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Growth of laryngeal glands in infants: a comparative study of the sub- and supraglottic larynx

Tadashi Nakashima, M.D., Hirohito Umeno, M.D., Shun-ichi Yonekawa, M.D., Atsushi Kikuchi, M.D., Sachio Tsuda, M.D., Kiminori Sato, M.D.

Abstract

It is well established that laryngeal secretions, produced in the glandular tissue, contribute to the local defence system of the larynx. In this study, the distribution of glandular tissues in the subglottic and supraglottic larynx was examined in 55 infants, using an image analysing system. The average area of the mucosa or glandular acini at the level of the false vocal fold was almost three times larger than that of the subglottis. At both levels, the area occupied by the glands increased according to the age at death and became more evident according to the total development score (TDS) which was estimated by adding the age at death and the gestational period. The results indicate that the glandular structure of the level of the sub- or supraglottic larynx develops constantly and equally after birth.

Key words: Larynx; Infant; Mucous Membrane; Developmental Biology

Introduction

The larynx is subjected to various stimuli such as air pollution as well as viral or bacterial infection. Recent advances in immunology and immunopathology have revealed that the larynx possesses an active mucosal immune system as in other respiratory and digestive organs.[1,2] We have been studying the development of the mucosal defence system by glandular tissue in the human adult[3,4] and infant[5] larynx. In particular, attention has been focused on the development of laryngeal glands before and after birth.[6]

We recently demonstrated changes in the laryngeal airway by measuring the subglottic structure using an image analysis system.[7] The area of the subglottic inner cavity and mucosa increases with age after birth and the subglottic airway becomes stable with advancing age. In the present study, the appearance of glandular tissue in the larynx was observed. By precisely measuring the submucosal glandular acini that are distributed at the subglottic level as well as at the level of the supraglottic larynx, the changes in the ratio of the distribution of the glandular tissues in the mucosa of each infant's larynx were compared.

Materials and methods

Materials

The materials consisted of laryngeal tissue specimens obtained from infant cadavers whose autopsies were carried out at Fukuoka Children's Hospital in Fukuoka City, Japan. Before extracting the larynx, the hospital records of the infant were carefully checked. Any infant whose disease or cause of death seemed to affect the development of the larynx were excluded from the study. The larynges of 55 infants (38 boys, 17 girls, Table I) were studied. The age at death ranged from one day to 104 weeks. The gestational period of the babies before birth ranged from 24 to 42 weeks. The weight at birth ranged from 1212 to 3800 g.

Because many of the babies examined had been premature births, the concept of a total development score (TDS) was adopted; it was estimated by adding together the age at birth and the gestational period.[7] The TDS of the examined babies ranged from 24 to

TABLE I
SUBJECTS STUDIED

Number of subjects 55
Age at death 1 day–104 weeks (average 12.7 week)
Gestational period 24–42 weeks (average 35.4 weeks)
Weight at birth 1212–3800 g (average 2283 g)
Total development score (TDS) 24–143 weeks (average 48.1 weeks)
Endotracheal intubation (none 7, 1 day: 11, 2–7 days: 19, 8–14 days: 6, 15–30 days: 8, 31 days or more: 4)

