,M.J., Cox, T.M., 1996. Mice lacking tartrate-resistant acid phosphatase (Acp 5)have disrupted endochondral ossification and mild osteopetrosis. Development122, 3151–3162.

[61]. Holmbeck, K., Bianco, P., Chrysovergis, K., Yamada, S., Birkedal- Hjertquist S-O Biophysical studies of epiphyseal growth zones and adjacent compact bone tissue in normal and rachitic dogs and rats. Acta Societatis Medicorum =Upsaliensis 1961; LXVI: 3-4: 202-16

[62]. Holmbeck, K., Bianco, P., Caterina, J., Yamada, S., Kromer, M., Kuznetsov, S.A., Mankani, M., Robey, P.G., Poole, A.R., Pidoux, I., Ward, J.M., Birkedal-Hansen, H., 1999. MT1- MMP-deficient mice develop dwarfism, osteopenia, arthritis, andconnective tissue disease due to inadequate collagen turnover. Cell 99, 81–92.

[63]. Hunziker, E.B., Kapfinger, E., Geiss, J., 2007. The structural architecture of adult mam-malian articular cartilage evolves by a synchronized process of tissue resorptionand neoformation during postnatal development. Osteoarthritis Cartilage 15,403–413.

[64]. Hellings, I.R., Dolvik, N.I., Ekman, S., Olstad, K., 2017. Cartilage canals in the distalintermediate ridge of the tibia of fetuses and foals are surrounded by differenttypes of collagen. J. Anat. 231, 615–625.

[65]. Hollberg, K., Hultenby, K., Hayman, A., Cox, T., Andersson, G., 2002. Osteoclasts frommice deficient in tartrate-resistant acid phosphatase have altered ruffled bordersand disturbed intracellular vesicular transport. Exp. Cell Res. 279, 227–238.

[66]. Hollberg, K., Nordahl, J., Hultenby, K., Mengarelli-Widholm, S., Andersson, G., Reinholt, F.P., 2005. Polarization and secretion of cathepsin K precede tartrate-resistant acid phosphatase secretion to the ruffled border area during theactivation of matrix-resorbing clasts. J. Bone Miner. Metab. 23, 441–449.

[67]. HORTON, W. A., MACHADO, M. A., CHOU, J. W. & CAMPBELL, D. (1987). Achondrogenesis type II: Abnormalities of extracellular matrix. Pediatric Research 22, 324-329.

[68]. Hunziker, E.B., Lippuner, K., Shintani, N., 2014. How best to preserve and reveal thestructural intricacies of cartilaginous tissue. Matrix Biol. 39, 33–43.

[69]. Jaramilloiego D, Olga L. Villegas-Medina, David K. Doty[2], Roberto Rivas

[70]. American Journal of RoentgenologFebruary 2004, VOLUME 182 NUMBER 2

[71]. Kampen W U ˙ ClaassenH , Kirsch T 1995:Mineralization and osteogenesis in the human first rib cartilage Ann Anat - Mar;177(2):171-7. doi: 10.1016/s0940-9602(11)80069-5.

[72]. Kang S -S , GuoY , Zhang D , Jiang D –Y 2015 Rib Cartilage Assessment Relative to the Healthy Ear in Young Children with Microtia Guiding Operative Timing Chin Med J (Engl) Aug 20;128(16):2208-14. doi: 10.4103/0366-6999.162505 PMID: 26265615

[73]. Kember NF, Sissons HA 1976 Quantitative histology of the human growth plate. J Bone Joint Surg 58B:426-434),

[74]. Kindig WM, Kent R W. 2013 Characterization of the centroidal geometry of human ribs J Biomech Eng-Nov;135(11):111007.-doi: 10.1115/1.4025329.-MID: 24008370

[75]. Kuettner, K.E., Pauli, B.U., 1983. Vascularity of cartilage. In: Hall, B.K. (Ed.), Cartilage Volume 1: Structure, Function, and Biochemistry. Academic Press, New York,

[76]. Kugler, J.H., Tomlinson, A., Wagstaff, A., Ward, S.M., 1979. The role of cartilage canalsin the formation of secondary centres of ossification. J. Anat. 129, 493–506.

