STATISTICAL METHODS IN THE EXPLORATORY STUDY OF DENTINE HARDNESS

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ABSTRACT

The aim of this study was to determine the hardness of dentin using the Vickers hardness test. For dentin were obtained hardness values between 40-78VHN. In the case of dentin, after lifting the load was found a change of indentation geometry, a phenomenon explained by the elastic return of dentin. The return of the material takes place mainly and significantly, in the normal direction of the surface, on which the load is applied. The diagonals of the indentations, remains almost unchanged. The depth of the indentation immediately after penetration and after at an interval of 10 hours showed significant returns, approximately between 20-25%, lower, as the load applied to the hardness test increases, confirming that the dentin has better elastic returns at low loads, and its ability to restructure decreases as local tensions increase.

Key words: dentine, microhardness test

INTRODUCTION

The quality of dentin depends on microstructure, mineral density and especially the particular location of the mineral in relation to the organic structures of the tissue. [1] The hardness of dentin was studied by a number of researchers who used spherical Vickers and Knoop indentors, from which Knoop was the most popular method. [2] Although dentin is modified by physiological processes, such as aging, it is a complex hydrated biological composite structure for which only limited structure-property relationships are available. [3]

Vicker Test allows the determination of the hardness of metallic [4], non-metallic materials, including biological samples (dentin, malt, etc.), being one of the most widespread resistance heads. The experimental device used is the HV-1000 Microdurimeter. The hardness can be calculated with the following relation:

$$HV = \frac{2F\sin(\frac{136^o}{2})}{d^2} \cong 1.854\frac{F}{d^2}$$

F = load measured in Kgf, d = arithmetic mean of the two diagonals d1 and d2 of indentations made in the material.

To measure the hardness of a sample, first it must be well preparated. Thus, the surface of the roof must be flat and clean, free of grease or dirt. The surface must be polished on a granularity ≤0.05.

MATERIAL AND METHODS

Sample preparation: 6 recently extracted third molars were used in the study. They were sectioned transversely and longitudinally with a diamond disc at the contra-angle piece, to include the cusp, the middle waist and the cervical waist in the case of the cross section. After sectioning, the samples were fixed in acrylic resin.

The samples were then prepared using 1200 to 4000 grit silicon carbide discs and washed with water after each preparation. The final preparation was done using 0.05 alumina paste, after which a preparation was performed with diamond tires used in dental practice when sanding the composite. The samples were washed with softened water for 10 minutes.

To determine the hardness, the sample is fixed in the vise of HV-1000 microdurimeter, located on its movable table, so that the surface of the sample is perfectly flat, thus avoiding possible measurement errors due to defective indentation.

Using the optical system of the microdurimeter (10x, 40x objectives), the image is set up to locate the area of interest in the sample, then the rotating head is rotated until the penetrator is brought to the located area. For dentin, the value of the load is set (10gf, 25gf, 50gf) and its duration (10s, 15s, 30s). After the indentation, the 40x objective is returned to position and the image is acquired and the diagonals d1 and d2 are determined in order to measure the hardness of the investigated sample.

Statistical data analysis: Experimental data were processed using the IBM SPSS Statistics 23 program. The procedures used were: descriptive statistics (calculating the measures of central tendency, measures of dispersion, measures of shape, finding and removing outleiers, with the recalculation of the above measures, verification if the sample data follow a normal distribution) graphs (Bar + Error Bar), parametric statistical tests (One-Way ANOVA test, Paired sample t test.

RESULTS AND DISCUSSIONS

Each dentin sample was subjected to loads (Load) of 10gf (A1-A3), 25gf (B1-B3), 50gf (C1-C3) for 10s and 20 impressions were performed for each sample at a distance between them of at least 2.5 times the diagonal of an indentation (fig. 1). Each indentation was carefully analyzed in terms of symmetry. The average Vickers hardness values and the standard deviations, for each dentin sample (A1-A3, B1-B3, C1-C3), are shown in Tables 1-3.

