DETERMINATION OF AGE FROM HISTOLOGY OF BONE

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ABSTRACT

BACKGROUND

Identification of human remains is a problem of great importance in crime investigation. Estimating age at death and finding sex of the individual from skeletal remains can be done with relative accuracy only when skeleton is complete and is difficult when only part of the remains is available.

MATERIALS AND METHODS

Osteons of femur are counted after preparing unstained slides. The values are subjected to statistical analysis to find out the correlation if any. The formula for age determination was determined using regression analysis. To predict age from the knowledge of the osteons count from femur a linear regression equation was formed y=a+bx.

RESULTS

This study indicates that the total number of osteons per field is positively correlated with age.

CONCLUSIONS

The formula for age determination was formulated using regression analysis and three formulae were obtained.

KEYWORDS

Age, Skeletal Remains, Osteon Count, Femur.

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BACKGROUND

Determination of age of a person is very important in establishing the identity of that individual. There are many methods which help in determining the age of an individual at death, like changes in skull sutures, fusion of diaphysis with epiphysis, appearance of ossification centre and eruption of teeth. Finding the age of incomplete skeletal remains is still a problem.

Since Kerley's dissertation in 1965 which describes the original method of estimating age at death for the microscopic structure of bone, several modification and variations to this technique have been introduced. Studies indicate that there is significant difference in the number of osteon and lacunar densities in different population which may be attributed to factors like socioeconomic status, nutritional status and effect of work related mechanical stresses.

Although many population specific studies have been conducted in the Europe and America, not many studies have been conducted in Indian population.

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This study aims at developing standards for estimating age at death of an individual from histology of femur of the dead brought for autopsy at Medical College Thiruvananthapuram.

Aims and Objective- To derive formulae for determining age at death from the osteon count of femur.

MATERIALS AND METHOD

Study Setting-The study was conducted in the Department of Forensic Medicine, Medical College Thiruvananthapuram

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

Inclusion Criteria- Identified dead bodies above the age of 18 brought for autopsy 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.

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

Exclusion Criteria

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

The exact age, history of any bone disease and nativity were verified from relatives. After extending the autopsy incision through the midinguinal point, neck of femur is exposed. Bone chips 1x1 cm divisions, periosteum to endosteum is carefully cut out using chisel and hammer. Then the bony chips are carefully cleaned to remove any soft tissue attachments. The bone fragments are decalcified using 7% nitric acid solution at room temperature for four to seven days. Then they are embedded in paraffin blocks, cut with a standard microtome (thickness of 4 micrometer) and unstained slides are prepared. The prepared slides are then examined under light microscope using 10 x wide field ocular eye piece lens. A micrometre is attached to eye piece lens for accurate counting and primary and secondary osteons 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. 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 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 age determination was determined using regression analysis. To predict age from the knowledge of the osteons count of femur a linear regression equation was formed y=a+bx

Where y=age (dependent variable), a=intercept, b=regression coefficient: represent the amount of change in the dependent variable (osteon count) and x=average osteon count per field.

RESULTS

The age of the male samples selected ranged from 20 to 90 years and the age of female samples ranged from 18 to 96 years. Maximum number of cases is in the age group of 41 to 50 years (21%). The maximum number of male cases is of age group 41 to 50 years (25.3%) and maximum number of female cases is of 21 to 30 years (20%) (Graph 1)

The results of this study indicate that the total number of osteons per field is positively correlated with age. The highest osteon count per field thus obtained from femur was 52.6 for a 52-year-old female. The lowest was 9 in the case of a 21-year-old male. The number of osteons per field showed increase with advancing age though aberrant values were obtained for some cases.

To start with it was assumed that the relationship between the osteon count and the age of the deceased would be nonlinear and it was null hypothesis. But after the study it has been found that the relationship between the age and the osteon count was linear. The fitted regression line could be of the form y=a + bx were 'x' was the independent variable that is the osteon count and 'y' was the dependent variable, here it is the age. So 'x' was the linear predictor and 'y' was the predicted variable. 'a' and 'b' are constants. Thus, the age of the person could be predicted, if one has the osteon count.

Fitted Regression Lines- The following regression lines have been fitted.

- **The Regression lines of Osteon Counts of Femur in both Males and Females Taken Together-** The regression line of osteon counts of femur in both males and females on their respective age is fitted. The plotted regression line is y = 10.106 +1.175 x.

  Correlation coefficient, which is the correlation between the age and the osteon count of femur in both sexes r= 0.619 and P value is 0.000 (<0.05) which is highly significant.

  Coefficient of determination, r^2 is 0.383 which means 38% variation in osteon count is accounted for variation in age. This clearly indicates that the osteon count of both sexes if taken together, femur showed a clear linear predictor for determining the age of the deceased.

