DEVELOPMENT OF HUMAN LARYNGEAL CARTILAGES

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ABSTRACT: Foetal anatomy of laryngeal cartilages has not been fully described. In the present study we viewed the embryogenesis and histogenesis of foetal laryngeal cartilages , in forty foetuses of varying gestational ages ranging from 75mm C.R.L.(crown rump length) to220mm C.R.L. Tissues were prepared for microtomy by Paraffin Wax Embedding method. Step sections (every fifth) were fixed and stained by Haematoxylin and Eosin (H&E) and Masson's trichrome method. In the present study laryngeal cartilages are seen arranged on either side of laryngeal cavity occupying some definite positions in relation to one another. Thyroid and Cricoid cartilages are first to appear and are well formed at 12 weeks of gestation. Ventral fusion of thyroid laminae is seen at 16 weeks of intrauterine life. At this stage thyroid cartilage appears better than cricoid cartilage. Mesenchymal condensation of Arytenoid and Epiglottic cartilage appears at 16 weeks of intrauterine life and starts chondrifying at 12 weeks and 19 weeks of intrauterine life respectively. Corniculate and Cuneiform cartilages are seen during 26 weeks of intrauterine life. The cartilages show caudocranial order of maturity. The anatomy of larynx is important for Otorhinolaryngologists, Anaesthetists, neonatal physicians etc. Increased care of foetal and neonatal airways has led to advances in neonatal medicine. The early diagnosis and treatment of respiratory diseases requires knowledge of foetal airway anatomy and development. Our aim was to determine the time of appearance of different types of laryngeal cartilages and their structural variability during foetal life.
KEY WORDS: Laryngeal cartilages, foetus, gestational age

INTRODUCTION: Larynx also called as Voice Box is an important escape valve for the emotions like anger, grief and affection which are essential for maintenance of our psychological equilibrium (1) so its study is important. With the advent of modern technology and diagnostic procedures it becomes imperative to have a sound knowledge about developmental anatomy. Shikinami in 1926 said" If one wishes to become acquainted with the structure and form of any organ, there is perhaps no better way than to trace its development, step by step, back into the early embryonic stage" as quoted by Harrison. D.F.N. (2).The lungs and larynx developed when air breathing vertebrates evolved from lower vertebrates that lived in water. The larynx is a sphincteric protective mechanism for the lower airways. Only after complete development of the protective role of the larynx does vocalisation evolves as a major, although secondary, function of that structure(3).The larynx can be a very challenging specimen to orient and dissect, with a multitude of anatomical structure intricately associated in a complex arrangement to achieve functionality(4). Although the development of larynx in the human embryo has been studied by many, interpretation is dependent on availability of large number of accurately staged specimens such as the Carnegie and Patten collections. Larynx develops from epithelio-mesenchymal interaction and is composed of all the three germ layers. It starts to develop during the 3rdweek of intrauterine life as an outcropping from the pharynx. The specific anatomy of the larynx develops from 3rd to 6thpharyngeal arch. Pharyngeal arch cartilages from 1st to 3rd arch develop from neural crest while that of 4th and 6th develop from lateral plate

mesoderm. Since laryngeal cartilages originate from 4th-6th arch, so they originate from lateral plate mesoderm. Primary laryngeal aditus or ventricular cavity is' T' shaped with three eminences. The hypobranchial eminence becomes the epiglottis and 2nd & 3rd eminences develop into arytenoids.

MATERIALS AND METHODS: The present study was carried out on 40 human foetuses from 75mm C.R.L. to220mm C.R.L. The foetuses were collected from Operation Theatres, Labour Room and Obstetric wards of Department Of Gynaecology And Obstetrics, Government Medical College, Jammu and various nursing homes operating in and around Jammu City after approval from Institutional Ethical Committee and written parental informed consent. Foetuses with Congenital Malformations were excluded from the study. Foetuses were preserved in 10% formalin and their Crown Rump length measured with Vernier Calliper. Assessment of the age of the foetuses was done according to the rule as described by Hamilton, Boyd and Mossman (5)(Table-1). Out of 40 foetuses dissected 15 foetuses were female and 25 were male. Two paramedian incisions were given on the anterior surface of the neck, skin was removed and larynx was removed enbloc along with epiglottis and some part of base of tongue. The tissue was fixed in 10% Formalin for 7 to 10 days and prepared for microtomy by Paraffin wax embedding method.7 micron thick step sections (every 5th) were taken for fixation and staining. Staining was done by Harris Haematoxylin and Eosin stain (H & E) and Masson's Trichrome stain.

