

CORRELATION BETWEEN MICRO-HARDNESS AND MINERAL CONTENT IN THE HEALTHY TOOTH ENAMEL OF HUMANS BELONGING TO DIFFERENT AGE GROUPS

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ABSTRACT

Objective: To find a linear relationship between micro-hardness and mineral content of the healthy tooth enamel which is particularly important in the clinical context.

Study Design: In-vitro experimental study.

Place and Duration of Study: This study was carried out at the Pakistan Institute of Engineering and Applied Sciences (PIEAS), from Jan 2019 to Mar 2019.

Methodology: This study was performed in-vitro in an experimental research laboratory with a controlled time series design. A total of 32 upper central incisors belonging to different age groups ranging from 21-60 years were extracted and categorized into four groups. The groups were named as A=21-30 years, B=31-40 years, C=41-50 years and D=51-60 years. The teeth of different age groups were utilized to make enamel specimens of 4 mm x 6 mm in poly-acrylic cylinders of 2 cm width and 3 cm height. These enamel specimens were finely polished. The enamel specimens were then evaluated for micro-hardness utilizing a Vickers micro-hardness tester. Eventually, the mineral content was analyzed with the help of energy dispersive x-ray spectroscopy. After that, the data attained was then assessed with a one-way analysis of variance.

Results: Tooth enamel of the younger age group ranging from 21-30 years exhibited the lowest Vickers micro-hardness and the mineral content. The level of Vickers micro-hardness and mineral content increased accordingly with age and was found to be highest in the tooth enamel of older age group ranging from 51-60 years.

Conclusion: The results demonstrated that the hardness of tooth enamel increases with age and is directly related to the mineral content.

Keywords: Enamel specimens, Energy dispersive x-ray spectroscopy, Mineral content, Vickers micro-hardness tester.

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INTRODUCTION

The tooth is one of the most substantial structures in the human body and consists of four tissues namely enamel, dentine, cementum and pulp¹.

Enamel in the tooth structure provides protection to the tooth crown as it is the hardest tissue in the human body and helps in achieving the functions of a tooth like mastication. This enamel layer has a variable thickness on the surface of the crown. Enamel layer is highly mineralized and consists of 96% by weight of inorganic material. It also contains 1% organic

material and 3% water. Calcium phosphate is the inorganic component mainly and is present in the enamel as hydroxyapatite crystals². Two groups of proteins namely amelogenins and non-amelogenins constitute the organic component in the developing enamel. Non-amelogenins are present in much smaller amounts².

It also consists of carbon (C), magnesium (Mg), sodium (Na), fluoride (F), potassium (K), zinc (Zn), lead (Pb), nitrogen (N), iron (Fe) and oxygen (O) as well as numerous other trace elements. Enamel is avascular and lacks a nerve supply. Moreover, it cannot be renewed. However, it is not a static tissue, and it can undergo some changes in mineralisation.

Furuhata and Yamamoto added various phenomena at different times that may be

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expedient in defining an age. They linked an increase in the specific gravity of teeth with aging especially after the period of twenty-five years. They further pointed out that the process of attrition, progresses with age and the tissues constituting the tooth namely enamel, dentin, and cementum harden with the increasing age³.

Several studies have established that the process of aging may affect the physical properties of enamel⁴. Because this process may influence the mineral concentrations of dental enamel, the mineral density of tooth enamel in younger age groups and older age groups was compared by He Bing and their colleagues⁵. The results depicted variations in the mineral density in the outer layer of enamel between the younger and older age groups. A large amount of mineral content in the enamel makes up for its strength and brittleness⁶.

Tooth enamel is the hardest biological structure present in the human body. This property allows the enamel to persist in the oral environment. Enamel hardness has been assessed previously and it has been used as an indicator to evaluate some of the properties of the tooth such as susceptibility to caries, resistance to wear, propagation of cracks and enamel mineralization. It has been noticed that teeth with lower hardness values are prone to demineralization, caries and crack development⁷.

The hardness of enamel varies in different individuals and in different teeth of the same individual. The factors such as age of the individual, orientation of prisms, size of crystals, amount of organic and inorganic content, crystallinity and water content affect the enamel hardness to a certain extent. Various methods can be utilized to assess the hardness of the enamel. Some of the most commonly used methods are Vickers and Knoop micro-hardness tests. However, an assessment through Vickers micro-hardness tester is much more convenient⁷.

It is prudent to note that the permeability of enamel decreases with age due to the deposition of minerals into the enamel from the saliva.

