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Shape Differences in the Auricula of the Human Foetus: A Geometric Morphometric Approach

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The aim of the study is to analyse the auricula of human foetus using geometric morphometric methods based on the foetal age groups and, thus, to determine the presence of shape differences. For this purpose, the foetuses having a gestational age of 23-40 weeks were divided in three groups based on intrauterine gestational age and analysed. After performing photography, landmarking and dataset formation stages for geometric morphometric analysis, the principal component, and discriminant and regression analyses were made. The first principal component accounted for 26.461% of the total shape difference based on foetal age. The most apparent shape changes were observed on helix (superior), crus helicis, tragus and antitragus points. Consequently, the variations concentrated especially around cavum concha.

Key words: auricle, hearing, morphoJ, tragus

Introduction

Humans have the most complex and efficient communication system among mammals and this ability is one of the fundamental concept for surviving during the evolution of humankind. Hearing is one of the key abilities for communication and can be examined by aid of malleus, stapes and incus (unique to mammals) in fossil specimens since auricula cannot be fossilized. Although it is known that *Homo sapiens* (anatomically modern human) has the most complex auditory system among human species, a recent study points out that the closest modern human relative, *Homo neanderthalensis*, could have had similar auditory capacity as modern humans [12].

Auricle is a cartilaginous and fibroelastic structure covered by a thin skin [3]. Auricle grows throughout lifetime and males have greater increase then females [30]. It is also highly variable body part among individuals and populations. The surface of this structure is characterized by protrusions and depressions [47]. Auricula is composed of three main components: helix-antihelix, cavum concha and lobulus auriculae [31].

The studies have emphasized [26, 44] that the six hillocks originating from the first and second pharyngeal arches are responsible for the development of auricula. These hillocks located as three on both sides of meatus acusticus externus, three on each side, form auricula by expanding and combining asymmetrically. Lobulus auriculae are the last part developing in auricula [29, 46].

It is a complicated process that the principal structures of auricula combine in the foetal periods. For this reason, the development anomalies of auricula are common [5]. It is an important question which structures contribute to the development of auricula by changing shape on which side. Therefore, it is important to know the normal shape changes of auricula in the foetal period to clearly figure out the effect of anomalies on auricula.

Geometric morphometrics is a method helping many different disciplines such as anthropology, anatomy, zoology and botany in the recent years. In this method, landmarks (LM) are digitized at *Cartesian* coordinates in accordance with the geometric structure of objects [33]. Thus, two- or three-dimensional shape of the sample and the shape changes are analysed using the location differences between objects [27, 48, 50]. Also, the analysis results are interpreted by being mapped based on the size and direction of the change in the coordinates of the landmarks [7].

There are a limited number of studies in the literature for auricula by using geometric morphometric methods in adult human beings [31, 33]. However, no study has been found in which auricula is analysed with geometric morphometric methods based on the foetal period. For this reason, the aim of the study is to analyse the auricula of human foetus by using geometric morphometric methods based on the foetal age groups.

Materials and Methods

Samples

The foetuses which were obtained from Isparta Maternity and Child Hospital and found in Süleyman Demirel University Medical Faculty Anatomy Department laboratory by obtaining the permission of the families between 1996 and 2010 were used in this study. Approval was obtained from the Medicine Clinical Research Ethics Committee. The ages of the foetuses kept in formaldehyde solution (10%) were between the 23-40 gestational weeks and they did not have any anomaly or pathological condition. They were divided into three groups based on the intrauterine gestational age. Twelve foetuses had a gestational age of 23-28 weeks in the first group, 9 foetuses had a gestational age of 29-34 weeks in the second group, and 11 foetuses had a gestational age of 35-40 weeks in the third group.

