Section: Forensic Medicine

Original Research Article

ESTIMATION OF AGE BY MICROSCOPIC EXAMINATION OF RIB- AN AUTOPSY BASED STUDY

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ABSTRACT

Background: Age estimation is a quintessential step for the identification and profiling of an unknown corpse in Forensic medicine. It is also critical for profiling of the population as i t can provide a new horizon of data on demographics from the bio- statistical context. **Objective:** To arrive at the regression formula for assessing the age, based on number of osteons and other findings microscopically in the sternal rib and to estimate the age at the t ime of death by using the regression formula.

Materials and Methods: The bone samples, used in this study composed of sections which have been taken from the shaft region near the anterior ends of fourth sternal ribs of 69 individuals. After getting proper permission, concurrence and clearance from the Institute Ethical Committee, the samples were selected randomly from the autopsied bodies with known age group at the Sri Ramachandra Institute of Higher Education and Research, Chennai.

Results: The number of true osteons, fragmentary osteons, resorption spaces and concentric lamellae kept increasing when the individual ages as per all the previous studies.[47,28,34] The least standard error for estimating age was found in true osteons (±3 . 51) followed by resorption spaces (±4 . 71), fragmentary osteons (±5. 19), non- haversian canal (±9 . 14) and then finally by concentric lamellae (±12. 19). The number of Non-Haversian canals tend to reduce with age in a linear fashion which is seen in the previous studies Kerley et al (1965) and Ericksen et al (1991) with coefficient of determination r=- 0. 81.

Conclusion: This study i l lustrates the variation in certain microscopic structures of bone are systematically affected by age. Using this conclusion, regression formulae were developed to aid in age estimation from rib.

Keywords: Estimation ff Age, Microscopic Examination, Rib.

INTRODUCTION

Whether we are trying to bridge a clear basis of understanding to age related changes in bone macroscopically and micro- anatomically or our specific goal is to create methods for practitioners to apply for estimating age, Forensic experts need an in depth understanding of the distribution, degenerative and quanta of biological processes related to skeletal age changes.

The manifestations and understanding of age indicators in the bones are essential due to intricate physiological processes which occur during rapid growth phase, that is from childhood to adolescence and a relatively slower growth rate later in adulthood making this complex association of age markers and individual´s chronological age non-linearly connected.[1] A competent age indicator (trait or character) should present a l inear and a

predictable change with age, demonstrating a fixed positive/ negative correlation to chronological age and varying co nstantly across individuals from different ethnic groups.[2] As predicted, this is not the usual findings we see for most of the age markers used in age estimation procedures and methods.[3] Throughou the years, the Forensic community has worked incessantly on the procreation of age estimation procedures and techniques. This work has also included checking the quality and reliability of the techniques with consistent improvement of existing age prediction methods.[4]

Once growth has stopped, the bones continue to maintain and protect its functional and physiological properties, but undergoes a slow but constant degenerative process over time. This process can be assessed both macroscopically and microscopically with the aid of multiple standard techniques for better visualization under a microscope.

Microscopic approach using histological methods was first applied to estimate chronological age on bones in the mid - 20th century certifying that it could be used for measuring and arriving at the age of unknown individuals with decent amount of reliability.[5] From then on, multiple techniques of staining and histological analysis were created and improvised for better age estimation.[6,7] The foundation and basis of bone remodeling comprises of the substitution of older bon e and the genesis of new bone taking place by synchronized and harmonious activity of bone cells (osteoclasts and osteoblasts). These two are often called together as bone multicellular unit (BMU) and also some t times as bone remodeling units.[8] The microscopic features which are created by change in osteonal structures is known as the remodeling events, number of secondary osteonal structures significantly correlated to age, rising with age and erasing complete evidence of the primary bone microscopic s structures present in young adults.[9]

Prior knowledge and training with various equipment' s are important for implementing microscopic methods for the processing and e valuating thin- sections of bone.[10] Histological slide preparations are to be constantly improved, modified and tested for better bone processing and enha nced evaluation of chronological age.[11,12] Additionally, fossilization and taphonomic processes can also affect the histopathological and micro - anatomical analysis of the remains. The chance of bacteria entering the cortex via the porous network and expanding differently throughout the cortex is highly possible after death.[13] Although taphonomic changes concern a lot to a forensic expert as many remains are found underground, diagenetic processes in the soil in which i t was buried can give a lo t of clues in forensic cases and can help to expound the associated changes observed and their subsequent explanations.

