Correlation between age and laryngeal ossification in Thai males

ความสัมพันธ์ระหว่างอายุและการเปลี่ยนแปลงเป็นกระดูกของกระดูกอ่อนกล่องเสียงในชายไทย

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Abstract

Objective: In personal identification, the age estimation was the most difficult. There is no method which is most accurate. Especially for the person who had complete epiphyseal fusion, many methods were applied together for age estimation for more narrowing and accurate results in forensic practice. In addition, there were many studies about the age estimation from cartilage. Thus, this research studied the degree of laryngeal ossification related to the chronological age for age estimation in Thai males. Radiopacity in laryngeal cartilage was classified to the degree of laryngeal ossification.

Materials and Methods: Two hundred and sixty-four laryngeal cartilages were obtained from Thai male cadavers who were 15 years and older. The samples were divided into 12 groups by five-year ranges per group. They were radiographed and evaluated the degree of laryngeal ossification for which the radiopaque ossified cartilages were confirmed by histopathological examination.

Results: The radiopacity of laryngeal cartilages were actually ossification. The degree of them correlated moderately with the chronological age (the average of correlation coefficient was 0.671). Furthermore, in those with the highest degree of ossification of each cartilage, one can predict for the lower limit of age in this group (the average of the correct prediction was 83.59%).

Conclusion: Because age estimation was determined from several methods on bone or cartilage in many parts of the body, the radiography of laryngeal cartilages was studied in present research. The results of this research showed that age estimation could be determined roughly from the laryngeal

ossification but it cannot be a definite standard. Thus, this method should be used in combination with other methods for more accurate result.

Keywords: Forensic science, Age estimation, Correlation, Laryngeal ossification, Prediction

บทคัดย่อ

วัตถุประสงค์: ใ นการตรวจพิสูจน์บุคคล การประมาณอายุเป็นสิ่งที่ตรวจพิสูจน์ยากที่สุดและยังไม่มีวิธีใดที่สามารถประมาณอายุได้ถูกต้องที่สุด ต้องใช้หลาย ๆ วิธีร่วมกัน ทั้งนี้ก็เพื่อให้การประมาณอายุของผู้เสียชีวิตนั้นแคบที่สุดและตรงกับอายุจริงที่สุดเท่าที่จะเป็นไปได้ โ ดยเฉพาะอย่างยิ่งในรายที่มีการเชื่อมปิดกันของ epiphysis ของกระดูกทุกส่วนของร่างกายแล้ว และปัจจุบันมีการศึกษากระดูกอ่อนเพื่อการประมาณอายุมากขึ้น ใ นการศึกษาครั้งนี้จึงมีจุดประสงค์เพื่อศึกษาความสัมพันธ์ระหว่างอายุและการเปลี่ยนแปลงเป็นกระดูกของกระดูกอ่อนกล่องเสียงในชายไทย โ ดยอาศัยความทึบแสงของกระดูกอ่อนกล่องเสียงจากภาพถ่ายรังสีในการแบ่งระดับการเกิดการเปลี่ยนแปลงเป็นกระดูก

วัสดุและวิธีการศึกษา: เก็บตัวอย่างกระดูกอ่อนกล่องเสียงจากศพเพศชายที่มีอายุ 15 ปีขึ้นไป จำนวน 264 ตัวอย่าง โดยแบ่งตามอายุออกเป็น 12 ช่วง ช่วงอายุละ 5 ปี แล้วนำไปถ่ายภาพรังสีและอ่านผลระดับการเปลี่ยนแปลงเป็นกระดูก จากนั้นศึกษาทาง Histopathology เพื่อยืนยันการเปลี่ยนแปลงเป็นกระดูกของกระดูกอ่อนกล่องเสียง