Data collection and Landmarking

The left auricle of samples was photographed in the way that the camera (18x55 lens, Canon Eos, 600D, Japan) and the focus were on the same plane (camera resolution

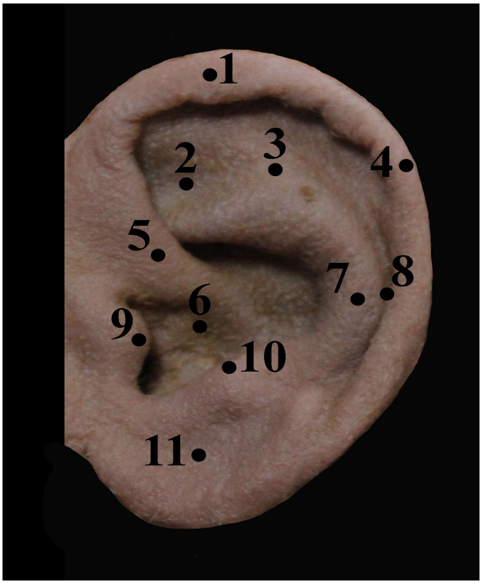

Fig. 1. Landmarks, 1. Helix (superior), 2. Fossa triangularis, 3. Crus antihelices (intersection of crus antihelices), 4. Helix (posterior, crus antihelices), 5. Crus helicis, 6. Cavum concha, 7. Antihelix, 8. Scapha (antihelix), 9. Tragus, 10. Antitragus, 11. Lobulus auriculae (mid point)

890 x 1065 pixels). Cavum concha was determined as the focus in the photographing. The distance between the focus and lens were standardized at 30 cm. These photos were converted into tps format using TpsUtil (Version 1.79) [41].

The eleven homolog LMs [25, 36] (**Fig. 1**) were marked on the photos by TpsDig2 programme (Version 2.31) [40]. Thus, the *x, y Cartesian* coordinates of LMs were determined. Before the statistical analysis, it was important to determine whether or not the new tangent space was small enough to demonstrate that it was a good representation of the Procrustes data in a Euclidean space. This confirmation test was made in TpsSmall (Version 1.34) [39] software by determining the correlations of the tangent and Procrustes distances. The test results (uncentred correlation: 0.999, root mean square error: 0.000149) confirmed that the correlation data were quite close for both space distances.

Statistical analysis

As there are differences in the auricula such as size, position and direction, General Procrustes Analysis (superimposition) was performed [45]. Principal component analysis (PCA) was performed on the new coordinates obtained as a result of Procrustes analysis. Thus, the degree of split upof samples based on the age groups between the factors was determined using covariance analysis [50].

The potential size and shape differences between the age categories were analysed with one-wayanalysis of variance (ANOVA). PAST (Version 4.02) [17] software was used for these analyses.

It was determined at which landmark and in which direction the shape difference was located based on the PCA. To assess the allometric effect based on the foetal age on the shape change, a multivariable regression of Procrustes coordinates was performed using a permutation test with 10.000 repetitions [13, 16]. As CS (centroid of size) corresponds to the square root of total of the distances of the squares from each turning point to the central point [38], the CS of the landmark configurations was used as a representative for the dimension measurement. Discriminant function analysis (DA) was performed on the procrustes coordinates to see the grouping properties of the samples. MorphoJ [28] software was used for all these analyses.

Results

Table 1 shows the results related to the PCA. Accordingly, the first principal component accounted for 26.461% of the total shape variance based on foetal age. The difference based on foetal age in terms of the first and second principal component was shown in the plot in **Fig. 2**. Based on the plot, it was observed that the foetuses in the first group fall mostly negative half of *y* axis and the individuals in the third group fall mostly positive half of *y* axis. The foetuses in the second group were located on the centre of the plot.

Table 1. Values obtained as a result of the PCA, PC: principal component

PC	Eigenvalue	% Variance	PC	Eigenvalue	% Variance
1	0,005162	26,461	12	0,000342	1,7546
2	0,003726	19,1	13	0,000238	1,2221
3	0,002559	13,116	14	0,000137	0,7029
4	0,002053	10,524	15	0,000112	0,57421
5	0,001232	6,3134	16	8,94E-05	0,45851
6	0,001036	5,3103	17	6,61E-05	0,33878
7	0,000694	3,5568	18	4,55E-05	0,23333
8	0,00061	3,1288	19	2,61E-05	0,13367
9	0,000528	2,7077	20	4,05E-16	2,07E-12
10	0,000492	2,5228	21	1,75E-16	8,97E-13
11	0,000359	1,8414	22	1,31E-16	6,71E-13