OBSERVATION: The human foetuses were divided into four groups (Table-2)

Group-1 (75mmC.R.L-125mm C.R.L)

Nine foetuses were dissected in this group. Ventricular shape ranges from circular to longitudinal depending upon the plane of section. The Thyroid and cricoid cartilages are observed which are well established. Small laminae of thyroid cartilages (fig- 1) with their cornu are observed on each side of ventricular cavity. Ventral fusion of thyroid lamina is observed at 112 mm C.R.L. Mesenchymal condensation of tissue in the region of the arytenoids is seen in foetus of 112mm C.R.L., which is above the cricoids cartilage on each side of the ventricular cavity. Due to these arytenoids masses, ventricular cavity assumes the shape of the letter T. A little amount of Mesenchymal condensation of epiglottic cartilage is observed in the epiglottic region at the top of the T shape ventricular cavity at 112 mm C.R.L (fig-2) There is no observance of the accordion effect which means that the hyoid, thyroid and the cricoids cartilage are in the close vicinity and may be overlapping one another.

Group -2 (128mm C.R.L. -145mm C.R.L.)

Eleven foetuses were dissected in this group. This group shows fully formed ventricular cavity. The size of thyroid laminae and cricoids cartilages has increased with well established perichondrium. Densely packed chondrocytes are seen which are uniformly scattered all over the cartilage within the perichondrium. No cell nests observed .The chondrification of the cricoids cartilage is well advanced than that of the thyroid cartilages and thyroid cartilages chondrify earlier to arytenoids. So developmental gradient of the cartilages is caudo-cranial. The condensation of the epiglottic cartilages shows progressive enlargement which is more in the centre as compared to the

tapering ends. Arytenoid cartilages show more maturity. Among the cartilages cricoid cartilage appears better developed then the thyroid cartilage in all the foetuses. (fig-3)

Group-3 (146mm -168mm C.R.L.)

Eight foetuses were dissected in this group which showed fully developed ventricular cavity. The cartilages are more mature as compared to the previous group. The cricoid, thyroid and arytenoids are fully developed. The two laminae of thyroid show full maturity and ventral fusion are observed in some foetal specimens. The arytenoid shows caudo-cranial order of maturity. The epiglottic cartilages are better developed and more mature then the previous group. (Fig-4) All the cartilages shows well formed perichondrium within which uniformly scattered large mature chondrocytes are seen. Mesenchymal condensation for corniculate and cuneiform cartilages is observed a little above the arytenoids on both sides of the ventricular cavity.(fig-5)

Group-4 (170mm -220mm C.R.L)

Twelve foetuses were dissected in this group showing same features as the previous group as regards the ventricular cavity. All the cartilages are well developed at this stage and have attained full maturity.(fig-6) The cartilages formed show the elongation of 'Accordion'. The two laminae of thyroid have acquired semicircular shape and their ventral fusion is observed clearly. The cricoid is seen in the form of lamina which is quite larger than thyroid cartilage. The two arytenoids are observed to be larger among the paired cartilages. Epiglottis is fully mature and has attained its final shape. (Fig-7) The corniculate cartilages make their appearance as small pieces of cartilages articulating with the apices of the arytenoids cartilages. Faint impression of the small bars of cartilages called as cuneiform cartilages are observed one in each aryepiglottic fold.