ผลการศึกษา: พบว่าการเปลี่ยนแปลงที่ปรากฏในภาพถ่ายรังสีของกระดูกอ่อนกล่องเสียงเป็นการเปลี่ยนแปลงเป็นกระดูกจริง และมีความสัมพันธ์กับอายุระดับปานกลาง (สัมประสิทธิ์สหสัมพันธ์เฉลี่ย = 0.671) นอกจากนี้ในกลุ่มที่มีระดับการเปลี่ยนแปลงเป็นกระดูกของกระดูกอ่อนกล่องเสียงที่มากที่สุดเราสามารถทำนายอายุขั้นต่ำของกลุ่มประชากรดังกล่าวได้ (ความถูกต้องในการทำนายเฉลี่ยร้อยละ 83.59)

สรุป: การประมาณอายุสามารถประมาณได้จากกระดูกหรือกระดูกอ่อนหลายส่วนของร่างกาย และหลากหลายวิธี ใ นการศึกษาครั้งนี้เช่นกัน ผู้ศึกษาใช้วิธีการถ่ายภาพรังสีกระดูกอ่อนกล่องเสียง และพบว่าการเปลี่ยนแปลงเป็นกระดูกของกระดูกอ่อนกล่องเสียงที่เกิดขึ้นนี้สามารถใช้ในการทำนายอายุได้อย่างหยาบ แต่ไม่สามารถนำวิธีนี้ไปใช้เป็นมาตรฐานได้ ควรใช้ประกอบกับวิธีการตรวจอื่น ๆ โดยวิธีนี้อาจเป็นเพียงการช่วยให้การประมาณช่วงอายุที่เป็นไปได้ให้แคบลง

คำสำคัญ: นิติวิทยาศาสตร์, การประมาณอายุ, สหสัมพันธ์, การเปลี่ยนแปลงเป็นกระดูกของกระดูกอ่อนกล่องเสียง, การทำนาย

Introduction

Whenever the death is apparently unnatural or if there is reasonable cause to suspect an unnatural death such as suicide, homicide, injury by animal, accident and unknown cause, according to the Criminal Procedural Code sections 148, 150 and 154, the postmortem examination is required, it is the

duty of the officers who are concerned, including an inquiry police officer of the locality where the body is located together with a forensic physician or a physician who has the authority under the law[1].

Frequently, suspicious remains or unidentified bodies were often found. Sometimes, dead bodies were difficult for identification e.g. skeleton, unidentified human remains or a serious injury of the face and head remains which were severely damaged, such as those in an explosion injury, the state of body will depend mainly on the degree of the explosion. Postmortem decomposition is a natural process whereby the dead body was decomposed gradually over time until skeletonized. The identification by visual cue becomes more difficult as the decomposition process advances. In circumstances where the body is fresh or, at least, has not yet lost its soft tissues through the natural process of decomposition, postmortem examination should include both external examination and internal dissection of the cadaver. Ideally, this procedure should be carried out by a forensic pathologist. In cases where the human remains consist only of the bony skeleton (or parts thereof), postmortem examination will benefit from the specialist knowledge of the forensic anthropologist[2].

Nevertheless, the analysis of skeletal, badly decomposed, or otherwise unidentified human remains leads to the personal identification process and create a biological profile i.e. sex, ethnic/race, stature (living stature), and age[3-4]. Especially, age is the most difficult to determine.

Although there are many methods of study about the age determination, there is no method which is most accurate. External examination, measurement, radiography and including microscopic examination in a part of body e.g. teeth, skull, pubis, long bones, vertebrae, and cartilages can be applied together for age estimation for more narrowing and accurate results in forensic practice.
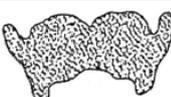
However, in Thailand, only xiphoid process of sternum and costal cartilage were studied but laryngeal cartilage ossification has not been performed. Thus, that is the reason why the present study is interested in studying the correlation between age and laryngeal ossification in Thai males. In addition to collect the data about correlation between pattern of laryngeal ossification and age of Thai males for forensic science and forensic medicine application.