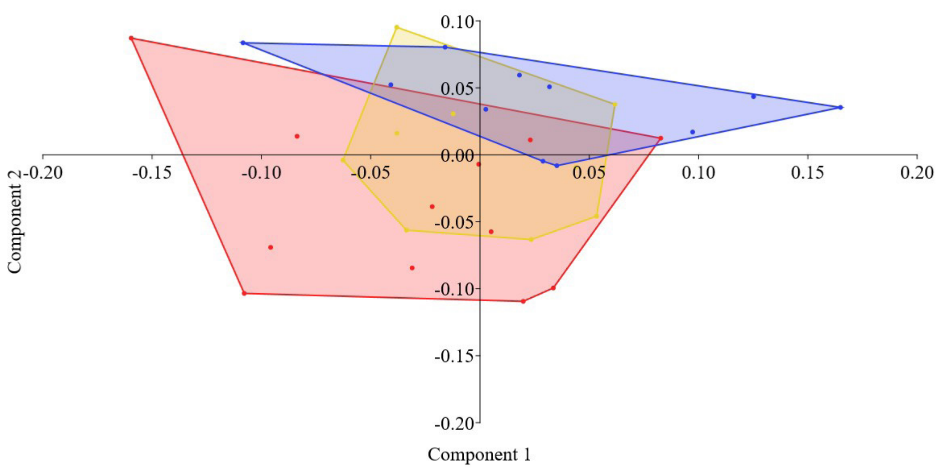

Fig. 2. In the graphic presentation of the results obtained based on the first and second principal component, the red points represent the first group individuals (foetal age of 23-28 weeks), the yellow points represent the second group (foetal age of 29-34 weeks) and the blue points represent the third group individuals (foetal age of 35-40 weeks).

Table 2 shows the results of ANOVA test made to determine the difference between the Procrustes coordinate values of the landmarks based on the foetal age groups. Table 3 shows the statistically significant landmark data ($p<0.05$) as a result of the Post-hoc (Tukey) test. Based on the tables, there were statistically significant differences between the groups related to the location of helix (posterior point), antitragus and lobulus auriculae on *x* axis and the location of crus helicis, scapha (antihelix) and lobulus auriculae on *y* axis.

Table 2. Results of Anova test. Co: Coordinates, SS: Sum of squares, Df: Degrees of freedom, MS: Mean square, F: F-value, P: P-value

Co	SS	Df	MS	F	P
X1	0,000558	2	0,000279	0,1779	0,8379
Y1	0,000722	2	0,000361	0,3107	0,7354
X2	0,001945	2	0,000973	2,745	0,08092
Y2	0,001804	2	0,000902	1,653	0,209
X3	0,002011	2	0,001005	2,867	0,07308
Y3	0,005954	2	0,002977	2,999	0,06548
X4	0,007927	2	0,003963	12,84	0,0001
Y4	0,001404	2	0,000702	1,23	0,3072
X5	0,004803	2	0,002402	2,861	0,07346
Y5	0,005209	2	0,002604	3,736	0,036
X6	9,61E+00	2	4,80E+00	0,06676	0,9356
Y6	0,002111	2	0,001055	1,224	0,3087
X7	0,000591	2	0,000295	0,5219	0,5989
Y7	0,002341	2	0,001171	1,627	0,2139
X8	0,004653	2	0,002326	2,575	0,09346
Y8	0,003848	2	0,001924	4,529	0,01943
X9	0,001917	2	0,000958	0,658	0,5255
Y9	0,001386	2	0,000693	1,147	0,3316
X10	0,017777	2	0,008888	7,527	0,00233
Y10	0,000422	2	0,000211	0,2347	0,7923
X11	0,005458	2	0,002729	4,761	0,0163
Y11	0,01378	2	0,00689	4,403	0,02138

Table 3. The landmarks with $p < 0.05$ as a result of the post-hoc test of Procrustes coordinates

