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Original Research Article

Sexual dimorphism in stapes by discriminant functional analysis

Kamal Singh*, Aarti Rohilla

Department of Anatomy, Pandit Bhagwat Dayal Sharma, PGIMS, Rohtak-124001, Haryana, India

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***Correspondence:**
Dr. Kamal Singh,
E-mail: drkamalanatomy@gmail.com

ABSTRACT

Background: Sexing of human skeleton is highly contested subject and is usually done by using morphological and metrical traits of big intact bones like skull, mandible, pelvis and long bones etc.[1] The stapes remains well protected by being housed in the middle ear, even in the mutilated bodies.
Methods: The morphometric study of the 120 human stapes bones from 60 unidentified cadavers was aimed to know the presence of sexual dimorphism.
Results: The weight and measurements of footplate could be a good criterion for determining percentage accuracy as male and female respectively and could be used as a potential tool for determination of sex.
Conclusions: The study of normal dimensions and indices of stapes will provide insight to the forensic experts for deciding the sex of an individual in the disputed cases. Our findings are the results for guidance and future manipulation in the forensic and legal medicine.

Keywords: Indices, Ossicles, Stapes, Sexing

INTRODUCTION

Our knowledge about these ossicles goes back to the 15th century, when Alessandro Achillini (1463-1513) for the first time noted the presence of the malleus and incus.[2] Giovanni Filippo Ingrassia, one of the leading Italian Physicians of the 16th century revisited and redefined some of Galeno's reports. The most important discovery in Ingrassia's study about the hearing organ was the first description of the third bone of the ossicular chain that he called "stapes".[3] The stapes is the stirrup like bone which lacks periosteum, having a head (articulating with the incus), a constricted neck, two diverging limbs from neck (processes or crura) and a flattened base (footplate). The footplate is attached to the margin of the fenestra vestibuli by a ring of fibres (the annular ligament). It articulated with incus to form incudostapedial joint which is a ball and socket articulation. The articular surfaces are covered with articular cartilage, and each joint is enveloped by capsule rich in elastic tissue and lined by synovial membrane.

The handle of the malleus faithfully follows all movements of the tympanic membrane moving the incus which pushes the stapedial footplate towards the labyrinth and the perilymph contained within the labyrinth. The three bones together act as a bent lever so that the stapedial footplate does not move in the fenestra vestibuli like a piston, but rocks on a fulcrum at its anteroinferior border, where the annular ligament is thick.

The rocking movement around a vertical axis, which is like a swinging door, is said to happen only at moderate intensities of sound. With loud, low-pitched sounds, the axis becomes horizontal, and the upper and lower margins of the stapedial footplate oscillate in opposite directions around this central axis, thus preventing excessive displacement of the perilymph.[4]

The stapes develops at the cranial end of the second branchial arch through an independent anlage of the cartilage of this arch. Between the stapedial anlage and the cranial end of the Reichert's cartilage there is a formation called the interhyale, the internal segment of which gives rise to the tendon of the stapedial muscle. The stapedial anlage is a unique formation with two distinct parts: the superior part that will comprise the base and the inferior part that will be crossed by the stapedial artery during embryonic development and will constitute the limbs and the head of the stapes.[5]

Arensburg et al, reported that the minimal metric variability and consequent morphologic stability was present in prehistoric and historic populations of entirely different ethnic and geographic origins, separated from each other by thousands of years. This statement was supported from the study where they measured 140 auditory ossicles including 36 stapes which were obtained from skulls belonging to three different populations:

- Epipalaeolithic (Natufian) from the Hayonim cave in the Galilee, Israel, dated 12,000-10,000 years B.P. (Before Present: 'x years ago')
- Roman, from the site of En Gedi, Israel, dated 100 years B.C. to 100 years A.D.
- recent population from India. Total height, length of footplate, width of footplate and index of stapes were measured.

This was so despite the considerable osteometric differences that characterizes the three samples: the Natufians were mainly dolichocranial or euryprosope, low statured, typical Proromediterraneans; the En Gedi people had a brachycranial, mesocranial or leptoprosope skull, and were medium statured; while the Indian sample was extremely heterogeneous and variable in its skeletal anatomy.

