Determination of Hardness of Bones and Importance of Calcium Phosphate in Bone Density.

Dr. Tasneem Jahan[1], Salma Yousuf Khan[2], R. Sahithi Rathod[3]

1.Assistant Professor,Department of Zoology, St. Ann's College for Women (Autonomous) Mehdipatnam, Hyderabad, India.

2-3. Students, St. Ann's College for Women (Autonomous) Mehdipatnam, Hyderabad, India.

ABSTRACT-

The framework of the body of an organism is constituted by the skeletal system. The key components of the skeletal system are bones. The human/mammalian bones are composed of *calcium phosphate crystals* in a protein matrix. Bones are nano composite materials where the hard inorganic component is known as *hydroxyapatite crystallites* and the organic component being known as *collagen fibril*. The quality of bone, is dependent on spatial distributions in the shape, size and composition of bone constituents i.e. *mineral, collagen* and *water*. The hardness of the bone is an important property which includes both elastic and plastic deformations. In this study, test for *the determination of hardness of bone* is performed on avian and mammalian bone samples (for a comparative study of hardness). The samples were prepared and treated with vinegar i.e. *acetic acid* (mild acid) and bleach i.e. *sodium hypochlorite* (mild base). The test was performed by soaking the samples in the different reagents (in the dark) for a certain period of time till structural changes were visually observed. The results indicated that the samples soaked in mild acid turned *elastic/flexible*. The samples soaked in mild base lost their composure resulting in loss in *tensile strength* which made them easily breakable. This is due to the different actions of acid and base on the calcium phosphate present in the bone respectively. The untreated bone was significantly harder with higher *resistance* showcasing more tensile strength when compared to the bones subjected to acid and alkali treatment. The effect of *acidosis* and *alkalosis* on bones was understood and a correlation between the *degenerative diseases* caused by acidosis and alkalosis on bones was made.

KEYWORDS-

Bone, skeleton, hardness of bone, calcium phosphate, collagen, collagen fibrils, hydroxyapatite, sodium hypochlorite, acetic acid, bone density, tensile strength, elasticity, resistance.

INTRODUCTION-

- BIOLOGY OF A BONE-

Bones are the living tissues of an organism which have their own blood vessels. They are made up of various cells, proteins, minerals and vitamins. This specific structure enables them to grow, transform and repair themselves throughout the life of an individual forming a skeleton for the body to stand erect. Bones provide structural support for the body and protect vital organs. They also provide an environment suitable for the niche enriching the production of RBC's and WBC's in the marrow.

- CHEMICAL COMPOSITION OF BONES

The nonliving intercellular material of bone consists of an organic component called *collagen*. It is a fibrous protein which is arranged in long strands or bundles similar in structure and organisation to the collagen of ligaments, tendons, and skin.*[Ref:34}* Along with collagen, small amounts of *protein polysaccharides*, *glycosaminoglycans* which are formerly also known as *mucopolysaccharides* are present which are chemically bound to proteins and dispersed within and around the collagen fibre bundles, and an inorganic mineral component present in the form of rod-shaped crystals. These crystals are arranged parallel to the long axes of collagen bundles with many lying in voids within the bundles themselves*{Ref:14}*

Organic material constitutes and gives 50% of the volume and 30% of the dry weight of the intercellular composite. The remainder is constituted by minerals. The major minerals of the intercellular composite are *calcium* and *phosphate*. At initial deposition, the mineral is amorphous crystallographically, after maturation it becomes typical of the apatite minerals with the major component being *hydroxyapatite*. Apart from this, carbonate is also present in a quantity that varies from 4% of bone ash in fish and 8% in most mammals. It occurs in two distinct

phases namely calcium *carbonate* and *carbonate apatite*. There is very little free water in adult mammalian bones except for that associated with its cellular elements (approximately 8 percent of total volume). This results in diffusion from surfaces into the interiors of the intercellular substances occurring at slow rates.