The micro- anatomical structures of a bone show a varied degree of complex association with chronological age which should be understood in depth (decrease, increase or lack of association).[14,15] The variation with a population must be taken into consideration along with internal and external factors such as nutrition, genetic disorders, sedentary l lifestyle which leads to a completely modified bone turnover rates.[16]

Ribs are considered to be a better biological option for estimation of age as the periosteal remodeling can be easily averaged in a smaller cortical area than longer bones l like the femur and humerus where methodological issues can arise because they have a larger cortical area and the periosteal remodeling averaging can be a huge concern. The rib is a better bone for estimating age and the second argument relates to varied remodeling tempo observed in weight bearing bones due to differential metabolic and mechanical stress, concluding these changes to be minimal in rib.[17,18]

This study is considering the increase in natural calamities and mass disasters in various locations which is being attributed largely to the climate change. The findings of my study are authentic and can be cumulated with other standard or conventional methods for age estimation of unknown dead bodies to obtain a better result with more precision and accuracy.

MATERIALS AND METHODS

The bone samples, used in this study composed of sections which have been taken from the shaft region near the anterior ends of fourth sternal ribs of 69 individuals. After getting proper permission, concurrence and clearance from the Institute Ethical Committee, the samples were selected randomly from the autopsied bodies with known age group at the Sri Ramachandra Institute of Higher Education and Research, Chennai. The materials are mostly from the male bodies and few from the female bodies. Since the autopsies for female body usually less in number, our samples also have a smaller number of female samples.

Inclusion and Exclusion Criteria

In this study, the inclusion criterion was when the given age of the individual is known by the inquest report and original age proof documents. The exclusion criterion was when the age of the individual is not known. The sample materials were taken from the already known age group. Samples with suspicious pathological condition were positively omitted from the study.

Procedures Followed for Taking Samples

After completion of autopsies of known age group individual, the portion of bone was removed from the shaft of the fourth sternal rib around 5 cm away from cost- chondral junction near their anterior ends because near the anterior end, the bone has maximum breadth and doesn't have cartilaginous tissue. And the further away, from the costo-chondral junction ends of fourth rib as when the

people get younger. The bone was cut across the longitudinal axis of the bone. The portion chosen should not have decomposition changes.

Step 1

The part of fourth rib, about 0. 5 cm long, was removed from the thoracic cage, 5 cm lateral from the costochondral junction, by the help of rib- cutter/ bone shear.

Step 2

After removing the soft t issues and muscle attachments, the part of rib was placed in 10 % formalin solution and with the sample details such as number and age was labelled in the bottle

Figure 1: a) The soft t issues and muscle attachments to the fourth rib is being removed.
b) The rib sample is placed in 10 % formalin solution and
labelled.

Step 3

After the sample was fixed in 10 % formalin solution for 24 hours, the sample was then removed and placed in decalcification solution. In this case, we used 10 % Formic acid for decalcification which is a weak acid as the bone size is small with lesser thickness.

Step 4

The bone sample's consistency was checked for 12 hours, until it becomes soft and malleable. Excessive decalcification will lead to improper staining and loss of i ts original structure in microscopy.

Step 5

Then, the bone sample was removed from the decalcification solution, proper full thickness of the bone sample was cut using a blade scalpel and was placed in a plastic cassette for making blocks.

Step 6

Then the cassette was placed in a series of alcohols from 70 % to 95 % to 100 % for dehydration. For clearing, it was placed in Xylene which is miscible with paraffin it was carried out in the automated t issue proces sor. Finally, the t issue is infiltrated with the embedding agent, paraffin. After solidification, i t was cut into sections that can be placed on a slide using a microtome.

With the help of non- toothed forceps, the section was placed on the glass microscope slide of dimensions 7. 4 x 2. 5 cm and later which was cleaned with 90 % alcohol in such a way that the long axis of the bone section is parallel to the long axis of the slid e (Fig.7 & 8). This process was followed for the convenience while counting the number of osteons.

Step 7

Hematoxylin and eosin staining were done and the bone section was mounted with DPX Mountant fluid and glass cover slip 2 .5 x 2. 5 cm.

Histological analysis of bone slides

The bone section in the slide was placed in the well illuminated ordinary transmission light microscope. With 10 X objective and 10 X ocular lens, the section of the bone and consequently, the osteons and other structures was focused. After focusing the osteons and other structures, the entire cross section of the bone was scanned completely. This enabled us to study the distribution of the osteons and microstructures in full thickness of the compact bone of the rib. Then, the number of

1. True osteons
2. Non- Haversian canals
3. Fragmentory osteons
4. Concentric lamellae
5. Resoprtion space was counted in 3 fields. The counting field was selected in such a way that the number of osteons should be relatively maximum in number.

True osteons were defined as 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 Have rsian canal and/ or share a scalloped reversal line due to the plane of sectioning including a branching event, then they were also counted as one system.