[77]. Kwon DS, Spevak MR, Fletcher K, Kleinman PK. 2002; Physiologic Subperiosteal New

Bone Formation: Prevalence, Distribution, and Thickness in Neonates and Infants. AJR:179: 985-8

[78]. Laval-Jeantet M, Balmain N, Juster M, Berbard J. 1968 [Relations of the perichondrial ring to the cartilage in normal and in pathological growth). Ann Radiol, 11:327-35.

[79]. Lui, J.C., Nilsson, O., Baron, J., 2014. Recent research on the growth plate: recentinsights into the regulation of the growth plate. J. Mol. Endocrinol. 53, T1–T9.

[80]. Lutfi, A.M., 1970. Mode of growth, fate and functions of cartilage canals. J. Anat. 106,135–145.

[81]. Marconi, A., Hancock-Ronemus, A., Gillis, J.A., 2020. Adult chondrogenesis and spontaneous cartilage repair in the skate, Leucoraja erinacea. Elife 12 (9), e53414, http://dx.doi.org/10.7554/eLife.53414.

[82]. Maruyama, Z., Yoshida, C.A., Furuichi, T., Amizuka, N., Ito, M., Fukuyama, R., Miyazaki, T., Kitaura, H., Nakamura, K., Fujita, T., Kanatani, N., Moriishi, T., Yamana, K., Liu, W., Kawaguchi, H., Nakamura, K., Komori, T., 2007. Runx2 determines bonematurity and turnover rate in postnatal bone development and is involved inbone loss in estrogen deficiency. Dev. Dyn. 236, 1876–1890.

[83]. Melton, J.T., Clarke, N.M., Roach, H.I., 2006. Matrix metalloproteinase-9 induces theformation of cartilage canals in the chondroepiphysis of the neonatal rabbit. J.Bone Joint Surg. Am. 88 (Suppl. 3), 155–161.

[84]. Minkin, C., 1982. Bone acid phosphatase: tartrate-resistant acid phosphatase as amarker of osteoclast function. Calcif. Tissue Int. 34, 285–290.

[85]. Nakashima, K., de Crombrugghe, B., 2003. Transcriptional mechanisms in osteoblastdifferentiation and bone formation. Trends Genet. 19, 458–466.

[86]. Nefussi, J.R., Sautier, J.M., Nicolas, V., Forest, N., 1991. How osteoblasts becomeosteocytes: a decreasing matrix forming process. J. Biol. Buccale 19, 75–82.

[87]. Oppenheimer SJ, Snodgrass JAI1980. Neonatal rickets: Histopathology and quantitative bone changes. Arch Dis Child; 55:945-9

[88]. Olsen BR, Reginato AM, Wang W. Bone development. Annu Rev Cell dev Biol 2000; 16: 191-220.

[89]. Ostreich AE, Ahmad BS. 1992The periphysis and its effect on the metaphysis: I. Definition and normal radiographic pattern. Skeletal Radiol; 21: 283-6.

[90]. Oestreich, A.E. 2014 The metaphyseal collar is also present until later childhood and at birth. Pediatr Radiol; 44, 1046–7

[91]. Omar, I., Guterman-Ram, G., Rahat, D., Tabach, Y., Berger, M., Levaot, N., 2018. Schlafen2 mutation in mice causes an osteopetrotic phenotype due to a decreasein the number of osteoclast progenitors. Sci. Rep. 8, 13005.

[92]. Park, E. A. (1954). The influence of severe illness on rickets. Arch. Dis. Childh., 29, 369.

[93]. Park, J., Gebhardt, M., Golovchenko, S., Perez-Branguli, F., Hattori, T., Hartmann,C., Zhou, X., deCrombrugghe, B., Stock, M., Schneider, H., von der Mark,K., 2015. Dual pathways to endochondral osteoblasts: a novel chondrocyte-derived osteoprogenitor cell identified in hypertrophic cartilage. Biol. Open 4,608–621.

[94]. Peltomäki T 1994 Histologic structure of human costochondral junction- Plast Reconstr Surg Oct;94(5):585-8. doi: PMID: 7938280 DOI: 10.1097/00006534-199410000-00003

[95]. Peltomaki T (1992) Growth of a costochondral graft in the rat temporomandibular joint. Journal of Oral and Maxillofacial Surgery 50, 851-857.

[96]. Peltomaki T, Isotupa K (1991) The costochondral graft: a solution or a source of facial asymmetry in growing children. A case report. Proceedings ofthe Finnish Dental Society 87, 167-176.