TDS: gestational period + age at death

From the Department of Otolaryngology – Head and Neck Surgery, Kurume University School of Medicine, Kurume, Japan.
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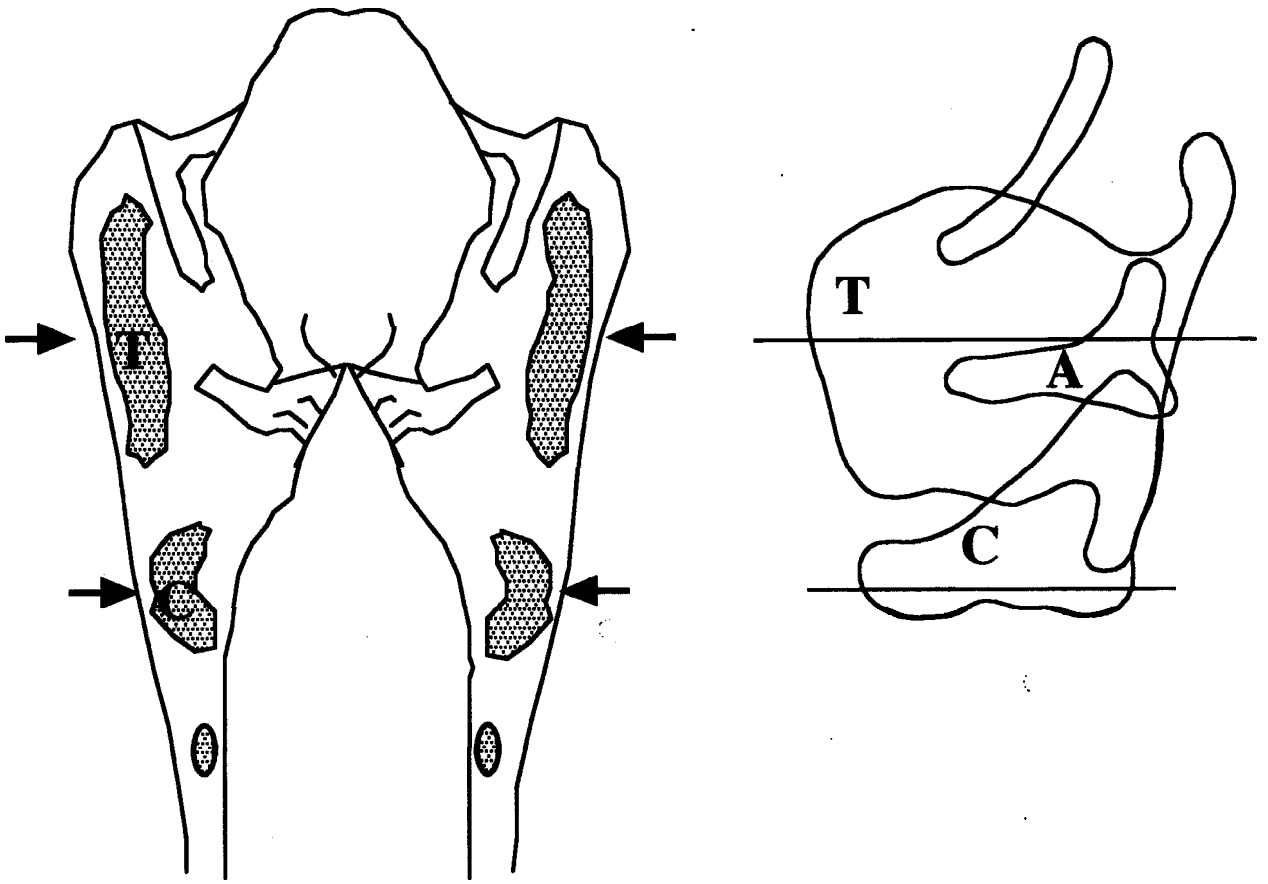

FIG. 1
Schematic presentation of the human larynx. A transverse section of the subglottic and supraglottic larynx was made at the level of the cricoid ring (C) and the apex of the arytenoid cartilage (A). T: thyroid cartilage.

143 weeks. The history of endotracheal intubation was found in the admission records of 48 babies aged from one day to 69 days (Table I). In 11 babies, however, such intubation had been performed immediately prior to death. After confirming there was no macroscopic findings such as traumatic injury or an ablation of the mucosa, the larynx was carefully separated from the cadaver. Each larynx was cut horizontally at the level of the false vocal fold and the subglottis (Figure 1). As shown in the figure, the subglottic and supraglottic level of the larynx was determined by cutting in a horizontal plane at the cricoid ring and arytenoid cartilage level.[8] Decalcification was not necessary in the examined infant larynges. Each tissue was conventionally embedded in paraffin, cut in 5 μm thick sections and then was stained with haematoxylin-eosin (Figures 2(a) and 3(a)).

Measurement of the laryngeal structure at both levels was performed using the image analysing system (WINROOF software, Mitani Corporation, Fukui, Japan) connected to a microscope (Nikon ECLIPS E800, CF 160 system). The microscopic view from the transverse section of the subglottic or supraglottic larynx stained with haematoxylin and eosin was transported to the image analysing system and then projected onto a visual display unit. The areas of the subglottic mucosa at the level of the cricoid ring were measured as reported previously.[7] Briefly, the inner lining of the cricoid ring as well as the mucosal surface line was traced using a tracing device (Figure 2(b)). By these procedures, the areas of both the maximum airway and the available airway was estimated. As a result, the area of the mucosal part (area of maximum airway minus area of available airway) could be determined. The measurement of each subject was repeated at least three times and then the average was used.

