ORIGINAL RESEARCH

Correlation Between Ear Morphology and DNA-Based Gender Identification: A Comparative Observational Study

Hema Kumari[1], Md Afzal Hussain[2]

[1]Assistant Professor, Department of Physiology, ICARE Institute of Medical Sciences & Research and Dr. Bidhan Chandra Roy Hospital, Haldi, Purba Medinipur, West Bengal, India
[2]Associate Professor, Department of Anatomy, ICARE Institute of Medical Sciences & Research and Dr. Bidhan Chandra Roy Hospital, Haldi, Purba Medinipur, West Bengal, India

Corresponding author
Hema Kumari
Assistant Professor, Department of Physiology, ICARE Institute of Medical Sciences & Research and Dr. Bidhan Chandra Roy Hospital, Haldi, Purba Medinipur, West Bengal, India
Email: jyotish404@gmail.com

Received: 29 August, 2016 Accepted: 24 September, 2016 Published: 18 October, 2016

ABSTRACT

Background: Gender identification is a vital component of forensic science, aiding in narrowing down biological profiles in criminal investigations and mass disaster scenarios.**Objective:**To assess the correlation between external ear morphometric parameters and DNA-based gender identification using the amelogenin gene.**Methods:**This cross-sectional observational study was conducted and a total of 85 individuals were recruited using non-probability consecutive sampling. The participants included both males and females, with efforts made to maintain an approximately equal gender distribution. **Results:**Males showed significantly higher values in ear height (62.4 ± 4.1 mm), width (34.6 ± 2.9 mm), and lobule dimensions compared to females (57.8 ± 3.7 mm and 31.5 ± 3.1 mm respectively, $p < 0.001$). Attached lobules and rolled helices were more frequent in males, while females more commonly had free lobules and flat helices ($p < 0.05$). Correlation analysis revealed strong positive associations between ear dimensions and male gender, particularly total ear height ($r = 0.61$) and ear width ($r = 0.57$).**Conclusion:**It is concluded that external ear morphology exhibits notable sexual dimorphism and shows a significant correlation with DNA-confirmed gender. Ear morphometry may serve as a supportive, non-invasive tool in forensic sex estimation, particularly when molecular methods are unavailable.

Keywords: Ear morphology, gender identification, forensic anthropology, amelogenin gene, DNA profiling, auricular biometrics

INTRODUCTION

The identification of an individual's gender is one of the foundational steps in forensic science, anthropology, and disaster victim identification (DVI). It not only assists in narrowing down missing person databases but also plays a crucial role in reconstructing biological profiles in medico-legal contexts. While numerous anatomical featuressuch as the pelvis, skull, and dental structuresare classically used for sex determination, these approaches often rely on skeletal remains and may be limited in cases involving soft tissue preservation or mutilation [1]. As such, there is a growing interest in exploring soft tissue traits like ear morphology as potential indicators of biological sex, particularly when supported by molecular techniques such as DNA analysis [2].The external ear, or auricle, is a uniquely structured and highly individualized anatomical feature that develops under strong genetic influence. It comprises distinct regions including the helix, antihelix, tragus, antitragus, concha, and lobule, each of which may exhibit size and shape variations across individuals [3]. Due to its cartilaginous composition, the auricle tends to resist postmortem decomposition longer than many other soft tissues, making it particularly valuable in forensic contexts. Importantly, some studies have indicated sexual dimorphism in ear parameterssuch as total ear height, width, lobular length, and attached versus detached lobuleswhich may offer a relatively non-invasive method of sex estimation [4].

Despite the anatomical uniqueness of ears, the reliability of ear morphology in gender identification has remained a topic of debate. The observed

variability due to age, ethnicity, environmental exposure, and genetic heterogeneity may reduce its discriminative power when used in isolation. Therefore, a need exists to validate such phenotypic assessments with more definitive techniquesmost notably, DNA profiling. DNA-based sex determination, particularly through amplification of the amelogenin gene located on the X and Y chromosomes, provides a highly accurate and conclusive method for gender identification [5]. The gene exhibits differential band sizes in males and females during gel electrophoresis or capillary electrophoresis, with the presence of both X and Y alleles indicating a male genotype, while females exhibit only the X allele [6].