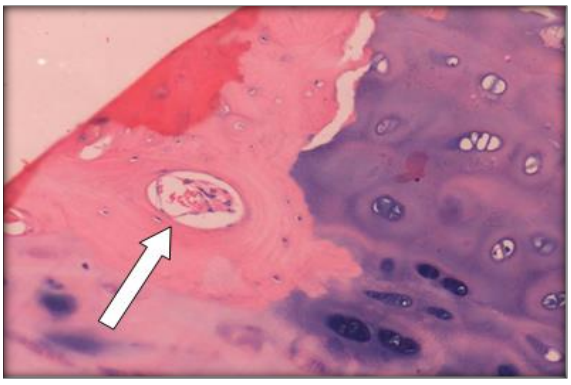


Figure 2: H& E Staining – Cross section of 4 th Rib- True Osteon

Non- haversian canals

All primary vascular channels, including those that have filled in partly with concentric lamellae to form primary osteons or pseudo - Haversian are vascular canals that was 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 represented areas of un-remodelled bone. The secondary osteon was formed in a space left by osteoclastic resorption and represents internal remodeling of the bone.

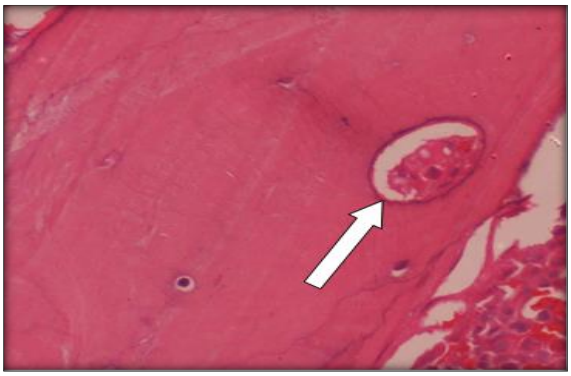


Figure 3: H& E Staining – Cross section of 4 th Rib- Non- Haversian Canals

Fragmentary Secondary Osteons or Osteonal Fragments

Number of secondary osteons with a partially visible Haversian canal that has been breached either by a neighbouring osteon or a resorptive bay and secondary osteons with no remnants of a Haversian canal was present. Osteon fragments that lack a Haversian canal was identified by concentric lamellar rings and the presence of a defined reversal line with a scalloped (irregular) margin.

Concentric lamellae

The total number of lamellae surrounding a true osteon are labelled as concentric lamellae in each field.

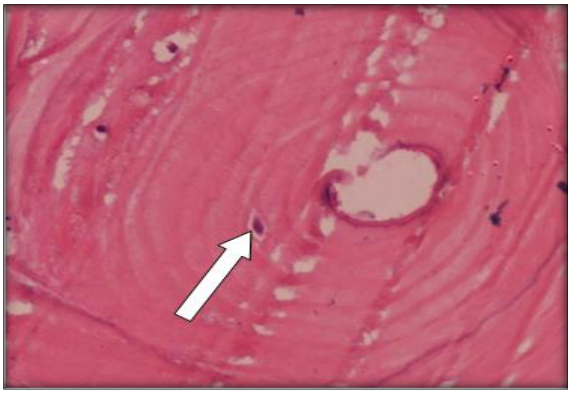


Figure 4: H& E Staining – Cross section of 4 th Rib– Concentric Lamellae

Resorption Spaces

These includes the first signs of new osteon formation and are characterized by scalloped edges. Osteoclastic giant cells presence with ragged edges.

Statistical Analysis

Statistical analysis was done in SPSS V 21 (Software Package for Social Science) for the study of correlation, regression analysis and to obtain the correlation coefficient for all the histological parameters. Regression analysis offers the mathematical formula used to predict one variable. Observed data has been documented in the scatterplot diagram. We could get visually analysable relationship between the variables. In this study, age was plotted in the y – axis and the number of Osteons and other variables were in the x – axis.

A correlation coefficient (r) was derived. It summarizes the significance in the relationship of two variables. The following observations were obtained. In the statistical studies, if ‘r’ value ranging from 0. 75 to 0. 99 means, the study was considered as having high correlation value. And if from 0. 5 to 0. 74, having moderate and if from 0. 25 to 0. 49 , having low correlation values. The l imitation of the ‘r’ value is the dependency of the sample size. So, the best way to determine the relationship is calculation of r 2. “ r2 ” is the coefficient determination. If the “r2” is closer to 1. 0, then the study is for positive relationship and if away from 1. 0, negative for relationship.

RESULTS

The rib samples from 69 cases were analysed which were collected with the informed consent. In this study consisting of 69 cases, the youngest victim was 6 months old and the oldest was 88 years. The highest number of cases was between the age of 41 - 50 and 51 to 60 each totaling up to 15 cases (22 %) respectively. The number of cases in the productive years i.e. 20 - 50 years was reported to be 38 cases which, constitute almost half the number of total cases taken in this study. The lowest number of cases is reported in 61 - 70 and 81 - 90 age group in this study which sums up to 2 cases (3 %) in each age group respectively. Out of 69, 61 were male (88. 4 %) and 8 were female (11. 6%).

The highest number of cases were seen among the Lower Middle category with 41 cases (59. 42 %) and the least was found among the Upper category with 3 cases (4.34%) and no cases in Lower socioeconomic category.