Materials and Methods

Two hundred and sixty four samples of laryngeal cartilages were obtained from male cadavers who underwent forensic autopsy at the Department of Forensic Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University and Institute of Forensic Medicine, Police General Hospital, Royal Thai Police. All of them were 15 years and older who had Thai nationality. Hyperparathyroidism, abnormal calcification of other parts of body and incomplete cartilages were excluded. During the forensic

autopsies, the larynx was routinely removed, the muscles and ligaments were carefully dissected until laryngeal cartilages were clearly seen. After that, cut the joint that join all three cartilages together (thyroid, cricoid, and arytenoid cartilage). Each clearly cleaned cartilage was subjected to radiography in an anteroposterior orientation at 50 mA, 40 kV for 100mSEC. These were performed at Department of Radiology, Faculty of Medicine Siriraj Hospital, Mahidol University by using Digital Radiography (DR).

The degree of laryngeal ossification was evaluated for each cartilage with the help of adjusted classification of Keen and Wainwright as follows[5]:

	Stage 0 : No opacity of ossification
	Stage 1 : Opacity in the inferior horns spreading upward in the lamina
	Stage 2 : Opacity extending anteriorly for a variable distance into the lamina
	Stage 3 : Opacity extending to the superior horns
	Stage 4 : Cartilage was almost entirely opaque
	Stage 5 : Complete opacity of the cartilage

Stages of thyroid cartilage ossification

	Stage 0 : No opacity of ossification
	Stage 1 : Opacity along the superior border of the lamina in one or two areas on each side
	Stage 2 : Opacity across the midline in the superior half of the lamina
	Stage 3 : Inferior half of the lamina showing varying degree of opacity along with the posterior half of arch
	Stage 4 : Complete or partially complete opacity of the cartilage

Stages of cricoid cartilage ossification

	Stage 0 : No opacity of ossification
	Stage 1 : Opacity in the muscular process
	Stage 2 : Most of the muscular process and body are radiopaque
	Stage 3 : Opacity spreading up to the posterior border of the apex

Stages of arytenoid cartilage ossification

Figure 1 Stages and appearance of thyroid, cricoid, and arytenoid cartilages ossification

Spearman's rank correlation was used to present the relation between the chronological age and the stage of laryngeal ossification. The statistical significance was set at $p<0.05$ level. Then, simple and multiple linear regressions were used to determine the best predictor valuable or combination of laryngeal cartilages for predicting the age. In addition, the age group for predicting was classified with the help of receiver operating characteristic (ROC) analysis.

After the radiographic analysis, histopathological examination was the method used to confirm laryngeal ossification. All ossified cartilages were decalcified and processed to paraffin sectioning and stained by Hematoxylin and Eosin (H&E) method.

Results

Two hundred and sixty-four laryngeal cartilages ranged in age from 15 to 87 years. The mean age was 44.9 years (SD = 17.7). A great number of ossified thyroid cartilages were classified into stage 2 (74 samples, 28%) and stage 3 (96 samples, 36.4%). For the cricoid cartilages, there were many ossified cartilages at stage 2 (78 samples, 29.5%) and stage 3 (83 samples, 31.4%). Almost half of arytenoid cartilages were classified into stage 2 (128 samples, 48.5%) and the age of this stage was the highest dispersed (SD = 14.306). The distribution of age correlated with the stage of thyroid, cricoid, and arytenoid cartilages was shown in Table 1 and Figure 2.

Stages of ossification	Number of subjects (Percentage)	Min Age, Max Age (Years)	Median (Years)	Mean, SD (Years)
Thyroid cartilage				
0	12 (4.5)	15, 22	19	18.58, 2.234
1	25 (9.5)	15, 57	22	23.04, 8.394
2	74 (28)	17, 74	34.5	36.8, 12.62
3	96 (36.4)	16, 87	53	52.32, 14.517
4	38 (14.4)	27, 78	57.5	57.84, 11.702
5	19 (7.2)	34, 83	61	58.05, 15.389
Cricoid cartilage				
0	18 (6.8)	15, 25	19	18.56, 2.915
1	37 (14)	17, 69	27	29.46, 11.647
2	78 (29.5)	17, 74	40	41.46, 14.436
3	83 (31.4)	16,78	52	51.54, 14.249
4	48 (18.2)	34, 87	62.5	60.62, 12.454
Arytenoid cartilage				
0	39 (14.8)	15, 40	20	20.85, 4.771
1	72 (27.3)	18, 75	35.5	38.4, 13.324
2	128 (48.5)	16, 87	53.5	52.6, 14.306
3	25 (9.5)	38, 83	61	61.4, 11.754

