

Bone Development in Hyoid Apparatus of Indigenous Sheep

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Abstract: The study included follow-up of bone development in (38) specimens of hyoid apparatus took from Awassi ewe's fetuses slaughtered in Mosul. These specimens prepared by double staining method (for small ages) and maceration with diluted potassium hydroxide (KOH) (for old ages). These specimens studied under dissecting microscope and magnifying lens to detect the time of appearance of primary ossification centers and to follow up the bone development in bones forming the hyoid apparatus. The study reveals that the 1st ossification center appear in the great cornu (stylohyoid bone) at 7th week, the paired ossification centers of basihyoid bone appeared at 14th week. At the age of 15th week a third ossification center appeared at the middle of basihyoid bone detect the origin of lingual process of the bone. While the ossification centers of both small and middle cornua (keratohyoid and epihyoid) appeared at 18th and 19th weeks respectively. At 20th week of fetal age the ossification center of thyrohyoid bone noticed at the distal end of this bone. The statistic analysis show presence of significant variance in the average total length of bones forming hyoid apparatus (especially the stylohyoid bone) during the successive studied weeks (12th – 20th weeks) of embryonic development.

Key words: Bone development, Ossification center, Hyoid apparatus, Sheep fetuses.

INTRODUCTION

The development of growing heads is necessarily an extremely complex process since organs and tissues having completely different functions are growing in close physical association with each other (Wenham and fowler, 1973).

The hyoid bone (os hyoideum) lies mainly between the vertical rami of the mandible. Caudally, it is attached by cartilaginous rods (the tympanohyoids) to the stylohyoid processes of the petrous part of the temporal bones. Rostrally, it is associated with the tongue, pharynx and larynx. It is composed of a number of parts, some single and other paired. These parts are the body, the lingual process, the thyroid processes, and the small, middle, and great cornua (May, 1970).

The basihyoid is short and flattened, and lies in a transverse plane. A short lingual process is situated centrally on the rostral side. The paired thyrohyoid is not firmly attached to the basihyoid. They are long (2.5 to 3 cm) and narrow, and articulate caudally with the rostral cornua of the thyroid cartilage of the larynx. The keratohyoid and epihyoid are small, rounded bones situated on each side between the basihyoid and the stylohyoid. The keratohyoid is about (4 mm) in diameter and the epihyoid is less (May, 1970).

The stylohyoid articulates rostrally with the epihyoid and caudally with the styloid process of the temporal bone. The bone is elongated, flattened, and about (6 cm) long and (5 mm) wide. The rostral end is slightly expanded, while the caudal end bifurcates. The dorsal part of the caudal end articulates with the styloid process of the temporal bone by the tympanohyoid, and the ventral part is the muscular process (May, 1970).

In species possessing a bony skeleton, ossification occurs in two different ways, intramembranous ossification and intracartilaginous ossification (Williams and Dyson, 1989). The hyoid bone developed from the second pharyngeal arch from six centers: two for the body, and one for each cornu. Ossification commences in the greater cornua toward the end of the fetal life, in the body shortly afterward, and in the lesser cornua during the first or second year after birth (Gray, 2009, 2011).

This study has been essentially aimed at revealing the mechanism of normal bone growth in the prenatal period of sheep fetuses development according to age by using many measurements.

MATERIALS AND METHODS

Thirty eight fetuses were collected from uteri of Awassi ewes slaughtered in Mosul. The crown rump length of each fetus was measured by using a measuring tape. This length used in Richardson formula to find the estimated age of each fetus (Arthur *et al.* 1989). The skulls of young fetuses separated and prepared by skinning, fixing in 90 % ethyl alcohol, then staining with modified double staining method by using mixed solution of alizarine red S and alcian blue, the specimens then macerated in 2 % potassium hydroxide solution, after that cleared by using glycerin (Ahmed, 1998), then examined by dissecting microscope, while the skulls of older ages prepared by using maceration with different concentrations of KOH according to the age (Mahmood,

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2007). The growth and development of hyoid bone followed up in (13) weeks (8th – 20th week) by examining the specimens of hyoid bone which removing carefully from the head of the fetuses during 13 weeks (8th – 20th week) of fetal age . the examination has been done by taking the following measurements, (Fig 1):

1- Total length of stylohyoid bone:represents by the longitudinal line reaching between the proximal angle attached with tympanohyoid cartilage and the distal end of bone attaching with the epihyoid bone.