Landmark	Group	Axis	P-value
LM4	1x2	X	0.001
	1x3		0,000
LM5	1x2	Y	0,048
LM8	1x2	Y	0,047
	1x3		0,036
LM10	1x3	X	0,002
LM11	1x2	X	0,033
	2x3		0,024
	1x3	Y	0,016

Figure 3 shows at which landmarks the shape differences occurred based on the PCA. Accordingly, an apparent shape differentiation was observed superioposteriorly in LM1, inferioposteriorly in LM5 and LM6, superioanteriorly in LM8 and LM10 and inferiorly in LM9.

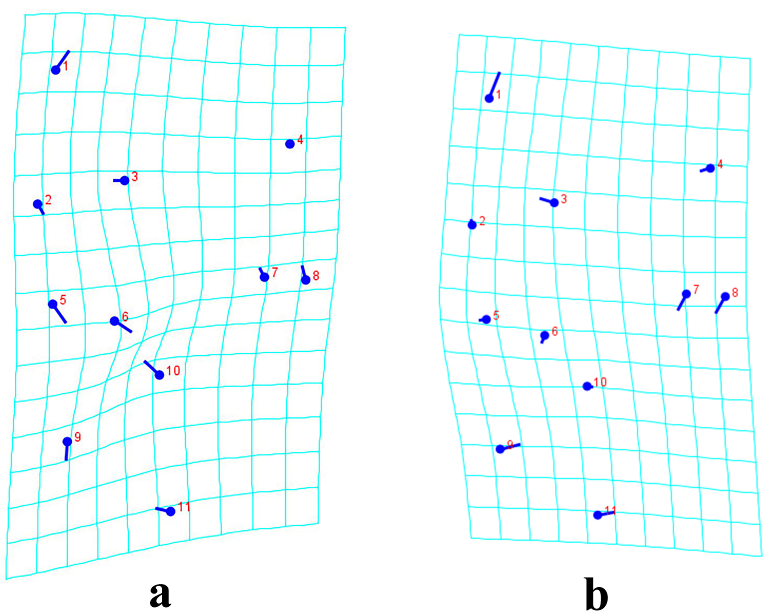

Fig. 3. The point demonstration of the shape differences occurred at the landmarks based on the PC1 (a) and PC2 (b). Point represents the average shape. Set scale factor: 0.1.

Table 4 shows the distinction obtained with the discriminant function analysis implemented to observe the classification of the foetal age groups. Accordingly, it was observed that group 1 was completely separated from the other groups. Also, **Fig. 4** shows the discriminant score graphic obtained as a result of the discriminant function analysis and **Fig. 5** shows the shape variation plot. Accordingly, among all the groups, apparent shape differences were observed at LM8, LM9 and LM11. In addition, in the comparisons of group 1 and group 3, a significantshape change was observed at LM6, LM7, LM8, LM9, and LM11 (**Fig. 5**).

Table 4. Discriminant function analysis of the foetal age groups. The number of the samples was presented for each group.

Group	**1 (23-28 week)**	**2 (29-34 week)**	**Total**
1 (23-28 week)	12	0	12
2 (29-34 week)	1	8	9
Group	**1 (23-28 week)**	**3 (35-40 week)**	**Total**
1 (23-28 week)	12	0	12
3 (35-40 week)	0	11	11
Group	**2 (29-34 week)**	**3 (35-40 week)**	**Total**
2 (29-34 week)	8	1	9
3 (35-40 week)	1	10	11