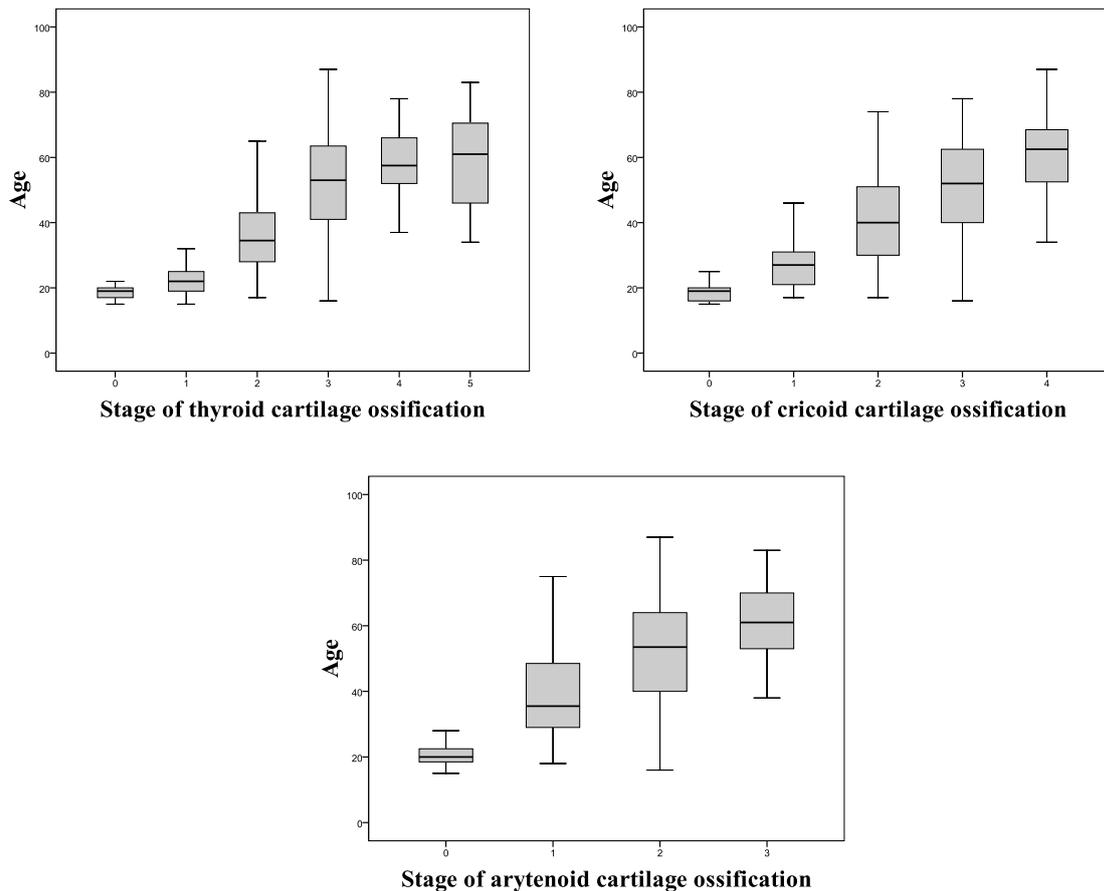Table 1 Distribution of age correlated with the stage of thyroid, cricoid, and arytenoid cartilage ossification

Figure 2 Box and whiskers plot for stage of thyroid, cricoid, and arytenoid cartilages ossification:
box represents interquatile range, thick black horizontal line represents median

Spearman's rank correlation was used for analyzing the correlation between age and three types of laryngeal cartilages, which revealed that the chronological age and the stages of laryngeal ossification were moderately significant6, correlation coefficient was 0.662 to 0.679 at a probability level less than 0.001. The chronological age was positively correlated with the stages of laryngeal ossification.

