

## Can Vascular Channels in the Bone Determine the Age of A Human Being?

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### Abstract

The accurate assessment of age-at-death from skeletal remains is a key factor in both forensic anthropology and bioarchaeology. Several methods of determining age at death are currently employed that utilize the age specific changes of several anatomical regions of the skeleton. However, as skeletal remains are often incomplete, it is useful to develop new methods based on previously unevaluated anatomy. This makes it more likely that sets of incomplete skeletal remains may include some feature that can be used to determine age-at death.

The purpose of this study was to develop standards for estimating age at death, using bone microstructure, that are applicable to a South Indian population. The sample consisted of 67 individuals (59 males and 8 females) of known age and sex. The sample was removed 5 cm lateral from the costo-chondral junction of the fourth rib and slides were prepared according to standard histological methodology.

It was found that the number of Non-Haversian canals tend to reduce with age in a linear fashion which is also seen in the previous studies with coefficient of determination being 0.6703. Then the regression equation was calculated for estimating age using the number non-haversian canal  $Y = -10.3637X + 70.37784$  with standard error of estimate being  $\pm 9.14$  years.

**Keywords:** Non-Haversian canal; Rib; Age estimation; Vascular channels.

### Introduction

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 it can provide a new horizon of data on demographics from the bio-statistical context. 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.

Macroscopic methods like examination of the bones grossly by looking at the degenerative changes are one of the commonly used methods as it can be easily visualized and requires no equipment.<sup>1,2</sup> But this approach is highly subjective according to the person and has a very high standard error of estimate and also gets higher when the person's age climbs above 50.<sup>3,4</sup> Moreover, age markers can start varying if the bones were buried and can cause a series of taphonomic and fossilization processes which may render the observation invalid and thus, can't be used in regular practice.<sup>5</sup>

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On the other hand, Microscopic approach using histological methods was first applied to estimate chronological age on bones in the mid-20<sup>th</sup> century certifying that it could be used for measuring and arriving at the age of unknown individuals with decent amount of reliability. From then on, multiple techniques of staining and histological analysis were created and improvised for better age estimation. The foundation and basis of bone remodelling comprises of the substitution of older bone 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 sometimes as bone remodelling units. The microscopic features which are created by change in osteonal structures is known as the remodelling events, number of secondary osteonal structures significantly correlated to age, rising with age and erasing complete evidence of the primary bone microscopic structures present in young adults.<sup>6</sup>

Non-Haversian canals are all primary and the main vascular channels, including those that have filled in partially with concentric lamellae to form primary osteones or false, pseudo-Haversian systems are vascular channels that were formed, by the addition or inclusion of minute, circumferential, tangential blood vessels into the bone by accelerated increase in size of the cortex in radius. Since these canals were created at the time the surrounding lamellar bone was formed, they are primary and indicate the places of unremodeled bone. The secondary osteon is created in the area left by osteoclastic resorption and represents internal remodelling of the bone microstructure. The primary osteon can be distinguished from the secondary osteon by the unremodeled bone or lamellar bone surrounding it. The primary osteon has no immaculate reversal or cement line surrounding it, and the surrounding lamellae curve smoothly around the external limits. In the secondary osteon, the lamellae surrounding it run towards the reversal line and curb the osteoclasts during the resorptive phase.<sup>7</sup>

### Methodology

The present study was conducted in Forensic Medicine and Toxicology Department of Sri Ramachandra Medical College & Research Institute about 67 samples of 4<sup>th</sup> rib taken from dead body brought for post-mortem examination in mortuary during the period of May 2019 to September 2019. Its particulars were recorded and

age was cross checked from relatives. The proposal for research was submitted to ethics committee, Sri Ramachandra Medical College & Research Institute, Chennai, Tamil Nadu. The research work was approved by the ethical committee. Before taking the sample from deceased the consent form was filled & signed by kin of deceased.

The specimens were separated from the body by cutting the fourth rib at a point i.e. five centimetre outer to costo-chondral junction using a ribcutter. The muscles attached to the ribs were cut using scissors. Then after labelling the sample fixation was done before decalcification. For decalcification, formic acid was used. After decalcification regular tissue processing & staining was done & sample was stained with H&E. The bone slides were examined under light microscope (Olympus), with a 10X objective & 10X ocular lens piece and Non-Haversian Canals were observed and recorded (Figure 1).

In present study simple linear regression analysis has been done. The standard error of the estimate (SEE) was also determined with help of SPSS software (Version 21).