[97]. Peltomaki T, Ronning 0 (1991) Interrelationship between size and tissue-separating potential of costochondral transplants. European Journal of Orthodontics 13, 459-465.

[98]. Petersen, W., Tsokos, M., Pufe, T., 2002. Expression of VEGF121 and VEGF165 in hypertrophic chondrocytes of the human growth plate and epiphyseal cartilage.J. Anat. 201, 153–157.

[99]. Politis C, Fosion E, Bossuyt M (1987) The use of costochondral grafts in arthroplasty of the temporomandibular joint. Journal of Craniomaxillofacial Surgery 15, 345-354.

[100]. Poswillo (1974) Experimental reconstruction of the mandibular joint. International Journal of Oral Surgery 3, 400-411.

[101]. Poswillo D (1987) Biological reconstruction of the mandibular condyle. British

[102]. Poole, K.E., van Bezooijen, R.L., Loveridge, N., Hamersma, H., Papapoulos, S.E., Löwik, C. W., Reeve, J., 2005. Sclerostin is a delayed secreted product of osteocytes that inhibits bone formation. FASEB J. 19, 1842–1844.

[103]. Proff, P., Romer, P., 2009. The molecular mechanism behind bone remodelling: a review. Clin. Oral Investig. 13, 355–362.
[104]. Roach, H.I., 1997. New aspects of endochondral ossification in the chick: chondro-cyte apoptosis, bone formation by former chondrocytes, and acid phosphatase activity in the endochondral bone matrix. J. Bone Miner. Res. 12, 795–805.
[105]. Roach, H.I., 2000. Bone anatomy and cell biology. Reviews of the European Calcified Tissue Society. http://www.ectsoc.org/reviews/011 roac.htm, 1/13-13/13.
[106]. Roach, H.I., Clarke, N.M., 1999. "Cell paralysis" as an intermediate stage in the programmed cell death of epiphyseal chondrocytes during development. J. Bone Miner. Res. 14, 1367–1378.
[107]. Roach, H.I., Baker, J.E., Clarke, N.M., 1998. Initiation of the bony epiphysis in longbones: chronology of interactions between the vascular system and the chon-drocytes. J. Bone Miner. Res. 13, 950–961.
[108]. Roach, H.I., Mehta, G., Oreffo, R.O., Clarke, N.M., Cooper, C., 2003. Temporal analysisof rat growth plates: cessation of growth with age despite presence of a physis.J. Histochem. Cytochem. 51, 373–383.
[109]. Romanes G.J. (2000):Cunningham, S. Manual of Practical Anatomy volume. Fifteenth Edition, Oxford University Press Walton Sreet, Oxford Ox.
[110]. Salhotra, A., Shah, H.N., Levi, B., Longaker, M.T., 2020. Mechanisms of bone develop-ment and repair. Nat. Rev. Mol. Cell Biol. 21, 696–711.
[111]. Scheimberg I, Perry L 2014Does Low Vitamin D Have a Role in Pediatric Morbidity and mortality? An Observational Study of Vitamin D in a Cohort of 52 Postmortem.Examinations. Pediat Develop Pathol.; 17:455–64
[112]. Shapiro F, Holtrop ME, Glimcher MJ. 1977 Organisation and cellular biology of the perichondrial ossification groove of ranvier: a morphological study in rabbits. J Bone Joint Surg Am.; 59:703-23.
[113]. Sandoz B, Badina A, Laporte S, Lambot K, Mitton D, Skalli W 2013 Quantitative geometric analysis of rib, costal cartilage and sternum from childhood to teenagehood doi: 10.1007/s11517-013-1070-5 Sep;51(9):971-9. Med Biol Eng Comput -Epub 2013 Apr 7. PMID: 23564222
[114]. Sadler TW (2012):Lang man Medical Embryology 12th edition. Wolters Kluwer, Lippincott Williams &Wikins.
[115]. Sadler TW (2019):Langman Medical Embryology 14th edition. Wolters Kluwer
[116]. Seidel, R., Lyons, K., Blumer, M., Zaslansky, P., Fratzl, P., Weaver, J.C., Dean, M.N., 2016.Ultrastructural and developmental features of the tessellated endoskeleton ofelasmobranchs (sharks and rays). J. Anat. 229, 681–702.
[117]. Seidel, R., Blumer, M., Pechriggl, E.J., Lyons, K., Hall, B.K., Fratzl, P., Weaver, J.C., Dean, M.N., 2017. Calcified cartilage or bone? Collagens in the tessellated endoskele-tons of cartilaginous fish (sharks and rays). J. Struct. Biol. 200, 54–71.
[118]. SHAPIRO, I. M. & BOYDE, A. (1987). Mineralization of normal and rachitic chick growth cartilage: vascular canals, cartilage calcification and osteogenesis. Scanning Microscopy 1, 599-606.
[119]. Shapiro, F., 1998. Epiphyseal and physeal cartilage vascularization: a light micro-scopic and tritiated thymidine autoradiographic study of cartilage canals innewborn and young postnatal rabbit bone. Anat. Rec. 252, 140–148.
[120]. Shapiro, I.M., Adams, C.S., Freeman, T., Srinivas, V., 2005. Fate of the hypertrophicchondrocyte: microenvironmental perspectives on apoptosis and survival in theepiphyseal growth plate. Birth Defects Res. C 75, 330–339.
[121]. Schlager B, Krump F, Boettinger J, Jonas R, Liebsch C , M Ruf, Meinrad Beer, Hans-Joachim Wilke 2022 Morphological patterns of the rib cage and lung in the healthy and adolescent idiopathic scoliosis J Anat Jan;240(1):120-130. doi: 10.1111/joa.13528. Epub 2021 Aug 3. PMID: 34346505 - PMCID: PMC8655162 DOI: 10.1111/joa.135 28
[122]. Schollmeier G, Uhthoff H-K, Lewandrowski KU, Fukuhara K. Role of bone bark during growth in width of tubular bones. Clin Orthop Relat Res. 1999; 367:291-9.
[123]. Seinsheimer F, Sledge CB (1981) Parameters of longitudinal growth rate in rabbit epiphyseal growth plates. Journal of Bone and Joint Surgery 63(A), 627-630.
[124]. Shimomura Y, Wezeman Fh, Ray RD (1973) The growth cartilage plate of the rat rib: cellular differentiation. Clinical Orthopaedics and Related Research 90, 246-254.
[125]. sillence, d. o., horton, w. a. & rimoin, d. l. (1979). Morphologic studies in the skeletal