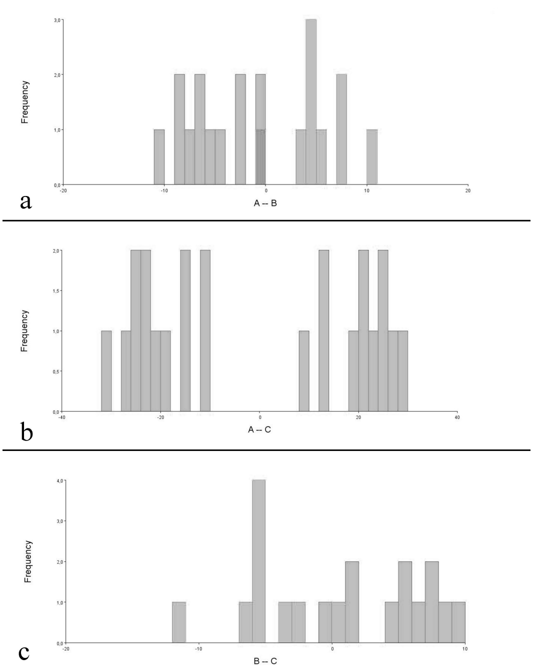

Fig. 4. The classification plot obtained with discriminant function analysis based on the foetal age groups, a. 1x2, b. 1x3, c. 2x3, A: Group 1 (23-28 weeks), B: Group 2 (29-34 weeks), C: Group 3 (35-40 weeks)

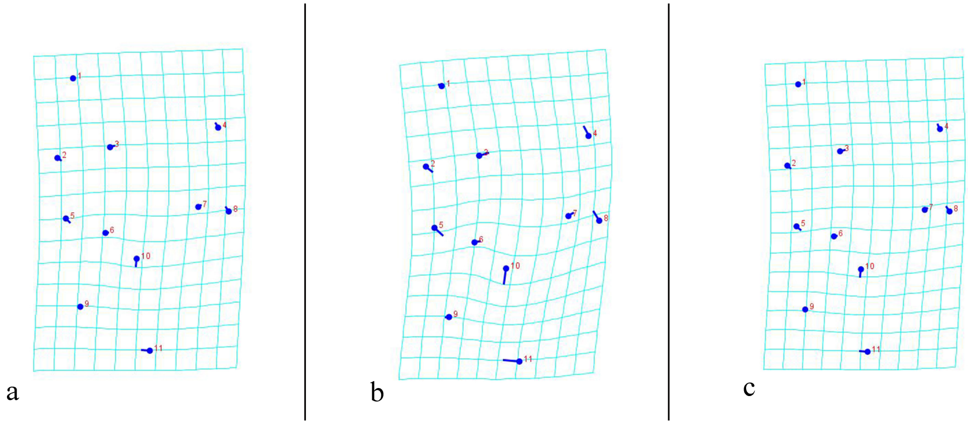

Fig. 5. The shape variation plot obtained with discriminant function analysis based on the foetal age groups, A. 1x2 (point represents group 1), B. 1x3 (point represents group 1), C. 2x3 (point represents group 2). Set scale factor: 1.0.

In the study, the statistical allometric effect of Procrustes coordinates (dependent variable) on dimension (log CS, independent variable) was observed only in group 2 (P value: Group 1: 0.652; Group 2: 0.0104; Group 3: 0.8722). Dimension explained 7.2556%, 24.9918%, and 4.7198% of the total shape difference in groups 1, 2, and 3, respectively and the groups were separated apparently (**Fig. 6b**).

In the assessment performed in terms of the rates of the allometric shape variation at landmark (**Fig. 6a**), it was observed that an apparent shape differentiation occurred in LM8, LM9, and LM11 together with the increase in the foetal age.