Reasoning from this fact, we can calculate that the degree of enamel maturity is by its permeability⁸. The tooth enamel experiences multi-directional stresses and withstands multi-million chewing cycles while protecting the dentin and pulp from loss due to mechanical overload and exposure to the harsh chemical environment of the oral cavity. Enamel mineralisation is a valuable property that is positively correlated with the mechanical behaviour of other tissues such as bone and teeth^{9,10}.

A reduction in mineral content of enamel may lead to many dental ailments. For example molar-incisor hypo mineralization may increase the sensitivity of the tooth to hot and cold food items. Thus, these thermal changes result in the failure of the restoration and structure of enamel. Furthermore, previous studies have established a link between the concentration of mineral content in the enamel and susceptibility to carious lesions¹¹.

This study aimed to evaluate the differences in micro-hardness and mineral content of healthy enamel among different age groups ranging from 21-60 years in Pakistan as there appears to be a deficiency of literature regarding age.

METHODOLOGY

This experimental study was carried out at PIEAS over a period of three months. A total of 32 periodontally involved upper central incisors of different age groups i.e. between 21-60 years were randomly collected from different dental hospitals of Pakistan over a period of 4 months. The research protocol was submitted for prior approval to the Board of Advanced and Secondary Education Research (BASR) and the ethics committee of Riphah International University.

Teeth included in this study were devoid of cracks and carious lesions. Moreover, no pre-treatments were performed on these teeth with any chemical agents such as hydrogen peroxide. The labial surfaces of the central incisors used in this study were intact and free from any defects. Teeth with any defects such as cracks, caries,

restorations and fractures were excluded from this study.

A total of 32 extracted central incisors belonging to different age groups were stored in thymol solution (Buffered 0.1% pH 7.00) for one week. Hard & soft deposits were removed with an ultrasonic scaler (Woodpecker), and the teeth were cleaned with prophylaxis paste in slow hand piece.

All 32 teeth of different ages were subdivided into four groups ranging from 21-60 years having 8 teeth in each group. The groups were named as A=21-30 years, B=31-40 years, C=41-50

and 0.05 um alumina polishing paste (allied high tech product, inc, USA) in a rotary polishing machine (J.p.s etudes constructions; 27 rue clock, 92 lucky 737.07.38). The surfaces of enamel specimens were verified utilising a reflection microscope (Nikon, Japan 514770) at a 10 x magnification. The enamel specimens presenting cracks and imperfections on the surface were discarded.

The specimens were immersed in de-ionised water in a beaker and placed in an ultrasonic bath (Ultrasonic cleaner Branson, a Smith Kline company, USA) for about 10 minutes and then stored in artificial saliva at 37°C throughout the experiment. Enamel specimens were embedded

Table-I: Comparison of mean values of vickers micro-hardness for all age groups (n=32).

Age Group	Micro-Hardness Levels (VHN)	Standard Deviation (VHN)	p-value
21-30 Years	320.300	1.147	0.000
31-40 Years	333.200	1.472	0.000
41-50 Years	344.743	3.181	0.000
51-60 Years	358.700	0.936	0.000
Total	339.236	14.550	0.000

Table-II: Comparison of mean differences in mineral content (n=32) in different age groups.

Age Group	Na K Levels ± SD	Mg K Levels ± SD	P K Levels ± SD	CaK Levels ± SD	C K Levels ± SD	N K Levels ± SD	O K Levels ± SD
21-30 Years	0.58 ± 0.06	0.21 ± 0.08	15.39 ± 1.67	32.13 ± 2.84	19.17 ± 0.12	6.32 ± 0.25	32.95 ± 0.74
31-40 Years	0.70 ± 0.04	0.24 ± 0.06	16.1 ± 2.12	33.16 ± 5.47	16.71 ± 0.74	5.37 ± 0.28	30.3 ± 0.79
41-50 Years	0.85 ± 0.06	0.27 ± 0.06	17.05 ± 1.78	41.55 ± 2.96	14.24 ± 1.23	4.42 ± 0.27	27.63 ± 0.42
51-60 Years	0.94 ± 0.24	0.3 ± 0.03	18.29 ± 1.56	48.19 ± 1.91	11.77 ± 0.51	3.47 ± 0.35	25 ± 0.38
Mean Difference	0.79 ± 0.15	0.26 ± 0.06	16.71 ± 2.02	38.76 ± 7.47	15.47 ± 2.9	4.9 ± 1.11	28.97 ± 3.07
p-value	<0.001	0.046	0.033	<0.001	<0.001	<0.001	<0.001

years and D=51-60 years. Then all the teeth were transversely cut at the cemento-enamel junction with the help of a low-speed digital cutting saw (Buehler, Isomet Panasonic, Japan), there by dividing the root and coronal portions. The coronal halves were then cut longitudinally to obtain standardized enamel specimens of 3mm thickness. Enamel specimens of approximately 4 mm (width) x 6 mm (height) were prepared for each age group.