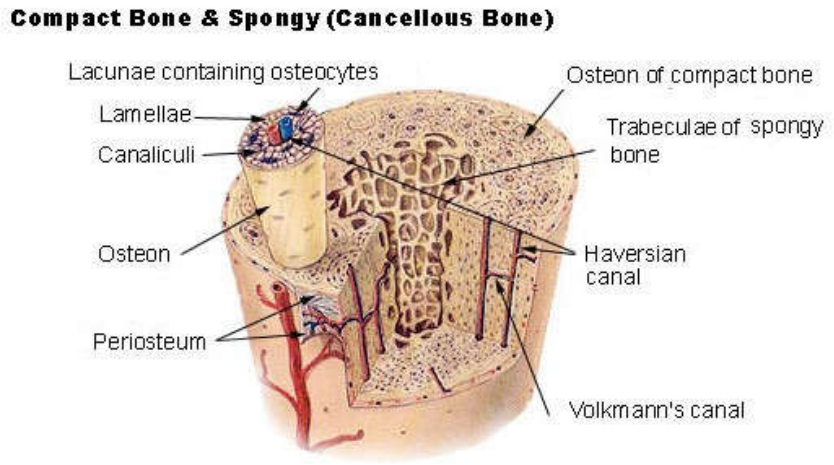
The hardness, rigidity, and compressive strength of bone is due to the presence of mineral crystals.*{Ref:12}* On the downside, these share a great weakness in tension, arising from the tendency for stress to concentrate and bring about defects including propagation of these defects with the presence of other crystalline substances. On the other end of the spectrum, the collagen fibrils present in the bone possess high elasticity, low compressive strength, and considerable tensile strength intrinsically. However the tensile strength of bone depends not on collagen alone but also on the intimate association of minerals with collagen, conferring many of the general properties exhibited by two-phase materials such as fibreglass and bamboo on the bone. The dispersion of a rigid but brittle material in a matrix of quite different elasticity prevents the propagation of stress failure through the brittle material and therefore allowing a closer approach to the theoretical limiting strength of single crystals.*[Ref:11}*

- MORPHOLOGY OF A BONE-

Tissue of the bone is organised into a variety of shapes and configurations which are adapted to the functioning of each bone i.e broad and flat plates such as the scapula, serve as anchors for large muscle masses, while the hollow thick-walled tubes such as the femur, radius, and ulna support weight by serving as a leverage.

Based on the tissue type, bones are classified into two types:

- Compact bone: It is also known as the cortical bone. It has a hard-outer layer which is strong and dense
- Cancellous bone: It is also known as the trabecular bone. It has a spongy inner layer consisting of a network of trabeculae. It is lighter and less dense than cortical bone.



[Source: ResearchGate.com}

The human/mammalian bone is composed of the following:

- Osteoblasts and Osteocytes: these are bone forming cells
- Osteoclasts: these are bone resorbing cells
- Osteoid: this is the non-mineral, organic part of the bone matrix made of collagen and non-collagenous proteins
- Inorganic mineral salts deposited within the matrix.

Specific cells in our bones are responsible for the formation of bone, resorption, maintenance and re-modelling:

- *Osteoblasts*: These cells are derived from mesenchymal stem cells. They are responsible for bone matrix synthesis and its subsequent mineralization. In the adult skeleton, the majority of bone surfaces that are not undergoing formation or resorption are lined by bone lining cells.

- *Osteocytes*: These cells are osteoblasts that get incorporated within the newly formed osteoid which eventually becomes a calcified bone. Osteocytes are situated deep in the bone matrix and maintain contact with newly incorporated osteocytes in osteoid, along with osteoblasts and bone lining cells on the bone surfaces. This is through an extensive network of cell processes called canaliculi. They are thought to be ideally situated to respond to changes in physical forces upon bone and to transduce messages to cells on the bone surface. This directs them to initiate formation or resorption responses.