The integration of morphometric analysis with molecular confirmation presents a novel and multidimensional approach in forensic identification. Establishing a strong correlation between ear morphology and DNA-based sex determination could significantly improve field-level triaging in mass casualty events, crimes, and unidentified body recovery, where resources for molecular testing may be delayed or unavailable [7]. In such scenarios, ear morphology could serve as an accessible, preliminary sex estimation toolespecially in cultures or regions where other anatomical exposures may be culturally restricted or impractical.Moreover, the use of digital imaging tools and 3D morphometry has significantly enhanced the precision and repeatability of ear measurements [8]. Software-assisted anthropometry now allows for accurate, reproducible, and operator-independent data collection. This technological evolution makes it more feasible to conduct large-scale population-based studies assessing sexual dimorphism in ear morphology and evaluating its predictive value when compared against molecular benchmarks [9].Several studies conducted in different populations have presented mixed results. These discrepancies underscore the importance of population-specific research, especially in underrepresented regions such as South Asia, where genetic diversity and anthropometric patterns may differ from those studied in Western literature [10].

Objective

To assess the correlation between external ear morphometric parameters and DNA-based gender identification using the amelogenin gene.

Methodology

This cross-sectional observational study wasconducted and a total of 85 individuals were recruited using non-probability consecutive sampling. The participants included both males and females, with efforts made to maintain an approximately equal gender distribution.

Data Collection

Digital photographs of the left and right ears were taken for each participant in a standardized environment, ensuring uniform lighting and background. The camera was positioned at a fixed distance, and subjects were instructed to sit upright with their head in the Frankfurt horizontal plane to maintain anatomical consistency. Using calibrated image analysis software such as ImageJ, the following parameters were measured: total ear height (from superaurale to subaurale), maximum ear width, lobule length and width, conchal dimensions, helix prominence, and lobule attachment type (free or attached). Measurements were taken independently by two trained observers to minimize bias, and the average of the two readings was recorded for analysis.

DNA-Based Gender Identification

Buccal swab samples were collected from each participant using sterile, single-use nylon swabs. DNA was extracted using a commercial kit such as the QIAamp DNA Mini Kit (Qiagen), following the manufacturer's protocol. The extracted DNA was quantified, and gender identification was performed using polymerase chain reaction (PCR) amplification of the amelogenin gene. This gene presents different banding patterns in males and females—males show both X and Y alleles (two distinct bands), while females show only the X allele (a single band). The PCR reaction was set up with specific primers targeting the amelogenin locus, along with Taq DNA polymerase, dNTPs, and reaction buffer. Thermocycling was performed using the following protocol: initial denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 58°C for 30 seconds, and extension at 72°C for 45 seconds. A final extension was performed at 72°C for 10 minutes. PCR products were visualized using 2% agarose gel electrophoresis stained with ethidium bromide and viewed under UV transillumination. Positive and negative controls were included to ensure accuracy and reproducibility of results.

Statistical Analysis

Data were analyzed using SPSS v 13. Continuous variables, such as ear height and width, were expressed as mean ± standard deviation, while categorical variables, such as lobule type, were presented as frequencies and percentages. Independent samples t-tests were used to compare ear measurements between male and female participants as confirmed by DNA analysis. A p-value of less than 0.05 was considered statistically significant for all comparisons.

RESULTS

A total of 85 participants were included in the study, consisting of 44 males (51.8%) and 41 females (48.2%), as confirmed by DNA analysis through

amelogenin gene amplification. The mean age of the study population was 28.6 ± 7.9 years, with no statistically significant age difference between males and females (p = 0.42).