The enamel specimens were then polished with sequential water cooled silicon carbide paper discs (1200, 2400, and 4000 grit; allied high tech product inc, USA) in a rotary polishing machine (Panasonic, Japan). The specimens were finally polished sequentially with 1 um, 0.3um

in self-cure poly-acrylic cylinders of about 2cm width and 3cm height so that 4mm x 6mm windows of enamel surfaces were exposed for the Vickers micro-hardness testing of each specimen belonging to different age groups¹².

Specimens were placed perpendicular to the long axis of the diamond indenter in the Vickers micro-hardness tester (401 Mvd, VD 414, Wolpert group) to record the Vickers micro-hardness. A load of 200g was applied for 15 seconds on each of the specimens. Three indentations were used to each sample at a distance of about 100µm, and an average of these readings was calculated and utilised. The mean micro-hardness values of all the specimens of different age groups were measured and then compared to each other¹³.

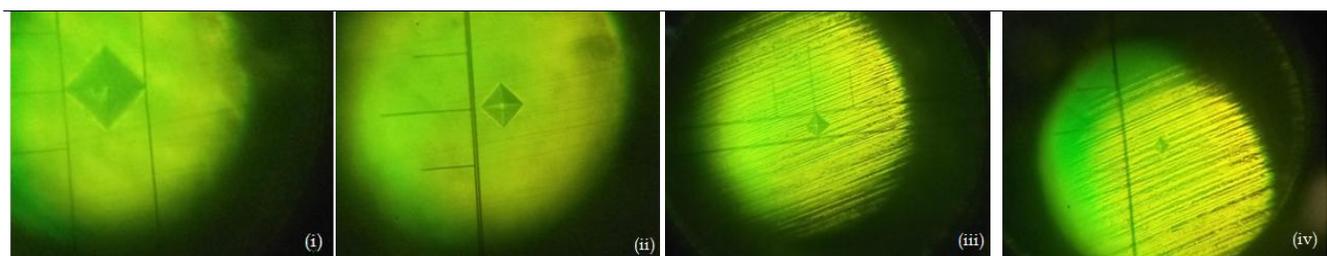
Enamel specimens were removed from the poly-acrylic cylinders and were assessed for their elemental composition utilising the energy dispersive x-ray spectroscopy that is incorporated into the scanning electron microscope (Nova Nano-sem 430; Fei company)^{14,15}. Emphasis was set on the concentration of calcium (Ca), phosphorus (P), sodium (Na), chloride (Cl), fluorine (F), sulphur (S) and magnesium (Mg) present in the samples¹⁶. Data regarding the level of mineral content and composition of specimens in the different age groups were statistically analysed and compared.

RESULTS

A significant difference in the mean micro-

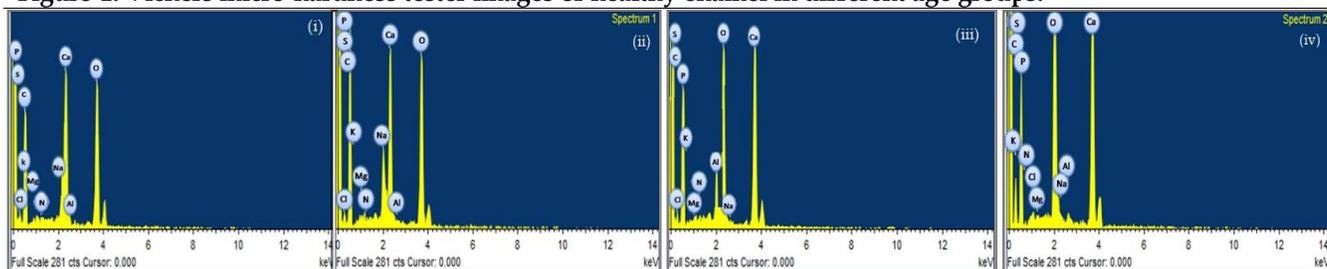
The process of aging also affects the physical properties of enamel making it more brittle and translucent¹⁷. Considering that this process may also influence the mineral concentrations of dental enamel, the mineral densities vary between the younger and older age groups. Differences in the mineral density of the surface layer of enamel in both the age groups were observed in this study¹⁸.