The survey of the supraglottic larynx was carried out at the level of the apex of the arytenoid cartilage (Figure 1). The ventricular fold and the upper portion of the laryngeal ventricle are located at this level.[8] The area of the supraglottis was determined by tracing the mucosa surrounded by the thyroid lamina and arytenoid cartilage (Figure 3 (b)). On the TV monitor, the mucosal lines of the inner cavity and ventricular evagination were traced and the area of each part was thus excluded. The area of the supraglottic mucosa at this level could then be estimated. When cavities from the ventricular evagination were found in the supraglottic plane, they were excluded from the area of the mucosa (Figure 3(b)) and thus the ratio of the glands per mucosa could be determined accurately.

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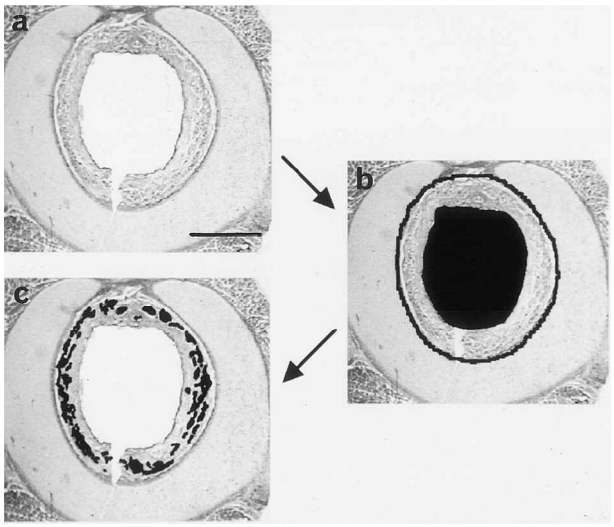

FIG. 2

Transverse section of the subglottic larynx (a). Stained by haematoxylin-eosin. Bar = 2 mm (b). The inner line of cricoid ring was traced on the TV monitor. The inner cavity of the larynx was also stained. Using an image analysing system, the area of each part was measured and, as a result, the area of the mucosa was determined (c). On the same image, the glandular acini were plotted minutely and the total area of the glands was calculated.

Measurement of the area of glandular acini

The glandular anici and ductules were minutely plotted in the same horizontal plane as seen on the TV monitor. Using an image binarization procedure, the plotted points were stained and the total stained area was calculated using a special software package (WIN ROOF, version 3.1) (Figures 2(c) and 3(c)).

Statistics

Significant correlations between the two groups and the coefficient of correlation were determined by Student's *t* test using the 'Stat view' software package (Abacus concepts, CA, USA). A *p*-value of less than 0.05 was considered significant.

Results

The mucosal area was measured from a transverse section at the level of the subglottis and supraglottis. In most of the examined materials, the larynx was cut from the posterior wall at the time of autopsy. Therefore the mucosal line was estimated by tracing it on the monitor. The average areas of the mucosa measured from the transverse section at the level of

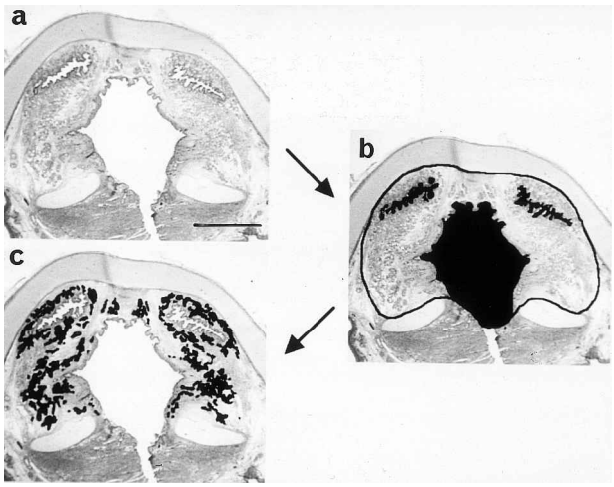

FIG. 3

A transverse section of the supraglottic larynx. (a) Stained by haematoxylin-eosin. Bar = 2 mm. (b) & (c) The area of mucosa (b) and glandular acini was also measured using the same method as shown in Figures 2(b) and 2(c).

subglottis (Figure 2(b)) and supraglottis (Figure 3(b)) were 13.74 mm^2 and 39.52 mm^2 (Table II). The average areas of the glandular acini at the same section were 2.78 mm^2 in the subglottis and 7.61 mm^2 in the supraglottis. The average area of each part (mucosa and glandular acini) in the supraglottis was almost three times larger than that of the subglottis.

