

Knowledge On Histology Of Bone Among Dental Undergraduates - A Cross-Sectional Survey

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ABSTRACT:

Background - Bone is constantly renewed by the coordinated action of osteoblasts and osteoclasts. Osteocytes have a prominent role in the onset of bone remodelling at selected sites of the skeleton.

Aim - The aim of the present study is to assess the knowledge about histology of bone among undergraduates.

Materials and Methods - This study is a questionnaire based survey to evaluate the knowledge about histology of bone among undergraduates. The data collected through google forms and analysed using SPSS software version 23.

Results -The present study includes 116 participants. 45% were first year 16% were second year, 16% were third year, 16% were fourth year and 5% were interns. Majority (14%) were the first years who were aware about the histology of bone when compared to second (9%), third (7%), fourth year(5%) and interns (1%) . Pearson chi square test was done and the p value is 0.017 and hence it is statistically significant.

Conclusion -The first year students have adequate knowledge when compared to other students. The knowledge gap can be fulfilled by effective learning through innovative theory and practical sessions to help students to know better about bone histology.

Keywords: osteoclast, osteoblast, osteocytes, osteoid, osteon, innovative technique.

INTRODUCTION

Bone is the primary structure of the human skeletal system. It is composed mainly of type-I collagen and inorganic salts. It protects vital organs like the skull, vertebra, ribcage and provides mechanical support. Bones are regarded with different shape, size, and location. Bone is composed of four different cell types. They are osteoblast, osteocytes, osteoclasts and bone lining cells. Osteoblasts, bone lining cells and osteoclasts are present on the bone surface and are called progenitor cells. Osteocytes permeate the interior of the bone and are produced from fusion of mononuclear blood-borne precursor cells. Bone resorption is the process by which bones are absorbed and broken down by the body. Bone remodelling is a lifelong process and controls the reshaping and replacement of bone. Alveolar bone is the part of the jaw that holds the teeth (1,2).

Bone helps in the formation of hematopoietic cells. Osteocytes survive for decades and are called the longest lived cells. The skeleton has an important role as a reservoir of minerals such as calcium and phosphate which can be released when demands are increased in the body and in maintaining serum homeostasis. These conventional functions of the skeleton depend on the homeostasis of bone itself (2,3).

Histology is the study of tissues, including their role in the body, their anatomy, their interaction with body systems and the ways they are affected by disease. The knowledge about histology of bone among first year students plays a vital role in incorporating better understanding. This microlevel perspective on biology and medicine can seem tedious, and some students may be frustrated and eager to move on to more generalized approaches to anatomy. Histology knowledge aids students in understanding the production of complex organs and organ systems. Students gain a better understanding of how organs and organ systems grow and evolve over time by examining tissue from humans and animals with complex organs. However, nothing else in biology makes sense without a knowledge of histology (4). Our team has extensive knowledge and research experience that has translated into high quality publications (5-24). Thus, the aim of the present study is to assess the knowledge of undergraduate students about bone histology.

MATERIALS AND METHODS :

This cross-sectional study was conducted in a private medical college among undergraduates in Chennai, from January 2021 to February 2021. 116 participants were included in this study. The participants were among first year, second year, third year and fourth year. A set of questionnaires was circulated among undergraduates through online surveys. Data are presented as mean standard deviation. Categorical variables are expressed as count and percentages. Continuous data were compared by using independent samples using descriptive frequency. Chi square tests were performed to determine significant factors associated with gender and education among undergraduates. Odd ratios (OR) with 95% confidence intervals were used for comparisons. P value of < 0.05 was considered statistically significant. All analyses were performed using the SPSS software version 23. Chi square test was used to analyze and comparative bar graphs were plotted and it is statistically significant only if the p value is less than 0.05.

RESULTS :

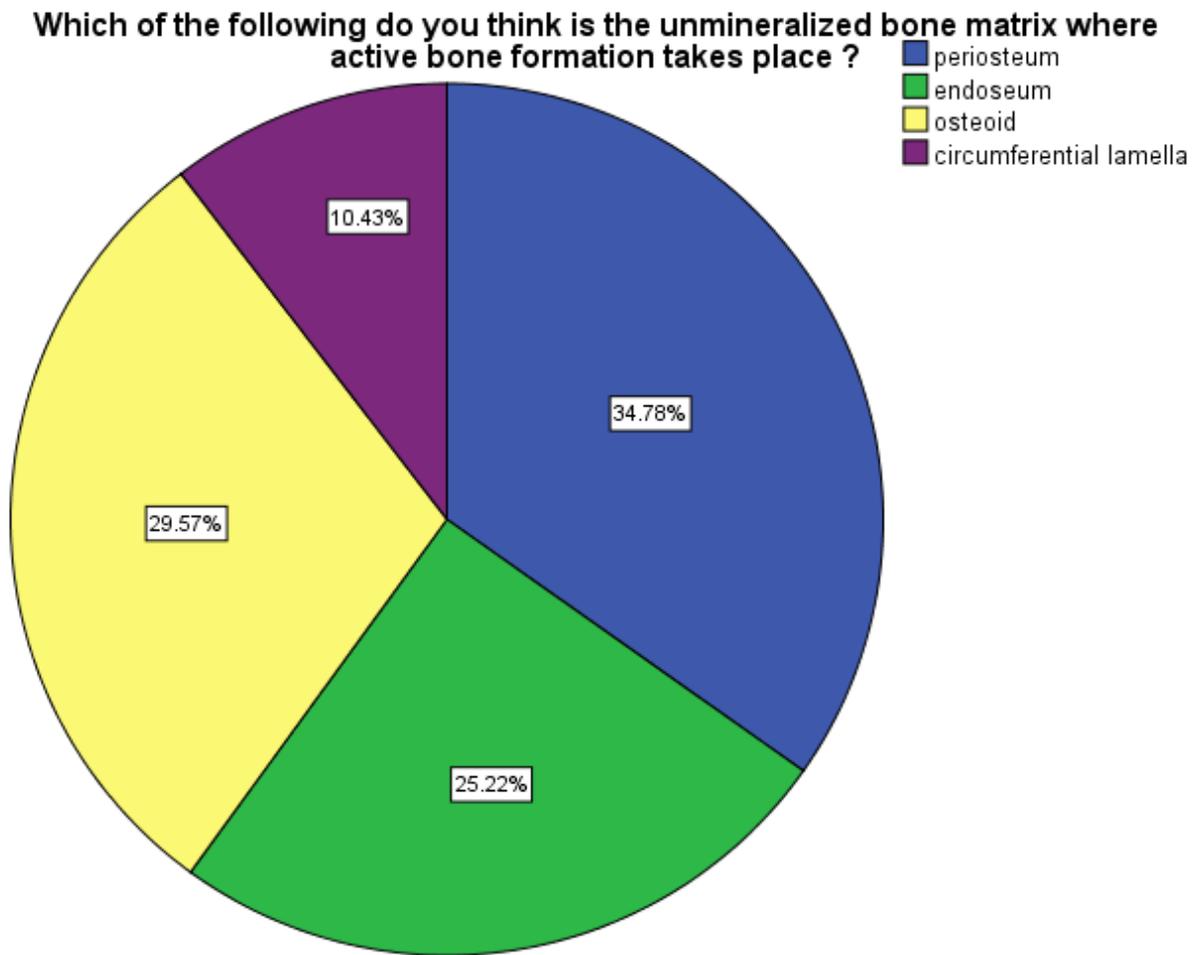
The present study has observed that out of 116 study participants and among them 45% were first year 16% were second year, 16% were third year, 16% were fourth year. Only 29.57% of the population were aware that osteon is the unmineralised bone matrix (**Figure 1**). According to the study, 25.22% of the population were aware that periosteum is the fibro collagen layer surrounding the compact bone whereas 29.57% (haversian canal), 34.78% (endosteum) and 10.43% (osteon) were unaware about the fibro collagen layer (**Figure 2**). Majority (34.78%) of the population were aware that the labelled parts are osteoclast and osteoblast (**Figure 3**). As shown by the study majority (48.70%) of the population were aware that reversal line marks the cessation of resorption (**Figure 4**). Only 26.96% of the population are aware about the osteon whereas 18.26% (hemopoietic tissue), 20.00% (trabeculae) and 34.78% (reversal line) were unaware about the osteon (**Figure 5**). Majority (43.48%) of the population were aware that the multinucleated cell is osteoclast (**Figure 6**).