It was reported that in the samples obtained, it was found that, 3 cases of hypothyroidism and 1 case of pancreatic carcinoma and the rest of the individuals didn’t seem to have any significant endocrine disorder.

ALCOHOLIC/ NON- ALCOHOLIC & SMOKER/ NON- SMOKER

In our sample size of 69 cases, 24 were involved in such habits of which 16 were alcoholic and 8 were chronic smokers and the rest 45 didn’t show or seem to have any of these habits

Table 1: This table reports the number of cases who are alcoholic and smokers along with the cases who aren't involved in such habits

CONSUMPTION OF ALCOHOL	NO. OF CASES
Alcoholic	16
Smoker	8
Non- Alcoholic & Non- Smoker	45
Total	69

INCIDENCE OF DEATH

The medicolegal cases which we have included in our study comprises of a variety of cases such as: -

Table 2: Descriptive table showing the various incidence type among the 69 reported cases

INCIDENCE TYPE	NO. OF CASES
Road Traffic Accident	28
Natural cause	17
Hanging	11
Poisoning	3
Drowning	3
Sudden death	3
Fall from height	2
Congenital Syndrome	1
Electrocution	1
TOTAL NO. OF CASES	**69**

The total number of cases taken for statistical analysis were 51 out of
69 cases because, 18 rib samples did not contain any bone sample and was mostly consisting of t issues, cartilaginous matter and hence could not be considered for the statistical analysis of this study to estimate the age using the fourth rib.

CORRELATION BETWEEN AGE AND TRUE OSTEON

The value of R is 0 . 9036.
This is a strong positive correlation, which means that high X variable scores (Age) go with high Y variable scor es (True osteons) (and vice versa).
The value of R 2, the coefficient of determination, is 0. 8165.
The P- Value is < . 00001. The result is significant at p < . 05.
Then, the regression equation was calculated for estimating age using the number of true osteons.

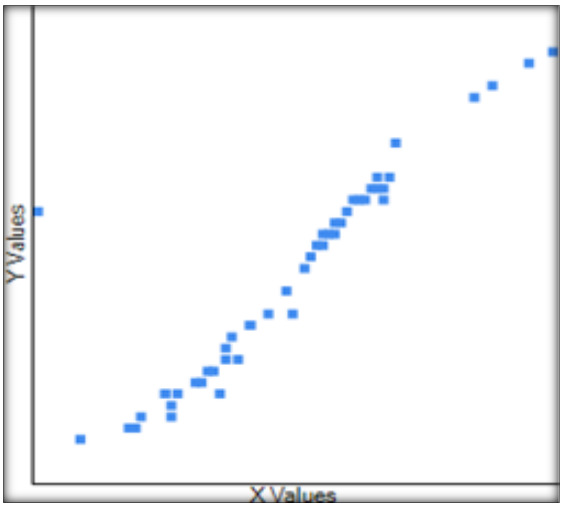


Figure 5 : Scatter plot which reports the positive correlation between age and true osteons.

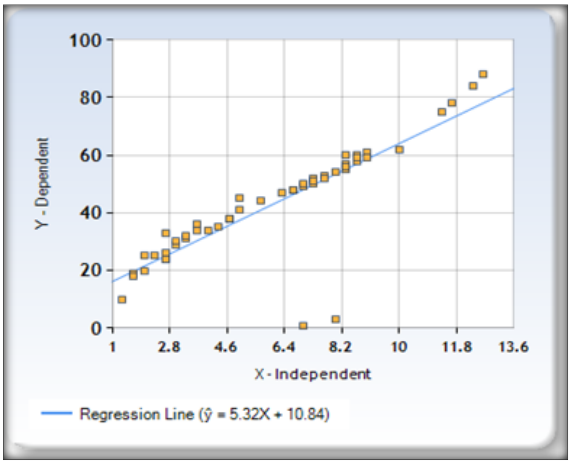


Figure 6: Scatter plot with the regression equation for true osteon

In the above graph, the number of osteons is taken as the variable in the X- axis and the age of the individual is taken as the variable in the Y - axis to arrive at a regression equation in which:
$\hat{y} = 5.31564\ X + 10.84238$
Standard Error when 51 cases included = 10. 28
Standard Error when 50 cases included = 9. 56 (removing case with 6 months of age)
Standard Error when 49 cases included = 3. 5 (removing case with 6 months of age and 3 years)

CORRELATION BETWEEN AGE AND RESORPTION SPACE

The value of R was 0. 9259, which was a strong positive correlation, which means that high X variable scores go with high Y variable scores (and vice versa).
The value of R 2, the coefficient of determination, is 0. 8573. The P- Value is <. 00001. The result is significant at p <. 05.

In the below graph, the number of resorption spaces is taken as the variable in the X- axis and the age of the individual is taken as the variable in the Y- axis to arrive at a regression equation given below.