The ear ossicles of all three of these populations, so distant in time and environment, present a striking similarity and stability in their morphological and metric traits, contrary to other parts of their anatomy. Since that time, extensive research has been carried out on the morphometry and morphology of ear ossicles.[2]

Various surgical procedures employed in the 20th century to overcome the ill effects of osteosclerosis out of which stapes mobilization attracted much attention for which the morphometric measurement of stapes in the various populations gained importance. In India, Dass et al, worked on and studied 165 human stapes. The study revealed that the stapes shows marked variations.[6]

Anson have also reported the average length of the base of the stapes and the average width of stapes. There is a general tendency for the length and breadth to diminish with increase in age, but no correlation has been observed between the weight of stapes and the age of the individual as reported by Anson et al and De vincentiis and Cimino.[7-9]

Harneja and Chaturvedi reported the detailed observations which were recorded on incus, malleus and stapes of 50 temporal bones of the adult human males with the additional importance for the weight of the bone.[10] Sarrat, studied both morphological and structural variations in a series of auditory ossicles from fresh cadavers and skulls from the Department's collection and found that individual deviations of ossicles are extremely rare. In a series of 100 cases, morphological variations of human ossicula tympani were studied and it was reported that the most striking diversity was in the malleus and the stapes. The great variations of the head, limbs and base of stapes were due to the age and some pathological events. These morphological variations of ossicles could be related to age, sex, race, and could bring out peculiarities in the acoustic transmission.[11]

Comparative study between ossicles from Antinoe Necropolis from the collection of Egypt and Dynastic Egyptian group was done. In stapes the measured parameters were anterior ramus length, posterior ramus chord, posterior ramus sagitta and footplate length. These measurements were analysed and subjected to statistical analysis and revealed that the metric traits of the ossicles were generally larger in the Antinoe Necropolis group and they differ significantly from the dynastic Egyptian group.[12] Otosclerosis is a hereditary localized disease of the bone derived from the embryonic otic capsule in which lamellar bone is replaced by woven bone of greater thickness and vascularity. The position of the focus of new bone formation determines its effect on the function of the ear, corrected by Stapedectomy which bypass the fixation of the stapes footplate caused by otosclerosis. The connection between the tympanic membrane and the inner ear is thus reconstituted and hearing restored.[13]

Although the stapes being the smallest bone among ossicles present in the middle ear cavity, it reaches an advanced state of maturity at birth.[14]

The review of literature revealed no study to utilize the stapes morphometry for sex determination. Sex identification in skeletal remains of the bodies is a challenging task as well as it is the first essential step in medicolegal autopsies.[15] The malformations of incudostapedial joint may also be associated with malformation of the external ear.[7-9] In this study we attempted to establish the sexual dimorphism by descriptive and discriminant functional analysis so that stapes could be of use as a possible tool for sex determination.

METHODS

The study was performed on 120 stapes bones retrieved from 60 unidentified cadavers in various stages of decomposition during medicolegal postmortem

examination by using Cobbler's Cut technique.[16] The parameters of cleaned and dried stapes bones studied were as follows (Figure 1 and 2).

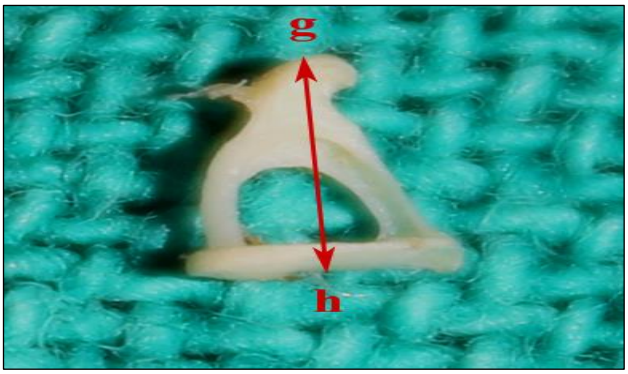

Figure 1: Stapes (enlarged view): (a) head, (b) neck, (c) anterior limb, (d) posterior limb, (e) footplate (base); total height (g-h).

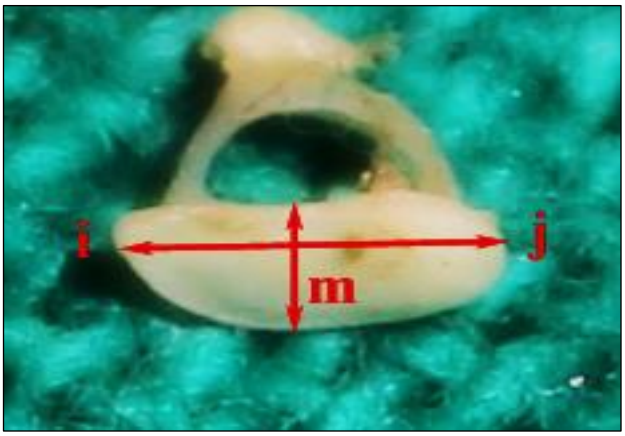

Figure 2: Stapes (enlarged view): (a) head, (b) neck, (c) anterior limb, (d) posterior limb, (e) footplate (base); length of footplate (i-j), width of footplate (m).