dysplasias. American Journal of Pathology 96, 813-870.
[126]. SIEGEL S (1958) Biological survival and growth of cartilage grafts. Plastic and Reconstructive Surgery 22, 11-28.
[127]. Silberberg, M., Silberberg, R. 1940The effect of thyroidectomy and administration of anterior pituitary extract of cattle on the growth of cartilage and bone of immature guinea pigs. Am J Pathol; 16 (4): 505-24.
[128]. Sillence, D. O., Horton, W. A. & Rimoin, D. L. (1979). Morphologic studies in the skeletal dysplasias. American Journal of Pathology 96, 813-870.
[129]. Shapiro, I. M. & Boyde, A. (1987). Mineralization of normal and rachitic chick growth cartilage: vascular canals, cartilage calcification and osteogenesis. Scanning Microscopy 1, 599-606.
[130]. Shimomura Y, Wezeman FH, Ray rd (1973) The growth cartilage plate of the rat rib: cellular differentiation. Clinical Orthopaedics and Related Research 90, 246-254.
[131]. Stempel, J., Fritsch, H., Pfaller, K., Blumer, M.J., 2011. Development of articular car-tilage and the metaphyseal growth plate: the localization of TRAP cells, VEGF,and endostatin. J. Anat. 218, 608–618. Tryfonidou MA, Hazewinkel HAW, Riemers FM et al. 2010 Intraspecies disparity in growth rate is associated with differences in expression of local growth plate regulators. Am J Physiol Endocrinol Metab; 299: E1044 –52.
[132]. Standring S et al., 2016: Grays Anatomy. The anatomical basis of clinical practice. 41 Edition. Elsevier
[133]. Streeter,G.L.(1920):Weight,sitting,height,head size, foot length and menstrual age of the human embryo.Contrib Embryol 11:143-170.
[134]. Streeter.G I (1949):Development a horizons in human embryos.(Forth issue).A review of histogenesis of cartilage and bone. Contrib. embryo carnegic Instt.Washington.33:149-167
[135]. Stickens, D., Behonick, D.J., Ortega, N., Heyer, B., Hartenstein, B., Yu, Y., Fosang, A.J., Schorpp-Kistner, M., Angel, P., Werb, Z., 2004. Altered endochondral bone development in matrix metalloproteinase 13-deficient mice. Development 131,5883–5895.
[136]. Tsang, K.Y., Chan, D., Cheah, K.S., 2015. Fate of growth plate hypertrophic chondro-cytes: death or lineage extension? Dev. Growth Differ. 57, 179–192.
[137]. Umlauf et al., 2010 Dec;67(24):4197-211. Cartilage biology, pathology, and repair Cell Mol Life Sci- doi: 10.1007/s00018-010-0498-0. Epub 2010 Aug 25. PMID: 20734104 DOI: 10.1007/s00018-010-0498-0
[138]. Vu, T.H., Shipely, J.M., Bergers, G., Berger, J.E., Helms, J.A., Hanahan, D., Shapiro,S.D., Senior, R.M., Werb, Z., 1998. MMP-9/gelatinase B is a key regulator ofgrowth plate angiogenesis and apoptosis of hypertrophic chondrocytes. Cell 93,411–422.