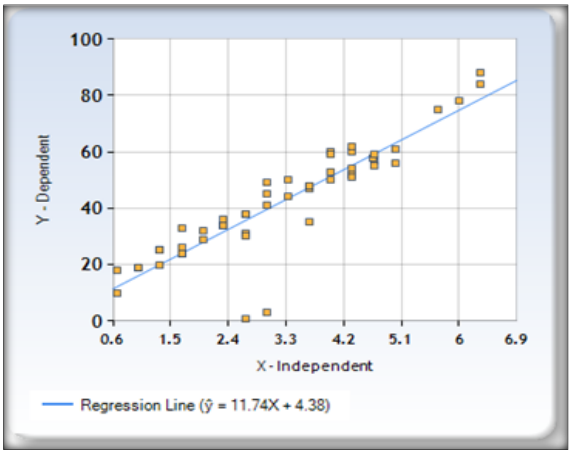


Figure 7: Scatter plot which reports the positive correlation between age and resorption space

Then the regression equation was calculated for estimating age using the number resorption spaces.

ŷ = 11. 73861 X + 4. 37501

SE when 51 cases included = 8. 47

SE when 50 cases included = 6. 97 (removing case with 6 months of age)

SE when 49 cases included = 4. 71 (removing case with 6 months of age and 3 years)

CORRELATION BETWEEN AGE AND CONCENTRIC LAMELLAE

The value of R is 0. 7284. This is a moderate positive correlation, which means there is a tendency for high X variable scores go with high Y variable scores (and vice versa).

The value of R 2, the coefficient of determina t ion, is 0. 5306. The P- Value is <. 00001. The result is significant at p <. 05.

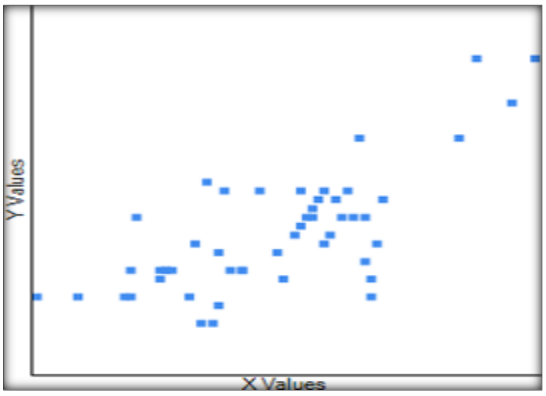


Figure 9: Scatter plot which reports the positive correlation between age and concentric lamellae

In the above graph, the number of Concentric lamellae is taken as the variable in the X- axis and the age of the individual is taken as the variable in the Y- axis to arrive at a regression equation given below.

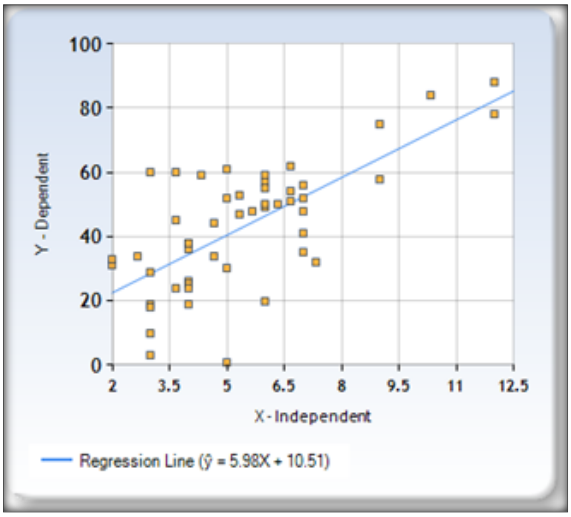


Figure 10: Scatter plot which reports the positive correlation between age and concentric lamellae

Then, the regression equation was calculated for estimating age using the number of concentric lamellae present in the ribs.

ŷ = - 10. 3637 X + 70. 37784

SE when 50 cases included = 10 . 46 (removing case with 6 months of age)

SE when 49 cases included = 9. 14 (removing case with 6 months of age and 3 years)

SE when 51 cases included = 11. 56

CORRELATION BETWEEN AGE AND FRAGMENTARY OSTEONS

The value of R is 0. 8929.

This is a strong positive correlation, which means that high X variable scores go with high Y variable scores (and vice versa).

The value of R 2, the coefficient of determination, is 0. 79 73. The P- Value is <. 00001. The result is significant at p < 0. 05.

Then the regression equation was calculated for estimating age using the number of true osteons.

ŷ = 8. 03969 X + 17. 04019

SE when 51 cases included = 10. 4

SE when 50 cases included = 8. 31 (removing case with 6 months of age)

SE when 49 cases included = 5. 19 (removing case with 6 months of age and 3 years)

CORRELATION BETWEEN AGE AND NON-HAVERSIAN CANAL

The value of R is - 0. 8187.