In our present study, the majority (40.00%) of the population were aware that 25 years is the average half life of human osteocytes (**Figure 7**). In the same study only 29.57% of the population were aware that canaliculi form channels of osteocyte lacunae (**Figure 8**). And, 27.83% of the population were aware that the bone shown is both compact and cortical bone (**Figure 9**) and 16.52% of the population were aware that osteoblasts are responsible for synthesis and secretion of bone matter (**Figure 10**). Majority (30.43%) were the first years who were aware about the reversal line when

compared to second (5.22%), third (5.22%), fourth year (2.61%) and interns (1.74%) . Pearson chi square test was done and the p value is 0.015 and hence it is statistically significant (**Figure 11**). According to the study, the majority (14.78%) were the first years who were aware of the average half life of human osteocytes when compared to second (9.57%), third (7.83%), fourth year (5.22%) and interns (0.87%). Pearson chi square test was done and the p value is 0.017 and hence it is statistically significant (**Figure 12**). Finally, in this survey, (**Figure 13**) majority (6.09%) were the first years who were aware about the osteoblast are responsible for synthesis and secretion of bone matter when compared to second (1.74%), third (2.61%), fourth year (1.74%) and interns (0.87%). Pearson chi square test was done and the p value is 0.015 and hence it is statistically significant.

The association between year of study and the question regarding osteoblast was evaluated using pearson chi square test and found out that first years were more aware about osteoblast and bone secretion when compared to other years and the p value is 0.015 and hence it is statistically significant (**Figure 13**)

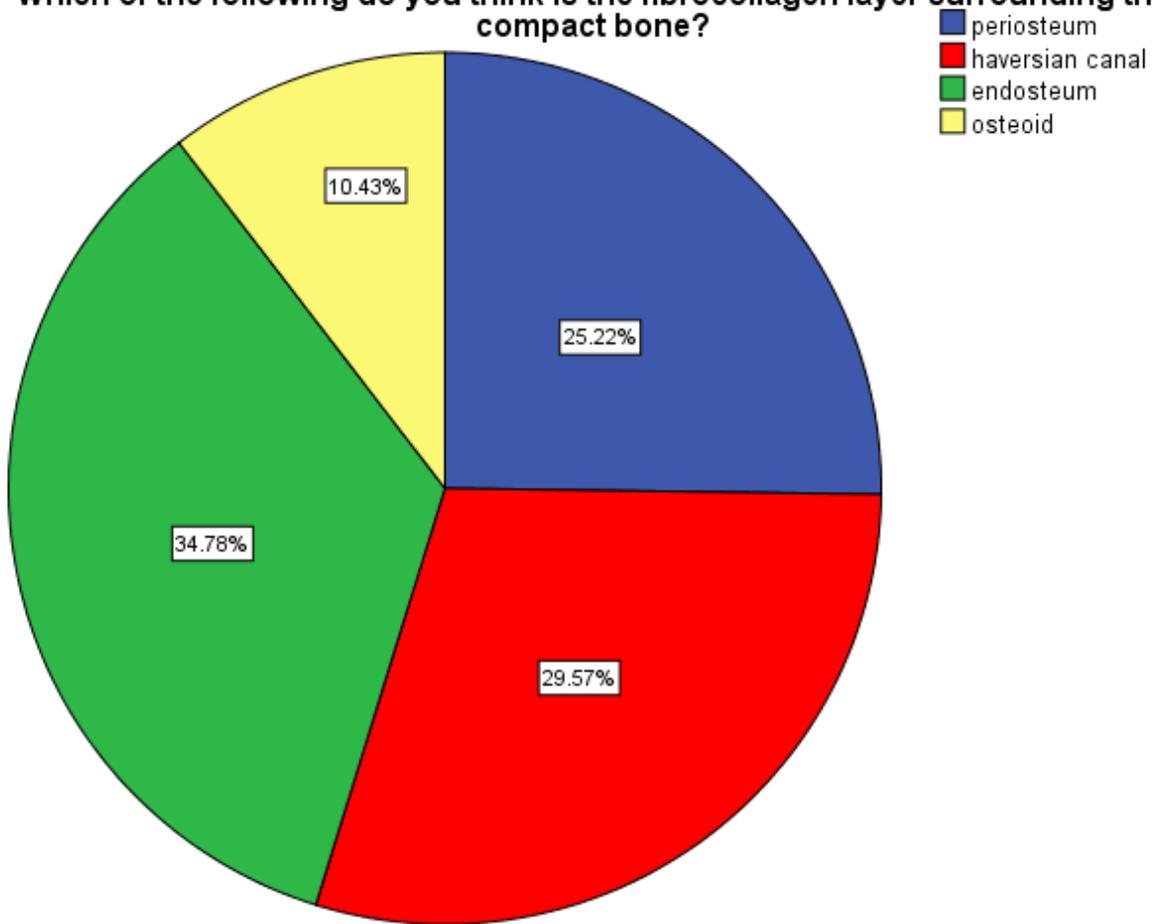
Fig 1



In Fig 1, Pie chart shows the percentage of response for unmineralised bone matrix. Blue denotes periosteum, Green denotes endosteum, yellow denotes osteoid and purple denotes circumferential lamellae. Only 29.57% of the population were aware that osteon is the unmineralised bone matrix whereas 34.78% (periosteum), 25.22% (endosteum) and 10.43% (circumferential lamellae) were unaware about the unmineralised bone matrix.

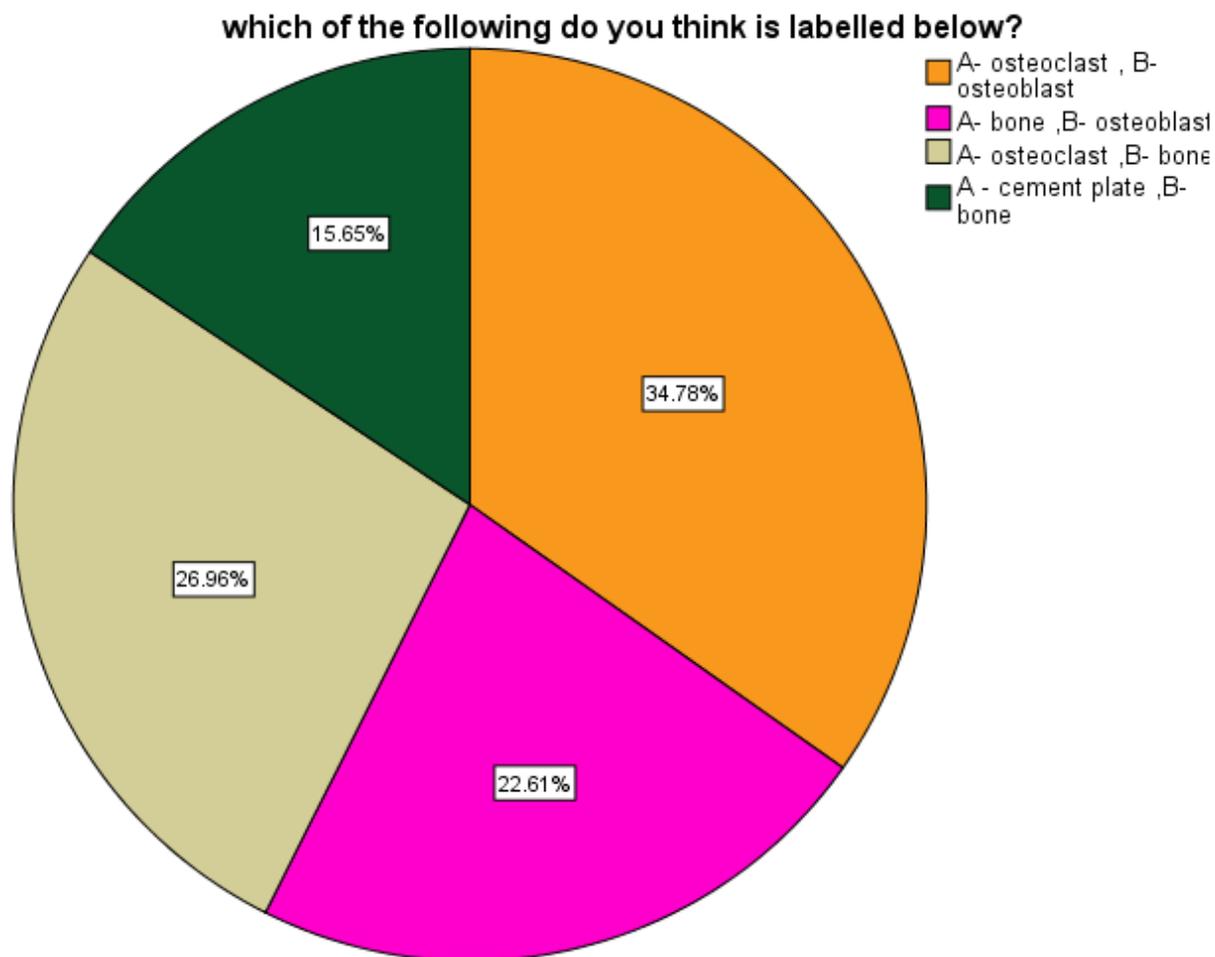
Fig 2

Which of the following do you think is the fibrocollagen layer surrounding the compact bone?