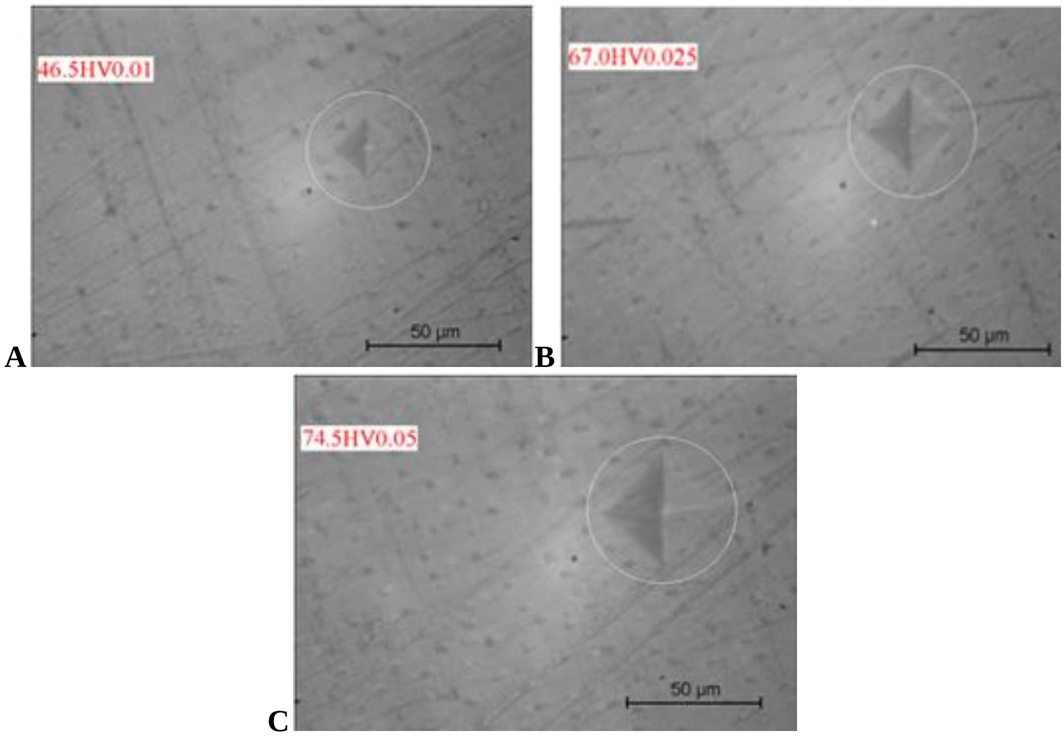

Figure 1. Dentin indentations, A1 sample (Duration = 10s, Load = 10gf), B1 sample (Duration = 10s, Load = 25gf), sample C1 (Timp = 10s, Încărcare = 50gf)

Table 1. Descriptive statistics of (A1-A3) samples: Load = 10gf, Duration = 10s.

Sample	*Count*	*Mean* (Kgf/mm^{-2})	*Std.Dev.*
A1	20	49,37	2,92
A2	20	48,32	3,66
A3	20	48,46	4,17

Table 2. Descriptive statistics of (B1-B3) samples: Load = 25gf, Duration = 10s.

Sample	*Count*	*Mean* (Kgf/mm^{-2})	*Std.Dev.*
B1	20	68,19	1,67
B2	20	68,82	2,10
B3	20	67,92	1,88

Table 3. Descriptive statistics of (C1-C3) samples: Load = 50gf, Duration = 10s.

Sample	*Count*	*Mean* (Kgf/mm^{-2})	*Std.Dev.*
C1	20	73,13	2,05
C2	20	72,60	1,85
C3	20	73,35	2,25

The one-way One-Way ANOVA analysis applied to dentin samples showed that for each load 10gf for 10s, 25gf for 10s or 50gf for 10s there are no significant differences ($F < F_{cr}$, $p > 0.05$) between the mean values of hardness obtained for the 20 indentations made for each sample (Table 4, Fig. 2, 3, 4).

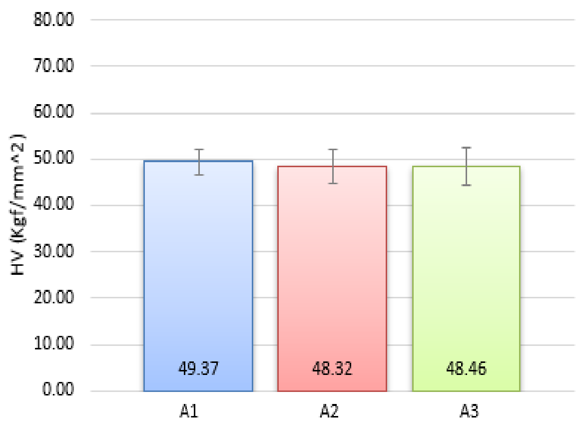

Figure 2. Bar+Error Bar graphic for the average hardness values obtained in the A1-A3 dentin samples for a load of 10 gf applied for 10s.