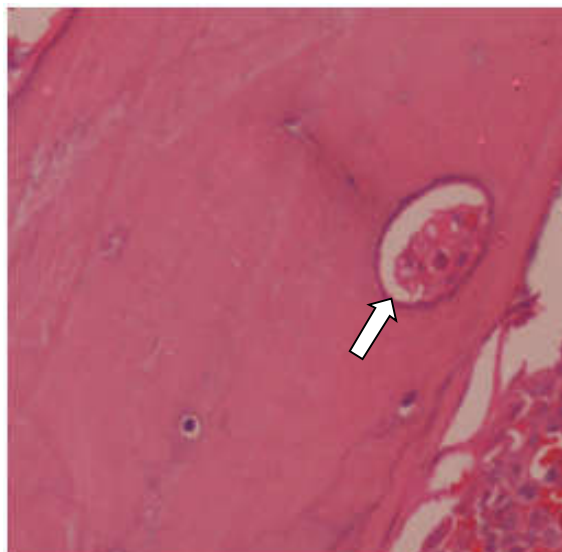


Fig. 1: H&E Staining - Cross section of 4<sup>th</sup> Rib -Non- Haversian Canals.

### Results

In the below graph, the number of Non-haversian canals was taken as the variable in the X-axis and the age of the individual is taken as the variable in the Y-axis (Figure 2) to arrive at a regression equation given below.

The value of R was -0.8187.

The value of R2, the coefficient of determination, was 0.6703. The P-Value was < .00001.

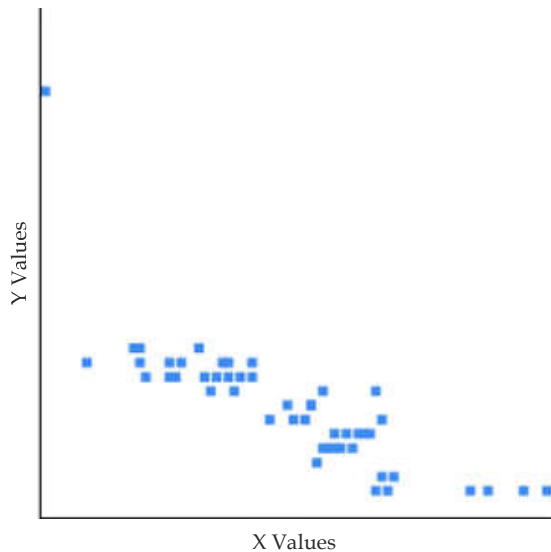


Fig. 2: Scatter plot which reports the negative correlation between age and non-Haversian canal.

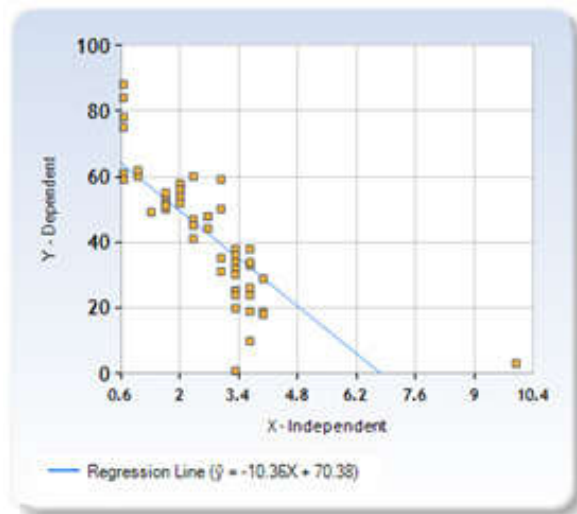


Fig. 3: Scatter plot which reports the negative correlation between age and non-haversian canal.

Then the regression equation was calculated for estimating age using the number of non-Haversian canal (Figure 3).

$\hat{y} = -10.3637X + 70.37784$  (SE of estimate =  $\pm 9.14$  years).

## Discussion

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.<sup>8</sup> 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 correlation which led to decrease in 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 but in this study, it 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.<sup>9</sup> 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. This indicated that even though resorption process is present throughout life, not all of the lamellar bone is replaced by true osteons until approximately 80 years of age. It was clear that there was no real difference between the correlation values for the male and female groups. Similar findings were seen in the study when Ericksen's (1991) results are examined.<sup>9</sup>

## Conclusion

The number of Non-Haversian canals tend to reduce with age in a linear fashion which is seen in the previous studies like Kerley et. al. (1965) and Ericksen et. al. (1991) and further research should be conducted to identify the changes occurring in sexual dimorphism, diseases altering bone metabolism and also diet.

The reason the rib was specifically chosen for this study is that it is more likely to survive and remain fairly undamaged after death.<sup>10</sup> At first, it is easily accessible and removable bone from the body in the post mortem examination, unlike the other long bones like femur, tibia etc. Removal of just one rib causes almost no mutilation or disfigurement of the body. Unlike other long bones, which are usually weight bearing bones, ribs are less vulnerable to

mechanical stress and in turn stress induced aging changes.

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