Estimation of Age from Sternal End of Fourth Rib in Western Rajasthan Population: A Microscopic Study

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Research Article

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ABSTRACT :

Estimating the age at death in the adult skeleton is problematic owing to the biological variability in age indictors and the differential skeletal response to environmental factors over an individual's life. Determination of age and sex play a pivotal role in identification of unknown skeletal remains. Age estimation from the recovered bones has been studied and analyzed by many workers. In the present study the sternal end of fourth ribs were used for determination of age by microscopic study. Currently there are different parameters available to determine the age of a person like study of teeth, ossification of bones and other ancillary data, but the accurate reliability of these measures is only limited to a particular age group i.e. 25± 5 years. For the age beyond this, many workers in different parts of the world have done their studies to accurately determine the age of a person from the skeleton. A random study of 100 cases for age determination from sternal ends of the ribs was carried out in the Department of Anatomy, SPMC, Bikaner. The aim of the study was to determine the age after death with minimal error.

INTRODUCTION:

Age has always been of great interest to everyone especially for forensic expert and anthropologists. Researchers have studied this process to understand its origin, evolution and consequences.

Chronological age assessment is an important part of medico legal practice. The procedures for age determination are complex and involve the consideration of many factors. A number of methods for age determination have been proposed. These can be classified in four categories, namely- clinical, radiological, histological and chemical analysis. In the living persons, any or all of the above methods can be used to determine age, in cases where actual age is not known or is to be confirmed. However, in case of a dead person, post-mortem changes such as decomposition, mutilation or skeletonisation may make identification progressively more difficult almost to the point of impossibility.[1]

While there have been a number of fairly accurate techniques developed on age determination from the skeleton such as cranial suture closure, pubic symphysial metamorphosis, morphologic changes of the sternal end of the rib; microscopic analysis of bone (osteon counting).[2,3] Microscopic analysis of structures within long bone cortical segments is another valuable technique. Among these, sternal end of the rib is argued to be more reliable for age estimation from late adolescence to old age.[4,5,6,7]

Bone histology for determining age at death has been in use for approximately 50 years. Since Kerley's dissertation[8] in 1965 which describes the original method of estimating age at death from the microstructure of bone several modifications and variations to this technique have been introduced. Stout also explained that different methods for age determination vary from each other due to different factors. These are sample location, which bone is used, which microscopic structure is observed & which method is used. This study also has some limitations as population variations, different age profiles among population, lack of authorized data, nutrition & disease.

The aim of this study was to develop regression formulae for estimating age at death using bone microstructure that is applicable to the Western Rajasthan population.

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METHODS:

The present study was conducted in Anatomy department of S. P. Medical college & associated hospitals on 100 samples of 4th rib taken from dead body brought for postmortem examination in mortuary during the period of 17-08-2012 to 07-02-2014 from department of forensic medicine, S.P. medical college, Bikaner, Rajasthan. Its particulars were recorded and age was cross checked from relatives.

The proposal for research was submitted to ethics committee, S.P. Medical College & Associated group of hospitals, Bikaner, Rajasthan. The research work was approved by ethical committee. Before taking the sample from deceased the concent form was filled & signed by kin of deceased.

The specimens were separated from the body by cutting fourth rib at two points i.e. three centimeter inner to and five centimeter outer to costochondral junction using a rib cutter without damaging the costochondral junction.

The muscles attached to the ribs were cut using scissors. The portion of ribs were labeled and kept in water containers for three to four weeks. Thus the soft tissues could be removed from the bone easily. The remaining soft tissue and cartilages if any were removed by keeping the bones in boiling water for ten to fifteen minutes. Then after labeling the sample fixation is done before decalcification. For that sample is cut into small pieces.

For decalcification EDTA is used with pH 7.0 with regular changes of solution. After decalcification regular tissue processing & staining has been done & sample was stained with H&E stained.

The bone slides were examined under light microscope (Nikon), with a 10X objective & 10X ocular lens piece. The Nikon microscope was also fitted with Nikon camera. The bone slides were marked at 4 measured points in each sample & then digital photograph had been taken for further observations.