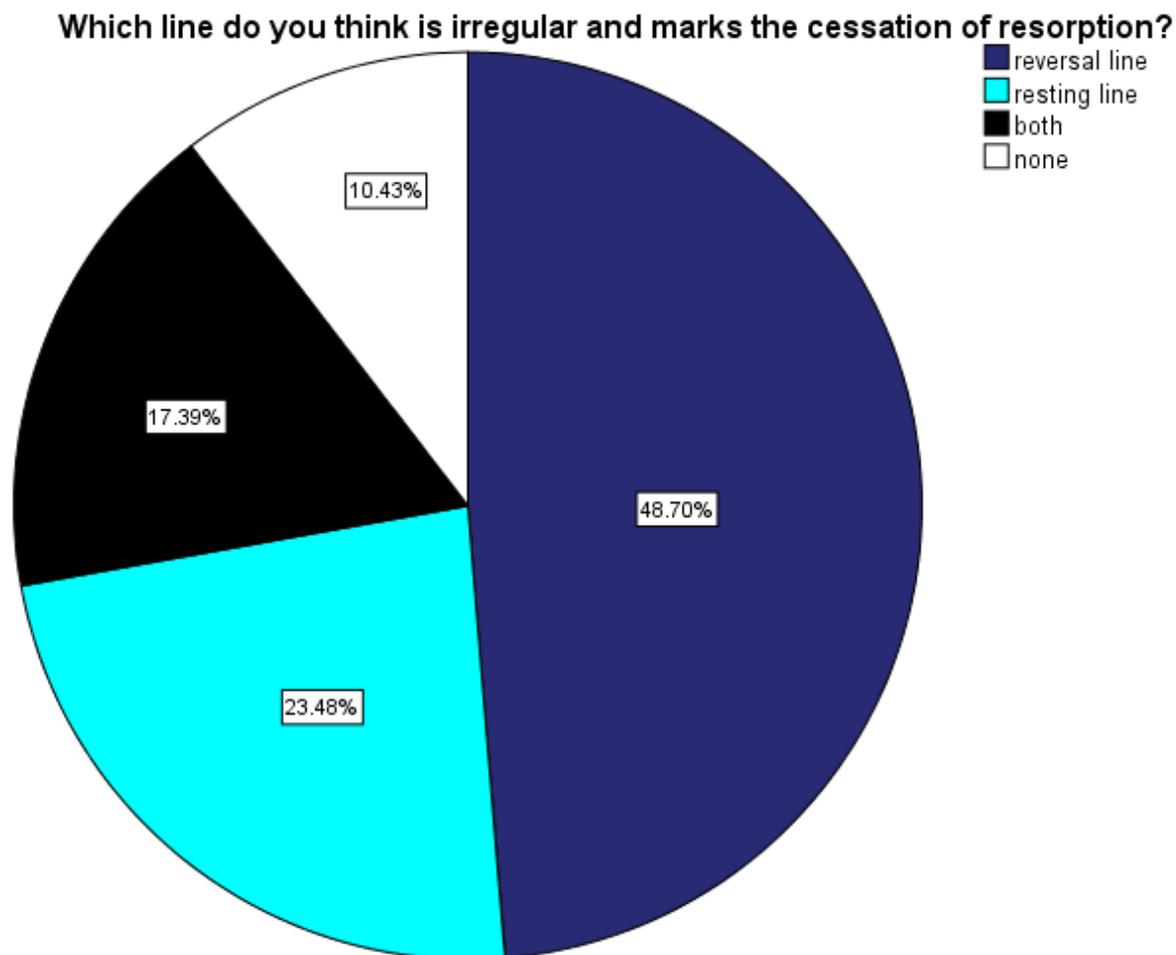
In Fig 2, Pie chart shows the percentage of responses for fibro collagen layer. Blue denotes periosteum , Red colour denotes Haversian canal, green colour denotes endosteum and yellow colour denotes osteoid. Only 25.22% of the population were aware that periosteum is the fibro collagen layer surrounding the compact bone whereas 29.57% (haversian canal), 34.78% (endosteum) and 10.43% (osteon) were unaware about the fibro collagen layer surrounding the compact bone.

Fig 3



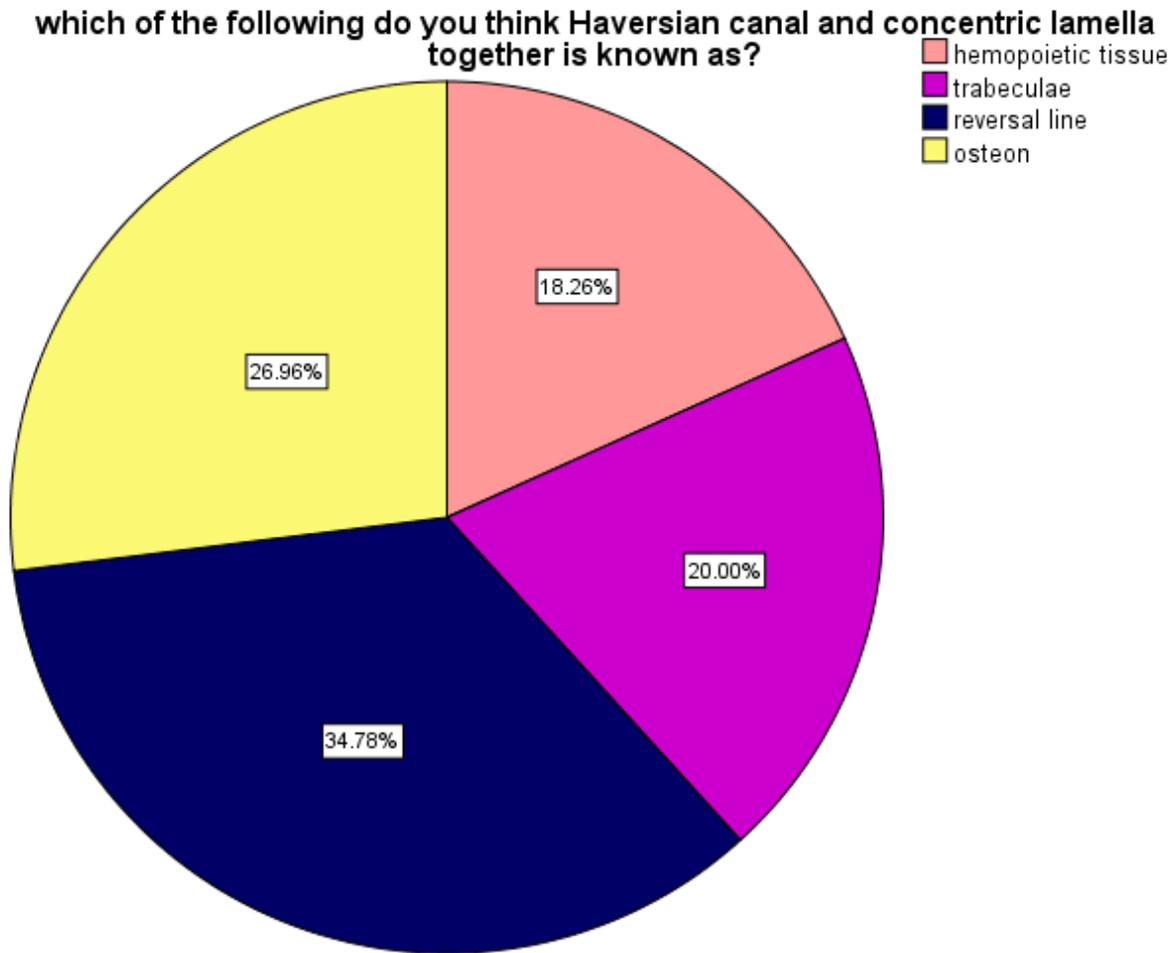
In Fig 3, Pie chart shows the percentage of responses for the labelled parts. Orange colour denotes osteoclast and osteoblast, Grey colour denotes bone and osteoblast, Pink colour denotes osteoclast and bone and dark green colour denotes cement plate and bone. Majority (34.78%) of the population were aware that the labelled parts are osteoclast and osteoblast whereas 22.61% (bone and osteoblast) , 26.96% (osteoclast and bone) and 15.65% (cement plate and bone) were unaware about the labelled parts.

