SEX DETERMINATION FROM HISTOLOGY OF FEMUR

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ABSTRACT

BACKGROUND

Sex of an individual can be known with much accuracy when the whole skeleton is available. Sex can be predicted using osteon count of femur.

MATERIALS AND METHODS

The study was conducted on 100 known males and 100 known females above the age of 18 years brought for autopsy in the Department of Forensic Medicine, State Medico-Legal Institute, Medical College, Thiruvananthapuram for a period of 9 months. Unstained slides were prepared from femur bits taken from right femur. Osteon counts were taken. The values were subjected to statistical analysis to find out the correlation if any. The formula for sex determination was done using discriminant analysis.

RESULTS

From the discriminant function coefficients, the formula for calculating the sex when osteon count per field of femur is known was obtained.

CONCLUSION

In this study, it is found that there is some difference in the osteon count of femur between males and females. Thus, sex can be predicted from the formulae derived from the present study using osteon count of femur with 59.1 % accuracy.

KEYWORDS

Sex, Skeletal Remains, Histology, Femur.

BACKGROUND

Studies indicate that there is significant difference in the number of osteons and lacunar densities in different population which may be attributed to factors like socio economic status, nutritional status and effect of work related mechanical stresses. Although many population specific studies have been conducted in the Europe and North America, not many studies have been attempted on Indian population.

It is said that every piece of bone tells a tale of its own role in the individuality of its owner; a tale if properly unfolded may result in an individual either partially or at times completely. This study aims at developing standards for finding sex of the individual from the histology of femur of the dead brought for autopsy at Medical College, Thiruvananthapuram.

Aims and Objectives

To derive formula for determining sex of the individual from osteon count of femur.

MATERIALS AND METHODS

Study Setting

The study was conducted in the Department of Forensic Medicine, State Medico-Legal Institute, Medical College, Thiruvananthapuram.

Study Period

Period of nine months from 15-3-2011 to 15-12-2011.

Sample Size

100 known males and 100 known females above the age of 18 brought for autopsy.

Inclusion Criteria

Identified dead bodies above the age of 18 years brought for medico legal autopsy to the Department of Forensic Medicine, State Medico -legal Institute, Medical College, Thiruvananthapuram during the above period were selected for study.

Exclusion Criteria

1. Unknown bodies.
2. Deep burns and extensively burnt bodies
3. Those with bone diseases or pathologic lesions in femur.
4. Prolonged weather exposed bodies.
5. Those with any dispute in age.

Consent for taking the bone from the dead body is taken from a close relative. After extending the autopsy incision through the midinguinal point, the neck of femur is exposed. Bits are collected from the neck of right femur.

The bone fragments are decalcified using 7% nitric acid solution at room temperature for duration of 4-7 days. Then they are embedded, in paraffin blocks, cut with a standard microtome (thickness of 4 micrometer) and unstained slides are prepared. The study group included 100 males and 100 females.

Of these, 9 samples from males and 15 samples from females had to be excluded from the study because of excessive decalcification which lead to interference with good quality slides. From the 176 valid samples, histopathology slides were prepared. The prepared slides are then examined under the light microscope using 10x wide field ocular eye piece lens. A micrometer is attached to eye piece lens for accurate counting and primary and secondary osteon in the field are counted. Four random fields were thus counted. The osteon lying over the dividing line is included in the segment containing the greater half of the osteon. The aggregate per segment and the average are calculated. The values thus obtained are entered into the proforma, the sample of which is appended. The values are subjected to statistical analysis to find out the correlation if any.

The formula for sex determination was done using discriminant analysis.

Discriminant Analysis

A discriminant function was developed for the purpose. The general structure is

$$Z_{jk} = a + w_1 X_{1K} + w_2 X_{2K} + ... + w_n X_{nK}$$

Where

Z_{jk} = Discriminant Z score of discriminant function j for object k

a = intercept

w_i = Discriminant weight for independent variable i

X_{iK} = independent variable i for object k.

Discriminant function is defined as a variate of the independent variables selected for their discriminatory power used in the prediction of group membership.

RESULTS

The objective of this study is to determine the sex of the individual from the osteon count of femur. The cases included in this study fall between the age groups 18 to 96 years, comprising of both sexes. The study group included 100 males and 100 females.

Histopathology slides of femur are prepared from 176 valid samples. Of the samples 91 are males (51.7%) and 85 are females (48.3%).

Of the 176 cases maximum number of samples is from hanging cases (28.4%) and minimum number of samples from superficial burns (6.3%). All the superficial burns cases are females (12.9%).