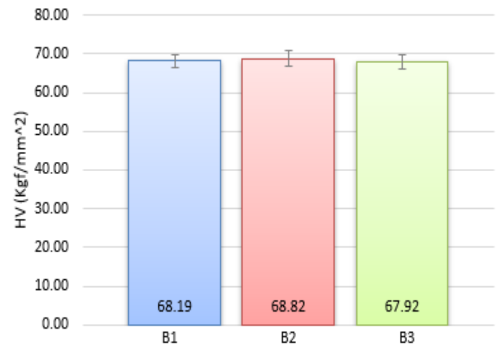

Figura 3. Bar+Error Bar graphic for the average hardness values obtained in dentin samples B1-B3 for a load of 25 gf applied for 10s.

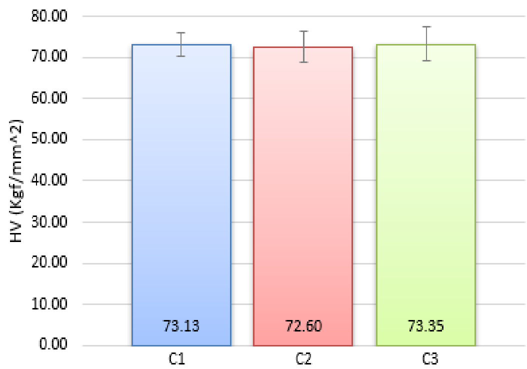

Figura 4. Bar+Error Bar graphic for the average hardness values obtained in the C1-C3 dentin samples for a load of 50 gf applied for 10s.

Taking into account the results previously obtained from the One-Way ANOVA unifactorial analysis we cumulated the data obtained for the (A1-A3) samples subjected to a Load = 10gf, Time = 10s thus forming an extended data set called Sample A. Similarly we proceeded with data for (B1-B3) samples subjected to a Load = 25gf, Time = 10s forming Sample B, respectively for (C1-C3) samples subjected to a Load = 50gf, Time = 10s forming Sample C, in order to compare now average hardness values obtained at different loads. The One-Way ANOVA one-factor analysis applied to the new data sets showed that there are significant differences ($F > Fcr$, $p < 0.05$) between the average hardness values depending on the applied force of 10gf, 25gf and 50gf at the load time of 10s (Table 5, Table 6, Fig. 5).

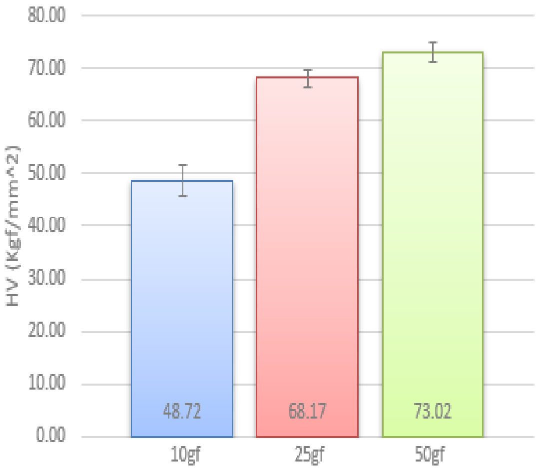

Figura 5. Bar+Error Bar graphic for the average hardness values obtained in dentin compared to 10gf, 25gf, 50gf for 10s.

Table 4. One Way Anova Test results for A1-A3, B1-B3, C1-C3 samples.

Sample	*Load/Time*	*F*	*P-value*	*F crit*
A1 A2 A3	10gf/10s	0,49	0,614	3,159
B1 B2 B3	25gf/10s	0,02	0,884	3,159
C1 C2 C3	50gf/10s	0,72	0,491	3,159

Table 5. Descriptive statistics of (A, B, C) samples: Load: 10gf, 25gf, 50gf for de 10s.

Sample	*Count*	*Mean* (Kgf/mm^{-2})	*Std.Dev.*
A	60	48,72	3,59
B	60	68,17	1,98
C	60	73,02	2,10

Table 6. One Way Anova Test results for A, B, C samples.

Sample	*Load/Time*	*F*	*P-value*	*F crit*
A B C	10gf, 25gf, 50gf /10s	1712,97	0,000	3,047

The indentations made in the dentin were carefully analyzed in terms of their symmetry. Ideally, the values of the two diagonals d1 and d2 measured using the microdurimeter reflection microscope should be equal if the surface of the material has been well prepared for the measurements. Unfortunately, this does not always happen.