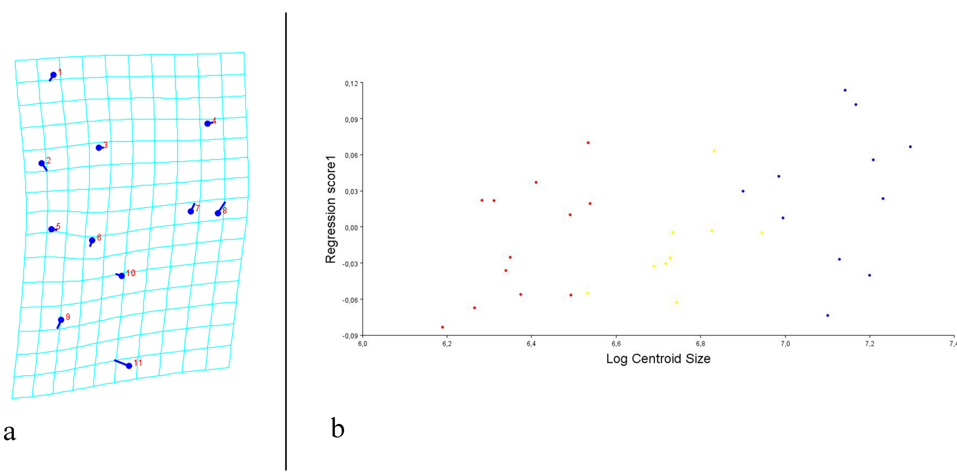

Fig. 6. Regression plot of Procrustes coordinates based on the foetal age groups (a, set scale factor: 0.5) and shape variation (b), Red: Group 1 (23-28 weeks), Yellow: Group 2 (29-34 weeks), Blue: Group 3 (35-40 weeks)

Discussion

At the end of the twentieth week of the foetal period, auricula takes its adult shape and this development continues averagely until the age of 9 in the postnatal period [49]. It has been suggested that the factors such as skin elasticity [35], strength of connective tissue [21], gravity force [37], genetics [32], age [2], sex[6], geographical location [14], chemicals and radiation exposure[18], uterus and placenta functions [4] may affect this development. However, the effects of these factors on the anatomic structures contributing the 'whole auricula shape' are not clear yet. In the present study, in the foetuses with the gestational age of 23-40 weeks, the first five components, based on the PCA, explained 75.5144% of the total shape variation. Different fetal age groups are separated in the PCA graph. Based on the PCA plot, it was remarkable that the foetus group with the gestational age of 29-35 weeks were gathered in the transition areas of the other two foetus groups. It was interpreted that this situation may be due to the fact that the shape change occurred gradually based on the foetal age groups. Also, considering that it is nearly impossible to standardize the above mentioned factors, affecting the general form of auricula, in practice, it was significant that the foetal age groups formed an important shape variation in terms of the "whole auricula shape" based on the PCA.

The effective and appropriate analysis of auricula shape helps us to understand the anatomic changes caused by the pathological disorders [15]. Foetal auricula, which is an important criterion for pediatrists, is useful in the assessment of some congenital malformations [10, 24, 37] and syndromes [37]. Kalcıoğlu *et al.* (2003) [23] reported in their study on auricula morphometry that the measures from tragus to helix and antihelix may be determinant in the diagnosis of abnormal auricula structure. Auricula is also an indicator of abnormal development in the pharyngeal region due to the fact that it has a close relationship with pharyngeal arcus and it develops from different origins [9, 43]. Therefore, the development of the foetus auricula and its general shape are highly important. We investigated which landmarks produced the most significant changes in the shape of the auricle in normal fetuses. In the present analysis performed for this purpose, it was observed that the most apparent shape changes were on helix (superior), crus helices, tragus and antitragus points.

Lobulus auriculae areolar, which has a connective tissue and fatty tissue quality [3] is the last part developing in auricula [5]. The most apparent changes in auricula together with the aging process occur in lobulus auriculae [8]. In the study, the general shape of auricula was focused rather than the general form of lobulus auriculae. For this reason, a single landmark representing lobulus auriculae was determined. In the present study, there was an anterior apparent variation in the landmark (LM11) representing lobulus auriculae based on the foetal age groups. However, whether or not there is a change in the general shape of lobulus auriculae or the degree of the variation may be examined in another study.

Among the hillocks, responsible for the formation of auricula, the first hillock contributes to the development of tragus, the second hillock contributes to the development of crus helices, the third hillock contributes to the development of helix, the fourth and fifth hillock contribute to the development of helix, scapha and antihelix and the sixth hillock contributes to the development of helix and antitragus. The combination of these hillocks occurs in the 6th and 8th weeks of the embryological development and they grow at different growth rates and provide that auricula takes its normal shape [22]. In accordance with the literature [22], it was also determined in the present study that the anatomic formations of foetus auricula had different growth rates with the result of the discriminant function analysis. Accordingly, the fastest and apparent variation was observed at tragus (LM9), scapha (antihelix, LM8) and lobulus auriculae (LM11).