- **The Regression line of Osteon Count of Femur in Males-** The regression line of osteon count of femur of males on their respective age was fitted.

  The plotted regression line is y = 13.948 + 1.037 x. Correlation between the age and the osteon count of femur in males, r =0.616. The P value was found to be 0.000(<0.05) which is highly significant. Coefficient of determination r^2 is 0.380 which means 38% variation in osteon count is accounted for variation in age. These two results indicate that osteon count of the femur of the males had a linear relationship with their age and also has a highly significant correlation showing that the osteon count is a very good linear predictor for determining age.

- **Regression line of osteon count of femur of females-** Regression line of osteon count of femur of females on their respective age was fitted.

  The fitted regression line is y = 6.998+ 1.292 x. The correlation between the age and osteon count of femur in females r=0.626 and P=0.000 which is highly significant.

  r^2= coefficient of determination = 0.392 which means 39.2% variation in osteon count is accounted for variation in age.

  This shows that the osteon count of femur of females also had a linear relationship with their age and also has a highly significant correlation. Using the formula 49% of the males are predicted correctly and 64.7% of the females are correctly predicted.

Sex	Bone	r	r^2	P
Both Sexes	femur	0.619	0.383	0.000
Male	femur	0.616	0.380	0.000
Female	femur	0.626	0.392	0.000

Table 1. The Correlation Coefficient (r), Coefficient of Determination of Males and Females for Femur

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 2. 59.1% of Original Grouped Cases were Correctly Classified

DISCUSSION

The aim of the study was to develop formulas for age estimation of an individual using the osteon count of femur. The results of this study indicate that the total number of osteons per field is positively correlated with age. Osteon count was done for femur which included both primary and secondary osteon, but excluding osteon fragments. The highest osteon count per field thus obtained was 52.6 for a 52-year-old female. The lowest was 9 in the case of a 21-year-old male. The number of osteons per field showed increase with advancing age though aberrant values were obtained for some cases.

The total osteon count has been used in most, as the osteon is the fundamental structure involved with the remodelling process. All relevant authors (Kerley 1965, Ahlquist and Damsten, Singh and Gunberg 1970, Erickson 1991) found that these structures increase throughout life[1,2,3,4].

Manual grinding of undecalcified bone is time consuming and finally provides an uneven thickness. Many studies by Martrille, Catherine Cannet showed that decalcification of bones and examination of the prepared slides can be used for age estimation at death[5,6]. Ahlqvist and Damsten noted that age determination using a single parameter was inferior[5]. Present study does not agree with Cohen and Harris who found no significant relationship between the number of osteons and age[7]. In a revised study by Kerley and Ubeleker, Kerleys original data for finding age from osteon count has been reanalysed to produce new regression equations[8]. According to David Burr, and Christopher Ruff no striking relationship were found between bone tissue changes and age[9]. Stout and Paine did age estimation from histology of rib and clavicle and found that age can be estimated with better accuracy using formula based upon rib and clavicle combined together[10]

The differences between the correlation coefficient of this study and those of other researchers could be attributed to other factors such as sample size, population groups, malnutrition, diseases and mechanical stress several variables including total osteons, osteon fragments, resorption spaces, Haversian canal diameter, percentage of unremodelled bone have been used to develop regression formulae in previous years. Of these variables the total osteon count appears to be the most highly correlated with age[1]. The accuracy of these formulae to estimate age of an individual outside the population has been questioned[2].

But the present study indicates that using osteon count per field, one could arrive at a reasonably reliable age at death in both sexes from femur.

CONCLUSIONS

The aim of this study is to develop formula for determining the age at death of the individual from the osteon count of femur. Bone samples are obtained from femur. The bone fragments are decalcified, and slides were obtained. The average osteon count per field was calculated. The values thus obtained were entered into the proforma, the sample of which is appended. The values were subjected to statistical analysis to find out the correlation if any.

1. The results of this study indicate that the total number of osteons per field is positively correlated with age.
2. The formula for age determination was formulated using regression analysis and three formulae were obtained.
   1) Applicable to both sexes in case of femur y=10.106 +1.175 x r=0.0619.
   2) Applicable to males in case of femur y= 13.948+ 1.037 x r =0.616.
   3) Applicable to females in case of femur y = 13.948+ 1.037 x r=0.626.

'y' is the calculated age and 'x' is the osteon count /field. 'r' is the correlation coefficient.

Limitations of this Study

From this it may be deduced that there is only a slight difference between the correlation coefficient between males and females. The number of osteons per field showed increase with advancing age though aberrant values were obtained for some cases. It is found that the using osteon count for age determination is less reliable for older age groups, over 60 years.

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