To check if there are size differences between the diagonals d1 and d2 of the impressions that cause problems in calculating the hardness of the sample we used a graphical procedure called Bland & Altman Plot. This procedure establishes the concordance between two methods of analysis, in this case, between the measured values of the diagonal d1 and the measured values of the diagonal d2 which do not normally have to differ from each other for each impression. The method consists in the graphical representation of the difference between the values of the two diagonals (d1 – d2) as a function of the arithmetic mean of the respective values (Mean of d1 and d2) for each imprint. The procedure allows the evaluation of the systematic and / or proportional differences between the measured values of the diagonal d1 and the measured values of the diagonal d2, respectively the identification of possible outliers.

For a load of 10gf for 10s, the mean of the differences $\bar{d}$ (bias) between the measured values of the diagonal d1 and the measured values of the diagonal d2 for each individual impression is -0.1661µm, with a standard deviation (s) of 1.0054 µm. It was found that the mean value of the differences $\bar{d}$ does not differ significantly from the ideal bias (zero value): $p = 0.2056 > \alpha = 0.05$ and the limits of the calculated concordance interval are L_i = -2.13 µm and L_s = 1.80 µm (Fig. 6).

For a load of 25gf for 10s, the average of the differences $\bar{d}$ (bias) between the measured values of the diagonal d1 and the measured values of the diagonal d2 for each impression is -0.2547µm, with a standard deviation (s) of 1.0770µm. It was found that the mean value of the differences $\bar{d}$ does not differ significantly from the ideal bias (zero value): $p = 0.1720 > \alpha = 0.05$ and the limits of the calculated concordance interval are L_i = -2.36 µm and L_s = 1.85 µm (Fig. 7).

For a 50gf load for 10s, the average of the differences $\bar{d}$ (bias) between the measured values of the diagonal d1 and the measured values of the diagonal d2 for each impression is 0.2015µm, with a standard deviation (s) of 1.2026 µm. It was found that the mean value of the differences $\bar{d}$ does not differ significantly from the ideal bias (zero value): $p = 0.1955 > \alpha = 0.05$ and the limits of the calculated concordance interval are L_i = -2.15 µm and L_s = 2.55 µm (Fig.8).

Since no problem-solving outliers were identified, respectively graphically, we cannot speak of systematic and / or proportional differences between the two methods, and the concordance limits (L_i, L_s) are small and do not create problems, we can conclude that the measured values of the diagonal d1 and the measured values of the diagonal d2 are concordant.

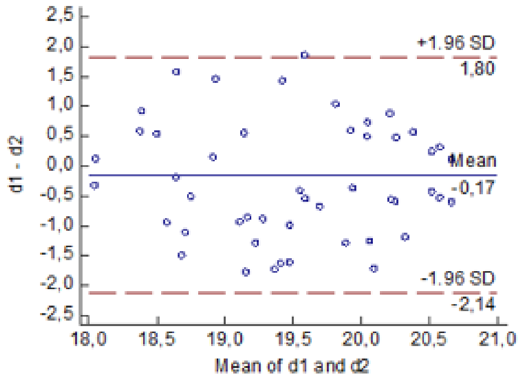

Figura 6. Bland-Altman plot graphic for the measured values of the diagonals d_1 and d_2 at a load of 10gf for 10s.

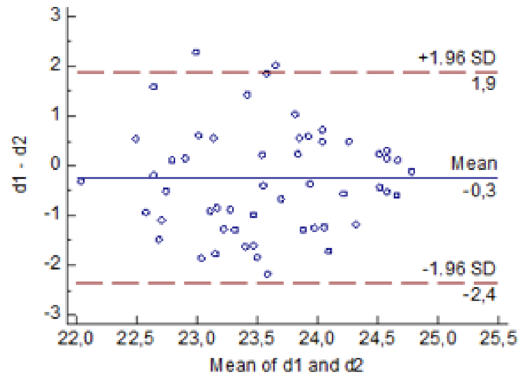

Figura 7. Bland-Altman plot graphic for the measured values of the diagonals d_1 and d_2 at a load of 25gf for 10s.