This is a strong negative correlation, which means that high X variable scores go with low Y variable scores (and vice versa).

The value of R2, the coefficient of determination, is 0. 6703. The P- Value is

<. 00001. The result is significant at p <. 05. the number of Non - haversian canals is taken as the variable in the X- axis and the age of the individual is taken as the variable in the Y- axis to arrive at a regression equation given below.

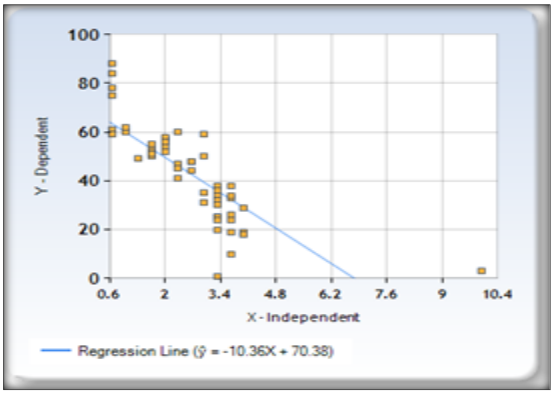


Figure 12: Scatter plot which reports the negative correlation between age and non- haversian canal

Then the regression equation was calculated for estimating age using the number non- haversian canal.

$\hat{y} = -10.3637X + 70.37784$

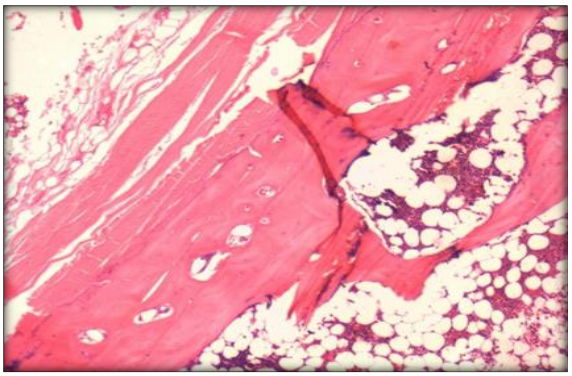
SE when 51 cases included = 11. 56

SE when 50 cases included = 10. 46 (removing case with 6 months of age)

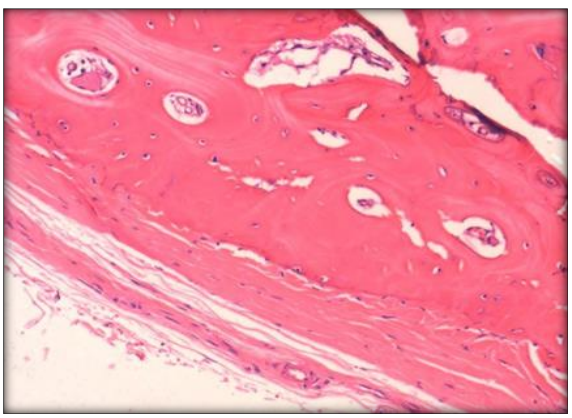
SE when 49 cases included = 9. 14 (removing case with 6 months of age and 3 years).

COLOUR PLATES

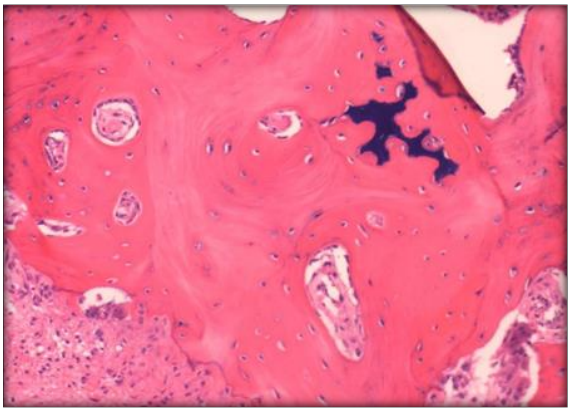
A Field showing all the 5 parameters and bone marrow of a 62 years male (PM NO. 168 / 19).



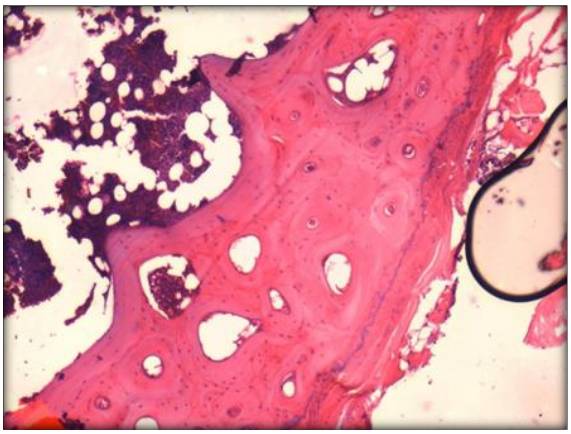
A Field showing all the 5 parameters and bone marrow of an 88-year-old male. We find increased number of true osteons, fragmentary osteons and resorption spaces. (PM NO. 197/ 19)