DISCUSSION: The present study elucidates the sequential development of laryngeal cartilage under light microscopy. On reviewing the literature regarding the development of laryngeal cartilages, controversial reports were available. In the present study, well formed laminae of thyroid and cricoids cartilages are found at 12 weeks of intrauterine life. Major portions of the cartilages were chondrified. This was in accordance with the observation of Meena Negi & Chandrama Anand(1987),(6) Manoukian J.J & Tan A.K.(1997)(7), Love(Jr)(1983)(3), Lisser(1911)(8), Tucker & Tucker(1975)(9) & Hast M.H.(1970)(10).

Besides Hast M.H.(1970)(10), Love(Jr)(1983) & Tucker & Tucker(1975)(9) reported that major topography of the foetal larynx was complete between 8-16 weeks of intrauterine life.

The present study reveals the Mesenchymal condensation for Arytenoid cartilages and epiglottic cartilages at 16 weeks of intrauterine life which is supported by the findings of Meena Negi and Chandrama Anand(1987)(6). However Tucker & Tucker (1975)(9) saw them at 8th& 6th weeks of intrauterine life respectively, whereas Hast M.H.(1970)(10) reported the cricoids and epiglottic development at the beginning of 3rdmonth and 2ndmonth respectively. According to them the cuneiform cartilages appear at 28 weeks of intrauterine life, but in the present study, they were seen at 26 weeks of intrauterine life, our finding being consistent with the findings of Meena Negi and Chandrama Anand(1970)(6)

Manoukian J.J. & Tan J.J (1997) (7)noticed the epiglottic maturation during 17-28 weeks of intrauterine life with simultaneous development of corniculate and cuneiform cartilages. The

development and maturation of epiglottis, cricoids and corniculate cartilages in the present study is seen at 18-20 weeks of intrauterine life and this finding is consistent with findings of Meena Negi and Chandrama Anand(1987)(6).

The present study shows ventral fusion of thyroid laminae at 16 weeks which is not coinciding with findings of Tucker & Tucker(1975)(9) and Manoukian J.J. & Tan A.K.(1997)(7) who observed it at 12 weeks of intrauterine life, but the rule of elongation of 'Accordian' described by them is seen in present study. Caudocranial order of maturity of laryngeal cartilages as observed by Meena Negi and Chandrama Anand (1987)(6) was seen in the present study.

SUMMARY: The present study shows the different stages in the development of laryngeal cartilages in 40 human foetuses varying from 75mm C.R.L. to 220mm C.R.L. In the present study thyroid and cricoids cartilages are first to appear and are well formed at 12 weeks of gestation. Ventral fusion of thyroid lamina is seen at 16 weeks of gestation. The Mesenchymal condensation of arytenoids and epiglottic cartilages is seen at 16 weeks of gestation which starts chondrification at 12 weeks and 19 weeks of gestation respectively. Corniculate and cuneiform cartilages are seen at 26 weeks of gestation. All the cartilages show caudo-cranial order of maturity. No congenital anomaly in the form of immaturity of cartilages, thickening of cricoids cartilage, cleft in the cartilages is seen. The Anatomy of larynx is extremely important for Anaesthesiologists for mastering skills of advance airway management and in assessing risk factors for laryngeal injury during tracheal intubation. Laryngeal structures are frequently manipulated while inserting an endotracheal tube. The endolaryngeal structures are subject to insult during this procedure. Understanding of laryngeal injury requires knowledge of laryngeal anatomy of which developmental anatomy forms an important part. Major skeleton of larynx is formed from laryngeal cartilages, so study of the development of laryngeal cartilages is important.

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Table -1 Showing Division of Foetuses in Four Groups

GROUP NO.	CROWN RUMP LENGTH (CRL) in mm	NUMBER OF FETUSES
I	75 to 125	09
II	128 to 145	11
III	146 to 168	08
IV	170 to 220	12