- *Osteoclasts*: These are large multinucleated cells, like macrophages. They are derived from the hematopoietic lineage. Osteoclasts function in the resorption of mineralized tissue and are found attached to the surface of bone at sites of active bone resorption. Their characteristic feature is a ruffled edge where active resorption takes place with the secretion of acid and bone-resorbing enzymes, which digest bone mineral and bone matrix.

- BONE MODELLING AND REMODELLING-

Modelling is done when bone resorption and bone formation occur on separate surfaces meaning formation and resorption are not coupled. An example of this process of modelling is the long bone increases in length and diameter. Bone re-modelling occurs during birth to adulthood and is responsible for gain in skeletal mass and changes in skeletal form.
With the size of our skeleton and the amount of bone contained in it changing significantly throughout life, overarching objectives to maintain good bone health at various stages of life are described as prevention throughout life.
Once peak bone mass has been achieved in adulthood, the bone mass and structural integrity is maintained by a process called remodelling.

According to the pattern of collagen forming the osteoid, we can identify bones as the following:

- Woven bone: It is characterised by a haphazard disorganised arrangement of the collagen fibres. It is mechanically weak.
- Lamellar bone: It is characterised by a regular parallel alignment of collagen into sheets called lamellae. It is mechanically strong.*[Ref:27}*

The woven type of bone is produced when osteoblasts produce osteoid rapidly. This initially occurs in all foetal bones, but the resulting woven bone is replaced by re-modelling and the deposition of more stronger and resilient lamellar bone. In adults, woven bone is formed when there is very rapid new bone formation, as it occurs in the repair of a fracture. Following a fracture, woven bone is re-modelled, and lamellar bone is deposited.
Bones such as vertebrae, subject to primarily compressive or tensile forces, are provided with structural rigidity contributed by trabeculae as they have thin cortices. In case of bones such as the femur, it is subject to prominent bending, shear, or torsional forces, having usually thick cortices and a tubular configuration, with a continuous cavity running through their centres (medullary cavity).

Gross structural features are commonly exhibited in the long bones which are distinctive of the body's extremities. The central region of the bone known as the *diaphysis* is most clearly tubular. At both ends most commonly and sometimes at one end, the *diaphysis* flares outward and assumes a cancellous internal structure predominantly. This region (known as the *metaphysis* functions to transfer loads from weight-bearing joints surfaces to the *diaphysis*.*[Ref:36}* At the end of a long bone is a region known as an *epiphysis*, which exhibits a cancellous internal structure and constitutes the bony substructure of the joint surface. *Epiphysis* is separated from the *metaphysis* by a cartilaginous plate called the *growth plate* or *physis* prior to structural and skeletal maturity; In bones with complex articulations such as the humerus at its lower end or bones with multiple protuberances such as the femur at its upper end there may be several separate epiphyses, each with its growth plate.

MATERIALS AND METHODOLOGY-

- Sample preparation-

Four bone samples, two each of avian bones [chicken bones] and mammalian bones [goat bones], 4% - 18% acetic acid [vinegar], 5%-6% sodium hypochlorite [household bleach], forceps, hammer, glass or non-degradable plastic containers, lids to cover the containers.

Initially, the bones obtained were boiled to remove any excess meat remaining in and around the surface. The bones were then cooled and washed off to remove any left out meat on the bone. Four clean and dry containers were taken. Acetic acid was poured in two of the containers and sodium hypochlorite was poured in the rest of the two containers. The cleaned and cooled samples of chicken bones and goat bones were placed one each in vinegar and bleach. *[Ref:22}*

Figure 1a

Figure 1b

[figure 1a depicts avian bones soaked in the reagents and figure 1b depicts mammalian bones soaked in the reagents.]