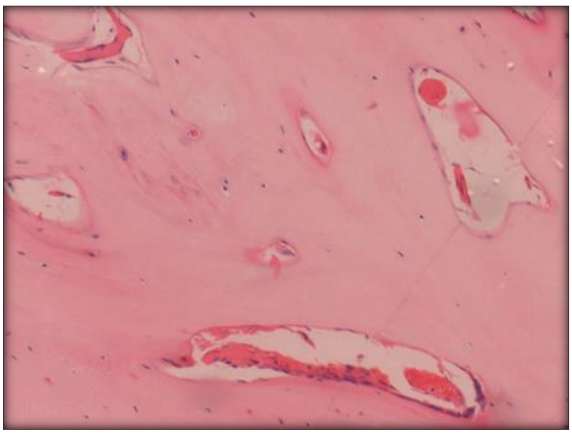
A field showing all the 5 parameters and Hyper cellular bone marrow of a 3-year-old child. It was also seen that the osteocytes were not present in the lacunae and the size of the osteons were comparatively smaller. (PM NO. 181 / 19)



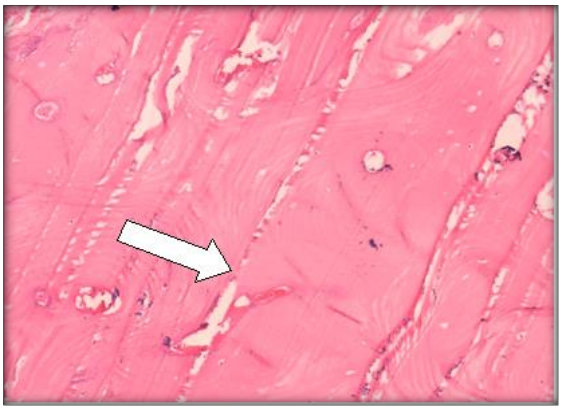
A field showing all the 5 parameters and Hyper cellular bone marrow of a 6 months old child. It was also seen that the osteocytes were not p resent in the lacunae and the size of the osteons were comparatively smaller. (PM NO. 190 / 19)



A Field showing 3 parameters of a 33 years male with no true osteons hence the number of concentric lamellas could not be calculated in this field. (PM NO. 91 / 19)



A Field showing all the 5 parameters of a 53 years male. To be careful about the artifacts while preparing the slide and tissue processing. (PM NO. 75 / 19)



DISCUSSIONS

Various researchers found different results depending on which bones were analyzed. It has been shown that the cranium19,20 demonstrated low correlations for the occipital bone (r 2 = 0. 44) and frontal bone (r 2 = 0 . 276) respectively, whereas the mandible21 appeared to yield the highest r- squared value (r2 = 0 . 979). The other bones (tibia, clavicle, humerus, fibula and ulna) have higher coefficients of determination (r2= 0. 67 – 0. 98) when compared to the results obtained from the femur (r 2 = 0. 42 – 0 . 95). The two studies that tend to have the lowest coefficients of determination are that of Cool et al. (1995) and Curtis (2003). Cool et al. used the occipital bone (r2 = 0. 44) and Curtis used the frontal bone (r2 = 0 . 276). The specific area from which the bone sections are sampled also plays an important role in the accuracy and consistency of the results.

Here, there is absence of sampling error, which occurs in using wedges of bone sample taken from the thick bone like tibia or femur. Absence of sampling error gives high positive correlation values. Next, the entire diameter of the rib is smaller (1 . 2 x 1. 0 cm). So, i t is completely well fit against the microscope glass slide which is measured 7 . 4 x 2. 5 cm. And so, cutting, taking sample from the rib for cross section and counting of osteons are simple, easy and less time - consuming procedures. It is very much important because of the less probability for inter observer variations in osteon counting.

The relationship for the linear analysis (single variables) from previously reported studies were compared with the correlations achieved in this study. The interest of using multiple linear regression equations and analysis is to increase the optimization of the variables that shows the b est association and relationship with age. In this study the regression equations calculated using linear regressions showed accurate results. The highest r-squared value was obtained when all the variables were taken into consideration (r = 0. 9152 - males). This implied that 81 % of the changes and variation seen within these variables can be related and attributed to age. The standard error of estimate for this equation was ± 3 . 51 years which was the most immaculate achieved by all the regression equations. The female sample did not yield very good results sexual dimorphism could not be identified using the variables.

The total osteon count is by far the most used technique, of the histological ageing as osteon is the basic and important fundamental structure with the process of remodeling. The number of osteons increased with age which is in correlation with many authors.[22,23] The results of this study indicate that the total number of true osteons positively correlated with age, 81 % of the variance in the predicted age was explained by this variable. The osteon count in males was found to have a greater and stronger correlation with age when compared to th e females, but this could be due to the small female sample compared to the relatively large male sample which was similar to Kerley (1965 who analysis yielded a correlation of 0 . 922 (r).

