ORIGINAL ARTICLE

Morphogenesis and Histogenesis of laryngeal Glands- A Prenatal Study

Ashwani Kumar Sharma, Rachna Magotra, Sanjay Raina, Shayama, Sundanda Raina

Abstract

Larynx is an important escape valve for the emotions like anger, grief and affection which are essential to maintenance of our psychological equilibrium. Phylogenetically it is eons older than those brain and muscle structures responsible for articulation and language. It is a very important organ for physicians, paediatricians, otorhinolaryngologists etc. The knowledge of anatomy of foetal airway and its development is important for the diagnosis and treatment of various diseases. In the present study the development of laryngeal gland during different stages of development of human foetal larynx was observed. Forty human foetuses of varying gestational ages ranging from 75mm to 220mm crown rump length were taken for this study. The foetuses were divided into four groups. Tissues were prepared for microtomy by "Paraffin wax embedding method". Serial sections were stained by Haematoxylin and Eosin (H&E) and Masson's Trichrome stain. It was observed that the lamina propria underneath the laryngeal epithelium was well developed and consisted of two clearly distinct zones in all the foetuses except the foetuses of group-I, where it was indistinct. There was well developed glandular tissue in the submucosa comprising predominantly of mucous gland and serous glands. This glandular formation was seen from the 12 weeks of intra uterine life and well established glands were seen in subsequent stages of development.

Key Words

Morphogenesis, Histogenesis, Foetus, Larynx, laryngeal Glands

Introduction

Larynx is a fine sphincter that orchestrates swallowing, breathing, coughing and voice (1). Most of laryngeal cavity is lined by ciliated pseudostratified columnar epithelium. The laryngeal mucosa has numerous mucus glands especially over the epiglottis and along the margins of the aryepiglottic folds where they are known as arytenoid glands (2, 3). However there are no mucus glands in vocal folds (4, 5). Abundance, distribution, and contents of secretory cells vary considerably among different kinds of species (6). Laryngeal glands, begins to develop from 12th week onwards and are formed by the 25th week of gestation (7). Most of the studies on larynx are limited to the study of cartilages, muscles and nerves. Very few studies describe the development of laryngeal glands. Scanning and thorough study of the available literature revealed a vast controversy and difference of opinion regarding the morphogenesis and histogenesis of human foetal laryngeal glands. The present study has been undertaken to study the time of appearance of glands in submucosa and their subsequent development and to compare the results so obtained with previous studies.

Material and Methods

The study was conducted on forty human foetuses of crown rump length ranging from 75mm-220mm. The foetuses were collected from operation theatres, labour room and obstetric wards of Department of Gynaecology and Obstetrics Government Medical College Jammu. The foetuses were obtained as the product of still births and abortions which were either induced or natural or from hysterotomy procedures. Foetuses having any type of congenital malformations were excluded. Assessment of the age of the foetus was done according to the rule described by Hamilton, Boyd, and Mossman (8). The

From the Department of Anatomy, Govt. Medical College Jammu, J&K- India

Correspondence to : Dr Aswani Kumar Sharma, Lecturer, Department of Anatomy, Govt. Medical College Jammu- J&K India

foetuses were subject to dissection after a week's time and larynx was removed enblock along with epiglottis and some part of base of tongue. The dissected specimens were fixed in 10% formal saline for 24 hours. The tissues were prepared for microtomy by the paraffin wax embedding method. 7 micron thick serial sections were stained with H & E and Masson's trichrome strain. These stained slides were then observed under the light microscope.

Results

The present study was carried out on 40 foetuses which were divided into four groups. (*Table 1*)

The lining epithelium of entire laryngeal cavity in all the foetuses was of respiratory type showing proximo-distal developmental gradient i.e. development was advanced in the proximal part of the ventricular cavity as compared to the distal end. The epithelium over the true vocal cords and dorsal surface of epiglottis was of stratified squamous type in group 3 and group 4 foetuses. Also flattening of epithelium over the true vocal cords due to desquamation was seen in group 3 and 4 foetuses.

Table 1. Showing CRL & Gestational Age of Foetuses

Group No.	*CRL (in mm)*	*Gestation in weeks*	*No. of Foetuses'*
I	75 to 125	13 to 17	09
II	126 to145	17 to 19	11
III	146 to 168	19 to 21	08
IV	169 to 220	21 to 26	12

Lamina propria was well developed in all the foetuses except in group-1. It consisted of two clearly distinct zones as inner dense zone and an outer loosely arranged zone of connective tissue studded with numerous connective tissue cells and fibres. Budding stage of glands was observed in submucosa of group-1 foetuses, which became more developed in group-2 foetuses and were more developed cranially than caudally. The glands were predominantly mucus with well established nuclei and were plentiful in ventricles. The glands were better developed caudally and were more mature in group-3 foetuses. Mixed glands in form of seromucinous glands were observed all over except in true vocal cords in this group. The submucosa was rich in mixed glands predominantly mucus with greater concentration on the ventricles and posterior surface of epiglottis in group-4 foetuses. Some tubuloacinus glands were also seen with ducts lined by stratified columnar epithelium in this group.