Morphologically, auricula is different in certain degrees in primates. It is known that this difference is one of the determinant criteria in terms of phylogenetic relations [11]. The auriculae of the anthropoid and non-anthropoid primates may be distinguished in terms of morphological differences but it is not known whether the auriculae of these species have the similar or different developmental stages in foetal period. When viewed from this point of view, no study similar to the present study was found in the literature not only on human beings but also on primate order.

The helix-antihelix complex of auricula allows collecting and directing sounds. Concha intensifies the collected sounds and increases their frequencies [1]. An adult human being has the ability to hear the sounds at the frequencies ranging between 20 Hz and 20 000 Hz. Some authors [42] have reported that the hearing frequency range in newborns was 500-1000 Hz. Hepper and Shahidullah (1994) [19] reported in their study that one foetus responded to 500 Hz tone of voice in the 19th gestational week. In the same study, it was revealed that as the foetal age increased, the degree of the sound frequency respond also increased. Hepper & Shahidullah (1994) [19] stated that all of the foetuses responded to 1000 Hz of tone in the 33th gestational week and 3000 Hz of tone in the 35th gestational week. In the present study, the shape variations of auricula, which is known to function as directing sound and increasing its frequency, based on foetal age groups were examined and it was observed that certain variations formed in many anatomic points. In the literature [19], it has been stated that the direct proportion between the age of responding to sound and frequency height may be related to the fact that all the structures included in the hearing function develop. The contribution of the shape variation in auricula to the relationship between the age of responding to sound and frequency is not known. However, in accordance with the present study, that the shape variation intensified around cavum conchae in terms of increasing foetal age suggested that the shape change in auricula may be one of the determinant factors in the relationship between the response age and frequency.

Özkoçak (2017) [33] stated in the study conducted with adult human beings that LM2, LM4, LM5 and LM6 included a shape change outwards, and LM10 and LM11 included a shape change inwards. In this study, it was assumed that the shape change stated to be outwards or inwards referred to the situation compared to the centre of the grid map. In the present study, the vectoral side of the shape changes was defined in accordance with the anatomic expressions.

When x or y coordinate values of the landmarks were statistically compared in terms of the foetal age groups in the present study, significant differences were observed in LM4, LM5, LM8, LM10, and LM11. Accordingly, it was concluded that the anterioposterior (x) or superioinferior (y) movement of helix (posterior point), antitragus, crus helicis, scapha (antihelix) and lobulus auriculae among the foetal age groups was significant. These differences also supported the variation plot data obtained as a result of the discriminant function analysis substantially.

Honkura *et al*., (2020) [20] stated that meatus acusticus externus of human foetuses had a funnel-like shape in the postnatal period together with cavum conchae based on the development of cartilage and muscles in the foetal period. The authors [20] have interpreted the variation using the knowledge that different cartilage reaches this shape with different growth rates [22]. In the present study, the shape variation, especially between group 1 and group 3, around cavum concha supports this information.

Conclusion

Consequently, the shape variations in the foetus auricula were investigated in the study based on the middle and late gestational ages. According to the results of the present study, there were apparent shape variations in different anatomic points based on the foetal age groups. These variations concentrated, especially, around cavum concha. Statistical allometric effect based on the foetal age in terms of size and shape was observed only in group 2.

The allometric effect was quite weak in the other two groups. We do not ignore that shape variations are assessed on more foetuses by including the factors such as symmetry or sex. However, we consider that the results obtained as a result of the study will make a new effect together with the limited number of auricula studies conducted with geometric morphometric method.

Author Contributions: Demiraslan Y, Aytek Aİ and Özgel Ö designed and directed the study. Demiraslan Y and Aytek Aİ conducted geometric morphometric application. Hız İ, Özdemir B and Albay S provided and prepared the material for study. Demiraslan Y, Aytek Aİ, Hız İ, Özdemir B, Albay S and Özgel Ö co-wrote the overall paper.

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