The appearance of submucosal glandular acini was estimated by measuring the area of the glands on the TV monitor system. The area of glands at the level of the subglottic larynx increased according to the age at death of the infants (Figure 4(a)). After applying the total development score (TDS) to our findings, the degree of increase with an advancing score was more evident (Figure 4(b)). The correlation coefficiency of the relationship with the area of the glands was higher in the case of TDS. The close relationship between the appearance of glands with advancing age was also seen at the level of the supraglottic larynx (Figure 5). The area of the glandular acini increased according to the age at death (Figure 5(a)) and became more evident as the TDS increased (Figure 5(b)). However, the correlation coefficiency of the relationship was not as high as for the subglottic larynx.

In order to anticipate the development of local immune function by the mucosal glandular system, we investigated the ratio of the glands (the percentage of the area occupied by the glandular acini per

TABLE II

AVERAGE AREA AND RATIO OF THE MUCOSA AND GLANDS

	Total (55)		Total development score (weeks) ≤40 (20)		40< ≤50 (18)		50< (17)	
	Sub	Supra	Sub	Supra	Sub	Supra	Sub	Supra
Area of mucosa (mm^2)	13.74	39.52	9.92	26.23	14.46	42.76	17.48	51.70
Area of glands (mm^2)	2.78	7.61	1.21	3.36	2.58	7.62	4.83	12.62
Area of glands / Area of mucosa	0.19	0.18	0.12	0.12	0.18	0.18	0.28	0.25

( ): numbers examined

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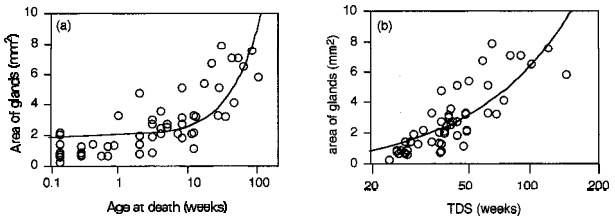

FIG. 4

Correlation between the total area of the glandular acini of the subglottic larynx and the age at death (a) or the total development score (TDS, b). Plotted on a logarithmic scale to the x-axis. The coefficient of correlation was 0.746 for (a) and 0.797 for (b).

area of the mucosa). In this analysis, the influence of endo-tracheal intubation according to the period of intubation of zero to one days (group A, n = 18), two to seven days (group B, n = 19) and more than eight days (group C, n = 18) was also examined. As a whole, the ratio of the glands increased as the TDS increased at both levels ($p<0.05$, Figure 6). However, no significant differences were observed between each intubation group.

The 55 cases were divided into three groups according to their TDS as follows: ⩽40, 40< ⩽50 and 50<. The average area of the mucosa of the subglottic or supraglottic larynx and the glandular acini at the same level increased with age (Table II). The average ratio of the areas occupied by the glandular acini per mucosal area in 55 infants was 0.19 for the subglottis and 0.18 for the supraglottis. Although the average ratios occupied by the glands increased from 0.12 to 0.28 in the subglottis and from 0.12 to 0.25 in the supraglottis according to each advanced TDS subgroup, the degree at both levels were consistently equal (Table II). These results indicate that the glandular structure of the larynx develops at an equal rate to the child's growth in the neonatal period.

Discussion

In a recent observation of the change of the subglottic structures in human infants, it has been reported previously that both the subglottic mucosa and available airway increase with age.[7]

Using the concept of the total developmental score (TDS), a significant correlation between the child's development and the stability of the subglottic airway was thus observed. In a previous survey of

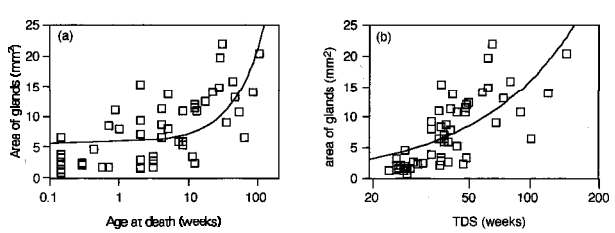

FIG. 5

Correlation between the total area of the glandular acini of the subglottic larynx and the age at death (a) or TDS (b), plotted on a logarithmic scale to the x-axis. The coefficient of correlation was 0.604 for (a) and 0.682 for (b).