Preliminary microscopic examination of the slides grouped in chronologic series revealed that the structural changes normally associated with age seemed better spaced to cover the total life span in the outer third of the cortex than in the middle or inner thirds. Also, the outer third is the least affected by resorptive changes in normal bone. After examining the slides following parameters have been counted:

1) The number of true osteones.
2) The number of non-Haversian canals
3) The number of fragments of old osteones.
4) The number of resorption spaces.
5) The average number of concentric lamellae.

In present study simple linear regression analysis has been done. Simple linear regression enables the researcher to observe the relationship of one predictor variable with the outcome variable. For the linear regression analysis the formulae are presented in the following form: [y = mx + c]

The standard error of the estimate (SEE) is also determined to allow calculation of a standard error range for each of the regression formulae. Correlation coefficient (r) (r^2) and linear & multiple regression equation was determined with help of appropriate statistical software.

RESULTS:

Table 1: Sex distribution of study sample (N = number of individuals)

S. No.	Age range	Male		Female		N (sexes pooled)
		N	% Distrubution	N	% Distrubution	
1	15-30	33	60	22	40	55
2	31-45	19	70	08	30	27
3	46-60	15	88	02	12	17
4	<60	00	00	01	100	01
	TOTAL	67	67	33	33	100

Table 2: Descriptive statistics among sexes (N = number of individuals)

S.No.	Sex	N	Mean (years)	SD (years)
1	M	67	34.38	12.46
2	F	33	29.36	11.75
3	TOTAL	100	32.73	12.40

After preparation of slide the tissue was examined under microscope for further study. These are:

- True osteons: Number of secondary osteons with an intact Haversian canal bounded by a scalloped reversal line. If connected to multiple osteons by a clearly defined Volkmann's canal the structures should be counted as separate osteons. If two or more structures appear to share a Haversian canal and/or share a scalloped reversal line due to the plane of sectioning including a branching event, then they are counted as one system.
- Non-haversian canals: All primary vascular channels, including those that have filled in partly with concentric lamellae to form primary osteones or pseudo-Haversian are vascular canals that were formed by the inclusion of small, peripheral blood vessels into the bone by the rapid expansion of the cortex in diameter. Since these canals were formed at the time the surrounding lamellar bone was formed, they are primary and represent areas of unremodeled bone. The secondary osteon is formed in a space left by osteoclastic resorption and represents internal remodeling of the bone.
- Fragmentary Secondary Osteons or Osteonal Fragments: Number of secondary osteons with a partially visible Haversian canal that has been breached either by a neighboring osteon or a resorptive bay and secondary osteons with no remnants of a Haversian canal present. Osteon fragments that lack a Haversian canal can be identified by concentric lamellar rings and the presence of a defined reversal line with a scalloped (irregular) margin.

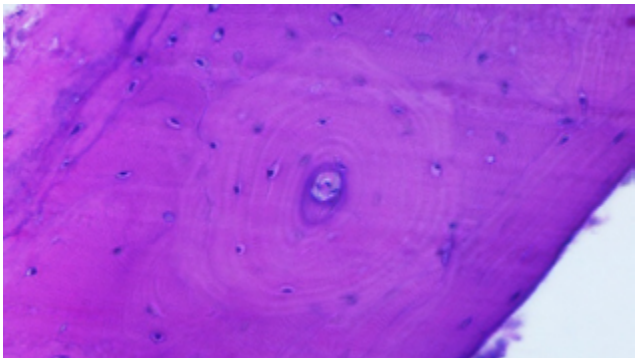

Fig1: Osteon with concentric Lamellae

- Resorption Spaces: These include the first signs of

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new osteon formation and are characterized by scalloped edges.

- Concentric lamellae: The total number of lamellae surrounding a true osteon are labeled as concentric lamellae in each field.