This was covered by a lid and kept in the dark till change in hardness was observed. The chicken bones were kept submerged in the different reagents for a week and the goat bones were kept submerged in the reagents for 13 days. This was kept till there was some change in texture and composition of the bones. There were periodic checks on the submerged bones for signs of degeneration and precipitated degenerated parts of the bones in the containers containing the bones.

Figure 2a

Figure 2b

[figure 2a shows result of chicken bone soaked in vinegar and figure 2b shows the result of bone soaked in bleach]

Figure 3a *Figure 3b*

[figure 3a shows result of goat bone soaked in bleach and figure 3b shows result of goat bone soaked in vinegar.]

RESULTS AND DISCUSSION-

In the experiment conducted, the bones soaked in acetic acid (vinegar) were observed to become flexible. Vinegar is a mild acid which is weakly ionised hence slowly reacting with the calcium phosphate present in the bone, the vinegar eliminates calcium, making the bone delicate and bendable. The bones soaked in sodium hypochlorite (bleach) lose their strength and become easily breakable. This phenomenon of action of acid and base on the bone making the bones flexible and breakable when soaked in acid and base is called *Acidosis* and *Alkalosis* respectively.*[Ref:06,10,15}*

ACIDOSIS:

As acid is induced into the bone, it prompts changes in bone mineral content which is predictable for proton support in the bone. In light of metabolic acidosis in an in-vitro bone organ culture framework, we noticed a fall in mineral sodium, potassium, carbonate and phosphate, which each proton acting as buffer in-vivo leading to increment of pH towards the physiologic normal. At first, metabolic acidosis animates physicochemical mineral disintegration and hence cell-intercepted bone resorption. Acidosis covers the development of bone-resorbing cells, osteoclasts. Metabolic acidosis actuates calcium efflux from bone and in the process cushions the extra hydrogen particles. At first metabolic acidosis animates

physicochemical mineral disintegration and afterward cell-interceded bone resorption. Acidosis assembles development of the bone resorbing cells, the osteoclasts, and reduces activity of the bone shaping cells, the osteoblasts. Osteoblastic quick reaction qualities are hindered as are qualities controlling lattice arrangement.

CAUSES:

Human bones depend on specific minerals to develop further and remain strong. In the event that the human body isn't getting enough of them,resulting in chances of a person getting *osteomalacia*. There are various reasons it can work out. The fundamental ones are:

If a person is not taking sufficient vitamin D. Nonattendance of vitamin D can adversely influence bone prosperity.

A person's body struggles with engrossing vitamin D. Gastric detour or different medical procedures that eliminate part of stomach or digestive tracts, celiac illness, and certain liver or kidney issues can all influence one's body's capacity to take in vitamin D or convert it to its dynamic structure.

Over the long haul, additional acid in the body liquids can gradually break up bone. However, certain individuals have a hereditary condition that causes *osteomalacia*.

Diagnosis-

It is done by blood tests to measure the degree of vitamin D in the body,X-Ray to check the bone structure,bone mineral density scan to examine how much calcium and phosphate in bones.

TREATMENT:

One of the principle components of osteomalacia treatment is to ensure that an individual gets the degrees of supplements they need to help the bone mineralization process.

Taking remedial measurements of enhancements, like vitamin D or phosphate, is a typical treatment system.

To forestall rickets or osteomalacia in youngsters, specialists suggest everyday portions of 600 IU vitamin D and dietary calcium in pregnant individuals and 400 IU day to day in newborn children from birth

ALKALOSIS -

Metabolic alkalosis is usually treated by replacing water and electrolytes (sodium and potassium) while treating the cause. Once in a while, when metabolic alkalosis is exceptionally serious, weakened corrosive such as a dilute acid is given intravenously. Human blood is composed of acids and bases.Keeping the right balance among acids and bases is significant. Even a slight change can cause

medical issues. Typically, human blood ought to have a somewhat higher measure of bases than acids.
Alkalosis happens when a person's body has such a large number of bases. It can happen because of diminished blood levels of carbon dioxide, which is an acid. It can also happen because of expanded blood levels of bicarbonate, which is a base. This condition might as well be connected with other fundamental medical problems like low potassium, or hypokalemia.