Fig 4



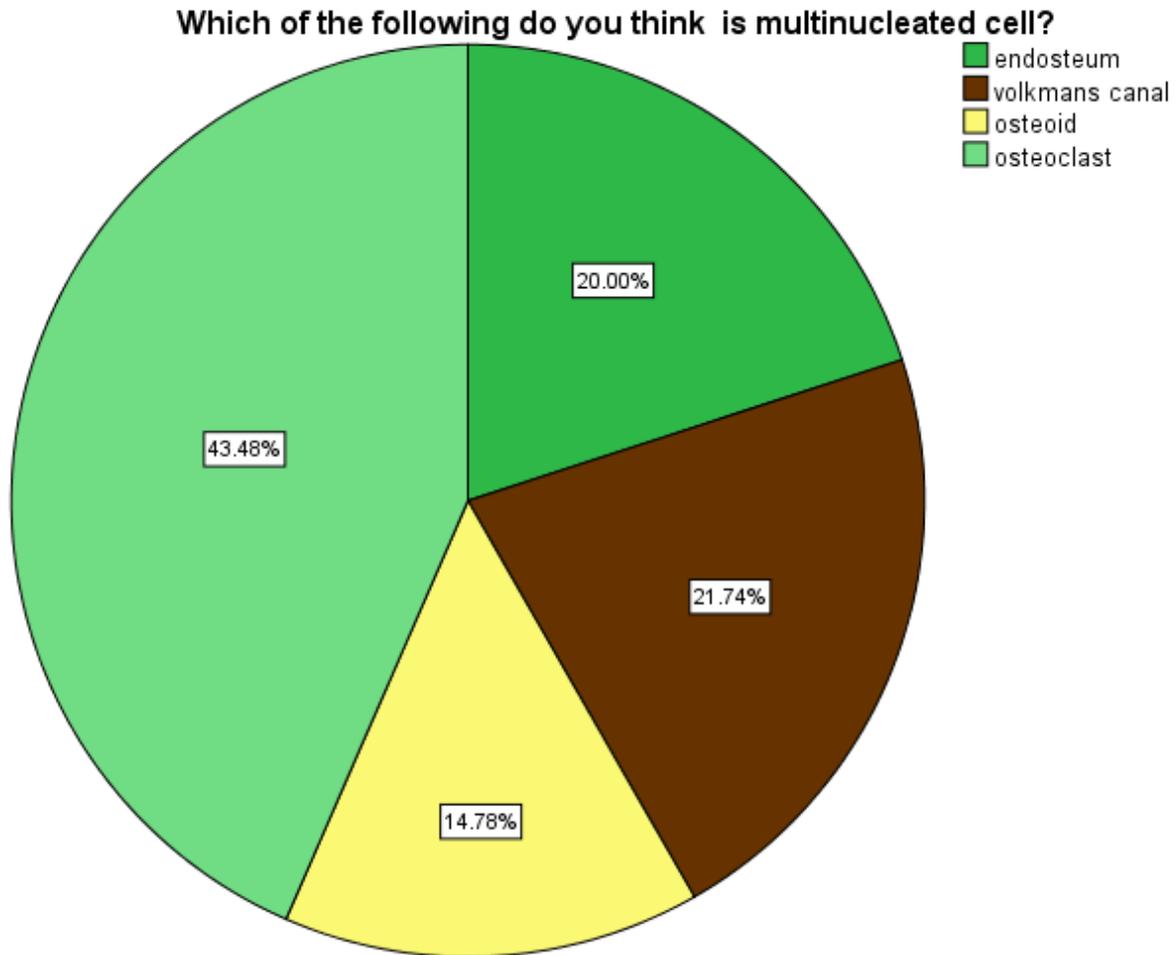
In Fig 4, Pie chart shows the percentage of responses for the reversal line. Dark blue colour denotes reversal line , light blue colour denotes resting line , black colour denotes both and white colour denotes none . Majority (48.70%) of the population were aware that reversal line marks the cessation of resorption whereas 23.48% (resting line), 17.39% (both) and 10.43% (none) were unaware about the reversal line.

Fig 5



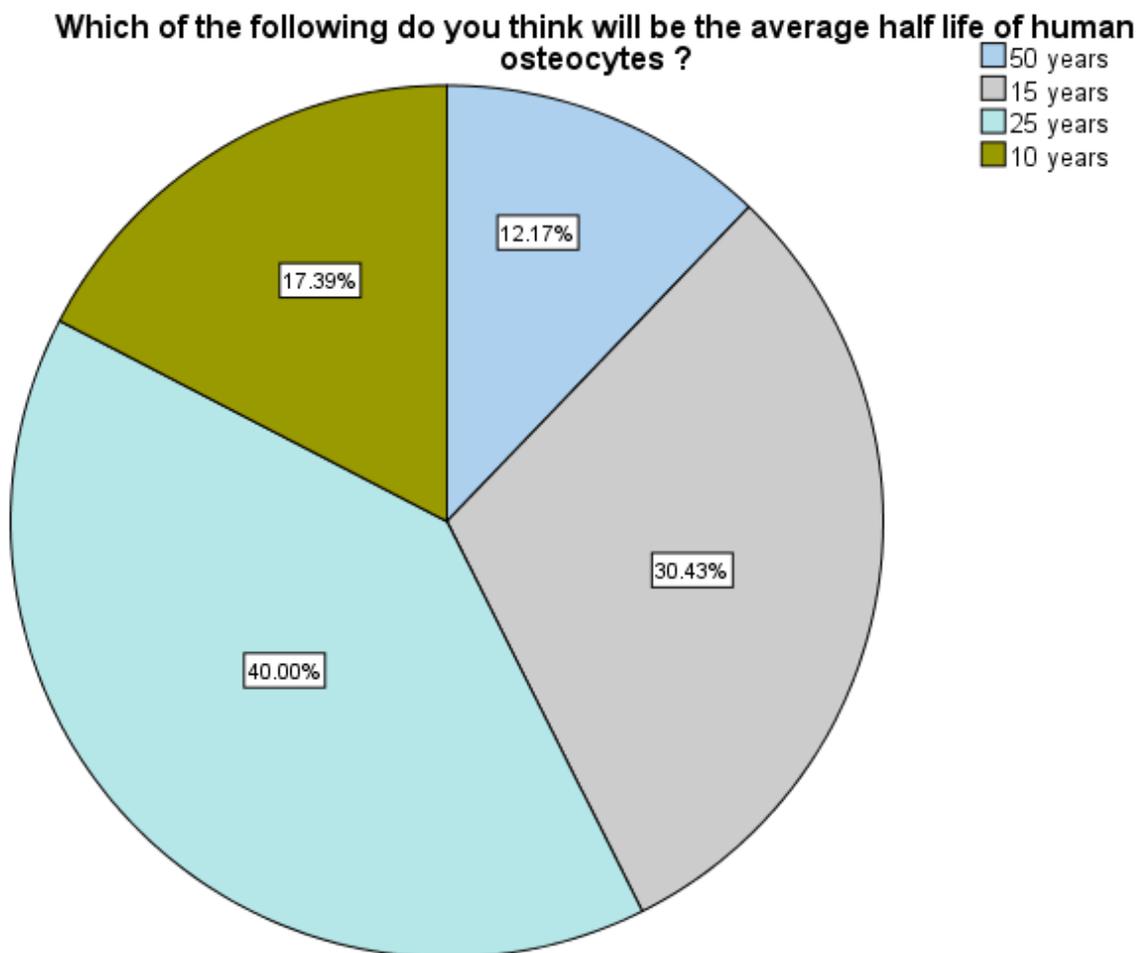
In Fig 5, Pie chart shows the percentage of responses for osteon. Peach colour denotes hemopoietic tissue, light violet colour denotes trabeculae, dark blue colour denotes reversal line and yellow colour denotes osteon. Only 26.96% of the population are aware about the osteon whereas 18.26% (hemopoietic tissue), 20.00% (trabeculae) and 34.78% (reversal line) were unaware about the osteon.

Fig 6



In Fig 6, Pie chart shows the percentage of responses for multinucleated cells. Green colour denotes endosteum, brown colour denotes volkmann's canal, yellow colour denotes osteoid and light green colour denotes osteoclast. Majority (43.48%) of the population were aware that the multinucleated cell is osteoclast whereas 20.00% (endosteum), 21.74% (volkmann's canal) and 14.78% (osteoid) were unaware about the multinucleated cell.