Table 1: Basic Demographics

Variable	Value
Total Participants	85
Males (confirmed by DNA)	44 (51.8%)
Females (confirmed by DNA)	41 (48.2%)
Mean Age (years)	28.6 ± 7.9

The mean total ear height in males was significantly greater than in females (62.4 ± 4.1 mm vs. 57.8 ± 3.7 mm, $p < 0.001$). Similarly, ear width was also larger in males (34.6 ± 2.9 mm) compared to females (31.5 ± 3.1 mm), with the difference being statistically significant ($p < 0.001$). Lobule length and width were greater in males (18.2 ± 1.8 mm and 16.1 ± 2.0 mm, respectively) than in females (16.4 ± 1.5 mm and 14.2 ± 1.7 mm), and both comparisons yielded p-values < 0.01.The conchal height and width also demonstrated gender-based differences. Males had a mean conchal height of 24.5 ± 2.1 mm compared to 21.9 ± 1.9 mm in females ($p < 0.001$), while conchal width was 21.3 ± 1.6 mm in males and 19.1 ± 1.7 mm in females ($p < 0.001$).

Table 2: Ear Morphometric Measurements by Gender

Parameter	Males (Mean ± SD)	Females (Mean ± SD)	p-value
Total Ear Height (mm)	62.4 ± 4.1	57.8 ± 3.7	<0.001
Ear Width (mm)	34.6 ± 2.9	31.5 ± 3.1	<0.001
Lobule Length (mm)	18.2 ± 1.8	16.4 ± 1.5	<0.01
Lobule Width (mm)	16.1 ± 2.0	14.2 ± 1.7	<0.01
Conchal Height (mm)	24.5 ± 2.1	21.9 ± 1.9	<0.001
Conchal Width (mm)	21.3 ± 1.6	19.1 ± 1.7	<0.001

Attached lobules were more frequently observed in males (59.1%) compared to females (39.0%), whereas free lobules were more common in females (61.0%) than in males (40.9%). This association was statistically significant ($\chi^2 = 4.38$, $p = 0.036$). In terms of helix shape, rolled helix was more prevalent in males (72.7%) whereas flat or less prominent helix was more frequently observed in females (63.4%), also showing a significant gender association ($\chi^2 = 5.62$, $p = 0.018$).

Table 3: Lobule and Helix Features by Gender

Feature	Males (n=44)	Females (n=41)	p-value
Attached Lobule	26 (59.1%)	16 (39.0%)	0.036
Free Lobule	18 (40.9%)	25 (61.0%)	0.036
Rolled Helix	32 (72.7%)	15 (36.6%)	0.018
Flat/Prominent Helix	12 (27.3%)	26 (63.4%)	0.018

Pearson's correlation analysis showed a strong positive correlation between ear height and male gender ($r = 0.61$, $p < 0.001$), as well as between ear width and male gender ($r = 0.57$, $p < 0.001$). Lobule dimensions also showed moderate positive correlations with male sex: lobule length ($r = 0.48$, $p = 0.002$) and lobule width ($r = 0.43$, $p = 0.004$). Similarly, conchal measurements demonstrated significant correlations with sex, particularly conchal height ($r = 0.51$, $p < 0.001$).

Table 4: Correlation Between Ear Parameters and Gender

Parameter	Correlation Coefficient (r)	p-value
Total Ear Height	0.61	<0.001
Ear Width	0.57	<0.001
Lobule Length	0.48	0.002
Lobule Width	0.43	0.004
Conchal Height	0.51	<0.001
Conchal Width	0.47	0.003

DISCUSSION

This study aimed to explore the potential correlation between external ear morphology and DNA-based gender identification, providing insights into the viability of using auricular measurements as supportive indicators in forensic sex determination. The findings revealed a statistically significant difference in most ear morphometric parameters