Table 2. Showing Histological Features

	Group-I	**Group-II**	**Group-III**	**Group-IV**
Epithelium	Ciliated pseudostratified columnar	Same as Group-I but stratified squamous on true vocal cords	Same as Group-II with stratified squamous on epiglottis also	Same as Group-III with desquamation on true vocal cords
Lamina propria	Two indistinct zones	Clearly distinct zones	Same	Same
Glandular development in submucosa	Budding stages of developing glands	In form of acini which are predominantly mucus	Well developed predominantly mucus	Some tubule acinus glands are also seen
Goblet cells in mucosa	Occasional	Occasional	More	Increased

Discussion

The generalized biological law that "Ontogeny recapitulates Phylogeny" is exemplified in the development of larynx. It is therefore, not surprising to observe the phylogenesis of the larynx from the African lungfish to the salamander to the alligator, in the ontogenesis of the human larynx

In the present study emphasis was layed on the submucosal glands of larynx as airway submucosal glands secrete mucous and bacteriocidal proteins like lactoperoxidase which are critical for normal airway function (9). Moreover submucosal glands also significantly influences bioelectric and fluid transport properties of the airway (10).

Meena Negi and Chandrama Anand (11) observed two zones of lamina propria which persisted till 25 weeks

of gestation. They observed glandular buds in foetuses of 13 to 14 weeks of intrauterine life. They also observed few goblet cells in the laryngeal mucosa. Such a finding was not reported earlier in the literature.

Nakashima T et al (12) while studying the local immune system in foetal larynx suggested the presence of plasma cells in lamina propria or periglandular connective tissue. They noted the glandular budding at 16 weeks of intrauterine life and observed that glandular distribution became apparent in submucosal connective tissue along the laryngeal cavity and trachea but they were unable to observe any goblet cell in any of the foetal specimens. In the present study glandular budding in submucosa was visualized in the foetuses of 13-14 weeks of intrauterine life which were seen as dark cluster of cells with H&E stain. Occasional goblet cells were also seen in the laryngeal mucosa.

These finding are in accordance with the findings of Meena N & Chandrama A (11). However these results do not coincide with the findings of Nakshima T et al (12) as they fail to demonstrate goblet cells in the laryngeal mucosa.

The maturation of glands happens to be gradual, passing through subsequent stages of development, before gaining maturity.

Conclusion

The present study on forty human foetuses ranging from 75mm-220mm crown rump length was done to study the development of human foetal laryngeal glands. We concluded that the entire laryngeal cavity was lined initially by respiratory epithelium that was ciliated pseudostratified columnar type. Later on the epithelium over the true vocal cords and dorsal surface of epiglottis changed into stratified squamous type. Lamina propria was well developed consisting of two distinct zones as inner dense and outer loosely arranged zone of connective tissue which was indistinct before 17 weeks of intrauterine life. There was well developed glandular tissue in the submucosa comprising predominantly of mucous glands and some serous glands as well. This glandular formation was seen from twelve weeks of intra uterine life and well established glands were seen in subsequent stages of development. A few goblet cells were seen in the laryngeal mucosa in few of the foetal specimens.

References

1. Silvia B. Development of Bioengineered human Larynx. *Biomaterials* 2011; 32 (19): 4433-42

2. Standring S. in larynx. Grey's anatomy. 40th Ed. Published by Churchil Livingstone Elsevier 2008 .pp. 577-94

3. Singh I. In respiratory system. Textbook of human histology. 6th Ed. Published by Jaypee brothers 2011 .pp. 217-26.

4. Singh V. In larynx. Textbook of anatomy head, neck & brain. 2nd Ed; Vol.3. Published by Reed Elsevier India Private Ltd. 2014 .pp. 218-30.

5. Garg K. in larynx. B D Chaurasia's human anatomy. 6th Ed; Vol.3. Published by CBS publishers & distributors pvt ltd. 2013 .pp. 252-63.

6. Bo Yang, Sijuj Yu, Yan Cui, *et al*. Histochemical and ultrastructural observations of respiratory epithelium and gland in yak (Bos grunniens). *Anat Rec* 2010; 293: 1259-69

7. Mohammad IO, Rachel MB, Jermy ES, Walter EF, Jonathen HW. Distribution and size of mucous glands in the Ferret tracheobronchial tree. *Anat Rec (Hoboken)* 2013; 296(11): :1768-74

8. Hamilton WJ , Mossman HW. In alimentary & respiratory system, pleural & peritoneal cavities. Hamilton, Boyd and Mossman's Embryology, 4th Ed. Published by Macmillan London 1978 .pp. 291-376.

9. Ballard ST, Inglis SK. Liquid secretion properties of airway submucosal glands. *J Physiol* 2004; 556; 1-10.

10. Wijkstrom-Frei C, El Chemaly S, Ali Rachedi R, *et al*. Lactoperoxidase and human airway host defence. *Am J Res Cell Mol Biol* 2003; 29: 206-12

11. Meena N, Chandrama A. Histology and Histo-chemical studies on developing human larynx. *J Ant Soc India* 1987; 36 (1): 1-7.

12. Nakashima T, Makishima K, Komiyama S, *et al*. Local immune system in the developing foetal larynx. *Laryngoscope* 1981; 91 (3):398-407.