CAUSES:
Bone alkaline phosphatase (BAP) is the bone-specific isoform of alkaline phosphatase. A glycoprotein that is found on the surface of osteoblasts, BAP reflects the biosynthetic activity of these bone-forming cells. BAP has been shown to be a sensitive and reliable indicator of bone metabolism.
Typical bone is continually going through redesigning in which bone deterioration or resorption is adjusted by bone arrangement. This interaction is important for keeping up with bone wellbeing. In the event that the cycle becomes uncoupled and the pace of resorption surpasses the pace of development, the subsequent bone misfortune can prompt osteoporosis resulting in greater weakness and affinity to breakage. Osteoporosis is a metabolic bone sickness described by low bone mass and unusual bone microarchitecture. It can result from various clinical circumstances including conditions of high bone turnover, endocrine issues (essential and auxiliary hyperparathyroidism and thyrotoxicosis), osteomalacia, renal disappointment, gastrointestinal infections, long haul corticosteroid treatment, different myeloma, and disease metastatic to the bones.

Diagnosis-
Alkaline phosphatase(ALP) is a protein that is found all throughout the body. A compound is a kind of protein in a cell that goes probably as an impulse and grants explicit genuine cycles to happen.ALP blood tests measure the level of ALP in blood that comes from liver and bones, and it's one of the tests associated with a total metabolic board. Raised levels of ALP in blood could show liver ailment or certain bone issues.
Treatment:
Alendronic acid is a sort of medication called a bisphosphonate. Bisphosphonates are recommended to assist a person's bones with remaining areas of strength for as long as conceivable. Taking alendronic acid can help assuming that a person has, or is in danger of creating, osteoporosis. This is a condition that makes one's bones get more fragile and be bound to break

CONCLUSION:

The skeletons of land vertebrates contain a gigantic hold of soluble mineral (hydroxyapatite), which is at last accessible to cradle metabolic H+ in the event that acid base equilibrium isn't kept up with inside tight cutoff points. The adverse consequence of acidosis on the skeleton has for quite some time been known however was remembered to result from latent, physicochemical disintegration of bone mineral. This concise, specific study sums up what is presently known about the direct utilitarian reactions of bone cells to extracellular pH. We found that bone resorption by refined osteoclasts is under direct stimulation of acid. The stimulatory impact is close maximal at pH 7.0, while above pH 7.4, resorption is turned off. In bone organ cultures, H+ stimulated bone mineral delivery is essentially osteoclast intervened, with an irrelevant physicochemical part. Acidification is the critical necessity for osteoclasts to unearth resorption pits in all species concentrated to date, and extracellular H+ may accordingly be viewed as the long-looked for osteoclast activation factor. Diets or medications that shift acid base equilibrium in the antacid bearing might give valuable medicines to bone misfortune issues.

REFERENCES-

1. Albert C, Jameson J, Toth JM, et al. Bone properties by nanoindentation in mild and severe osteogenesis imperfecta. Clin Biomech (Bristol, Avon). 2013;28(1):110–6.

2. Ator GA, Andrews JC, Maxwell DS. Preparation of the human skull for skull base anatomic study. Skull base surgery. 1993; 3: 1- 6.

3. Boivin, GY., Doublier, A., Farlay, D. et al. The role of mineralization and organic matrix in the microhardness of bone tissue from controls and osteoporotic patients. Bone 2008, vol. 43, no. 3, p. 532-538

4. Carvahlo, AAF., Louzada, MJQ. and Riso, NDM. Hindlimb unloading producing effects on bone biomechanical properties in mature male rats. Brazilian Journal of Morphological Sciences 2007, vol. 24, no. 3, p. 175-179.