There is a linear relationship between the hardness of the tooth enamel and mineral content of the tooth. Most commonly found ions are Calcium (Ca), Phosphorus (P), Magnesium (Mg) and Sodium (Na). Moreover, considerable emphasis was laid on the levels of calcium (Ca) and



Vickers micro-hardness of healthy enamel depicting different ages. (i) 21-30 years, (ii) 31-40 years, (iii) 41-50 years and (iv) 51-60 years.

Figure-1: Vickers micro-hardness tester images of healthy enamel in different age groups.



X-ray diffraction pattern of healthy enamel depicting different ages. (i) 21-30 years, (ii) 31-40 years, (iii) 41-50 years and (iv) 51-60 years.

Figure-2: X-ray diffraction pattern of healthy enamel in different age groups.

hardness levels was found between all the inter-age micro-hardness values (table-1). The increase in the mean micro-hardness values between the age group ranging from 21-30 years and the age group ranging from 51-60 years' was (38.400, 11.99%).

DISCUSSION

In this study, the level of hardness and mineral content of healthy enamel in the younger age group ranging from 21-30 years was compared to the hardness and mineral content in the older age group ranging from 51-60 years.

Phosphorus (P) because a relationship exists between the loss of calcium from the tooth enamel and a consequent change in the micro-hardness of enamel. The level of calcium, phosphorus, magnesium and sodium in the tooth enamel increases with the increase in the age from younger to the older age groups in the healthy enamel whereas the level of carbon, nitrogen and oxygen decreases with the increase in age from younger to the older age groups in healthy coating. These results co-related with several previous studies. The hardness levels of superficial

enamel increases with the age as observed by Park *et al*. By the age of 55 and above, both hardness and the elastic modulus of the old enamel can increase by over 12% to 16%. It is a direct result of enamel mineralisation as a consequence of aging. This process occurs at the enamel surface which causes a reduction in its permeability and increases its hardness thus making it less porous¹⁷.

When the tooth erupts in the oral cavity, it has not yet undergone post-eruptive maturation due to which it is more susceptible to demineralisation considering it to be porous. Thus enamel maturation is much dependent on enamel permeability, and solubility as these parameters change throughout life. It is because a decrease in water content occurs in the tooth with the process of aging. Kastelic and thus permeability change indicated the maturational effects in human teeth as reported by Bodecher and Lefkowitz³.

Enamel does not undergo further mineral deposition after it has been laid down by the ameloblasts. However, its surface can be grossly modified by the phenomena of abrasion, attrition, erosion, dental caries and at a crystalline level by the process of ion exchange, de-mineralisation and re-mineralisation¹⁵.

In this study, the increasing trend of mean values of Calcium, Phosphorus, Magnesium and sodium was seen in the enamel of younger age group ranging from 21-30 years which was 32.130 ± 2.844 , 15.390 ± 1.666 , 0.210 ± 0.078 , 0.576 ± 0.059 to the tooth enamel in the older age group ranging from 51-60 years where it was 48.188 ± 1.914 , 18.290 ± 1.561 , 0.300 ± 0.034 , 0.942 ± 0.024 . The decreasing trend of mean values of Carbon, Nitrogen and Oxygen was seen in the enamel of younger age group ranging from 21-30 years where it was found to be 19.17 ± 0.12 , 6.32 ± 0.25 , 32.95 ± 0.74 as compared to the enamel of older age group ranging from 51-60 years where it was 11.77 ± 0.51 , 3.47 ± 0.35 , 25.00 ± 0.38 . Which correlated with the previous studies¹⁸.

The mean Vickers micro-hardness of 320.300 ± 1.147 VHN was observed in the younger age

group ranging from 21-30 years that was increased to a mean micro-hardness of 358.700 ± 0.936 VHN in the older age group ranging from 51-60 years as a consequence of aging. There was a 12% increase in the mean Vickers micro-hardness of healthy untreated enamel in the older age group ranging from 51-60 years as compared to the younger age group ranging from 21-30 years which collaborated with the previous studies¹⁷.