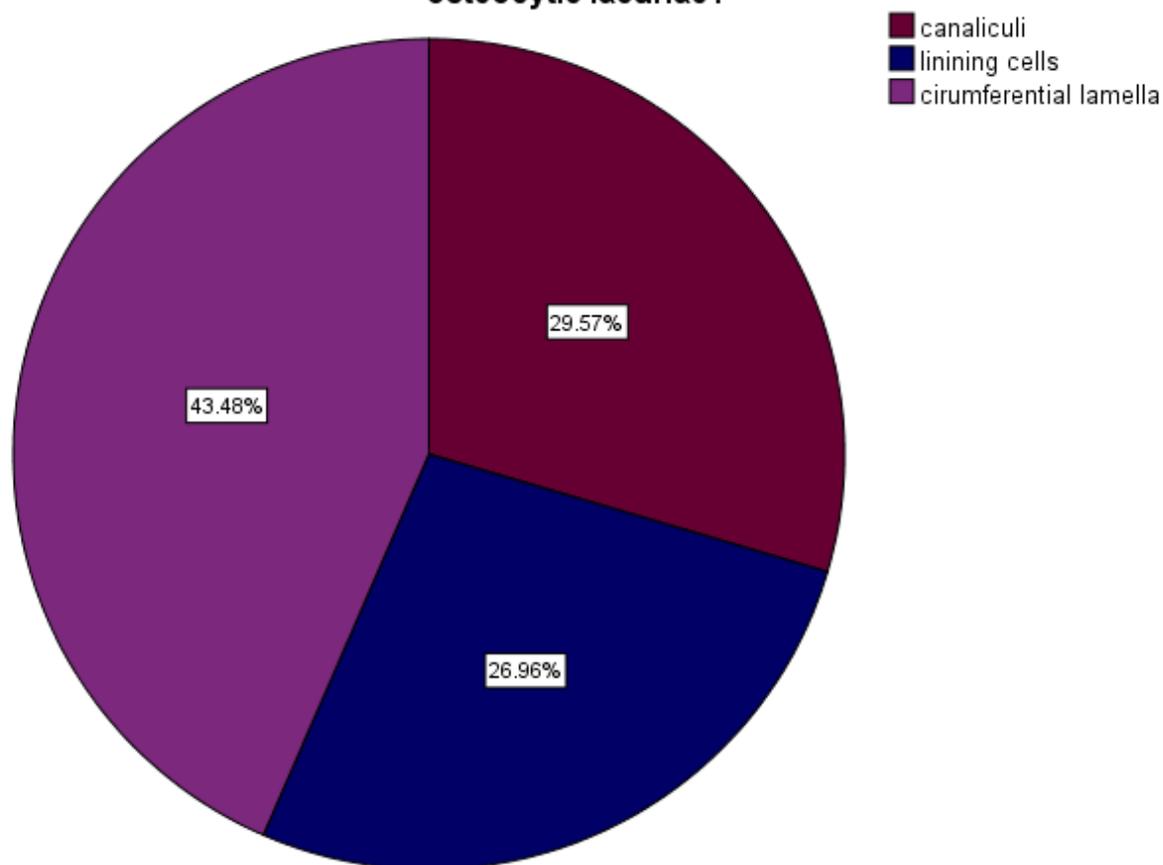
Fig 7



In Fig 7, Pie chart shows the percentage of responses for human osteocytes. Sky blue denotes 50 years, light grey colour denotes 15 years , crimson green colour denotes 25 years and dark yellow denotes 10 years. Majority (40.00%) of the population were aware that 25 years is the average half life of human osteocytes whereas 12.17% (50 years) , 30.43% (15 years) and 17.39% (10 years) were unaware about the average half life of human osteocytes.

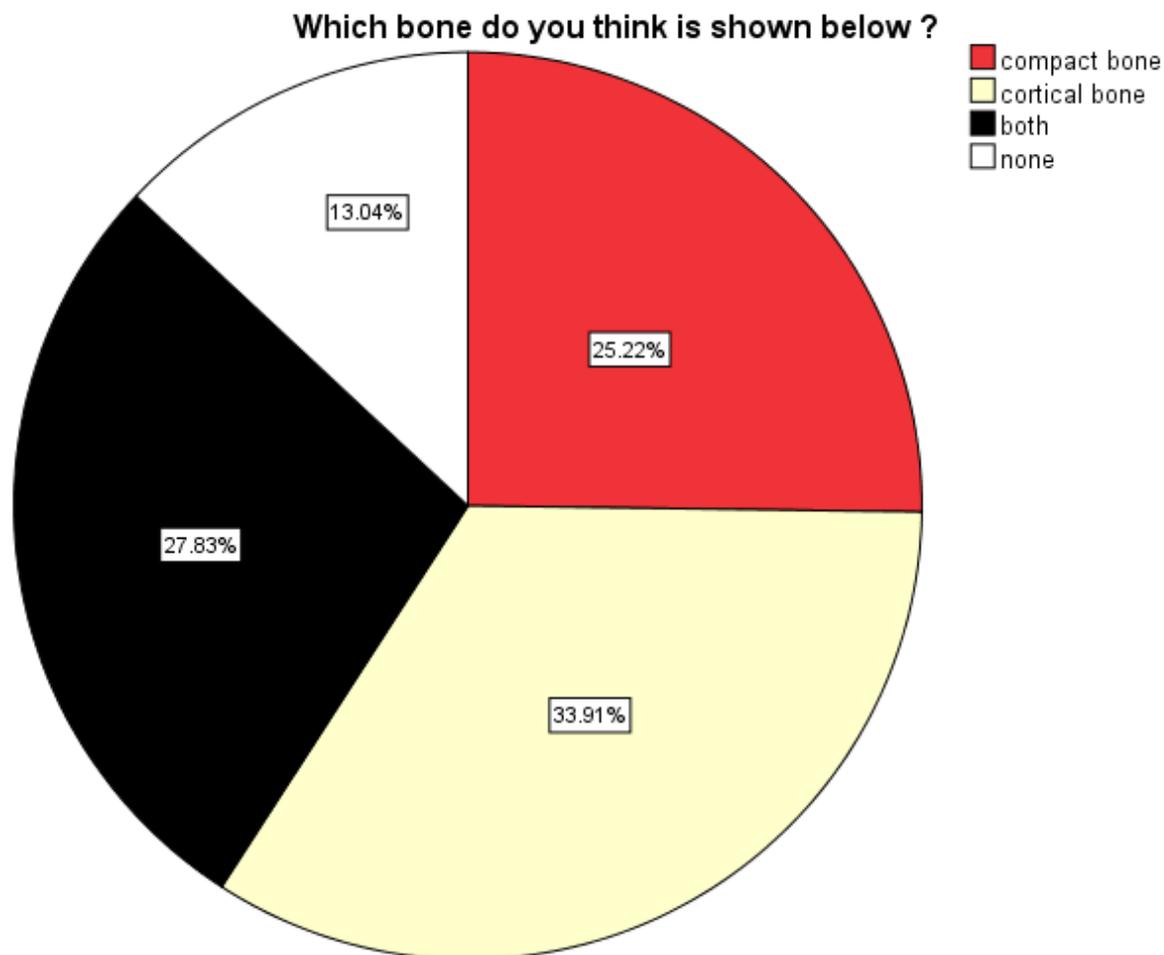
Fig 8

Which of the following do you think form channels of narrow extensions of osteocytic lacunae?



In Fig 8, Pie chart shows the percentage of responses for canaliculi. Maroon colour denotes canaliculi, Indigo colour denotes lining cells and purple colour denotes circumferential lamellae. Only 29.57% of the population were aware that canaliculi form channels of osteocyte lacunae whereas 26.96% (lining cells) and 43.48% (circumferential lamellae) were unaware about canaliculi.

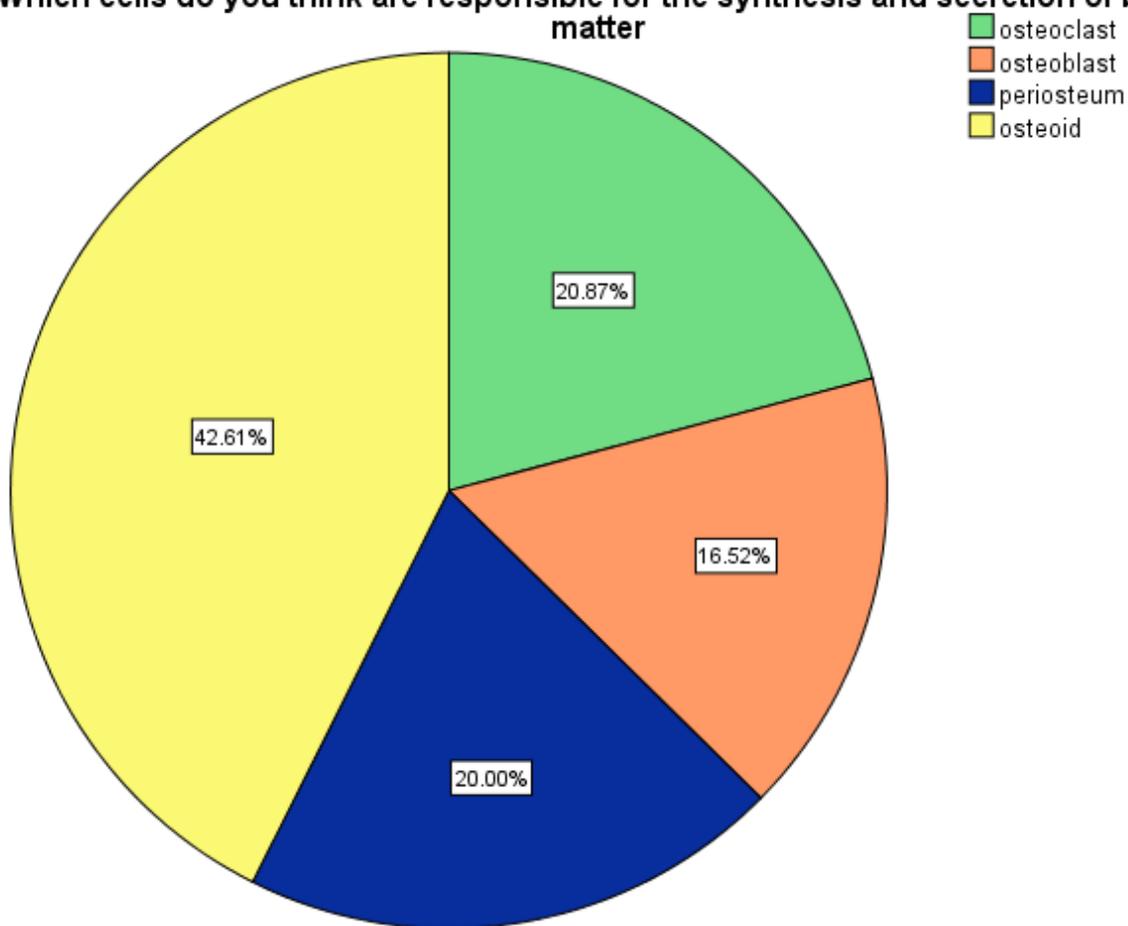
Fig 9



In Fig 9, Pie chart shows the percentage of responses for the bone shown. Crimson red colour denotes compact bone, light yellow colour denotes cortical bone, black colour denotes both and white colour denotes none. Only 27.83% of the population were aware that the bone shown is both compact and cortical bone whereas 25.22% (compact bone), 33.91% (cortical bone) and 13.04% (none) were unaware of the bone shown.