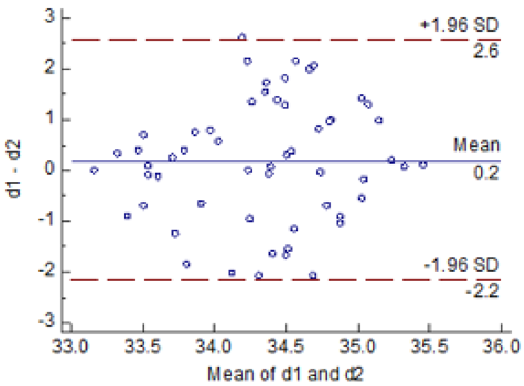

Figura 8. Bland-Altman plot graphic for the measured values of the diagonals d_1 and d_2 at a load of 50gf for 10s.

In the case of dentin, after the lifting of the load, was found the modification of the impression geometry, a phenomenon explained by the elastic return of the dentin.
In order to be able to take into account the elastic return of the material when stating a micro-hardness value measured by

impression, it is necessary to define an indicator called Elastic Recovery Index.

$$I_{re} = \frac{H_t - H_{măs}}{H_t} \cdot 100\%$$

Because the size of the diagonals, directly involved in the calculation of the HV hardness index, changes the least during the elastic return, it was proposed to normalize this value using the elastic return index, calculated in relation to the depth of the impression, for which significant returns were observed. up to 50%. As proof of the nonlinearity between the hardness of the material and its elastic recovery, fractional powers of 4th order are used:

$$HV_n = \frac{1{,}854 \cdot P}{d^2} \cdot \sqrt[4]{I_{er} \cdot C}$$

C is a constant whose value is specified in the literature C = 2.5, and P is the load on the measurement, and d is the arithmetic mean of the measured diagonals.

Table 7. Elastic Recovery Index (%), HV și HVn values after normalisation.

Load		N	Minim	Maxim	Medie	Dev.Std.
10gf	HV (Kgf/mm²)	60	43.42	56.94	48.72	3.59
	HVn (Kgf/mm²)	60	41.23	54.83	46.72	2.13
	Ire (%)	60	24.34	41.09	32.25	3.82
25gf	HV (Kgf/mm²)	60	66.84	69.31	68.17	1.98
	HVn (Kgf/mm²)	60	57.36	67.84	63.90	2.19
	Ire (%)	60	22.51	35.01	28.63	2.41
50gf	HV (Kgf/mm²)	60	68.500	76.61	73.03	2.10
	HVn (Kgf/mm²)	60	57.423	66.18	61.47	1.16
	Ire (%)	60	15.435	22.03	18.46	1.26

The returns are higher at lower loads. By applying the Independent Samples Test, it is observed that between the measured hardness values HV (Kgf / mm-2) and the normalized values HVn (Kgf / mm-2), taking into account the Return Index elastic I_{re} for each value of the applied indentation force (10gf, 25gf and 50gf) at 10s, there are significant differences p (Sig.) <0.001.

The majority of hardness measurements on dentin have been made with microindentation techniques. Reported values for hardness of dentin range from about 250 to 800 MPa depending upon the location of measurement with respect to the enamel and the pulp. [5-7] Ou results of microhardness measurements were confirmed by the data from microscopic studies [8,9].

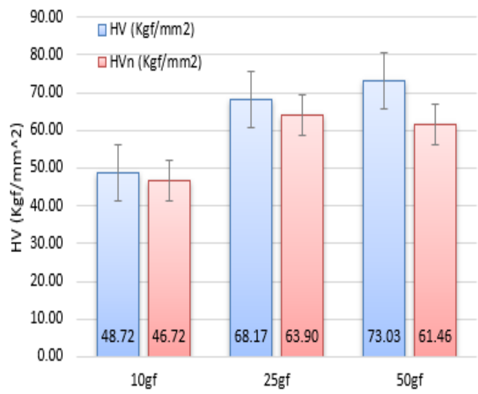

Figure 9. HV si HVn values. Loadings: 10gf, 25gf and 50gf.

CONCLUSIONS

1. Hardness values between 40-78VHN for dentin were obtained, results that were in accordance with the literature.

2. The one-way analysis of One-Way ANOVA applied data sets showed that there are significant differences ($F > F_{cr}$, $p < 0.05$) between the average hardness values depending on the applied load force of 10gf, 25gf and 50gf at the duration of 10s charge.

3. After lifting the load, the modification of the impression geometry was found, a phenomenon explained by the elastic return of the dentin.

4. Statistical methods are mandatory for a complete study of dentine hardness.

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