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CALCIUM LEVELS IN THE DEVELOPING TALI OF HUMAN FETUSES

[1]*Fazal-ur-Rehman and [2]Sheeba Nuzhat Khan

[1]Department of Anatomy, JNMC, A.M.U., Aligarh-202002, India
[2]Department of Kulliyat, A.K.T.C., AMU, Aligarh 202002, India

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ABSTRACT

The mineral component is the main constituent of bone and is important as it confers much of the hardness and rigidity to bone. Among all mineral, calcium is the major determinant of its mechanical properties. In the present study human fetal tali have been analyzed for calcium concentration, information is lacking in literature. None of the earlier studies considered calcium concentration in talus of human fetuses. Some scientist did consider the human fetal bones but were confined to femur and parietal bones. Most of the human studies related to skeleton mineralization process are based on information in postnatal life. There are different theories to explain the manner in which the bone matrix becomes impregnated with carbonate and phosphate salt of calcium. In our study, 16 human fetuses with or without any apparent anomalies of foot were obtained from the museum section of Department of Anatomy, J .N. Medical College, Aligarh, and divided into two groups of normal and with apparent foot deformity corresponding to 2nd and 3rd trimester of pregnancy. Tali were dissected out and cleaned by separating the soft tissue. Each talus was weighed and dissolved in 100ml concentrated nitric acid and the solution thus obtained was used to determine calcium. Results were analyzed by using Student's' test. In our study of normal fetuses tali mean value of total calcium increase (rise) was noticed in fetuses of both second and third trimesters, but the calcium concentration per gram of talus was less in larger fetuses of 3rd trimester only. So the most exciting result of normal fetuses' tali in our study was reduction in aforementioned calcium level relative to talar mass, indicating marked increase in matrix formation in talus than the rate of mineralization. In fetuses with apparent foot deformity there was reduction in total bone mass but the calcium concentration / gram of talus increases in larger fetuses of 3rd trimester, indicating decrease in matrix formation in talus than the rate of mineralization that could be due to mechanical stresses in uterus. Therefore, our study aimed measuring the calcium level in tali of developing human fetuses in different age group of normal and with apparent foot deformity, to find pattern, if any during development in normal and those affected by mechanical stresses in uterus and also useful for medico legal purpose.

INTRODUCTION

The quantitative make up of the bodies of fetuses in pregnancy is of value for investigation by chemical method. In early gestation a fetus has fibrous or cartilaginous framework from which gradually bones are formed throughout the remaining gestation period and for years after birth in a process called as ossification. So during development as growth occurred the cartilage grows and slowly replaced by bone with the help of calcium salts. Primarily the bones consist of collagen, phosphorus, sodium and calcium. Linear fetal growth is high during the last two trimesters of pregnancy and fetal bone mineralization increases towards the end of pregnancy due to increased rate of maternal fetal calcium, phosphorus, magnesium transfer across the placenta. The embryonic period is divided into 23 horizons or stages. At 4 weeks, in horizon 13 a minute lower limb bud germinates opposite the 5 lumber and first sacral myotome. Horizon 23 marks the end of the embryonic period proper. It corresponds to the end of the eighth embryonic week and average C-R length of 30mm. There are three stages in the formation of skeletal elements: mesenchymal, cartilaginous, and osseous. Mesenchymal- In horizon 17 and 18, the foot plate is already present. The mesenchyme condenses, differentiates, and forms the analage of the foot. The differentiation of the tarsus follows that of the metatarsals. Cartilaginous- Cartilage cells form in the mesenchymal –prochondral analage. As the process of chondrification advances, the skeletal elements become clearly

Corresponding author: Fazal-ur-Rehman
Department of Anatomy, JNMC, A.M.U., Aligarh-202002, India

identifiable; morphogenesis, aiming toward the adult form, occurs. Thus the chodrification of the foot is initiated in horizon 18, and the last element, except for the sesamoids, chondrifies in horizon 23 which represents the end of embryonic period proper. The chronologic sequence of chondrification occurs in 14 stages by senior (Senior, 1929). The central three metatarsal chondrify first, followed by fifth metatarsal and the cuboid. The chondrification of the tarsus continues with the calcaneus, the talus and 2^{nd}—3^{rd} cuneiforms. The 1^{st} cuneiform and 1^{st} metatarsal follows. The navicular is the last tarsal element to chondrify. Osseous- The forefoot ossifies before the hindfoot. In hind foot, the calcaneus is the first to ossify. The talus may begin to ossify during the eighth lunar month. Oliver G describes that the talus is delineated at horizon 18 (Oliver, 1962). At 27 mm (horizon 22) there is over lapping of talus and calcanium. The talus is narrower and longer, only superior and lateral articular surface observed but separated apart. Only the lateral third of the lower surface establish contacts with the calcaneus. At 34 mm (horizon 23) talus more or less resembles the adult structure i.e. the sustentaculum tali is well developed. The dorsal, lateral and medial particular surfaces of the talus have merged. Thus fetuses start developing bones during the second trimester and continue developing for years after birth. Fetal bones begin forming around 15-16th week after conception. Before this period bones are composed of cartilage and connective tissue then begin the process of ossification, or forming into bones.