In regression analysis, the best accurate formula for predicting the estimated age of an individual was comprised of three cartilages, 53% of variation of predicted age can be explained by thyroid, cricoid and arytenoid cartilages. It could correctly predict 35.99% (95/264) for the age range of ±5 years and about 52.27% (138/264) for the age range of ±8 years.

Because the results of correlation coefficient and regression analysis showed moderate correlation and moderate coefficient determination, Receiver Operating Characteristic curve (ROC) was used for declaring the cutoff point and predictive value. The predictive value of thyroid cartilage was 100% at the age lower than 22 years if there is no ossification (stage 0). If it was stage 1, the probable age was lower than 28 years and 88% for predictive value. The probability of 51 years and

older was 75.44% at stage 4 or 5 of thyroid cartilage. For cricoid cartilage, if it had no ossification or stage 0, the possibility of age was 20 years and younger and predictive value was 77.78%. Meanwhile, the predictive value was 83.33% at the age of 48 years and older if the ossification of cricoid cartilage was stage 4. If arytenoid cartilage had no ossification (stage 0), the possible age was 23 years and younger with 84.62% for predictive value. Predictive value was 92% for age 44 years and older at stage 3 of ossification.

For histopathological examination, the radiological opacity of the cartilage was proved to be true ossification as shown in Figure 3.
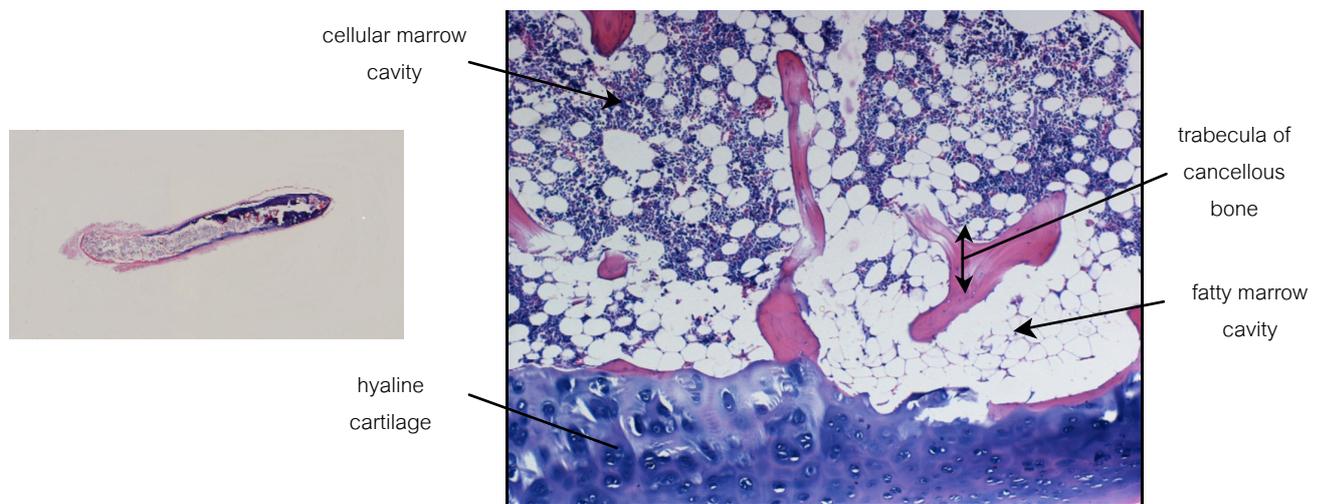

Figure 3 Photomicrography shows the ossification of thyroid cartilage, age 47 years (H&E x10).