5. Chen W, Lv H, Liu S, *et al.* National incidence of traumatic fractures in China: a retrospective survey of 512 187 individuals. *Lancet Glob Health*, 2017, 5: e807–e817.

6. Christensen, C.E.; McNeal, S.F.; Eleazer, P. Effect of lowering the pH of sodium hypochlorite on dissolving tissue in vitro. J. Endod. 2008, 34, 449–452.

7. Chung KC, Spilson SV. The frequency and epidemiology of hand and forearm fractures in the United States. *J Hand Surg Am*, 2001, 26: 908–915.

8. compared with NHANES III. J Bone Miner Res. 2010;25(1):64–71.

9. Dall'Ara E, Schmidt R, Zysset P. Microindentation can discriminate between damaged and intact human bone tissue. Bone. 2012;50(4):925–9

10. Dumitriu, D.; Dobre, T. Effects of temperature and hypochlorite concentration on the rate of collagen dissolution. J. Endod. 2015, 41, 903–906.

11. Fantner, G.E.; Birkedal, H.; Kindt, J.H.; Hassenkam, T.; Weaver, J.C.; Cutroni, J.A.; Bosma, B.L.; Bawazer, L.; Finch, M.M.; Cidade, G.A.; et al. Influence of the degradation of the organic matrix on the microscopic fracture behaviour of trabecular bone. Bone 2004, 35, 1013–1022.

12. Fonseca H, Moreira-Goncalves D, Coriolano HJ, et al. Bone quality: the determinants of bone strength and fragility. Sports Med. 2014;44(1):37–53.

13. Franzoso, G.; Zysset, P.K. Elastic anisotropy of human cortical bone secondary osteons measured by nanoindentation. J. Biomech. Eng. 2009, 131, 021001.

14. Gautieri, A.; Vesentini, S.; Redaelli, A.; Buehler, M.J. Hierarchical structure and nanomechanics of collagen microfibrils from the atomistic scale up. Nano Lett. 2011, 11, 757–766.

15. Kerbl, F.M.; DeVilliers, P.; Litaker, M.; Eleazer, P.D. Physical effects of sodium hypochlorite on bone: An ex vivo study. J. Endod. 2012, 38, 357–359

16. Labonte, D.; Lenz, A.K.; Oyen, M.L. On the relationship between indentation hardness and modulus, and the damage resistance of biological materials. Acta Biomater. 2017, 57, 373–383.

17. LA Moher D, Tetzlaff J, et al. Preferred reporting items for systematic reviews and meta-analyses—the PRISMA statement. Int J Surg. 2010;8(5):336–41.

18. Lees S. A model for bone hardness. *J Biomech*, 1981, 14: 561–567

19. Looker AC, Melton LJ 3rd, Harris TB, et al. Prevalence and trends in low femur bone density among older US adults: NHANES 2005–2006

20. Martin, R.B.; Boardman, D.L. The effects of collagen fibre orientation, porosity, density, and mineralization on bovine cortical bone bending properties. J. Biomech. 1993, 26, 1047–1054.

21. McGlamry MC, Robitaille MF. Analysis of screw pullout strength: a function of screw orientation in subtalar joint arthrodesis. *J Foot Ankle Surg*, 2004, 43: 277–284.

22. Modi BS, Puri N, Patnaik VVG. Evaluation of techniques for cleaning embalmed cadavers's bones. International Journal of Anatomy and Research. 2014; 2: 810-813.

23. Ohman C, Zwierzak I, Baleani M, Viceconti M. Human bone hardness seems to depend on tissue type but not on anatomical site in the long bones of an old subject. *Proc Inst Mech Eng H*, 2013, 227: 200–206.

24. Osterhoff G, Morgan EF, Shefelbine SJ, et al. Bone mechanical properties and changes with osteoporosis. Injury. 2016;47:S11–20.