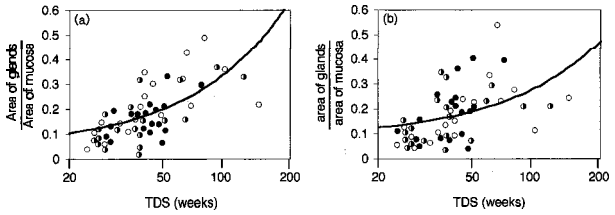

FIG. 6

Correlation between the ratio of the areas occupied by the glands per mucosa and the TDS in the subglottic (a) and supraglottic larynx (b). Plotted on a logarithmic scale to the x-axis. In the analysis, the infants were divided into three groups according to the period of intubation of 0-1 day (○: group A, n = 18), 2–7 day (◑: group B, n = 19) and more than 8 days (●: group C, n = 18). The coefficient of correlation was 0.618 for (a) and 0.419 for (b).

the pattern of subglottic glandular distribution, the glandular acini of human foetuses were observed to be sparse but tended to increase after birth.[5] The significant increase in the number of glandular acini as the TDS increases based on an examination of the subglottic larynx in the present study confirmed such results. The increase in the ratio of the glandular tissue per mucosal area strongly indicated the developing local immune function in the human neonatal larynx.

The present study investigated the development of glandular tissues of the supraglottic region in the neonatal larynx. In comparison to the subglottic larynx, the level of which was relatively easy to determine by estimating the plane at the centre of the cricoid ring, it was difficult to determine the same supraglottic plane in each laryngeal specimen. We therefore specified the horizontal level of the infant larynx at the plane which includes the apex of the arytenoid cartilage (Figure 1).[8]

It was interesting to note that the glandular tissues of the supraglottic larynx also gradually increased with age, particularly as the TDS increased. Although the coefficient of correlation was smaller than that of the subglottic larynx, these findings indicate that the laryngeal glands along the laryngeal mucosal line develop constantly in accordance with the child's growth. As the local immune system of the mucosa is known to take effect mainly by the production of secretory immunoglobulins in the secretory glandular acini or epithelium,[1–3] these findings strongly support the hypothesis that the local immunity of the larynx constantly develops after birth.

The increase in the ratio of the area occupied by the glands per area of the mucosa (Figure 6) seems important for the mucosal immune system of the human infant larynx. In particular, the subglottic larynx is a point at which the lumen is fixed by being surrounded with the cricoid ring and the available airway can thus easily change due to swelling of the mucosa.[9,10] The subglottic larynx therefore often causes dyspnoea due to a swelling of the mucosa because of either a viral or bacterial infection. It was previously reported that the subglottic larynx becomes stable with age because of the relative

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increase of the ratio of available airway per maximum airway.[7] The increase of the glandular tissues in the relatively thin subglottic mucosa in higher TDS infants, therefore, increases the advance of the local immune response by the locally distributed glandular system.

There was no significant difference in the ratio of available airway per maximum area between the groups with, and without, a history of intubation.[7] In the present study, the influence of endotracheal intubation in both the supraglottic and subglottic region was observed by dividing the infants into three groups depending on the ratio of the glands per mucosa. In a limited number of examined infants, no differences were detected between the periods of intubation. Further quantative analyses regarding the constitution of the serous or mucosa-type glands at both levels of the glands are therefore required.

Conclusions

By making a transverse section at the level of the subglottic and supraglottic larynxes from 55 infants, the area of the mucosa and the glandular structures were examined using an image analysing system. The area of the mucosa and glands increased with advancing age. The degree of increase was more evident when the analysis was done using the total development score (TDS). The percentage of area occupied by the glands in the mucosa at both levels increased constantly. These results strongly indicate that the local defence system due to the presence of glandular tissue along the mucosal line develops with age in the human infant larynx.

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Address for correspondence:
Tadashi Nakashima, M.D.,
Department of Otolaryngology – Head and Neck Surgery,
Kurume University School of Medicine,
67 Asahi-machi,
Kurume 830-0011,
Japan.

Fax: +81-942-37-1200
E-mail: orlkaku@med.kurume-u.ac.jp

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