Discussion

From the general characteristics of each cartilage and stage of ossification, the stage of ossification increases, the age also increases. Therefore, if the age increases, laryngeal cartilages also change to bone as a normal age change called "ossification"7. Correspondingly, many researchers studied the relation of laryngeal ossification and age, for which the ossification occurred at increasing age 8 – 18.

According to the moderately significance of correlation coefficient, the researcher tried to establish the regression equation that might be able to predict the age from the stage of laryngeal ossification. The result of regression analysis showed the highly predictable regression equation by using three cartilages. The formula indicates the moderate prediction (35.99% (95/264) for the age range of ±5 years and 52.27% (138/264) for the age range of ±8 years). A wide inter individual

variability of age in the same class may be the causation. Accordingly, some specimens were ageing but the cartilage did not progressively ossify with age and vice versa so that the accuracy of formula was moderate to poor. In addition, the minimum age that was calculated from this equation did not correspond to the chronological age if all cartilages were stage 0. It might be a result of the stage that was classified roughly.

In considering pattern of laryngeal ossification, most of these specimens and the other specimens that described in the previous literatures are alike. Ossified thyroid cartilage usually begins in the inferior horns and inferior border of the lamina and then spread along the posterior border into the superior horns and midline of the lamina until the cartilage ossify completely. In some cases, the pattern of ossification was unlike in their appearance. The beginning of ossification was usual, inferior horns and inferior border of the lamina were the first to ossify, but transilient to the superior horns, discontinuous sequence in ossification ensued. This pattern was similar to third stage (20 – 30 years) of Vlcek stages of thyroid ossification[13] but Vlcek stages had no ossification at midline of the lamina. Moreover, ossification of some cases was almost entirely in the lamina but the superior horns did not ossify, thus evaluation of the stage of thyroid cartilage was very difficult. The authors think that these different patterns may have influence on the accuracy of the regression equation. However, for the cricoid and arytenoid cartilage, the ossification process in this study followed Keen and Wainwright stages[5].

In addition, radiographic appearance of the ossification may be confused with foreign body. Some research[19] reported that the sites likely to be mistaken is the area in the posterior lamina and inferior horn of thyroid cartilage, especially in the early stages of ossification but the confusion is less in the fully pattern. In the cricoid cartilage, the dense superior tip or curvilinear and vertical posterior margin of the lamina are the confusion sites. Compare the method with the present study; the specimens of Hately' s study were the radiographs of the neck while the specimens of this study were the radiographs of the clean cartilages that were removed from the corpses, thus this study can eliminate such mistake.

The results of histopathological method for confirmation of laryngeal ossification examined after complete radiolographic examination indicate that the specimens were indeed ossified because the structure appears eosinophilic for the bone matrix that is left intact after decalcification process. In addition, ensuring the bone authenticated by osteocytes in this structure, namely trabeculae which enclosed with bone marrow. This degenerative progress changes the cartilage to cancellous bone. Conclusively, the radiopacity of cartilage seen on radiograph was actually ossification.

Conclusion

The correlation between the degree of laryngeal ossification and chronological age in this study revealed moderate correlation with positive significance. There was moderate accurate regression equation that is composed of all cartilages and the age calculation from this equation was not correct if all cartilages are stage 0, thus the ranging predictive age was classified by using ROC Curve and could be most correctly predictable in the lowest and highest stage of each cartilage. Furthermore, histopathological examination could confirm that the radiopaque areas seen on the radiograph were really ossified cartilages.

Recommendation

From this study design, it was a subjective study that the observer who influences stage evaluation on radiography was the important factor. Thus, degree of laryngeal ossification depends on individual's interpretation. In addition, this method yields wide age range. However, when using this method, the radiological interpretation can be controlled by the classification of stage guideline adherence. This method may be useful in forensic application as a supplementary study for age estimation when combined with other methods.

Acknowledgements

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