Fig 10

Which cells do you think are responsible for the synthesis and secretion of bone matter



In Fig 10, Pie chart shows the percentage of responses for bone matter. Light green colour denotes osteoclast, coral colour denotes osteoblast, blue colour denotes periosteum and yellow colour denotes osteoid. Only 16.52% of the population were aware that osteoblast are responsible for synthesis and secretion of bone matter whereas 20% (periosteum), 20.87% (osteoclast) and 42.61% (osteoid) were unaware about the synthesis and secretion of bone matrix.

Fig 11

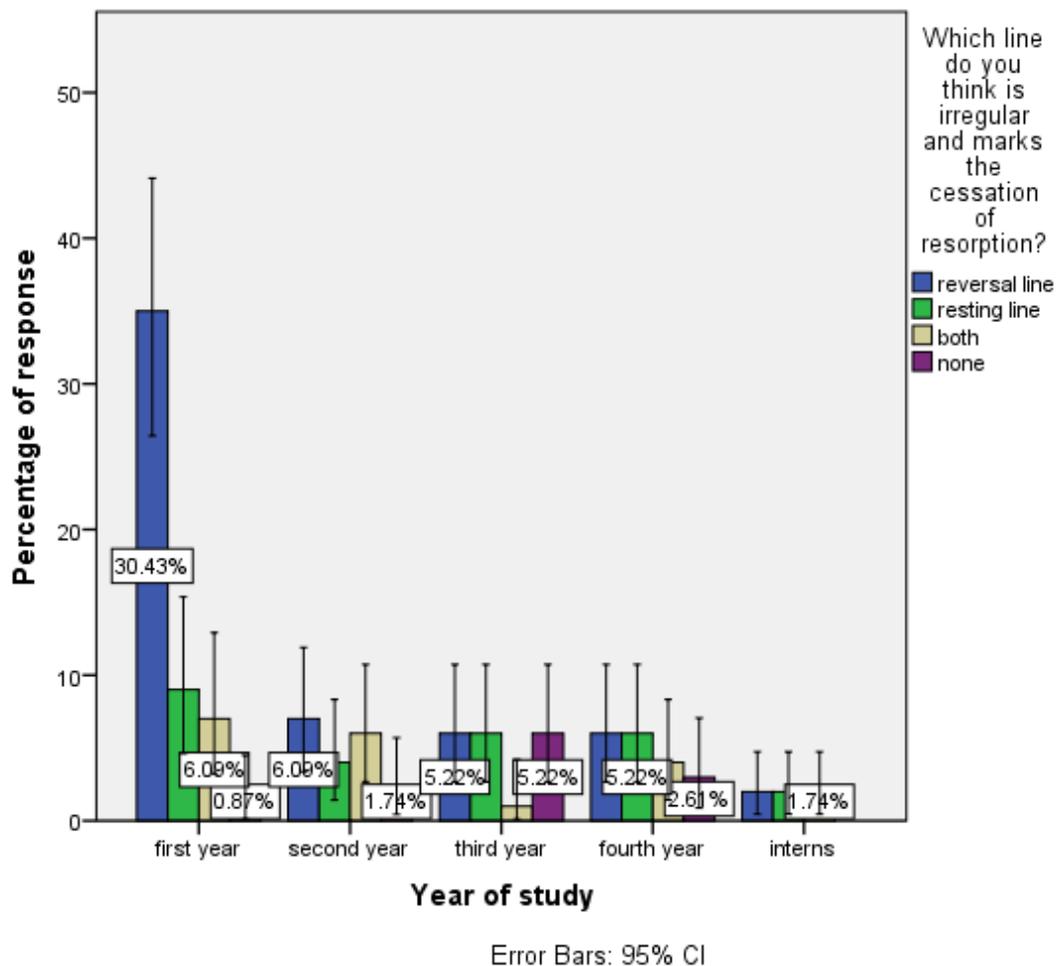


Fig 11 : Bar graph represents the association between year of study and the respondents who were aware about the reversal line. X-axis represents the year of study and y-axis represents the percentage of responses overall. Dark Blue colour depicts reversal line ,light blue colour denotes resting lines, black colour denotes both and white colour denotes none. Reversal line was the most commonly marked the cessation of resorption by all year. Majority (30.43%) were the first years who were aware about the reversal line when compared to second (5.22%), third (5.22%), fourth year (2.61%) and interns (1.74%). Pearson chi square test was done and the p value is 0.015 and hence it is statistically significant.

Fig 12

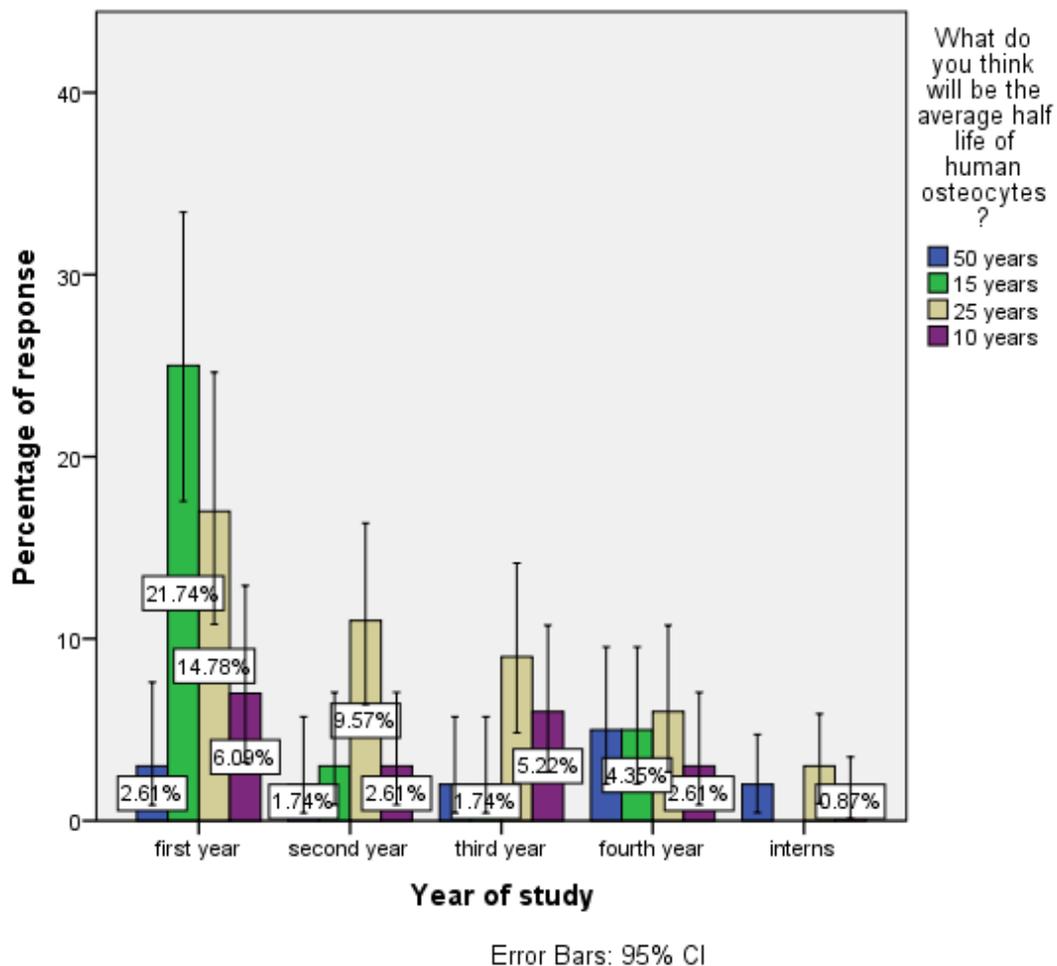


Fig 12: Bar graph represents the association between year of study and the respondents who were aware about the average half life of human osteocytes. X-axis represents the year of study and y-axis represents the percentage of responses overall. Sky Blue colour depicts 50 years , light grey colour denotes 15 years, crimson green colour denotes 25 years and dark yellow colour denotes 10 years. 25 years was the most commonly marked as the average half life of human osteocytes by all year. Majority (14.78%) are the first years who were aware about the average half life of human osteocytes when compared to second (9.57%), third (7.83%), fourth year (5.22%) and interns (0.87%). Pearson chi square test was done and the p value is 0.017 and hence it is statistically significant.