A fetal bone structure is fully formed by 29th week of fetal development. As a fetus grows his/ her bones begin to calcify with the addition of vitamin D and calcium that he/she receives from her mother's milk or through infant formula then later through milk. Calcium is one of the major components of the body mineral which account about 99-99.9%of the total body store and provides hardness and strength to body skeleton. It starts deposing into the membranous or cartilaginous model of bone right from intrauterine life and approximately 200 to 300 mg of calcium is deposited daily in the skeleton of developing human fetus during 3rd trimester of pregnancy (Watt, 1923). This calcium hardens the bones so they are strong enough to ensure body weight. There are a number of factors that can affect development of fetal bone that is growth of bones is influenced not only by chemical factors (such as hormones or vitamins), and physical factors like mechanical stresses but also by some hereditary and congenital conditions. Movement, one of the factors that influences bone development in fetuses, is their ability to move around in the uterus.

This may help to stimulate bone modeling (formation of new bone) and may also affect bone remodeling, which is a process that continues throughout life and involves the destruction and reformation of bone in response to stress. A study in "Calcified Tissue International" found that fetuses that could not move normally in uterus result in a reduction of bone thickness, and showed signs of osteopenia, a precursor to osteoporosis (Jose Rodriguez *et al*., 1988). The bones of these babies also fractured easily and showed mechanical defects. Thus mechanical stresses accelerate the thickness of the limb bones. Congenital postural abnormalities were a group of anomalies of the musculoskeletal system caused by intrauterine molding of a previously normally formed part (Dunn, 1972). All parts of the infant might be affected including skull, face, jaw, thorax, spine, and limbs, the most important deformities from the medical viewpoint being those of the feet like congenital telipes equinovarus, congenital dislocation of the hips etc. Controversy exists regarding mechanism involved in precipitation of calcium salts in different bones. Majority of information available regarding bodily calcium are based on experimental studies on lower animals (Chan and Swaminathan, 1998; Feaster *et al*., 1956; Graham and Scothorne, 1970). Some of information related to calcium in human skeletal system is very much available but these throw light on postnatal life. Some scientists have determined calcium contents in postnatal human mandible (Goret-Nicaise and Dhem, 1985). Calcium contents were also measured in ribs (Tzaphlidou and Zaichick, 2003) and facial skeleton of human adults (Fischer *et al*., 2009). Studies on fetal bone calcium were performed earlier (Macdonald, 1954; Mokrzyn´ski, 1994; Tobin, 1972) but none of them considered fetal tali.

Aim of the study

- To determine the level of calcium in fetal tali during 2^{nd} and 3^{rd} trimester of normal and abnormal fetuses.
- To find out whether there is statistically significant difference between the calcium levels of tali during aforementioned periods of developing normal and abnormal fetuses.
- To confirm whether the movement in uterus influences the bone development in fetuses or not (mechanical stresses).

Accurate information regarding calcium contents in fetal tali of normal and abnormal developing fetuses during intrauterine life will not only establish the normalcy but also help in determining the age of fetuses which will be of great medico-legal importance.

MATERIALS AND METHODS

The bones for this study were obtained from sixteen human fetuses with or without any apparent anomalies (Still birth or from infants who had died very soon after birth) preserved in the museum of department of Anatomy, J. N. Medical College, Aligarh. Fetuses were divided into two groups of eight fetuses each of normal and deformed foot corresponding to 2^{nd} and 3^{rd} trimester (Table 1). Postgraduate medical students had already been using these fetuses for their research work and sought permission from institutional ethics committee for this purpose. Tali were dissected out and removed. Each talus was weighed and dissolved in 100ml of concentrated nitric acid. The solution thus obtained was used to measure the calcium by the method of Henry (1963). Results were analyzed by using Student’s, t, test.

Table 1. Sub-grouping of normal human fetuses without any foot anomaly

Groups	Periods	Number of fetuses	Number of normal tali
I.	2^{nd} trimester	4	8
II.	3^{rd} trimester	4	8

Preparation of reagents solutions

For the determination of calcium, bone samples were dissolved in 1M HNO_3 solution, while EDTA (dried at 45 ^0C

for about half an hour) solution was prepared in demineralized water. Murex oxide indicator was prepared by dissolving 0.2 gm of murex oxide in 40 g per 250 ml of K_2SO_4 solution.