Table – 2 Showing Crown Rump Length of Embryos Along With Their Estimated Ages

S. NO.	CROWN- RUMP LENGTH (mm)	ESTIMATED AGE (In days)	SEX	NUMBER OF CASES STUDIED
1	75	86	M	2
2	97	101	M	1
3	100	103	F	2
4	112	111	F	1
5	115	113	F	1
6	120	116	M	1
7	125	120	M	1
8	128	122	M	1
9	130	123	M	1
10	134	126	M	2
11	135	127	F	2
12	136	127	F	1
13	140	130	M	1
14	142	131	F	1
15	143	132	M	1
16	145	133	F	1
17	146	134	M	1
18	150	136	M	2
19	158	142	M	2
20	160	143	F	1
21	168	148	F	2
22	170	150	M	1
23	172	151	F	1
24	176	154	M	1
25	180	156	M	1
26	187	161	M	2
27	190	163	F	2
28	200	170	M	2
29	220	183	M	2

Fig 1- Lamina of Thyroid cartilage (A), Inferior cornu (B) & Cricoid cartilage (C) seen in 125mm C. R. Length foetus H & E x 100

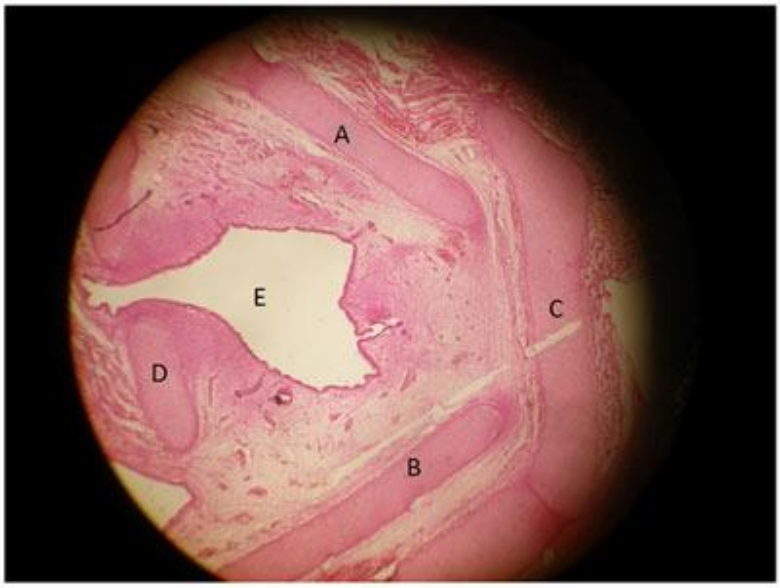

Fig . 2 Two thyroid laminae (A & B) before fusion and Hyoid bone (C), Arytenoid condensation (D) and ventricular cavity (E) seen in 100 mm C. R. L. foetus H. & E X 10

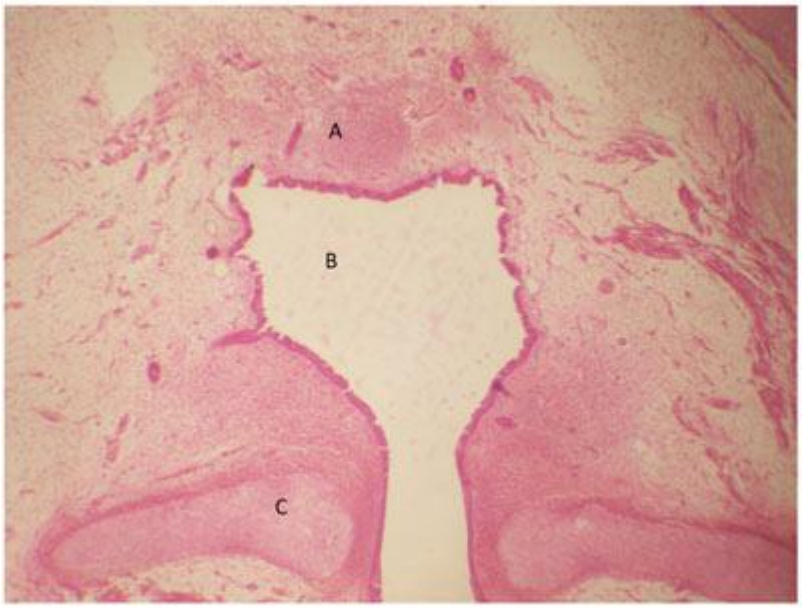

Fig 3 Epiglottic condensation (A) in epiglottic region at top of ventricular cavity (B) and arytenoids condensation (C) seen in 145 mm C.R. L. Fetus H & E. X 100