2-Total length of keratohyoid bone : represents by the longitudinal line reaching between the proximal and distal ends of the bone.

3-Total length of thyrohyoid bone : represents by the longitudinal line reaching between the rostral end of bone attaching with basihyoid bone and caudal free end attacing rostral cornu thyroid cartilage of larynx.

4- Total length of basihyoid bone: represents by the transverse line reaching between right and left ends of the bone.

5- Total length of lingual process : represents by the line reaching between the origin of lingual process and it's free end. (Parsons, 1908)

The data of these measurements submit to the statistical analysis to find the analysis of variance then followed by least significant test (Steel, 1981) to demonstrate the significance of variance present in these measurements during the studied weeks (12th – 20thweek) of fetuses age.

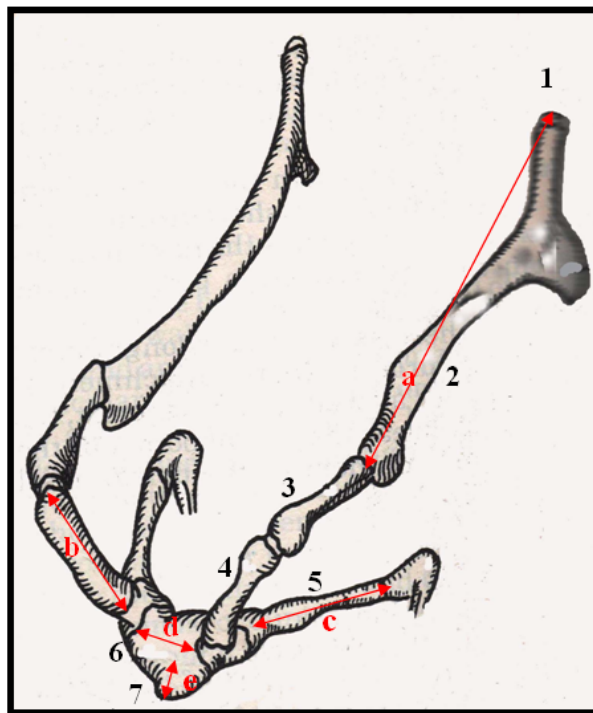


Fig. 1: Adult hyoid bone of sheep,show the parts of hyoid bone and the way of measuring the total length of each bone used in statistic study.

1. Tympanohyoid
2. Stylohyoid
3. Epihyoid
4. Keratohyoid
5. Thyrohyoid
6. Basihyoid
7. Lingual process
- a. Total length of stylohyoid bone.
- b. Total length of keratohyoid bone.
- c. Total length of thyrohyoid bone.
- d. Total length of basihyoid bone.
- e. Total length of lingual process.

Results:

Bones formed hyoid apparatus of indigenous sheep fetuses ossified by intracartilaginous ossification. The first initiation of ossification begin in the hyoid apparatus at 7th week this clearly appeared in the stylohyoid bone (great cornue) at 7th week of embryonic age (fig 2). The ossification appeared in basihyoid bone at the

beginning of 14th week as paired ossification centers (fig 3). At 15th week another center appeared at the middle of the bone represent the origin of the lingual process ,while the ossification of both small and middle cornua (keratohyoid and epihyoid) initiate at 18th and 19th week of embryonic age respectively. The last ossification center appeared at the distal end of thyrohyoid bone at 20th week of embryonic age (fig 4).