between males and females, with values consistently higher in the male group. These results were validated against DNA profiling using the amelogenin gene, which served as the gold standard for sex determination.The results demonstrated that total ear height and width, lobule length and width, as well as conchal dimensions, were significantly larger in males than in females ($p < 0.01$) [11]. This is consistent with existing literature, such as the findings of Purkait and Singh (2007), who reported similar sexual dimorphism in ear dimensions among Indian populations. The larger auricular dimensions in males may be attributed to the influence of androgens during puberty, which enhance cartilage growth and secondary sexual traits [12].Furthermore, the type of lobule and helix shape also showed significant gender associations. Attached lobules and rolled helices were more common in males, while females more frequently exhibited free lobules and flatter helix contours [13]. These phenotypic patterns are in line with prior anthropological observations, suggesting that certain ear shape characteristics can exhibit sex-linked inheritance patterns [14].Importantly, the correlation analysis revealed moderate to strong associations between specific ear parameters and male gender, with total ear height ($r = 0.61$) and ear width ($r = 0.57$) showing the strongest positive correlations. These findings reinforce the hypothesis that ear morphology can serve as a secondary biomarker for gender estimation, especially in cases where DNA extraction is delayed, infeasible, or degraded.The integration of morphometric and molecular methods provides a dual-layered approach that enhances the reliability of forensic identification [15]. While ear morphology alone is not definitive for gender classification due to overlapping ranges and individual variability, its use as a preliminary screening tool can help prioritize samples for molecular confirmation in mass disaster settings or resource-limited environments [16].Some limitations must be acknowledged. The sample size, though adequate for a pilot investigation, may limit the generalizability of findings across diverse ethnicities and age groups. Additionally, environmental factors such as mechanical pressure (e.g., from prolonged headphone use or cultural adornments) may subtly alter ear dimensions over time, potentially affecting measurement reliability.

CONCLUSION

It is concluded that ear morphology demonstrates significant sexual dimorphism, with males exhibiting greater measurements in parameters such as total ear height, ear width, lobule size, and conchal dimensions compared to females. These anatomical differences show a strong correlation with gender as confirmed by DNA-based identification using the amelogenin gene.

REFERENCES

1. Azaria R, Adler N, Silfen R, Regev D, Hauben DJ. Morphometry of the adult human earlobe: a study of 547 subjects and clinical application. Plast Reconstr Surg. 2003;111(7):2398–2402.
2. Barut C, Aktunc E. Anthropometric measurements of the external ear in a group of Turkish primary school students. Aesthetic Plast Surg. 2006;30(3):255–259.
3. Bozkır MG, Karakaş P, Yavuz M, Dere F. Morphometry of the external ear in our adult population. Aesthetic Plast Surg. 2006;30(1):81–85.
4. Brucker MJ, Patel J, Patrick S. A morphometric study of the external ear: age- and sex-related differences. Plast Reconstr Surg. 2003;112(2):647–652.
5. Murgod V, Angadi P, Hallikerimath S, Kale A. Anthropometric study of the external ear and its applicability in sex identification: assessed in an Indian sample. Aust J Forensic Sci. 2013;45(4):431–444.
6. Verma K, Joshi B, Kumar V. Morphological variation of ear for individual identification in forensic cases: a study of an Indian population. Res J Forensic Sci. 2014;2(1):1–8.
7. Ogawa Y, Imaizumi K, Miyasaka S, Yoshino M. Discriminant functions for sex estimation of modern Japanese skulls. J Forensic Leg Med. 2013;20(4):234–238.
8. Singh P, Purkait R. Observations of external ear—an Indian study. HOMO. 2009;60(5):409–422.
9. Ahmed A, Omer N. Estimation of sex from the anthropometric ear measurements in a Sudanese population. J Forensic Res. 2013;6(5):1–4.
10. Eboh DE. Morphological changes of the pinna in relation to age and gender among the Urhobo people in South-South Nigeria. J Exp Clin Anat. 2013;12(2):68–74.
11. Deopa D, Thakkar HK, Prakash C, Niranjan R, Barua MP. Anthropometric measurements of external ear of medical students in Uttarakhand Region. J Anat Soc India. 2013;62(1):79–83.
12. Meijerman L, Van Der Lugt C, Maat GJ. Cross-sectional anthropometric study of the external ear. J Forensic Sci. 2007;52(2):286–293.
13. Dharap AS, Than M. Five anthroposcopic traits of the ear in a Malaysian population. Anthropol Anz. 1995;53(4):359–363.
14. Ekanem AU, Garba SH, Musa TS. Anthropometric study of the external ear of adult Nigerians resident in Maiduguri metropolis. J Med Sci. 2010;10(6):176–180.
15. Ito I, Sando I, Balaban CD. Postnatal development of the human external ear: a histological study. J Otolaryngol. 2001;30(5):276–284.
16. Sforza C, Grandi G, Binelli M, Tommasi DG, Rosati R, Ferrario VF. Age- and sex-related changes in the normal human ear. Forensic Sci Int. 2009;187(1-3):110.e1–110.e7.