When compared to the SEE obtained from this study for the total number of osteons, Kerley and Ubelaker (1978) achieved a low value of ± 9 . 19 years whereas the present study yielded a value of ± 3. 51 years which has a vast difference. Ericksen's (1991) study yielded correlation coefficients of r = 0 . 49 (sex combined for the linear regression analysis which is lower compared to this study which had a coefficient of r = 0 . 91.

Singh and Gunberg et al (1970) established that there was an increase in the average number of lamellae per osteon as age increased and the similar finding was found in this study. The correlation coefficient in this study for this variable was r = 0.294, which indicated a moderate positive correlation. The correlation obtained by Singh and Gunberg for the average number of lamellae per osteon was r = 0. 890, indicating a strong positive correlation with age.

The results and correlation of this study (average Haversian canal size) agree with the results obtained by Yoshino et al. (1994), Watanabe et al. (1994) in suggesting that the size of the Haversian canal does not display any significant age- related changes but seems to be constant overall throughout the life time.

When the total number of non- haversian canals were calculated and analyzed, it was seen that the numbers generally tend to decrease with age. This result coincides and confirms with the analysis by Kerley et al (1965) and Ericksen et al (1991) whose results indicated that there was a strong negative co-relationship which led to decrease i n the number of non- haversian canals with rise in age. Kerley et al (1965) mentioned that non- haversian canals were not seen in individuals above the age of 55 years and theorized that non-haversian canals had been completely remodeled by osteons which would

occupy the majority of the lamellar bone. In this study, i t was found that non-haversian canals were present even at the age of 88 years, with some of the other older individuals having similar numbers of non- haversian canal.

Although there were still non-haversian canals present in these individuals, the total number drastically declined after 70 years of age. In the male sample, non-haversian canals were not observed in one or two fields in the individuals who were 70 years of age and above.

Similar findings were seen in the study When Ericksen' s (1991) results are examined, with the aid of scatterplot and the findings from the study it was noted that the number of osteonal fragments increased with chronological age. These results agree with previous authors,[23,24] and are in concurrence with the process of bone remodeling theories. Ericksen (1991) achieved a high correlation value (r = 0. 71) as did Kerley (r = 0 . 86) for the osteon fragments but this study yielded a strongly positive correlation of r = 0 . 9.

This indicated that the formation of osteon fragments is highly correlated with age in this sample when compared to other samples. This could be due to the fact that constant remodeling of the bone is taking place and conversion of primary osteons to secondary/ true osteons occurs in somewhat l inear and continuous process. Osteons are formed at random areas in the lamellar bone and their sizes are also variable, so the possibility that bigger osteons could be present even when they are replaced by a new smaller osteon.

The occurrence presence of resorption spaces was present throughout the sample. This variable was included by Ericksen (1991) and Yoshino et al. (1994). In this study it was seen that the appearance or formation of resorption spaces does follow a linear and a positive correlation with age pattern. Resorption of bone is not totally dependent on age alone but incidence of resorption space depends upon the area of sampling and the resorption cavities move distally from the origin of formation. While the resorption cavities are being formed, they are also simultaneously being filled with vascular channels and filled with concentric lamellae rings.

CONCLUSION

This study illustrates the variation in certain microscopic structures of bone are systematically affected by age. Using this conclusion, regression formulae were developed to aid in age estimation from rib.

Five variables were observed on the microscopic sections and from these observations linear regression analyses were conducted. The l inear regression involved analysis of the independent variables.

It was proved that the number of true osteons, fragmentary osteons, resorption spaces and concentric lamellae kept increasing when the individual ages as per all the previous studies (47 ,28, 34). The least standard error for estimating age was found in true osteons±3.51) followed by resorption spaces (±4.71), fragmentary osteons (±5.19), non- haversian canal (±9.14) and then finally by concentric lamellae ±12.19).

It was also found that the number of Non-Haversian canals tend to reduce with age in a linear fashion which is seen in the previous studies Kerley et al (1965) and Ericksen et al (1991) with coefficient of determination r=- 0. 81.

There was no difference between Alcoholics, smokers and Non - alcoholic, non – smokers as the statistical strength was less due to lesser number of samples similar to the study done by Keough et al (2007).

This study also contributes to the understanding that some structures (resorption spaces, lamellae per osteon, Haversian canal diameter) may not be completely dependent on age. Furthermore, remodeling of bone may be affected by external factors such as disease and mechanical stress.

These factors (mechanical loading, disease) cause alterations in the remodeling process of bone, thus potentially influencing histological techniques if these factors are not identified before analysis is conducted. This misidentification could result in over and under estimation of the ages of these individuals.

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