The results of statistic analysis reveals presence of difference in the average of the total length of bones forming hyoid apparatus among successive studied weeks (12th-20th weeks).There is clear significant variance between total length of stylohyoid bone and the same length of other bones forming hyoid apparatus, among all studied weeks. This result also reveals with the lingual process in compared with the other bones (table 1).

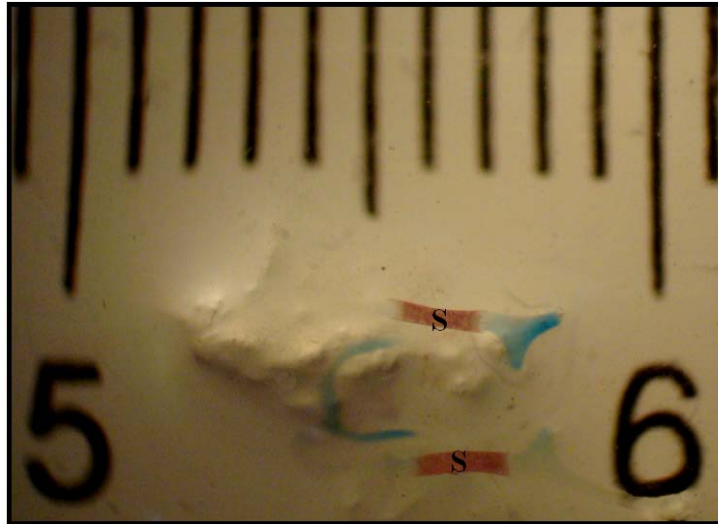


Fig. 2: Hyoid apparatus of indigenous sheep fetus with an estimated age (7) weeks,prepared by double staining method of alizarin red S and alcian blue. Notice the ossification extend in about half of stylohyoid bone (S).

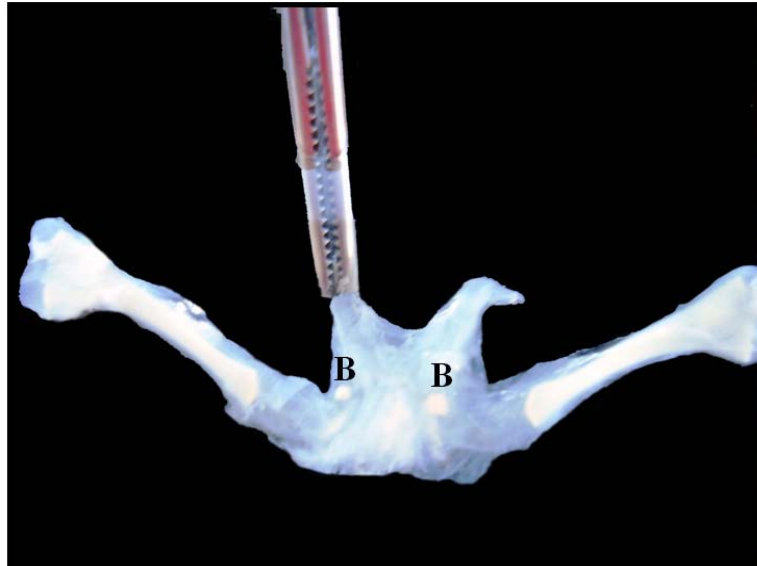


Fig. 3: Hyoid apparatus of indigenous sheep fetus with an estimated age (14) weeks, prepared by maceration with diluted KOH. Notice the paired ossification centers in basihyoid bone (B) , 2.7 X.