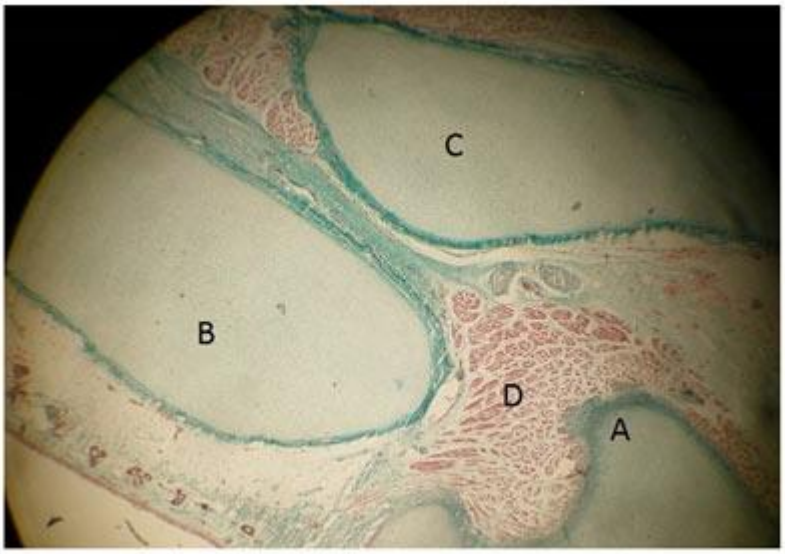

Fig -4 Arytenoid cartilages (A)showing less chondrification than cricoid (B) and Thyroid (C) Cartilages along with cricoarytenoid muscle (D) seen in 146mm C.R. Length foetus. Masson's Trichrome X100.

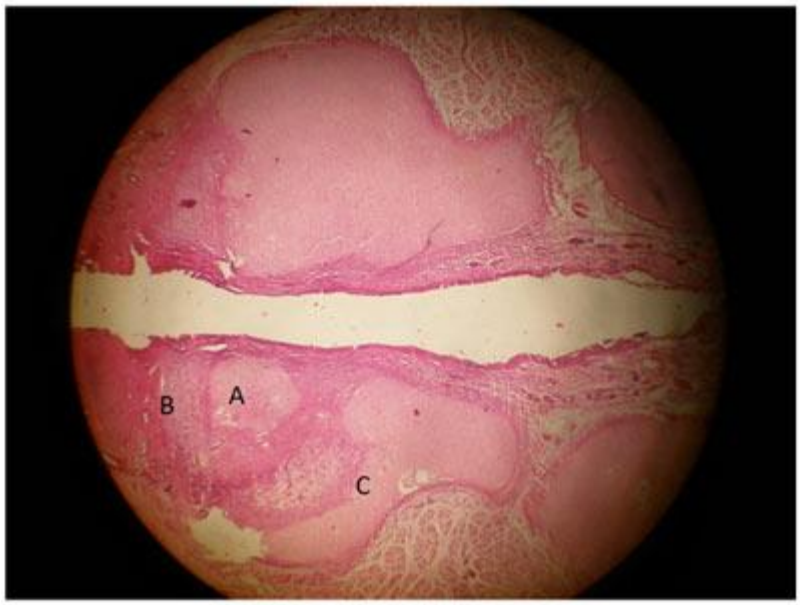

Fig 5. Mesenchymal condensation of corniculate (A) and cuneiform cartilages (B) just above Arytenoid cartilages (C) seen in 168 mm C.R. L. Fetus H & E X 100