- Total height (g-h) mm: Maximal distance between the top of the head and the vestibular aspect of the footplate.
- Length of footplate (i-j) mm: Maximal length of the long axis of the footplate.
- Width of footplate (m) mm: Maximal length of the short axis of the footplate.
- Index: Length of the footplate x 100/ total height of stapes.
- Weight of stapes (mg)

The parameters were studied by digital Vernier caliper with the least count of 0.01 mm. The weights of the bones were measured by the electronic micro-balance of 'Sartorius CP224S' model with the minimal readability of 0.1 mg. All the measurements were taken by one setup and each reading was taken thrice by the same observer at different timings and statistically analyzed for any intra-observer error. Insignificant intra-observer error was found after statistical analysis, so the method adopted was accurate for evaluation. The results of the study were computed and analyzed with SPSS software. Normal descriptive statistics i.e. means, standard deviation and P-Value as well as Canonical discriminant function coefficients in discriminant function analyses were performed. The discriminant function formula is as follows:

$$F(X) = A_1X_1 + A_2X_2 + ... + A_NX_N + C$$

Where F(X) represents the discriminant function score, X_1 to X_N are the measured variables, A_1 to A_N are the unstandardized coefficients of each variable and C is the function's constant.

The sectioning point (Z0) for each Discriminant function is calculated from the weighted mean of values at the group centroids for males and females using the formula provided by Xavier.[17]

$$Z0 = [(Z_m \times N_f) + (Z_f \times N_m)]/(N_m + N_f)$$

Where Z_m and Z_f are the group centroids for male and female groups, N_m and N_f being the number of stapes bones of males and females respectively. Any value above the sectioning point will be classified as male and the values below the sectioning point will be classified as female.

RESULTS

Descriptive statistics of both the sexes for left and right sides of stapes bone were analyzed and compared respectively (Table 1 and 2). The only statistical significance was found in weight of stapes on the left side only. Rest all parameters were found to have no statistical significance on comparison of both the sexes for left and right side respectively. But, discriminant function analysis of the different parameters of stapes showed that these parameters provide us a good criterion in determining the group centroids and significance of percentage accuracy in determination of sex (Table 3 and 4).

On applying DFA on the individual parameters for the left side has produced a sex determination accuracy of 76.7% in males with weight, and 60.0% in females with width of footplate of stapes bone. Whereas a 65.0% accuracy for sex determination was being observed with weight for a total of both (Table 3). On applying DFA on the combination of all the parameters for the left side it is observed that has produced a sex determination with maximum accuracy of 80.0% in males, 63.3% in females and 71.7% in total of both respectively.

On applying DFA on the individual parameters for the right side has produced a sex determination accuracy of 66.7% in males with weight, and 63.3% in females with length of footplate of stapes bone. Whereas a 60.0% accuracy for sex determination was being observed with

length of footplate for a total of both (Table 4). On applying DFA on the combination of all the parameters for the right side it is observed that has produced a sex determination with maximum accuracy of 73.3% in males, 63.3% in females and 68.3% in total of both respectively.

Table 1: Descriptive statistics of the left stapes bone.

Parameter	Male (Mean±SD)	Female (Mean±SD)	P-value
Total height (mm)	3.3993±0.21043	3.3093±0.23730	0.126
Length of foot plate (mm)	2.8783±0.24398	2.8923±0.24103	0.824
Width of foot plate (mm)	1.3327±0.17848	1.3080±0.06386	0.479
Index	84.8318±7.23316	87.7319±8.57332	0.162
Weight (mg)	2.7133±0.58882	3.1067±0.63514	0.016

Table 2: Descriptive statistics of the right stapes bone.

Parameter	Male (Mean±SD)	Female (Mean±SD)	P-Value
Total Height (mm)	3.4003±0.20876	3.3003±0.30184	0.141
Length of Foot Plate (mm)	2.8607±0.25868	2.7683±0.22110	0.143
Width of Foot Plate (mm)	1.3450±0.17449	1.3333±0.09575	0.749
Index	84.1748±6.31983	84.2795±7.36919	0.953
Weight (mg)	2.6633±0.71703	2.9133±0.77936	0.201

Table 3: Discriminant function analysis showing percentage accuracy for sex determination by taking individual and combined parameters of left stapes.

Sr. No.	Parameters	Unstandardized Coefficients	DF's Constant	Wilk's Lambda	Group centroids		Correctly assigned (%)		
					M	F	M	F	T
A	Total height (mm)	4.459	-14.957	0.960	+0.201	-0.201	46.7	56.7	51.7
B	Length of foot plate (mm)	4.124	-11.898	0.999	-0.029	+0.029	50.0	36.7	43.3
C	Width of foot plate (mm)	7.460	-9.850	0.991	+0.092	-0.092	46.7	60.0	53.3
D	Index	0.126	-10.878	0.967	-0.183	+0.183	73.3	43.3	58.3
E	Weight (mg)	1.633	-4.752	0.904	-0.321	+0.321	76.7	53.3	65.0
A+B+C+D+E		1.380 -6.383 -3.042 0.195 1.947	-4.710	0.731	-0.597	+0.597	80.0	63.3	71.7

Table 4: Discriminant function analysis showing percentage accuracy for sex determination by taking individual and combined parameters of right stapes.