Table 3- Descriptive statistics of study samples according to their TTO, TNHC, TOF, TRS, ACL

S. No.	Parameters	Mean	SD
1	TTO	10.24	7.81
2	TNHC	6.21	4.70
3	TOF	11.95	4.94
4	TRS	3.3	2.19
5	ACL	5.427	2.47

Simple Linear Regression Analysis

Data was charted in order to identify possible outliers. Data was also charted in order to demonstrate relative differences when comparing true osteons, non haversian canals, osteonal fragments, resorption spaces & concentric lamellae to actual age. Regression analysis was undertaken in order to evaluate apparent trends for each of the five variables examined.

Each independent variable has been discussed and scatter plot graphs for each of these variables studied. The scatter plot graphs give a visual indication of what the correlation of these variables are with age. For each variable the regression equation also has been derived after statistical analysis of data.

Table 4:- Simple linear regression Equation (single variable only) for pooled sexes

S. No.	VARIABLES	REGRESSION EQUATION
1	**TTO**	AGE= 0.7781*TTO+24.622
2	**TNHC**	AGE= -1.2968*TNHC+40.643
3	**TOF**	AGE= 1.2597*TOF+17.536
4	**TRS**	AGE= 1.6562*TRS + 27.125
5	**ACL**	AGE= 2.4345*ACL + 19.377

Table 5:- Results of the linear regression analysis (single variable only) for pooled sexes (SEE = standard error of the estimate, t value= students t-test, p value= significance coefficient)

S. No.	Variables	R square	Std. error	SEE (years)	t-value	p-value
1	**TTO**	0.232	0.143	11.11	5.444	0.000
2	**TNHC**	0.234	0.237	11.10	-5.469	0.000
3	**TOF**	0.243	0.224	11.03	5.615	0.000
4	**TRS**	0.083	0.555	12.14	2.985	0.004
5	**ACL**	0.229	0.452	11.14	5.388	0.000

The single variable that is best related to age for this pooled sexes sample is the total number of osteonal fragments (TOF). The coefficient of determination (r^2) for this formula is 0.243 and the standard error of the estimate is ± 11.03 years.

From these results it is clear that the total number of true osteons, total number of non haversian canals, the total number of osteonal fragments and the average number of lamellae per osteon are all reliable factors. This indicates that these variables are easy to score and thus prove useful in age estimation techniques.

The variables that show to be unreliable is the total number of resorption spaces. This variable is difficult to score and thus may not prove useful in age estimation techniques.

DISCUSSION:

Positive identification involves matching of an "unknown" individual to a "known" individual. The identification of skeletal and other decomposed human remains is very important for legal and humanitarian reasons.

The use of the microscopic analysis of bone for the estimation of age at death has become a popular technique, stimulated by modern technical advances.[3] To date, the methods that have been established appear to be highly reliable for estimating age at death with standard error of estimates of less than 10 years. Several variables, including total osteons, osteon fragments, resorption spaces, Haversian canal diameter, percentage of unremodeled bone, have been used to develop regression formulae in previous years.

The total osteon count has been used in most, if not all, of the histological ageing techniques thus far. The reason for this is that the osteon is the fundamental structure involved with the remodeling process. All relevant authors[8,9,10] found that these structures increase in number throughout life. The results of this study indicate that the total number of osteons is positively correlated with age, but in present study only 23% of the variance in the predicted age is explained by this variable. The difference between the results of this study and those of other researchers could be attributed to factors such as sampling location, malnutrition, disease and mechanical stress.

According to the previous studies on ribs (Sam D stout et al 1994)[11] and clavicle (U Young Lee 2014)[12] mostly authors used the variable for their study was osteon population density. Osteon population density was total of true osteons and fragmentary osteons. In the study of Sam D stout et al[11] calculated r value 0.832 and SEE was 10.43 years & U Young Lee[12] noted r value 0.76 and SEE was 11.93years. They noticed the best result for their study.

Of the five variables examined the ones that showed the highest correlation with age were the, (1) total true osteon (TTO), (2) total number of non-haversian canals (TNHC), (3) total number of osteonal fragments (TOF).The remaining two variables showed low significant correlation with age and should be excluded.

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