Preparation of $CaCO_3$ solution

1 g of pure $CaCO_3$ was taken in 250 ml conical flask and dissolved in HCl in (1:1) ratio to get the solution. The solution was boiled for 5 minutes to eliminate CO_2. The excess acid was neutralized using ammonium hydroxide. The solution was transferred into 1L conical flask and make up to neck using double distilled water. Each ml of this solution contained 1 mg of $CaCO_3$ equivalents.

Standardization of EDTA

Burette was rinsed and filled with EDTA solution. 50 ml of standard $CaCO_3$ solution was transferred in a conical flask. To which was added 10-15 ml of buffer solution and 2-4 drops of indicator. EDTA solution was used to titrate the solution till wine red color changed to clear blue. Volume used was noted.

Titration of un-known bone sample solution

Unknown bone sample solutions were titrated against EDTA solution using murex oxide as indicator, till wine red color changes to clear blue. The volume of EDTA was noted and used for the calculation of Ca equivalents.

RESULTS AND DICUSSION

Normal' fetal development is difficult to ascertain because a number of variable factors determine growth rate and weight. Mechanism of calcification and ossification of any bone in the body is still a mystery in spite of enormous literature available on subject (Watt, 1923; Feaster *et al*., 1956; Fischer *et al*., 2009). Our study on calcium levels in tali of human fetuses of different gestational age groups provides information only regarding pattern of calcium deposition instead of detailed mechanism involved. Mean value of total calcium during second trimester was only 1.95 mg which increased to 5.31 mg during 3rd trimester [Table 3]. Sudden rise in calcium concentration in late fetal life was first described in parietal bone which might be due to its relative importance in protection of brain (Toverud and Toverud, 1933). The possible explanation for such increase in talus seems to be relatively more growth of lower limbs in late fetuses. Commencement of ossification of talus at six month of intrauterine life might be an additional factor for said increase in talar calcium level (Faruqi, 2007). The most exciting result in our study were (1) reduction in calcium level relative to talar mass (Table 3, Fig 1) in normal fetus tali. The calcium concentration was reduced from 15.1mg/g of talus in smallest fetus of 20th weeks to 3.2mg/g of talus in largest fetus of 40th week. This result was clear indication of marked increase in matrix formation in talus than the rate of mineralization in normal fetuses. The only parallel report available was that of Ghaus (Ghaus *et al*., 2011) who reported similar result in maxillae of human fetuses.

Table 2. Sub-grouping of human fetuses with apparent foot anomaly (CTEV)

Groups	Periods	Number of fetuses with (CTEV)	Number of deformed tali
I.	2nd trimester	4	8
II.	3rd trimester	4	8

Table 3. Calcium level in tali of normal human fetuses (Mean ± SD)

Gestational period	Number of tali	Total calcium per talus (mg/100 ml)	Percent changes	Calcium level /g of talus (mg)	Percent change
2nd trimester	8	1.95±0.50		12.55±3.42	
3rd trimester	8	5.31±1.27*	+120	6.15±1.60**	-59

P value * < 0.01, ** < 0.001

Table 4. Bilateral variations in calcium level in tali of normal human fetuses

Side	Number of tali	Total calcium level/talus (mg/100 ml mean ± SD)	Calcium level /g of talus (mg, mean ± SD)
Right	8	3.91±1.43	10.16±5.28
Left	8	2376±1.55*	9.28±4.36*

P value * < 0.5 (Not significant), ** < 0.001

Table 5. Calcium level in tali of fetuses with apparent foot anomaly (Mean ± SD)

Gestational period	Number of tali	Total calcium per talus (mg/100 ml)	Calcium level /g of talus (mg)
2nd trimester	8	1.30±0.50	8.31±0.5
3rd trimester	8	2.20±0.50	2.31±0.85

Table 6. Bilateral variations in calcium level in deformed tali of human fetuses with apparent anomaly

Side	Number of tali	Total calcium level/talus (mg/100 ml mean ± SD)	Calcium level /g of talus (mg, mean ± SD)
Right	8	2.19±1.01	7.62±3.99
Left	8	2.41±0.50	8.00±3.30

(2) In fetuses with apparent anomaly of foot (congenital talipes equinovarus), there was reduction in total bone mass of talus but the calcium concentration of talus increases in larger fetuses of 40th wk as compared to smaller fetus of 2nd trimester. This result indicates that in deformed talus there is decreased bone mass formation than the rate of mineralization. These findings are true by the facts that congenital talipes equinovarus feet are smaller and deformed due to arrest of the normal growth. Our study is in progress to reach some definite conclusion of gestational age by estimation of phosphorus, calcium-phosphorus ratio, as the percentage of calcium and phosphorus steadily increases with fetal age.

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