[139]. Wang, Y., Menendez, A., Fong, C., ElAlieh, H.Z., Kubota, T., Long, R., Bikle, D.D., 2015.IGF-I signaling in osterix-expressing cells regulates secondary ossification cen-ter formation, growth plate maturation, and metaphyseal formation duringpostnatal bone development. J. Bone Miner. Res. 30, 2239–2248.
[140]. Weigele, J., Franz-Odendaal, T.A., 2016. Functional bone histology of zebrafishreveals two types of endochondral ossification, different types of osteoblastclusters and a new bone type. J. Anat. 229, 92–103.
[141]. Wilsman, N. J. & Van sickle, D. C. (1970). The relationship of cartilage canals to the initial osteogenesis of secondary centers of ossification. Anatomical Record 168, 381-392.
[142]. Wilsman, N.J., Van Sickle, D.C., 1972. Cartilage canals, their morphology and distribution. Anat. Rec. 173, 79–93.
[143]. Yang SS, Kitchen E, Gilbert EF, Rimoin DL 1986 Histopathologic examination in osteochondrodysplasia: time for standardization. Arch Pathol Lab Med 110:lO-12
[144]. Yang, L., Tsang, K.Y., Tang, H.C., Chan, D., Cheah, K.S., 2014. Hypertrophic chondro-cytes can become osteoblasts and osteocytes in endochondral bone formation.Proc. Natl. Acad. Sci. U. S. A. 111, 12097–12102.
[145]. Yan H · ZhangG· LiuW· Wang N· Z Liu 2019 Different Methods of Fabricating Cartilaginous Ear Framework in Children With Microtia According to the Length of the Eighth Costal Cartilage Intraoperatively. J Craniofac Surg Jul;30(5):1425-1429.
[146]. PMID: 31299736
DOI: 10.1097/SCS.0000000000005282
[147]. Yousefzadeh D K, Doerger K, Sullivan C 2008": The bloo d supply of early, late, and nonossifying cartilage: preliminary gray-scale and Doppler assessment and their implications Pediatr Radiol Feb;38(2):146-58. PMID: 18040678 DOI: 10.1007/s00247-007-0655-2. Epub 2007 Nov 27.
[148]. Zelzer, E., McLean, W., Ng, Y.S., Fukai, N., Reginato, A.M., Lovejoy, S., D'Amore, P.A., Olsen, B.R., 2002. Skeletal defects in VEGF

(120/120) mice reveal multiple rolesfor VEGF in skeletogenesis. Development 129, 1893–1904.
[149]. Zelzer, E., Mamluk, R., Ferrara, N., Johnson, R.S., Schipani, E., Olsen, B.R., 2004. VEGFAis necessary for chondrocyte survival during bone development. Development131, 2161–2171.
[150]. Zhou, Z., Apte, S.S., Soininen, R., Cao, R., Baaklini, G.Y., Rauser, R.W., Wang, J., Cao, Y., Tryggvason, K., 2000. Impaired endochondral ossification and angiogenesis inmice deficient in membrane-type matrix metalloproteinase I. Proc. Natl. Acad.Sci. U. S. A. 97, 4052–4057.
[151]. Zhou, X., von der Mark, K., Henry, S., Norton, W., Adams, H., de Crombrugghe, B., 2014.Chondrocytes transdifferentiate into osteoblasts in endochondral bone duringdevelopment, postnatal growth and fracture healing in mice. PLoS Genet. 10(12), 1–20.

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