25. Pajamaki, I., Sievanen, H., Kannus, P. et al. Skeletal effects of estrogen and mechanical loading are structurally distinct. Bone 2008, vol. 43, no. 4, p. 748-757

26. Patel PSD, Shepherd DET, Hukins DWL. The effect of screw insertion angle and thread type on the pullout strength of bone screws in normal and osteoporotic cancellous bone models. *Med Eng Phys*, 2010, 32: 822–828.

27. Reznikov N, Shahar R, Weiner S. Three-dimensional structure of human lamellar bone: the presence of two different materials and new insights into the hierarchical organization. *Bone*, 2014, 59: 93–104.

28. Rho, J.Y.; Kuhn-Spearing, L.; Zioupos, P. Mechanical properties and the hierarchical structure of bone. Med. Eng. Phys. 1998, 20, 92–102.

29. Reitman CA, Nguyen L, Fogel GR. Biomechanical evaluation of relationship of screw pullout strength, insertional torque, and bone mineral density in the cervical spine. *J Spinal Disord Tech*, 2004, 17: 306–311.

30. Shinohara R, Ueda K, Watanabe F. Influence of the difference between implant body and screw materials on abutment screw loosening. *Dent Mater J* 2019, 38: 150–156.

31. Svedbom A, Hernlund E, Ivergard M, et al. Osteoporosis in the European Union: a compendium of country-specific reports. Arch Osteoporos. 2013;8:137.

32. Tartari, T.; Bachmann, L.; Maliza, A.G.A.; Andrade, F.B.; Duarte, M.A.H.; Bramante, C.M. Tissue dissolution and modifications in dentin composition by different sodium hypochlorite concentrations. J. Appl. Oral Sci. 2016, 24, 291–298

33. Tingart MJ, Lehtinen J, Zurakowski D, Warner JJP, Apreleva M. Proximal humeral fractures: regional differences in bone mineral density of the humeral head affect the fixation strength of cancellous screws. *J Shoulder Elbow Surg*, 2006, 15: 620–624.

34. Viguet-Carrin, S.; Garnero, P.; Delmas, P.D. The role of collagen in bone strength. Osteoporos. Int. 2006, 17, 319–336

35. Walden SJ, Evans SL, Mulville J. Changes in Vickers hardness during the decomposition of bone: possibilities for forensic anthropology. *J Mech Behav Biomed Mater*, 2017, 65: 672–678.

36. Weiner, S.; Wagner, H.D. The material bone: Structure-mechanical function relations. Annu. Rev. Mater. Sci. 1998, 28, 271–298.

37. Wu, W.W.; Zhu, Y.B.; Chen, W.; Li, S.; Yin, B.; Wang, J.Z.; Zhang, X.J.; Liu, G.B.; Hu, Z.S.; Zhang, Y.Z. Bone Hardness of Different Anatomical Regions of Human Radius and its Impact on the Pullout Strength of Screws. Orthop. Surg. 2019, 11, 270–276

38. Yin, B.; Guo, J.L.; Wang, J.Z.; Li, S.; Liu, Y.K.; Zhang, Y.Z. Bone Material Properties of Human Phalanges Using Vickers Indentation. Orthop. Surg. 2019, 11, 487–492.

39. Zioupos, P., Currey, JD. and Casinos, A. Exploring the effects of hyper mineralisation in bone tissue by using an extreme biological example. Connective Tissue Research 2000, vol. 41, no. 3, p. 229-248.

40. Zysset, P.K.; Guo, X.E.; Hoffler, C.E.; Moore, K.E.; Goldstein, S.A. Elastic modulus and hardness of cortical and trabecular bone lamellae measured by nanoindentation in the human femur. J. Biomech. 1999, 32, 1005–1012.

Online sources- https://my.clevelandclinic.org/

https://www.ncbi.nlm.nih.gov/

https://pubmed.ncbi.nlm.nih.gov/

https://g.co/kgs/n7WNMNhttps://www.researchgate.net/search/researcher?q=