Sr. No.	Parameters	Unstandardized coefficients	DF's constant	Wilk's lambda	Group centroids		Correctly assigned		
					M	F	M	F	T
A	Total height (mm)	3.853	-12.910	0.963	+0.193	-0.193	53.3	60.0	56.7
B	Length of foot plate (mm)	4.156	-11.697	0.963	+0.192	-0.192	56.7	63.3	60.0
C	Width of foot plate (mm)	7.105	-9.515	0.998	+0.041	-0.041	43.3	60.0	51.7
D	Index	0.146	-12.270	1.000	-0.008	0.008	30.0	23.3	26.7
E	Weight (mg)	1.335	-3.724	0.972	-0.167	+0.167	66.7	46.7	56.7
A+B+C+D+E		10.067 -16.367 -2.585 0.466 1.368	-27.304	0.819	-0.463	+0.463	73.3	63.3	68.3

Thus, weight alone could be the good criteria for determining percentage accuracy for labelling bone as a male and measurements of footplate could be fairly good criteria for determining percentage accuracy for labelling bone as a female making it to be a potential tool for determination of sex.

DISCUSSION

Morphometric analysis of ear ossicles had been the subject of interest since the mid fifteenth century and earlier researchers have observed insignificant variations in the morphology of stapes not only in both the sexes but also on both sides.[2] Studies on embryogenesis of the hearing have shown that the ear differs from one individual to another with a saying that "no two ears are the same".[5,9,18] The review of Indian as well as international literature revealed that morphometry of ossicles especially stapes has been studied by very few many investigators more so in males.[10,20,21]

This study represents an endeavor by the investigators to provide a comprehensive coverage of measurement of the various parameters of the stapes. The literature, so far as available, is remarkably devoid of extensive observations even on the gross anatomy of the ossicles. Earlier studies by Unur et al and Wadhwa et al reveal various morphometric measurements of stapes.[20,21] Even though the total height, length and width of footplate of stapes was studied widely.[10,17,21,22] The study of index of stapes was carried out in three different population Natufian, En Gedi and Recent India.[2,20]

The stapes weight was exclusively done and reported by Harneja and Chaturvedi only.[10] These reported studies from Indian and other research workers throughout the world have studied the parameters of the ear ossicles particularly in males only.[21,23] The various morphometric results of bisexual and bilateral values of stapes were compared with previous researchers are shown in Table 5. Although results of morphometric analyses did not reveal much statistically significant level of sexual dimorphism, but on discriminant functional analysis it is found that stapes bone can be used as a tool for determination of sex with a good accuracy similar to the findings of Singh et al.[24,25]

Table 5: Comparison of morphometric data of stapes from present study and some previous studies.

Authors	Morphometric parameters of stapes				
	Total height (mm)	Length of foot plate (mm)	Width of foot plate (mm)	Index	Weight (mg)
Present data (2016)	3.352	2.85	1.33	85.254	2.849
Wadhwa, Kaul and Agarwal (2005)	3.41	2.97	0.39		
Unur, Ulger, Ekinci (2002)	3.2	2.6	1.3	80.1	
Siori et al (1995)					
Gebelen/Asiut*		2.84			
Antinoe*		2.93			
Schuknecht and Gulya (1995)	3.26	2.99			
Arrensburg et al (1981)					
Natufian*	3.3	2.9	1.5	90	
Roman (En Gedi)*	3.3	2.8	1.3	86.9	
Recent India*	3.2	2.8	1.3	85.1	
Harneja and Chaturvedi (1973)	3.12	2.68	1.26		3.17
Angel (1972)	3.3				
Bouchet and Giraut (1968)	3.5				
Dass et al (1966)	3.29				
Anson and Bast (1958)	3.06	2.75	2		
De vincentiis and Cimino (1957)	3.61				
Bast and Anson (1949)	3.26	2.99	1.41		
Anson, Karabin and Martin (1939)			1.5		
Heron (1923)	3.4				
Urbantschitsch (1876)	3.7				

* Population

CONCLUSION

Thus, this study may turn out to be a potential source of forensic investigation for evaluation of sex by stapes in severely mutilated and decomposed bodies during postmortem examination. The results in present study revealed subtle sex differences by accuracy and suggested that the assumptions of previous investigators in this

regard needs to be reassessed. The dimensions of the stapes may provide insight to the ENT surgeons for implications in reconstruction of ossicular chain to regain the original mechanics. This study will also give a basis for teaching the medical students about the basic and detailed structure of the ear (external, middle, internal) as well as its related significance in clinical and forensic medicine.

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