Analysis for sex was done with discriminant analysis. The mean osteon count of femur in males is 28.85 and that in females is 25.09 (Table No-1)

The significance value of femur is 0.021 (<0.05) (Table No-2)

Wilk's Lambda shows the proportion of the variance in the discriminant scores not explained by differences among groups. Statistical significance for the Chi-square test indicates that there is a significant difference between group means.

The two tables (table No 1 & 2) show that the average osteon score from femur show significant difference between males and females. The canonical correlation measures the association between the discriminant scores and the groups. This is the method used for calculating the formula for determining the sex.

Sex from femur

From the discriminant function coefficients, the formula for calculating the sex when osteon count per field of femur is obtained.

-2.528+0.093 (Osteon count/field of femur)

Table No-3 shows the mean of the discriminant score for each group. The assignment of the predicted group membership will assign discriminant scores ≥0 to males and negative scores for females.

The table- 4 shows that 59.1% of original grouped cases were correctly classified. Using the formula 49% of the males are predicted correctly and 64.7% of the females are correctly predicted.

Sex	Sample	Mean	Standard Deviation
Male	femur	28.855	11.252
Female	femur	25.096	10.0694
Total	**femur**	**27.040**	**10.832`**

Table 1. Group Statistics

Bone	Wilks Lambda	Significance
femur	0.970	0.021

Table 2. Tests of Equality of Group Means

Sex	Function
Male	0.170
Female	-0.182

Table 3. Unstandardized Canonical Discriminant Functions at Group Centroids

Sex		Predicted Group Membership		Total
		Male	**Female**	
Original count	Male	49	42	**91**
	Female	30	55	**85**
Percentage	Male	53.8	46.2	**100.0**
	Female	35.3	64.7	**100.0**

Table 4. Classification Results

DISCUSSION

In the present study it is found that there is some difference in the osteon count of femur between males and females. Thus, sex can be predicted from the formulae derived from the present study using osteon count of femur with 59.1 % accuracy. The results of this study also indicate that the total number of osteons per field is positively correlated with age.

The number of osteons per field showed increase with advancing age for both sexes though aberrant values were obtained for some cases.

Thompson found that females have larger Haversian canals in the femoral midshaft than males, but males seem to have more canals in general. Thompson (1981), however, suggested that this difference may be due to sampling distribution[1.] Kerley (1965) found that sex did not have a significant role when estimating age.[2] Singh and Gunberg who worked in accordance with Kerley, specified that there is no significant differences between sexes, but they also indicate that future studies should include possible differences observed in individuals due to sex or population affinity.[3]

Thompson and Erickson both separated the regression formulae to incorporate sex-specific equations.[4,5] Erickson found that the sex-specific equations yielded better results than the combined equations. He found that there were significant differences in relation to the number of osteons and the number of osteon fragments.[5] Burr et al, indicated that the osteons in females increase in size with age, while the osteons in males seem to decrease in size with age.[6] A recent study conducted by Maat *et al* indicated that there is no statistically significant difference between males and females with respect to the percentage of unremodelled bone when dealing with a Dutch sample.[7]

Another factor that could play a fundamental role is the differences seen between males and females is the skeletal maturation and timing of growth cessation. It is well known that epiphyseal closure in males and females varies and this could account for the differences seen between males and females, particularly in younger individuals.[8]

A more common factor playing a role in the difference between the bone structure of male and female individuals is microdamage. Microdamage is the result of continuous strain on the skeletal system throughout everyday life. Norman and Wang (1997) concluded in their research that micro damage of bone is significantly greater in females, and that micro damage is more prevalent in the midshaft of the femur and tibia than in other bones. The relevance of this to histological ageing techniques is that micro damage promotes an increase in remodeling and is known to be significantly age and sex dependent, thus influencing the appearance of bone structures.[9] According to Goldman et al. (2003), males tend to show a higher rate of bone turnover than females. Thus, male individuals have more frequent and more efficient renewal of bone. The authors (Goldman *et al,* 2003) believe that this variability in the degree of mineralization is an important factor to consider when understanding the biomechanical adaptation of bone and its age-related changes.[10]

CONCLUSION

The formula for determining the sex was made using discriminant analysis and a formula was made.

Formula for sex determination from the osteon count of femur is

Discriminant score = -2.528+0.093 (osteon count/field of femur)

In this study it is found that there is some difference in the osteon count of femur between males and females. If the value of discriminant score is above or equal to zero, the sex can be predicted as male and if the value is negative then the sex can be predicted as female

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