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CONCLUSIONS

There was a direct relationship between hardness and the mineral content of healthy enamel among all the age groups under study. An increase in the micro-hardness and mineral content of the tooth enamel specimens was observed with aging. Hence, the Vickers micro-hardness and mineral content were perceived to be the lowest in the healthy enamel of the younger age group ranging from 21-30 years which then increased accordingly with the increase in age and was found to be maximum in the healthy enamel of the older age group ranging from 51-60 years.

These results proved the hypothesis that with the advancing age micro-hardness increases by 12% from young to old age. Furthermore, it also proved that with the increasing age Ca, P, Mg and Na increased from young to old age. On the other hand, with aging C, O and N decreased from young to old age.

CONFLICT OF INTEREST

This study has no conflict of interest to be declared by any author.

REFERENCES

1. Mundra S, Mohan V, Gwyer J, Young N, Franklin SE, Gerhardt LC. Hardness, friction and wear studies on hydrogen peroxide treated bovine teeth. *Tribol Int* 2015; 89(1): 109-18.

2. Chun KJ, Choi HH, Lee JY. Comparison of mechanical property and role between enamel and dentin in the human teeth. *J Dent Biomech* 2014; 5(1): 17587360.
3. Petty WD. Effects of Aging on Human Dentin, as Related to Age Determinations. *Semantic Scholar* 1974; 1(1): 1-7.
4. Arola DD, Gao S, Zhang H, Masri R. The Tooth: Its Structure and Properties. *Dent Clin North Am* 2017; 61(4): 651-68.
5. He B, Huang S, Zhang C, Jing J, Hao Y, Xiao L, et al. Mineral densities and elemental content in different layers of healthy human enamel with varying teeth age. *Arch Oral Biol* 2011; 56(10): 997-1004.
6. Nanci A, TenCate AR. Ten Cate's oral histology development, structure, and function Antonio Nanci, 9th Edition, PhD (McGill), PhD Honoris causa (University of Messina), 2018. Available on <https://www.worldcat.org/title/ten-cates-oral-histology-development-structure-and-function/oclc/1012845679>
7. Gökduman K. Effects of hydrogen peroxide bleaching on human dentin and enamel microstructure and function. Sep 2005, <https://etd.lib.metu.edu.tr/upload/12606645/index.pdf>.
8. Salazar MDPG, Gasga JR. Microhardness and chemical composition of human tooth. *Mater Res* 2003; 6(3): 367-73.
9. Osterhoff G, Morgan EF, Shefelbine SJ, Karim L, McNamara LM, Augat P. Bone mechanical properties and changes with osteoporosis. *Injury* 2016; 47(Suppl-2): S11-20.
10. Lopes FC, Roperto R, Akkus A, Akkus O, Souza-Gabriel AE, Sousa-Neto MD. Effects of different lasers on organic/inorganic ratio of radicular dentin. *Lasers Med Sci* 2016; 31(3): 415-20.
11. Akkus A, Karasik D, Roperto R. Correlation between microhardness and mineral content in healthy human enamel. *J Clin Exp Dent* 2017; 9(4): 569-73.
12. Al-Salehi SK, Hatton PV, Miller CA, Mcleod C, Joiner A. The effect of carbamide peroxide treatment on metal ion release from dental amalgam. *Dent Mater* 2006; 22(10): 948-53.
13. Magalhães JG, Marimoto ÂRK, Torres CRG, Pagani C, Teixeira SC, Barcellos DC. Microhardness change of enamel due to bleaching with in-office bleaching gels of different acidity. *Acta Odontol Scand* 2012; 70(2): 122-26.
14. Relevance C. Enamel and dentin microhardness and chemical composition after experimental light-activated bleaching. *Open Dent* 2015; 1(1): 1-10.
15. Jeon JG, Rosalen PL, Falsetta ML, Koo H. Natural products in caries research: Current (limited) knowledge, challenges and future perspective. *Caries Res* 2011; 45(3): 243-63.
16. Cakir F, Korkmaz Y, Firat E, Oztas S. Chemical analysis of enamel and dentin following the application of three different at-home bleaching systems. *Oper Dent* 2011; 36(5): 529-36.
17. Park S, Wang DH, Zhang D, Romberg E, Arola D. Mechanical properties of human enamel as a function of age and location in the tooth. *J Mater Sci Mater Med* 2008; 19(6): 2317-24.
18. Farah RA, Swain MV, Drummond BK, Cook R, Atieh M. Mineral density of hypomineralised enamel. *J Dent* 2010; 38(1): 50-58.

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