Fig13

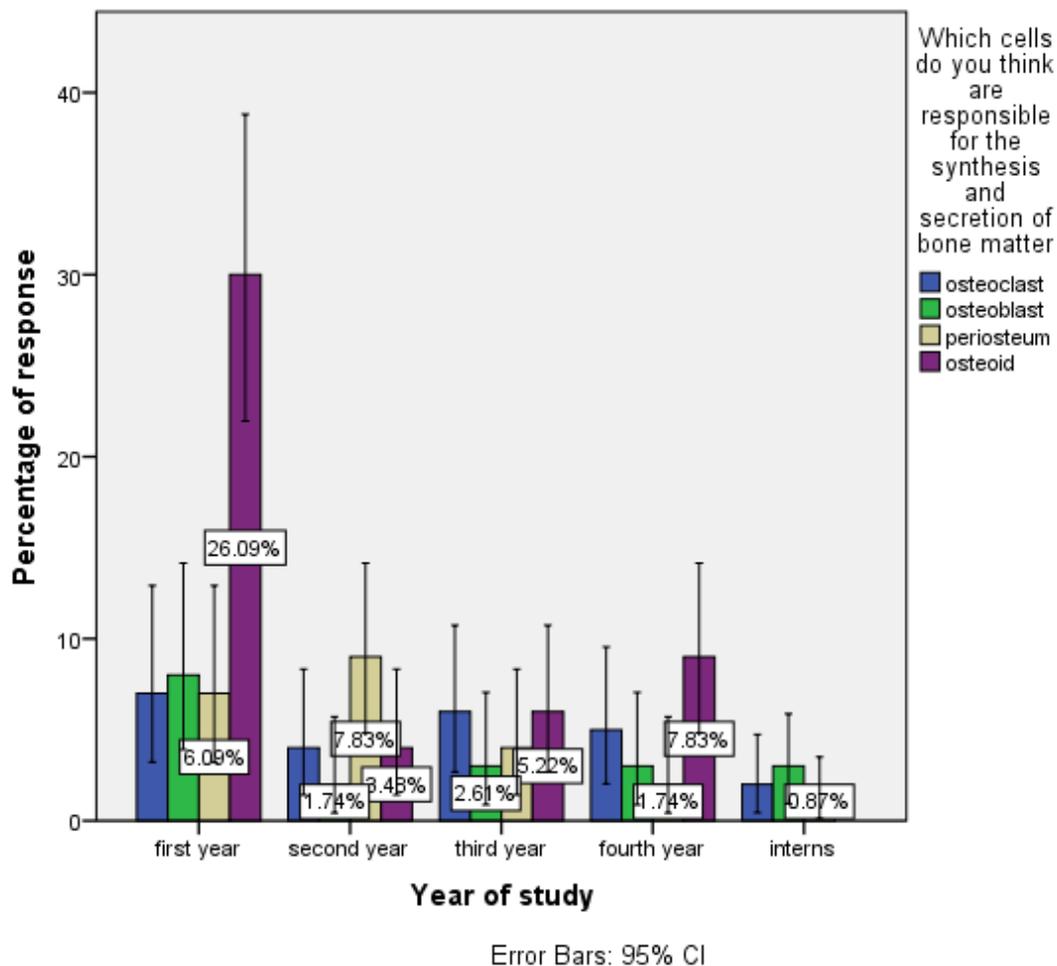


Fig 13: Bar graph represents the association between year of study and the respondents who were aware about osteoblast. X-axis represents the year of study and y-axis represents the percentage of responses overall. Light green colour depicts osteoclast ,coral colour denotes osteoblast, blue colour denotes periosteum and yellow colour denotes osteon. Majority (6.09%) are the first years who were aware about the osteoblast are responsible for synthesis and secretion of bone matter when compared to second (1.74%), third (2.61%), fourth year (1.74%) and interns (0.87%). Pearson chi square test was done and the p value is 0.015 and hence it is statistically significant.

DISCUSSION :

In our present survey, the majority of students' responses were from their first year (14.78%), pursued by their second (9.57 %), third (7.83 %), fourth year (5.22 %) and interns (0.87%). This study found that 6.09% of first-year students were aware of osteoblast, followed by 1.74% of second-year students,

2.61% of third-year students, 1.74% of fourth-year students and 0.87% of interns students. As a result of the current study, it is evident that first-year students are more aware of the histology of bone.

The most prevalent cell type in bones is osteocytes. In adult human bones, there are roughly ten times as many osteocytes as osteoblasts, and the number of osteoclasts is only a fraction of the number of osteoblasts (25,26). Bone turnover, when osteoclasts resorb bone and liberate osteocytes, is likely to have a substantial role in osteocyte lifespan (27). If the bone in which they live has a sluggish turnover rate, osteocytes can have a decade-long half-life (28,29). Osteocytes have an average half life of 25 years. In the present study, 40% of the population have knowledge about the average half life of human osteocytes and 43.48% of the population were aware that the multinucleated cell is osteoclast.

The reversal line separates the onset of osteoblast activity at a remodelling location in bone from the cessation of osteoblast activity (30,31). It's a connection between bone segments that were developed at separate times. The reversal line, we believe, carries regulatory signals that control osteoblast activity in part (32). In the present study, 48.70% of the population have knowledge about the reversal line that marks cessation of resorption.

Compact bone is also known as cortical bone. Compact bone forms the cortex and hard outer hard shell of most of the bones in the body. On the periosteal, endosteal, and haversian canal surfaces, cortical bone is altered. The periosteal surface is in charge of bone width expansion. Periods of bone resorption alternate with phases of bone growth in the endosteum. The overall thickness of the bone cortex is determined by the combined actions of the periosteum and endosteum (33). In the present study, 25.22% of the population have knowledge about compact bone, 33.91% of the population have knowledge about cortical bone and 27.83% of the population have knowledge about both. 25.22% of the population have knowledge that fibro collagen layer surrounds the compact bone.

Osteons (the Haversian system) are cortical bone structural and functional units. The osteon structure, which includes osteocytes, lamellae, the lacuno canalicular network and Haversian canals has been proven to play a significant role in bone mechanics and turnover in recent years. Cortical bone regeneration requires the restoration of the osteon structure (34,35). In the present study, 26.96% of the population have knowledge that haversian canal and concentric lamellae together is known as osteon and on our study 29.57% of the population also have adequate knowledge that osteon is the

unmineralized bone matrix where active bone formation takes place. The sample size was small and more sample size would be beneficial to assess the knowledge about histology of bone more accurately. The survey can be conducted in offline mode rather than online so that the knowledge of the subjects could be analysed accurately. Equal number of male and female samples can be evaluated without difficulty.

CONCLUSION :

It is concluded that first year students when compared to other years were more aware about histology of bone from the study. This knowledge gap can be minimised by conducting more pictorial lectures and giving innovative handworks on histology of bone for the students to make them understand the subject in depth.

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CONFLICTS OF INTEREST:

The authors declare that there are no conflicts of interest in the present study.

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REFERENCES :

1. Rochefort GY, Benhamou C-L. Osteocytes are not only mechanoreceptive cells. Int j numer method biomed eng. 2013 Oct;29(10):1082–8.
2. Nakashima T, Hayash M, Takayanagi H. [Regulation of bone resorption by osteocytes]. Clin Calcium.