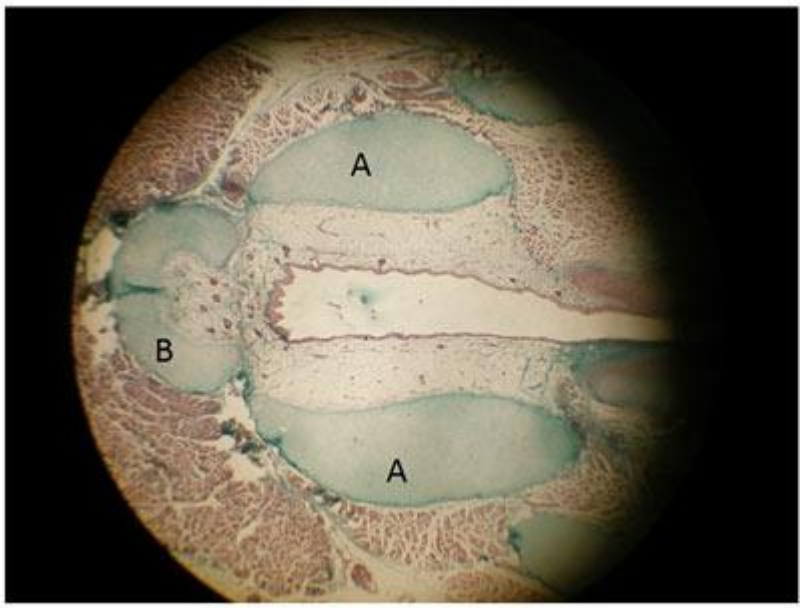

Fig - 6 Laminate of Cricoid cartilage (A) along with cricoid arch(B) seen in 200 mm C. R. Length foetus Masson's richrome X 10

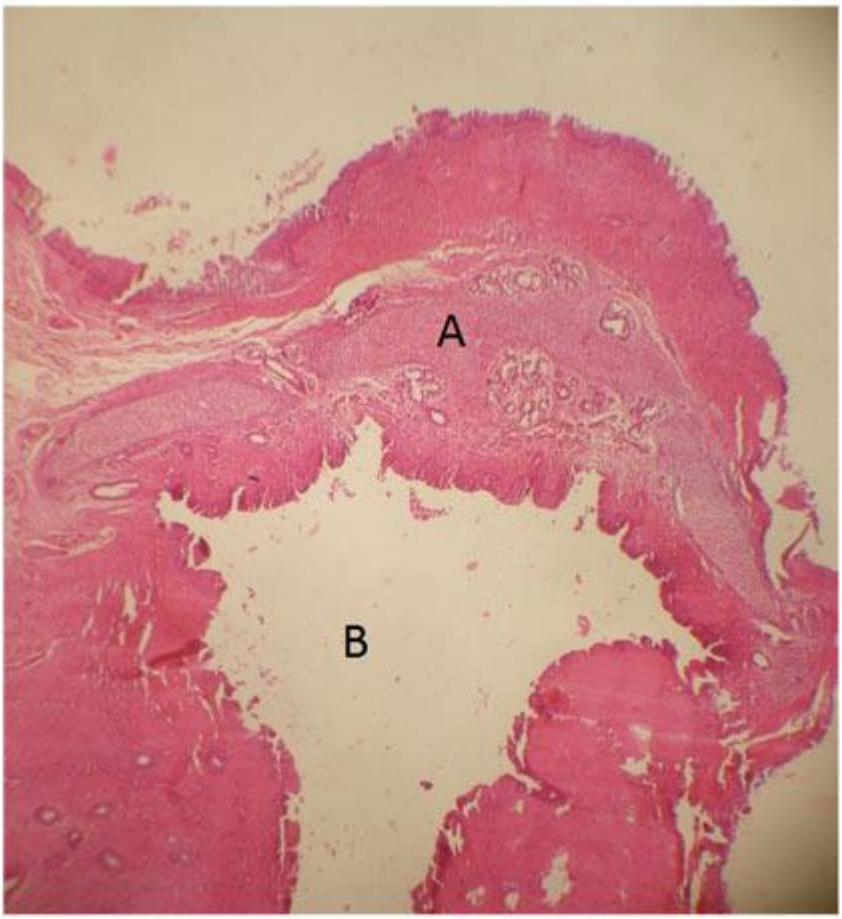

Fig 7 Chondrified & mature epiglottic cartilage (A) seen at the top of Laryngeal cavity (B) in 220 mm C.R. length foetus H & E X 100

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