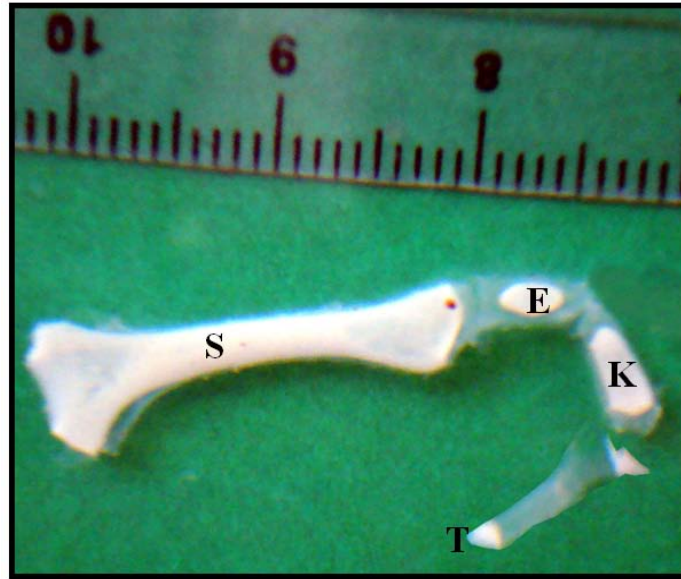


Fig. 4: Hyoid apparatus of indigenous sheep fetus with an estimated age (20) weeks, prepared by maceration with diluted KOH. Notice : the ossification locus at the end of thyrohyoid bone ,ossification centers in both small and middle cornues, while the ossification in large cornue extend to most of stylohyoid (S), keratohyoid (K) and epihyoid (E) bones.

Table 1: The length averages of bones forming the hyoid apparatus during nine successive weeks (12th -20th weeks) of indigenous sheep fetuses age.

Fetus age	Total length (mm) of bones forming hyoid apparatus				
	Stylohyoid	Keratohyoid	Thyrohyoid	Basihyoid	Lingual process
12	12 ± 0.408	2.625 ± 0.125	4.125 ± 0.0722	3.875 ± 0.125	1.375 ± 0.125
13	13.250 ± 0.479	3.125 ± 0.125	4.750 ± 0.144	4.500 ± 0.289	1.813 ± 0.120
14	16 ± 0.0	3.625 ± 0.125	5.375 ± 0.239	5.125 ± 0.125	2.188 ± 0.120
15	18.25 ± 0.479	4.250 ± 0.144	5.625 ± 0.239	6.5 ± 0.289	2.688 ± 0.120
16	21.250 ± 0.433	5 ± 0.0	6.750 ± 0.250	7.625 ± 0.239	3 ± 0.0
17	22.5 ± 0.204	5.375 ± 0.072	8.750 ± 0.250	8.375 ± 0.239	3.375 ± 0.0722
18	23.875 ± 0.125	6 ± 0.0	9.875 ± 0.125	9.563 ± 0.329	3.750 ± 0.102
19	25.375 ± 0.239	6.375 ± 0.125	10.750 ± 0.144	10.875 ± 0.125	4.375 ± 0.125
20	26.375 ± 0.239	7 ± 0.0	11.625 ± 0.125	11.563 ± 0.157	4.875 ± 0.125

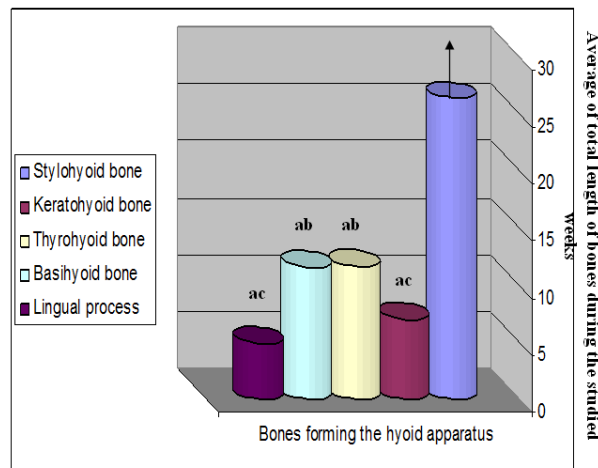


Fig. 5: The averages of total length of hyoid bones of indigenous sheep fetuses during the nine studied weeks. a: significantly differ from the average of total length of stylohyoid bone at $P < 0.05$
 b: significantly differ from value of keratohyoid bone and lingual process at $P < 0.05$
 c: significantly differ from the average of total length of keratohyoid bone and thyrohyoid bone at $P < 0.05$

Discussion:

Bones formed hyoid apparatus of indigenous sheep fetuses ossified by intracartilaginous ossification because the bone model originating from the connective tissue develops first to cartilaginous tissue and is subsequently to mature bone tissue (Williams and Dyson 1989).The blue staining of the hyoid apparatus of

indigenous sheep fetuses, an indicator of presence of cartilaginous tissue suggests that the mode of bone formation is intracartilaginous type ossification, this result agrees with (Atalgin *et al* 2007). The first appearing of hyoid apparatus in indigenous sheep fetuses begins at 7th week of the embryonic age. This result agrees with Wenham 1981 in his study on sheep fetuses where he said that the first ossification centers of hyoid apparatus appeared in the great cornu in sheep embryo with 48 day age and agree with (Horisnwa *et al* 1998) in the period of appearing of hyoid apparatus in human in which appeared at the beginning of 7th week of gestation. and disagree with (Farag *et al* 2012) in the position and the age of appearance of first ossification center in hyoid apparatus in rabbit which appeared in thyrohyoid and basihyoid bones at 10th week of embryonic age of rabbit, this result belongs to species of animal where the hyoid apparatus of rabbit missed the stylohyoid and the great cornu in rabbit is represented by the thyrohyoid while in this study on sheep fetuses the first primary ossification center noticed in the stylohyoid bone which represents the great cornu in the 7th week of embryonic age. This result agrees with result of (Atalgin *et al.*, 2007, Barone *et al.*, 1973, Popesko *et al*, 1992) who told that the thyrohyoideum (which is the greater cornu in the New Zealand white rabbit because hyoid apparatus of rabbit lacks the stylohyoid component) were observed to start the ossification at the prenatal stage and with (Parsons FRSC, 1909) who told that the great cornua are the first part that ossify from the hyoid apparatus in human.

The ossification of the small cornu (keratohyoid bone) appeared to ossify as small center in the distal end of the bone at the 12th week of embryonic age, this result agrees with the result of (Atalgin 2007) who told that the ossification center in keratohyoid bone seen in the 4th and 5th periods of New Zealand white rabbit.

The ossification in the basihyoid bone starts in the middle of the as paired centers at the 14th week of embryonic age, the same age ossification starts at the free end of the thyrohyoid bone, this result disagrees with the result of (Atalgin *et al* 2007) who told that the basihyoid bone was observed to start ossifying at the prenatal stage and agrees with them about that the basihyoideum were observed to start ossifying at the same stage (prenatal stage) which indicates that they are indeed the main skeletal structure of the hyoid apparatus and agrees with the result of (Parsons FRSC, 1909) who told that the ossification center for the body probably appears just before or after birth in the human, and disagrees with the result of (Harris HA) in the age of appearing of ossification center in the body of the hyoid bone of sheep fetuses (*ovis ovis*) which appears at 128 day of embryonic period. At 15th week of embryonic age the basihyoid bone has three ossification centers; two at the middle of the bone and the third present at the origin of the lingual process, at this age the ossification of great cornu (stylohyoid bone) extends approximately along the whole bone, this result agrees with the result of (Atalgin *et al* 2007) who told that the primary ossification centers fused totally among themselves in the fifth period of embryonic age of New Zealand white rabbit. and the fusion among the components of the hyoid bone showed a similar pattern to other bony fusion. The development and growth of the ossified parts of the studied bones continued subsequently and these results agree with the ossification period of other components of the skeleton in rabbit.

The results of statistical analysis reveal presence of difference in the average of the total length of bones forming the hyoid apparatus among successive studied weeks (12th -20th) and there is clear significant variance between the total length of stylohyoid bone and the same length of other bones forming hyoid apparatus among all studied weeks, this result also reveals with the lingual process in comparison with the other bones, these results agree with the result of (Atalgin *et al* 2007) who told that the numerous data obtained for the ossified and cartilaginous parts of the hyoid apparatus summarize the gradual increase in the ossified area and consequent decrease in the cartilaginous area.

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