2012 May;22(5):685–96.

3. Tang SY, Herber R-P, Ho SP, Alliston T. Matrix metalloproteinase-13 is required for osteocytic perilacunar remodeling and maintains bone fracture resistance. *J Bone Miner Res.* 2012 Sep;27(9):1936–50.
4. Hedgecock NL, Hadi T, Chen AA, Curtiss SB, Bruce Martin R, Hazelwood SJ. Quantitative regional associations between remodeling, modeling, and osteocyte apoptosis and density in rabbit tibial midshafts [Internet]. Vol. 40, *Bone*. 2007. p. 627–37. Available from: <http://dx.doi.org/10.1016/j.bone.2006.10.006>
5. Princeton B, Santhakumar P, Prathap L. Awareness on Preventive Measures taken by Health Care Professionals Attending COVID-19 Patients among Dental Students. *Eur J Dent.* 2020 Dec;14(S 01):S105–9.
6. Mathew MG, Samuel SR, Soni AJ, Roopa KB. Evaluation of adhesion of *Streptococcus mutans*, plaque accumulation on zirconia and stainless steel crowns, and surrounding gingival inflammation in primary molars: randomized controlled trial. *Clin Oral Investig.* 2020 Sep;24(9):3275–80.
7. Sridharan G, Ramani P, Patankar S, Vijayaraghavan R. Evaluation of salivary metabolomics in oral leukoplakia and oral squamous cell carcinoma. *J Oral Pathol Med.* 2019 Apr;48(4):299–306.
8. R H, Hannah R, Ramani P, Ramanathan A, Jancy MR, Gheena S, et al. CYP2 C9 polymorphism among patients with oral squamous cell carcinoma and its role in altering the metabolism of benzo[a]pyrene [Internet]. Vol. 130, *Oral Surgery, Oral Medicine, Oral Pathology and Oral Radiology*. 2020. p. 306–12. Available from: <http://dx.doi.org/10.1016/j.oooo.2020.06.021>
9. Antony JVM, Ramani P, Ramasubramanian A, Sukumaran G. Particle size penetration rate and effects of smoke and smokeless tobacco products - An invitro analysis. *Heliyon.* 2021 Mar 1;7(3):e06455.
10. Sarode SC, Gondivkar S, Sarode GS, Gadbail A, Yuwanati M. Hybrid oral potentially malignant disorder: A neglected fact in oral submucous fibrosis. *Oral Oncol.* 2021 Jun 16;105390.
11. Hannah R, Ramani P, WM Tilakaratne, Sukumaran G, Ramasubramanian A, Krishnan RP. Author response for “Critical appraisal of different triggering pathways for the pathobiology of pemphigus

- vulgaris—A review” [Internet]. Wiley; 2021. Available from: <https://publons.com/publon/47643844>
12. Chandrasekar R, Chandrasekhar S, Sundari KKS, Ravi P. Development and validation of a formula for objective assessment of cervical vertebral bone age. *Prog Orthod*. 2020 Oct 12;21(1):38.
 13. Subramanyam D, Gurunathan D, Gaayathri R, Vishnu Priya V. Comparative evaluation of salivary malondialdehyde levels as a marker of lipid peroxidation in early childhood caries. *Eur J Dent*. 2018 Jan;12(1):67–70.
 14. Jeevanandan G, Thomas E. Volumetric analysis of hand, reciprocating and rotary instrumentation techniques in primary molars using spiral computed tomography: An in vitro comparative study. *Eur J Dent*. 2018 Jan;12(1):21–6.
 15. Ponnulakshmi R, Shyamaladevi B, Vijayalakshmi P, Selvaraj J. In silico and in vivo analysis to identify the antidiabetic activity of beta sitosterol in adipose tissue of high fat diet and sucrose induced type-2 diabetic experimental rats. *Toxicol Mech Methods*. 2019 May;29(4):276–90.
 16. Sundaram R, Nandhakumar E, Haseena Banu H. Hesperidin, a citrus flavonoid ameliorates hyperglycemia by regulating key enzymes of carbohydrate metabolism in streptozotocin-induced diabetic rats. *Toxicol Mech Methods*. 2019 Nov;29(9):644–53.
 17. Alsawalha M, Rao CV, Al-Subaie AM, Haque SKM, Veeraraghavan VP, Surapaneni KM. Novel mathematical modelling of Saudi Arabian natural diatomite clay. *Mater Res Express*. 2019 Sep 4;6(10):105531.
 18. Yu J, Li M, Zhan D, Shi C, Fang L, Ban C, et al. Inhibitory effects of triterpenoid betulin on inflammatory mediators inducible nitric oxide synthase, cyclooxygenase-2, tumor necrosis factor-alpha, interleukin-6, and proliferating cell nuclear antigen in 1, 2-dimethylhydrazine-induced rat colon carcinogenesis. *Pharmacogn Mag*. 2020;16(72):836.
 19. Shree KH, Hema Shree K, Ramani P, Herald Sherlin, Sukumaran G, Jeyaraj G, et al. Saliva as a Diagnostic Tool in Oral Squamous Cell Carcinoma – a Systematic Review with Meta Analysis [Internet]. Vol. 25, *Pathology & Oncology Research*. 2019. p. 447–53. Available from: <http://dx.doi.org/10.1007/s12253-019-00588-2>

20. Zafar A, Sherlin HJ, Jayaraj G, Ramani P, Don KR, Santhanam A. Diagnostic utility of touch imprint cytology for intraoperative assessment of surgical margins and sentinel lymph nodes in oral squamous cell carcinoma patients using four different cytological stains. *Diagn Cytopathol.* 2020 Feb;48(2):101–10.
21. Karunagaran M, Murali P, Palaniappan V, Sivapathasundharam B. Expression and distribution pattern of podoplanin in oral submucous fibrosis with varying degrees of dysplasia – an immunohistochemical study [Internet]. Vol. 42, *Journal of Histotechnology.* 2019. p. 80–6. Available from: <http://dx.doi.org/10.1080/01478885.2019.1594543>
22. Sarode SC, Gondivkar S, Gadbail A, Sarode GS, Yuwanati M. Oral submucous fibrosis and heterogeneity in outcome measures: a critical viewpoint. *Future Oncol.* 2021 Jun;17(17):2123–6.
23. Raj Preeth D, Saravanan S, Shairam M, Selvakumar N, Selestin Raja I, Dhanasekaran A, et al. Bioactive Zinc(II) complex incorporated PCL/gelatin electrospun nanofiber enhanced bone tissue regeneration. *Eur J Pharm Sci.* 2021 May 1;160:105768.
24. Prithiviraj N, Yang GE, Thangavelu L, Yan J. Anticancer Compounds From Starfish Regenerating Tissues and Their Antioxidant Properties on Human Oral Epidermoid Carcinoma KB Cells. In: PANCREAS. LIPPINCOTT WILLIAMS & WILKINS TWO COMMERCE SQ, 2001 MARKET ST, PHILADELPHIA ...; 2020. p. 155–6.
25. Bonewald LF. The Role of the Osteocyte in Bone and Nonbone Disease [Internet]. Vol. 46, *Endocrinology and Metabolism Clinics of North America.* 2017. p. 1–18. Available from: <http://dx.doi.org/10.1016/j.ecl.2016.09.003>
26. Nijweide PJ, Burger EH, Klein-Nulend J. The Osteocyte [Internet]. *Principles of Bone Biology.* 2002. p. 93–107. Available from: <http://dx.doi.org/10.1016/b978-012098652-1.50108-6>
27. Hall BK. Osteoblast and Osteocyte Diversity and Osteogenesis In Vitro [Internet]. *Bones and Cartilage.* 2015. p. 401–13. Available from: <http://dx.doi.org/10.1016/b978-0-12-416678-3.00024-0>
28. Rochefort GY, Pallu S, Benhamou CL. Osteocyte: the unrecognized side of bone tissue. *Osteoporos Int.* 2010 Sep;21(9):1457–69.
29. Vatsa A, Smit TH, Klein-Nulend J. Extracellular NO signalling from a mechanically stimulated

osteocyte. *J Biomech.* 2007 May 18;40 Suppl 1:S89–95.

30. Romano PR, Caton JG, Puzas JE. The reversal line may be a key modulator of osteoblast function: observations from an alveolar bone wound-healing model [Internet]. Vol. 32, *Journal of Periodontal Research.* 1997. p. 143–7. Available from: <http://dx.doi.org/10.1111/j.1600-0765.1997.tb01396.x>
31. Everts V, Delaissé JM, Korper W, Jansen DC, Tigchelaar-Gutter W, Saftig P, et al. The Bone Lining Cell: Its Role in Cleaning Howship's Lacunae and Initiating Bone Formation [Internet]. Vol. 17, *Journal of Bone and Mineral Research.* 2002. p. 77–90. Available from: <http://dx.doi.org/10.1359/jbmr.2002.17.1.77>
32. Wittig NK, Laugesen M, Birkbak ME, Bach-Gansmo FL, Pacureanu A, Bruns S, et al. Canalicular Junctions in the Osteocyte Lacuno-Canalicular Network of Cortical Bone. *ACS Nano.* 2019 Jun 25;13(6):6421–30.
33. Qiu S, Sudhaker Rao D, Palnitkar S, Michael Parfitt A. Differences in osteocyte and lacunar density between Black and White American women [Internet]. Vol. 38, *Bone.* 2006. p. 130–5. Available from: <http://dx.doi.org/10.1016/j.bone.2005.07.004>
34. Chang B, Liu X. Osteon: Structure, Turnover, and Regeneration. *Tissue Eng Part B Rev* [Internet]. 2021 Mar 8; Available from: <http://dx.doi.org/10.1089/ten.TEB.2020.0322>
35. Martiniaková M, Grosskopf B, Omelka R, Dammers K, Vondráková M, Bauerová M. Histological study of compact bone tissue in some mammals: a method for species determination [Internet]. Vol. 17, *International Journal of Osteoarchaeology.* 2007. p. 82–90. Available from: <http://dx.doi.org/10.1002/oa.856>